Special Issue
Geriatric Pharmacotherapy

Guest Editor
Tommaso Cassano

Reviews
- Nutraceutical intervention in ageing brain
- Mechanisms and potential treatments for declining olfactory function and neurogenesis in the ageing brain
- From redox proteomics to clinical practice: search for therapeutic targets
- Extending lifespan through autophagy stimulation: a future perspective
- Bisphosphonates and osteoarthritis

Clinical Observations in Geriatrics
- Sensible prescribing for older adults: illustrated cases

Geriatrics and Gerontology Elsewhere
- The treatment of the elderly is a tricky and hard job
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Geriatrics and Gerontology Elsewhere

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Human brain ageing is associated with a number of specific neurobiological changes. As we age, the reduced neurogenesis in specific brain areas contributes to neural loss and brain shrinkage with an increased incidence of stroke, white matter lesions, and dementia. With age, myelin loss contributes to the decline of sensorimotor and cognitive processes. Moreover, β-amyloid storage, neurofibrillary tangle formation, lipofuscin accumulation, are well known physio-pathological age-related disorders. In neurons, ageing-related metabolic changes involved a switch from aerobic glycolysis to oxidative phosphorylation. Mitochondrial dysfunction and oxidative stress are two damage mechanisms that play a central role in brain ageing. Dietary interventions have been identified as potential means to prevent biological ageing and the related cognitive decline. Increased consumption of specific nutrients such as polyphenols, fish and seafood, vitamins and oligoelements has demonstrated protective effects by targeting specific cellular markers and cellular functions. The work presented here describes patho-physiological alterations associated with brain ageing, with insights into the cellular mechanisms underlying this process. We also review recent relevant experimental and clinical data regarding the effects of supplemental substances (i.e., polyphenols, vitamins, oligoelements, ω-3 polyunsaturated fatty acids) that have demonstrated encouraging therapeutic properties on neurodegenerative processes implicated with brain ageing.

**Key words:** Ageing, Brain, Alzheimer’s disease, Nutraceuticals, Oxidative stress, Parkinson’s disease

**INTRODUCTION**

The human brain is constituted by a multitude of cell types each of which further divided into additional subclasses with distinct morphological and molecular differences, and different degrees of specialization. These cells include resident innate immune cells, the microglia, as well as other glial cells of support, such as astrocytes and oligodendrocytes, and highly differentiated cells, the neurons. Neurons have a significant homeostatic control of the essential physiological functions like propagation and generation of electrical and chemical signals, while glia function mainly to modulate neural functions. Insults and injuries that affect this homeostatic maintenance are observed during ageing. The connection between these modifications and the onset and progression of age-related diseases is profound as the incidence of such diseases like neurodegenerative disorders rise steeply with age. This raises the possibility to reduce or postpone the incidence of these pathologies by targeting ageing. Molecular, cellular, anatomical, and neurochemical changes are all associated with ageing and age-associated diseases. Understanding the molecular mechanism underlying these processes could provide a wealth of opportunities for the development of anti-ageing therapies.
STRUCTURAL AND METABOLIC CHANGES IN BRAIN DURING AGEING

The effects of ageing on the brain include specific structural changes, such as thinning of the cortex and reduced volume. Thickening of the arachnoid and prominence of the arachnoid granulations in the meninges as well as increased ventricular volume have been described 2. As we age, the volume of the brain and/or its weight decline 3. The shrinking of the grey matter can be due to different aspects such as death of neuronal cells and/or decline in neuronal volume, decrease in dendritic arbour, spines and synapses. Selective loss of neurons in specific neuroanatomical areas like hippocampus and prefrontal cortex has been described by several authors 4. The most substantial shrinkage is observed in the prefrontal cortex, where the evidence of a relation between volume shrinkage and ageing deficits in executive and working memory function has been found 4. Neuronal loss is closely associated with microglia-mediated neuroinflammation and post mortem studies have indeed suggested that activated microglia may be present in the ageing brain 5.

Histopathological and imaging studies reveal that brain ageing is associated with the degradation of white matter in specific brain regions. Myelin sheaths deteriorate during normal ageing, with a regional predominance in late myelinating regions of the frontal lobes followed by the temporal lobes 1. White matter loss might represent the predominant neuroanatomic change in normal human ageing. Myelin loss contributes to the cognitive deficits in aged individuals, since myelin is necessary for the efficient and rapid conduction of impulses along axons 6. These and other studies clearly correlate the cognitive impairment observed in the elderly with the decline in grey and white matter structures in the brain, indicating a complex interplay between specific brain structures and cognitive decline.

Studies in rodents demonstrated that ageing is associated with a reduction of hippocampal neurogenesis that is responsible for the decline in memory functions 7. In fact, hippocampus plays an important role in learning and memory and in spatial navigation. However, other studies do not support this correlation and propose that defects in the dendritic structures as well as changes in the rest of the neuronal network may be responsible for the ageing-related cognitive decline 7.

Various transmitter systems are affected by ageing, with implications on cognitive declines that are associated with normal and pathological ageing. During ageing, changes in cholinergic, serotonergic, dopaminergic, noradrenergic and glutamatergic systems render neurons vulnerable to impaired neurotransmissions 8-10. In rats, the expression of ionotropic Gamma-Amino Butyric Acid (GABA) receptors, glutamate dehydrogenase, the rate-limiting enzyme synthesizing GABA, and GABAergic neurons decrease with age 11. GABA is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS), with an important role in regulating neuronal excitability. Age-related cognitive decline could be explained, at least in part, by decrements in GABA inhibitory neurotransmission 12.

Studies also suggest age-related changes in dopamine levels and decline in dopaminergic neuromodulation 8-13. This affects the fidelity of neural information and gives rise to less distinctive neural pattern representations that may underlie various facets of ageing cognitive and, possibly also, sensorimotor phenomena 13.

Serotonin (5-hydroxytryptamine, 5-HT) is a well known monoamine neurotransmitter that modulates/regulates different neural and behavioural activities 14-18. Both the level of 5-HT 19 and 5-HT receptor 20 has been found to decrease during ageing 9. Ageing also reduces the activity of different 5-HT receptor subtypes 21 such as 5-HT2A which is involved in learning 22, neuroendocrine function 23 and sleep behaviour 24. In mammals, decline in serotonin function with ageing may account for several behavioural disturbances, such as sleep, sexual behaviour and mood 19.

Like brain serotonin, alterations of another endogenous dipeptide (β-Ala-L-His), known as carnosine, have been observed during ageing 25. The decreased level of carnosine in olfactory bulb has been associated with the lost of smelling sense 26 27. Aerobic glycolysis, i.e., the exclusive use of glycolysis despite the presence of oxygen, predominates in the developing brain during embryogenesis to provide biosynthetic materials necessary for the proliferation of neuronal and astrocytic stem cells 28. Within certain regions of CNS, aerobic glycolysis seems to be associated with long-term memory formation and the maintenance of long-term potentiation of synaptic strength 29. It is likely that astrocytes can be the main site of aerobic glycolysis into the brain and lactate produced by these cells can be then utilized by mitochondrial neurons as a fuel source for oxidative phosphorylation (OxPhos) (astrocyte-neuron lactate shuttle hypothesis) 30. During ageing, a shift from aerobic glycolysis to OxPhos has been demonstrated in human brain 31, with a consequent loss of cell survival mechanisms that counter pathogenic processes underlying neurodegeneration 28. Thus, aerobic glycolysis is predominately used to support axon growth and myelination during childhood whereas OxPhos is primarily used throughout human lifespan to support synaptic activity with a progressive increase in activity during ageing 28.

However, glucose may be also taken up by neurons and routed preferentially toward the pentose phosphate
pathway for antioxidant production, in addition to bio-
synthetic pathways required for growth and synaptic
remodelling. 32 33.
The delicate balance between glycolysis and cellular
respiration might have a central role in ageing, inflam-
mation and disease. 34-36. Such changes may also be
induced by a reduced input of glucose or oxygen as a
consequence of falls cerebrovascular efficiency in the
ageing brain. Mildly impaired glucose availability in the
synapse may be sufficient to impair cholinergic neu-
rotransmission, being acetylcholine synthesis acutely
sensitive to brain glucose metabolism. 37 38.
Ageing is also associated to changes in sex hormone
status, which in turn can affect cognitive performance. 1.
This aspect is particularly relevant in women at men-
opause. It has been suggested that the decline in es-
trogens at menopause can be responsible in failing
memory and that estrogen therapy may increase dopa-
minergic responsiveness and play a protective part in
Alzheimer’s disease (AD). 1.

AGE-ASSOCIATED
BRAIN PATHOLOGICAL CHANGES

The ageing process can perturb molecular pathways
regulating cellular homeostatic mechanisms, ultimately
promoting disease states. Various changes such as se-
nile plaques, cerebral β-amyloid (Aβ) angiopathy, neu-
rofibrillary tangles, corpora amylacea and mineralization
develop with age in the nervous system of mammals. 39.
Moreover, the age-related increase in blood pressure,
especially due to brain shrinking in specific brain region
including the frontal cortex, can increase the risk of
stroke and ischaemia.

In humans, the incidence of several neurodegenerative
diseases including AD, Parkinson’s disease (PD) and
amyotrophic lateral sclerosis (ALS) raise sharply with
age. 40. Molecular alterations that occur in the nervous
system during normal ageing set the stage for the onset
of such conditions. Several studies, for example, de-
scribed the association between brain ageing and AD.
During normal ageing, elderly individuals can develop
anatomical and molecular features of AD including neu-
ritic plaques and neurofibrillary tangles, still maintaining
their cognitive abilities. 41.

AD is a progressive and irreversible brain disorder with
the highest incidence in people aged over 65. AD pa-
tients experience progressive cognitive and memory
deficits as well as dementia. AD neuropathological hall-
marks include the creation of extra-cellular Aβ peptide
plaques and the intracellular deposition of Tau protein
tangles. Both alterations are frequently observed in
brains over 60 years of age, particularly in the most
vulnerable areas, such as hippocampus and entorhi-
nal cortex. 42. Deposition of the microtubule-associated
protein Tau occurs in AD but also during normal brain
ageing. 43. Tau protein has been identified as a major
constituent of paired helical filaments (PHFs) and AD
is characterized by a major redistribution of the nor-
mal Tau protein pool into PHFs. In neurodegenerative
conditions, hyperphosphorylation of Tau may result in
microtubule destabilization, in addition to the deposi-
tion of toxic aggregates. 39.

PD is the second most common neurodegenerative
disorder after AD, which afflicted nearly 1% of the
population above the age of 60. A relationship between
PD and ageing has been proposed. 45. PD results in
movement, balance, and fine motor control changes as
a consequence of cell death of dopamine-containing
neurons of the substantia nigra pars compacta. 44. The
dopaminergic cell death is induced by reactive oxygen
species (ROS) overproduction and mitochondrial dys-
function among other factors. 45 46.

Pathways that lead to neural death in these conditions
include local inflammatory response, accumulation of
damaged molecules, mitochondrial alterations. 10 40 47.
In ageing, microglia undergoes phenotypic changes
compatible with its activation. Glial activation can lead
to neuroinflammation, which is increasingly accepted
as part of the pathogenesis of neurodegenerative dis-
eases, including AD. 48. Furthermore, during ageing
mitochondria in microglia accumulate DNA oxida-
tive damage an increased ROS production. ROS can
activate the redox-sensitive nuclear factor kappa B,
which promotes more neuroinflammation and cognitive
impairment. 49. Dysfunctions in mitochondrial function
have been associated with an increased activity of spe-
cific enzymes including monoamine oxidase (MAO). 49.
MAO is localized in the mitochondrial outer membrane
and catalyzes the oxidative deamination of biogenic
amines. The reaction accounts for a quantitatively large
production of H2O2 that contributes to an increase in
the steady state concentration of ROS within the mito-
chondrial matrix and cytosol. Thereby a role of mono-
amine oxidation and MAO activity as causative factors
in increased oxidative stress during physiological age-
ing has been underlined. 50. The amount of neuronal
lipofuscin pigment in pyramidal neurons increases with
age. 51. One of the most widely alterations that occur
in neurons during ageing, is the accumulation of dam-
aged molecules which form insoluble aggregates within
the cell. 40. Lipofuscin consists of undigested carbohy-
drates, proteins, and lipids that are present in residual
bodies derived from the lysosomal system. The accu-
mulation of lipofuscin contributes to abnormal intracel-
ular protein accumulation and elimination. 51.
MITOCHONDRIAL DYSFUNCTIONS AND OXIDATIVE STRESS IN AGEING BRAIN

Neurons, as much as all mammalian cells, depend on mitochondria for their survival and ATP production. Mitochondrial function generally declines during ageing due to a number of modifications that compromise the electron transport chain proteins. In old animals, brain mitochondria show reduced rates of electron transfer in complexes I and IV, decreased membrane potential, increased content of oxidation products of phospholipids and proteins, and increased size and fragility. The complex I inactivation together with oxidative damage is usually named “complex I syndrome”, a condition associated with mammalian normal brain ageing and neurodegenerative diseases.

The amount of cardiolipin, an acidic phospholipid associated to mitochondrial membranes, has been shown to decrease with age. This phospholipid is known to have optimal electrical insulating properties, thereby contributing significantly to the transmembrane potential that drives the formation of ATP via ATP synthase. The mitochondrial decline in ATP synthesis and concomitant damage caused by ROS seems to play a key role in ageing.

It was in the 1972 that mitochondria were for the first time associated with the process of ageing. According to this theory, free radicals generated through mitochondrial metabolism can act as causative factors of abnormal function and cell death. Mitochondria are known to be the main site of ROS production. Depending of their amount, ROS can regulate cell survival or apoptosis. Basal level of ROS can act as a second messenger to promote proliferation, whereas higher levels of ROS induce cellular damage and promote apoptosis.

It has been reported that old cell mitochondria do not properly control the generation of ROS and this can in turn generate damage to mitochondrial mtDNA and membranes resulting in alterations of mitochondrial morphology and progressive impairment of mitochondrial functions. Moreover, oxidation renders mtDNA more susceptible to mutation because oxidized bases are misread during replication and this leads to nucleotide substitution. The increased in ROS production is also associated to a decrease age-related in the free radical scavenging system so that increased escape of ROS occurs. Indeed, a decrease in the activity of several ROS-scavenging enzymes including superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase was also measured in the brain of AD patients.

As a consequence of ROS production, different mutations can accumulate with advancing age because of the number of replications of mtDNA that occur independently of the cell cycle. Several studies have found increased levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of the oxidative DNA damage, in mtDNA in the aged brain. High levels of 8-OHdG have been found in both nuclear DNA and mtDNA in post-mortem brains of aged subjects.

Recent data have also described the importance for naturally occurring replication errors, such as large deletions, in the formation of age-associated mtDNA mutations, suggesting that a model solely based on ROS does not properly explain the natural history of mtDNA mutations. Consistent with this hypothesis, other cellular and metabolic mitochondrial alterations may contribute to the ageing process. This enlarges the nutritional targets to a large spectrum of cellular pathways.

Furthermore, it is clear the association between mitochondrial dysfunctions, mainly as decreases in respiratory chain complex activities, with the onset and progression of ageing-related neurodegenerative disorders including PD and AD. Thus, mitochondria-targeted protective compounds, that prevent or minimize mitochondrial dysfunctions, constitute potential therapeutic compounds in the prevention and treatment of these CNS diseases.

NUTRACEUTICAL INTERVENTIONS

Several researches and results of both laboratory and clinical studies suggest that traditional herbs and derived phytochemicals may mitigate the decline in cognitive functions associate with ageing and disorders like dementia, AD and PD. Nutraceutical interventions slow physiological or pathological progression due to their anti-oxidative, anti-inflammatory and anti-amyloidogenic properties. Nutraceuticals also regulate mitochondrial stress, apoptotic factors, free radical scavenging system, and neurotrophic factors by targeting specific cellular targets. Here, we summarized a series of nutraceutical compounds and the main studies reporting the beneficial function of nutraceuticals on brain functions. Main results are listed in Table I.

(Poly)phenols

Phenolic compounds have at least one aromatic ring with several hydroxyl groups attached. These molecules are classified in flavonoids and non-flavonoids, but it is common referred to as (poly)phenols. Based on variations in the saturation of the ring system, their alklylation and/or glycosylation and the hydroxylation...
Table I. Nutraceutical compounds with potential effects on age associated brain alterations.

<table>
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<th>Experimental model</th>
<th>Effect</th>
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<td>Acetyl-L-carnitine</td>
<td>Animal study</td>
<td>Reverses decline in mitochondrial functions.</td>
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<td>Improves clinical features of AD.</td>
<td>183-185</td>
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<td>Improves energy to nerve terminals.</td>
<td>181, 183</td>
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<td>Taurine</td>
<td>Cell cultures</td>
<td>Ameliorates neuroinflammation.</td>
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<td>Mouse</td>
<td>Ameliorates age-dependent decline in spatial memory.</td>
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pattern, flavonoids may be divided into seven subclasses: flavonols, flavones, flavanones, flavanols, anthocyanidins, and isoflavones. Regular and moderate consumption flavonoid-rich plant foods such as wine, tea, berries and cocoa may result in cognitive benefits 74-77. Preclinical and epidemiological studies suggest that (poly)phenols can reverse neurodegenerative pathology and age-related loss in memory, learning and neurocognitive performance. They can act in several ways regulating the peripheral and cerebrovascular blood flow, interacting with intracellular signalling and reducing neuronal damage induced by neurotoxins and neuroinflammation 78-82. Indeed, three main processes may account for flavonoids effects. First, they present a selective interaction with a number of protein kinase and lipid kinase (such as the phosphatidylinositol 3-kinase, PI3K/Akt, and mitogen-activated protein kinase, MAPK) involved in signalling cascades that regulate pro-survival transcription factors and gene expression. This action at the brain site leads to the inhibition of apoptosis caused by neurotoxic species and promotes neuronal survival and differentiation 74. Second, flavonoids induce angiogenesis, and new nerve cell growth in the hippocampus promoting in this way the peripheral and cerebral vascular blood flow 83. Third, these phenolic molecules react directly with and scavenge neurotoxic species and pro-inflammatory agents produced in the brain as a result of both normal and abnormal brain ageing 84.

A discussed topic regards the possibility of flavonoids of exerting their functions in specific brain regions. Some experimental works describe their localization in the brain and their direct neuroprotective and neuro-modulatory actions 85. Flavanones such as hesperetin, naringenin and their in vivo metabolites, some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, have been shown to traverse the blood-brain barrier (BBB) in vitro and in vivo models 85. Their ability to penetrate the BBB is dependent on compound lipophilicity 86 and their interactions with specific efflux transporters expressed in BBB such as the P-glycoprotein 87.

As regards to their action on cognition and memory, studies show a beneficial effect of procyanidin-rich pine bark extracts on perception and oxidative stress in older individuals 77,88 and flavan-3-ol-rich cocoa on cognitive function in young healthy female adults 89. Consuming of flavonoid-rich foods such as grape juice, blueberry or cocoa results in positive effects on cognitive outcome measures 90-92. Moreover, grape, pomegranate, strawberry and blueberry (1%-2% [w/w freeze-dried] juice) affect several aspects of memory and learning 93. Blueberry-derived flavonoids may enhance the efficiency of spatial memory acting on a hippocampal sub-region (the dentate gyrus) most sensitive to ageing; in rats, blueberry-supplementation increase the proliferation of precursor cells in this region 94,95.

Ginkgo biloba extract EGB 761, rich in flavonoids and terpenes, improves cognitive performance in AD patients with mild to moderate cognitive impairment 96. Moreover, the same extract given for short-term period (i.e., 6 weeks) demonstrated its efficacy in enhancing certain neurocognitive functions/processes of cognitively intact old adults 97. In rats, the oral administration of EGB 761 is effective in preventing the appearance of enlarged mitochondria, decreased membrane potential and increased levels of mtDNA damage 98.

Soy extracts contain isoflavones such as genistein, diadzein and glyceitin, which are structurally similar to estrogen. The well reported efficacy of isoflavones on cognition and memory is related to their potential to mimic the action of estrogens in the brain and to influence the synthesis of acetylcholine and neurotrophic factors 99,100. Moreover, supplementation with soy isoflavones in post-menopausal women improves ratings of quality of life and has positive effects on neurocognitive function and mood 101-104.

Resveratrol (3,5,4’-trihydroxytrans-stilbene), is a naturally occurring phenolic compound that is found in a variety of food sources, including wine, soy, peanuts, and peanut products 105,106. Resveratrol can modulate neuroinflammation and neurodegeneration within the brain. The neuroprotective effect of resveratrol has been investigated in several in vitro and in vivo models of AD 107. An in vitro study performed on microglia cells, demonstrated that resveratrol suppressed the mRNA expression of the pro-inflammatory mediator TNF-α, and inhibited the activation of the transcription factor NF-κB. Resveratrol exerts their beneficial effects also promoting the mRNA expression of the anti-inflammatory molecule interleukin-10 (IL-10) 108. Resveratrol has been proposed as promising therapy for PD, because of its neurotrophic effects on dopaminergic neurons and the induction of neurotrophic factors release 109.

Curcumin is a phenolic compound and main constituent of the spice turmeric (Curcuma longa). Curcumin intake is positively related to cognitive function in healthy elderly individuals 110 with evidence that concentrations of Aβ and Tau are lower in populations that consume large amounts of curcumin 111,112. There is also evidence supporting the use of curcumin as a potent antioxidant and anti-inflammatory agent in neurodegenerative conditions and its ability to reduce amyloid plaque burden and disaggregating preformed Aβ fibrils 113-116. (-)-Epigallocatechin-3-gallate (EGCG) is the main and active flavonol in green tea, together with (-)-epigallocatechin, (-)-epicatechin and (-)-epicatechin-3-gallate. Despite the relatively small number of investigations
into the neuroprotective properties of EGCG in humans, epidemiological evidences report that higher consumption of tea/green tea, rich in EGCG, is associated with a reduced risk of neurodegenerative disorders and a lower prevalence of cognitive impairment decreasing the incidence of dementia, AD and PD. The mechanism by which tea chatechins exert their effects are very broad, due to their ability to chelate metal ion, above all iron, to promote an anti-inflammatory response, to facilitate cholinergic transmission and to enhance neurite outgrowth.

**Vitamins**

Ascorbic acid is the reduced form of vitamin C, with important antioxidant properties. A clear link between ascorbic acid deficiency and oxidative-induced neuronal death during neurodegeneration has been demonstrated. Ascorbic acid inhibits ROS production that is generated in neurons during synaptic activity and CNS metabolism. Glial cells function as ascorbic acid reservoir; under brain activity the vitamin is released in the synaptic cleft and taken up by neurons. Moreover, ascorbic acid can switch neuronal metabolism from glucose to lactate consumption to sustain synaptic activity.

Vitamin E is a dietary antioxidant that includes a group of structural-related antioxidants: four tocopherols and tocotrienols. The molecule, when administered to rodents has multiple effects including an improvement of cognitive behaviours, a neuroprotective action in apoE-deficient mice, a reduction of Aβ toxicity in cultured hippocampal neurons, and improved neurological and brain mitochondrial function in ageing rodents.

Although clinical trials have produced controversial results, a study conducted in 2004 has demonstrated that a supplement of vitamin E and vitamin C in combination is associated with reduced prevalence and incidence of AD in an elderly population.

Vitamin D levels are lower in people with AD than normal subjects, and patients with severe deficit in vitamin D have a significant increased risk of cognitive decline over 6 years. Moreover, the hormone 1,25-dihydroxyvitamin D(3) seems to be involved in the maintenance of brain homeostasis, inhibiting in a concentration-dependent manner the production of TNF-α, IL-6, and nitric oxide by stimulated microglial cells. In animal models, α-lipoic acid is able to prevent age-related decline in neurological functions and oxidative damage in brain mitochondria.

Folates are vitamins (B9 family) essential to the development of the CNS. A compromised folic acid status is common in older people due to a reduced dietary intake, and intestinal malabsorption. Cognitive decline, dementia and depression in healthy and neuropsychiatric older individuals have been associated to folate deficiency. The mechanisms underlying include hyperhomocysteinemia, lower occurrence of methylation reactions and tetrahydrobiopterin levels in neurons, increased incorporation of uracil into DNA, and shorter telomere length. No consistent evidences support the effect of folic acid supplementation, alone or in combination with other B vitamins, in the prevention of cognitive decline or neuropsychiatric diseases in old patients.

**Zinc**

Zinc (Zn) is the most abundant trace metal in the brain. Zn deficiency is quite common in elderly due to an inadequate food chewing, intestinal malabsorption, and other psychosocial factors. Despite this, the beneficial effect of Zn supplementation for brain function is controversial. Current investigations suggest that zinc may rapidly induce Aβ aggregation in senile plaque leading to AD development. Moreover, Zn can accumulate in post-synaptic neurons causing neuronal toxicity, cell-death and necrosis. On the contrary, other results support the neuroprotective role of Zn supplementation through the improvement of myelination, and an increased function of Zn-related proteins that contribute to maintain brain compensatory capacity. In addition, Zn metabolism and homeostasis have been suggested to play a major role in many processes related to brain ageing and in the onset and development of age-related neurodegenerative diseases.

**Ω-3 long-chain polyunsaturated fatty acids**

ω-3 PUFA (polyunsaturated fatty acids), including EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are dietary fats found in oily fish and seafood, with small amounts of DHA found in algae. DHA is highly incorporated into the brain with beneficial neuroprotective effects. Several epidemiological studies have established that moderate fish consumption is associated with a reduced risk of impaired cognitive functions in both healthy aged individuals, and patients with AD. Lower DHA and ω-3 PUFA levels were detected in plasma as well as in brain of elderly and AD demented patients where a widespread loss of synaptic contacts takes place in neurons. In longitudinal observation studies, an inverse relation between fish intake/DHA serum concentrations and cognitive impairment was reported. DHA (900 mg/d) administration for 6 month was able to improve learning memory function in age-related cognitive decline in healthy adults. However, it is likely that DHA may delay the onset of age-related cognitive decline, but not in individuals with already diagnosed AD progression.
Encouraging results come from a study in which the combination therapy (i.e., in combination with other dietary compounds or supplements, such as lipoic acid) stabilized or improved memory scores in patients with mild AD. Epidemiological and clinical studies have shown that elevated intake of DHA or higher intakes of food rich in ω-3 PUFAs is associated with reduced AD risk. Dietary DHA could be protective against Aβ production and increases cerebral blood volume in AD mouse model. In an AD mouse model, DHA also protects against dendritic pathology. DHA supplementation was shown to attenuate oxidative stress, specifically lipid peroxidation, and protect against memory loss in various rat models of AD and ageing as well as reduce interneuronal Aβ and Tau accumulation. Animal models of PD supplemented with DHA showed diminished Parkinsonism symptoms and decreased dopaminergic neuronal death, moreover DHA supplementation protects dopaminergic neurons in experimental rat model of PD by targeting inflammatory signalling pathways and by enhancing the expression of two neurotrophic factors glial-derived neurotrophic factor (GDNF) and neurturin (NT-3). A meta-analysis of 21 cohort studies demonstrated that DHA and fishery products are associated with lower risk of cognitive impairment, a lower risk of dementia and AD but without a linear dose-response relation.

**OTHER COMPOUNDS**

Carnosine is a naturally occurring dipeptide (β-Ala-L-His) present in muscle, brain and circulation. Carnosine has antioxidant and antiglycating properties, and neuroprotective effect towards brain diseases caused by oxidative stress. When used in combination with EGCG, L-carnosine prevents neurodegenerative diseases by reducing the neuronal age-associated damage caused by oxidative stress. In addition, carnosine attenuates the ageing-induced increase in the activity of MAO, thus indicating that carnosine may bring about oxidative stress and changes in brain regional serotonin level and hence behaviour. It has been demonstrated that carnosine might be used to control AD for its ability to suppress the toxic effect of Aβ in cultured cells and in transgenic mice. Additionally, carnosine has been shown to suppress mitochondrial dysfunction in animal models of AD. A similar efficacy was observed in PD disease, through the modulation of some biochemical events associated with this pathology.

Creatine is a molecule that is produced both endogenously in the liver, and acquired exogenously from foods. It plays a crucial bioenergetic role in several tissues acting as a spatial energy buffer. In older adults, creatine ingestion can improve quality of life, and ultimately may reduce the disease burden associated with sarcopenia and cognitive dysfunction. Current literature suggests that exogenous creatine supplementation is most efficacious as a treatment paradigm in PD improving patient mood and leading to a smaller dose increase of dopaminergic therapy. Acetyl-L-carnitine (ALC) is a metabolic intermediate that functions as an important trans-mitochondrial membrane transporter of long-chain fatty acids for β-oxidation. ALC is produced through endogenous biosynthesis of lysine and methionine, primarily in the brain, liver, and kidneys, and can also be consumed through foods and supplementation. It has been reported that ALC significantly reverses the age-associated decline of mitochondrial membrane potential and of cardiolipin level. Moreover, ALC increases cellular oxygen consumption, which declines with age, to the level of young rats. Additional studies and reviews showed that ALC can slow pathologic decline in young patients with AD, improve clinical features of AD and, when administered as a component of a vitamin formula, can delay cognitive decline in both early- and late-stage of AD. Chronic administration of ALC to animals induced a positive modulation of the synaptic structural dynamics through improvements in energy provision at nerve terminals.

ALC chronically administered to rats induced a lower age-dependent decline in the mitochondrial oxidation rate of NAD-dependent substrates and in the mitochondrial gene expression of complexes I, IV, and V and of adenosine nucleotide translocase. Taurine or 2-aminoethanesulfonic acid, is an organic compound that is widely distributed in animal tissues. It has been reported that taurine may ameliorate neuroinflammation sustained by activated microglia by switching the microglia from a M1 to a M2 activation status. Moreover, chronic supplementation of taurine to aged mice significantly ameliorated the age-dependent decline in spatial memory.

**CONCLUDING REMARKS**

Normal ageing is associated with deficits in cognitive performance, even in healthy individuals. Neurological and cellular modifications observed during ageing represent risk factors for certain pathological conditions. For this reason, a major understanding of the mechanisms underlying age-related changes in neuronal functions can help to reduce risk for neurological disease. Two principal processes are under investigation for possible and promising therapeutic interventions: neuroinflammation and neurodegeneration. Basic research into the
molecular mechanisms of these two processes allowed to identify specific cellular targets and more general cellular processes and biological functions altered, among these the role that oxidative stress has in ageing brain. In consideration of this, many studies suggest that a balance between pro-oxidants and antioxidants to delay oxidative damage to the brain results in decrements in neuronal and behaviour decays. Approaches such as nutritional interventions have demonstrated to reverse or delay the onset of pathophysiological modifications associated with normal ageing. It is quite likely that this approach would impact on neurodegenerative diseases.

Most of the molecules discussed above emerged as potential nutrients capable of improving health during ageing and neurodegenerative processes. Research has demonstrated that supplementing diets with fruits or vegetables has slowed and, in some cases, even reversed deficits in brain function, motor performance, and memory in old animals and humans. Increasing dietary intake of fruits and vegetables high in antioxidant activity may be an important component of a healthy living strategy designed to maximize neuronal and cognitive functioning into old age. How phytochemicals exert their beneficial effect is not only related to their anti-oxidant activity, but also to their capacity to bind in a selective way with specific intracellular targets. Examples of molecules and pathways targeted by neuroprotective phytochemicals include (i) protein and lipid kinase that regulate pro-survival transcription factors (such as PI3K/Akt and MAPK), (ii) the inactivation of pro-inflammatory mediator TNF-α and NF-κB transcription factor, (iii) acetylcholine and neurotrophic factors induced by hormone-like molecules and finally (iv) mtDNA, Tau and Aβ. These markers regulate a complex set of cellular processes that are important in neuronal plasticity. Despite these results, specific cellular targets remain largely unknown for a large class of nutrients. For this reason, there is yet an unexplored potential for investigating in detail these molecular targets and the molecular concentrations required to obtain these beneficial effects.

Moreover, some of promising molecules exhibited efficacy only in cellular or preclinical models and further in vivo studies are necessary to demonstrate their clinical potential. Nutraceuticals that have demonstrated to protect cells against ageing and age-disorders in human studies include a large number of phytochemical compounds, such as phenols (anthocyanidines, catechins, isoflavones and curcumin) and vitamins (C and E), and some animal derived molecules (PUFAs and creatine). At the moment, they represent an important pool of molecules for the development of therapeutic strategies.

References

17. Dalal A, Poddar MK. Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity


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73 Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr 2009;89:693S-6S.


79 Spencer J, Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. Proc Nutr Soc 2010;69:244-60.


84 Vauzour D. Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects. Oxid Med Cell Longev 2012;2012:1-16.


Reznichenko L, Amit T, Youdim MBH, et al. Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue


Hipkiss AR, Brownson C, Carrier MJ. Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. Mech Ageing Dev 2001;122:1431-45.


The role of olfactory function in maintaining quality of life and as a potential surrogate marker of neurogenic activity in the elderly brain is an underappreciated topic. The olfactory system is complex and is unusual in that its function is maintained by neurogenesis at multiple sites throughout the lifetime of an organism, which in humans may be over 80 years in length. Declines in olfactory function are common with advancing age and this is associated with reductions in the quality of life, the perception of flavour and neurogenesis. These reductions in neurogenesis may simply be a consequence of advancing age or may reflect the nascent development of underlying neurological dysfunction. There are a number of potential therapeutic interventions that can result in a gain of olfactory function, these range from behavioural modification, to dietary supplementation and pharmaceutical intervention but they are all thought to work in part by increasing hippocampal and olfactory neurogenesis. This review discusses the mechanisms underlying olfactory decline in the elderly, reviews a number of potential strategies for improving olfactory function and hypothesizes that increasing the rate of neurogenesis in the ageing brain would improve the quality of life not only by improving olfaction but by improving a range of cognitive processes that are dependent on neurogenesis including mood.

Key words: Olfaction, Ageing, Neurogenesis, Depression, Flavour

INTRODUCTION

A declining ability to detect, identify and correctly interpret the environmental significance of olfactory cues is a common feature of advancing age. This often leads to a decline in the quality of life, reduced environmental awareness and a reduction in the appreciation of “taste” and “flavour”, which is thought to result in a diminished appetite and nutritional neglect. In elderly human populations attempts to definitively link olfactory decline with nutritional neglect have yielded ambiguous results. This is despite the fact that a high incidence of “taste” dysfunction is actually reflective of declines in olfactory function. And that the perception of “flavour” is thought to be generated by a multimodal integration of taste, olfactory, textural and temperature information in a distributed network encompassing the tractus nucleus solitarius, insula, operculum, orbitofrontal, pyriform, and anterior cingulate cortices. Despite a degree of ambiguity concerning the exact relationship between olfactory decline and nutritional neglect a number of studies have shown that declines in olfactory function are associated with an increased risk of mortality in the elderly. One recent study in particular demonstrated that declines in olfactory function were strongly associated with an increased risk of mortality but declines in visual or auditory function were not. It has long been presumed that a decline in olfactory function is part of the normal ageing process that parallels a generalised decline in sensory and cognitive function that occurs in the absence of obvious neurological disease states and impaired olfactory function does predict generalised cognitive decline in the elderly. However, a number of diseases common in the
elderly, are also associated with olfactory dysfunction. These include depression 22-25, Alzheimer’s disease 24-27, prion disease 26, epilepsy 28-29, Parkinson’s disease 30-31, stroke 32-33, physical trauma 34 and diabetes 35-36. In some instances olfactory decline precedes the onset of clinical symptoms by a considerable margin 30-31, suggesting that the olfactory declines seen in “normal” ageing patients may also be contributed to by the nascent development of neurological diseases often seen in the elderly.

The obvious questions that arise from these observations are: What mechanisms contribute to a decline in olfactory function in the “normal” ageing brain? Are there practical strategies for improving olfactory function? And would improving olfactory function lead to an increase in the quality of life and improvements in health in the elderly population? In this review we focus on the importance of a number of mechanisms that contribute to olfactory decline and examine the development of a number of strategies that may lead to a gain in olfactory function and subsequent improvements in the quality of life.

CHANGES IN THE OLFACTORY SYSTEM DURING THE AGEING PROCESS

An appreciation of how the human olfactory system extracts physiologically and behaviourally salient information from the environment is complicated by the fact that most research into olfactory function is conducted in rodents who possess two functional and neuroanatomically distinct olfactory systems. The vomeronasal system in rodents is thought to be primarily responsible for the transmission of inter-species reproductive, social and physiological salient information transmitted by pheromones contained in urine. The alternative main olfactory system is considered to function as an analyser of “general” volatile odours. In primates, the vomeronasal system becomes evolutionarily redundant around the time that trichromic colour vision evolves 37 and as a consequence the human olfactory system relies on the main olfactory system only. This can make extrapolation of data derived from rodents problematic, but the main olfactory and vomeronasal epithelial systems in rodents are functionally very similar in that their receptor neurons are generated from stem cells throughout the life of the organism and differentiate into neurons that express 1-2 single olfactory receptors per cell.

THE OLFACTORY EPITHELIUM

In humans the main olfactory epithelium is localised in the nasal cavity and is positioned around the cribiform plate. Its olfactory receptor neurons are unusual in that they continue to be generated throughout life from stem/ progenitor cells, which differentiate into odorant-detecting olfactory receptor neurons which send projections into the mucous layer to sample the olfactory environment and unmyelinated axons through the cribiform plate to transfer information to the olfactory bulb 38-39. On differentiation, each olfactory receptor neuron expresses one olfactory selected from around 1,000 subtly different G-protein coupled odorant receptor genes 39-41. The proximal cues that influence this selection process are poorly understood but in the functionally similar but closely studied murine vomeronasal system the differentiation and selection process the cue is urinary pheromones, which act to initiate differentiation and influence the receptor selection process 42-43. During ageing a number of changes are thought to occur to the olfactory epithelium primarily the number of differentiating stem cells are reduced 44-47, a process that in rodents occurs in parallel with reductions in the differentiation of vomeronasal stem cells 48-50. This decline in neural differentiation is accompanied with a decline in the expression of Asc11, a pro-neural gene required for generation of olfactory sensory neurons 51 and epidermal growth factor signalling 52. The olfactory epithelium also demonstrates remarkable anatomical 53 and functional 54 regenerative capabilities. Following olfactory lesions anatomical recovery is evident with ~45 days and odorant expression patterns are re-established within 90 days 54. It has been hypothesised that there may be a reduction in the regenerative capacity of the olfactory epithelium as an animal ages and this is supported by evidence in the mouse. The efficacy of the regenerative process following a methimazole-induced lesion at the age of 16 months was considerably lower than that occurring at 10 days or 3 months old, the number of olfactory receptor neurons being around a third of those in the younger groups 55.

Two potential consequences of age that these studies fail to address is that the epigenetic stimuli that induce the transformation of olfactory stem cells into functional receptor neurons are unclear, in the mouse vomeronasal system this stimulus is urinary pheromones 42-43. The mouse vomeronasal organ also expresses 44 main olfactory receptors with the same 1 olfactory receptor per neuron expression pattern displayed in the main olfactory epithelium and the differentiation and olfactory receptor expression of these neurons is also under the control of urinary pheromones 43. In both systems differentiation is reduced with age, in the vomeronasal system this may reflect a reduction in the sensitivity of vomeronasal stem cells to the transformative epigenetic effects of urinary pheromones, and this reduction is sensitivity to the environmental epigenetic cues that influence differentiation may also occur in the main olfactory
Mechanisms and potential treatments for declining olfactory function and neurogenesis in the ageing brain

epithelium. Delivering drugs targeted to the olfactory epithelium by an aerosol is technically very feasible and investigation of the epigenetic mechanisms that control the differentiation of olfactory stem cells into functional olfactory receptor neurons may lead to treatments that support increased olfactory function in the elderly. One other problem that these studies do not address is that olfactory receptor neurons send unmyelinated projection to the glomerular layer of the olfactory bulb, this is a difficult process requiring successful axonal targeting and transport over a considerable distance. Although this is an underexplored topic in the olfactory system, these axonal processes in other models decline considerably with age 56-58 and it is likely that a similar process occurs in the olfactory system.

Primary olfactory processing. The olfactory bulb

The main olfactory system for an underappreciated sensory modality is surprisingly complex and many of the important but subjective experiences with an olfactory component including the perception of “palatability” or “flavour” rely on multisensory integration of olfactory with other inputs including taste, texture and temperature 16, which adds a further layer of complexity to attempts to appreciate olfactory function. A diagram illustrating the basic structure of the main olfactory system is outlined in Figure 1.

Axonal processes from olfactory receptor neurons synapse on the dendrites of the primary projection neurons of the olfactory bulb, the mitral and tufted cells, which together form a structure called the glomerulus. They are excitatory glutaminergic neurons, their dendrites project to a single glomerulus, and as well as receiving input from olfactory receptor neurons they have extensive input from lateral dendrites. Despite being excitatory neurons, the modification of these cells occurs by reciprocal dendrodendritic connections with γ-aminobutyric acid (GABA)ergic granule cells, the activity of which is modulated by centrifugal input from neurons outside the olfactory bulb. The incoming axons from the olfactory receptor neurons also synapse on local GABAergic interneurons (periglomerular cells) that are activated by glutamate released from mitral and tufted cells and provide an inhibitory influence within the glomerulus. Glomeruli are the first synaptic relay in the olfactory pathway and play a basic role in smell perception. A second level of olfactory processing occurs at the granular layer of the olfactory bulb by inhibitory GABAergic neurons that are activated by glutamate released from lateral dendrites of mitral cells, which act to inhibit the network in contrast to enhancement between mitral cells 59. The olfactory bulb is also unusual in that its neurons are replenished throughout life by neuroblasts generated in the sub ventricular zone and transported via the rostral migratory stream 60.

The volume of the olfactory bulb and the number of its laminae are thought to decline with age, but this decline in volume is thought to interact with a number of environmental influences including smoking 61-63. Olfactory bulb volume is a gross measurement but correlations have been found between volume and odour recognition thresholds in the normal ageing brain 62. Changes in the expression of proteins normally thought to be associated with dementia may also increase in the “normal” ageing brain. The proliferation of neurodegenerative fibrillatory tangles occurs in 35.5 to 40.5% of non-demented older patients 64 and the expression of α-synuclein increases as a consequence of ageing in the marmoset brain 65. The efficient function of the olfactory bulb is also dependent on the continuous replenishment of neurons generated in the sub-ventricular zone, a process that is reduced in number and disrupted with regard to spatiotemporal organisation as a consequence of age 66 67.

Figure 1. Simplified structure of the main olfactory system, showing basic interconnectivity and convergence on the frontal cortex.
SECONDARY AND TERTIARY OLFACTORIAL PROCESSING.
A DISTRIBUTED NETWORK

Axons from the olfactory bulb project into a distributed olfactory processing network. The engagement of each part of this network contributes to the processing of olfactory cues, the formation and recall of olfactory memories, the exact contributions of each stage of this network are beyond the scope of this review and are extensively covered elsewhere. The first relays in this network are the anterior olfactory nucleus, olfactory tubercle and the pyriform cortex, (a large planar cortical olfactory area). The functional interconnectivity of the network is complex but olfactory information from these three areas are then routed through a distributed network encompassing the amygdala, entorhinal cortex, hippocampus and the frontal cortex. Many of these areas send projections back to the olfactory bulb where they terminate primarily in the granule layer.

A number of changes occur within this network as a consequence of ageing, these include a reduction in the volume of the hippocampus, amygdala, pyriform cortex and accessory olfactory nucleus. The volume, neuronal organisation and localisation of the Islands of Calleja is altered by advancing age and is thought to contribute to a decline in olfactory function. Neurofibrillary and α-synuclein pathology in the olfactory bulb is associated with olfactory dysfunction in elderly non-demented patients, which is likely to compromise the ability of mitral and tufted cells to project to their appropriate targets. The olfactory system with its dependence on lifelong neural replenishment and the requirement that these new neurons be correctly integrated into existing neural structures is a classic example of extreme neuroplasticity. The mechanisms underlying neuroplasticity are extremely complex and warrant a review of their own but the mechanisms mediating neuroplasticity do decline with age. The expression of brain derived neurotrophic factor also declines with age which is likely to compromise the ability of new neurons to integrate successfully into pre-existing networks.

THE AGEING OLFACTORIAL SYSTEM
AS A THERAPEUTIC TARGET

The olfactory system is unusual in that its function is dependent on the continuous replenishment of neurons throughout the lifetime of an individual. In elderly humans, this process therefore needs to be maintained over an 80 year period, which given the relative decline in other cognitive processes is an impressive feat. This occurs in four levels of the olfactory system: the olfactory epithelium, the olfactory bulb, sub ventricular zone, which provides replacement GABA-ergic interneurons to the olfactory bulb and the dentate gyrus of the hippocampus. Increasing neurogenesis does provide a route to improve olfactory function in the elderly, which also has the advantage that it may improve other indices of life quality, including mood. The importance of olfaction to human wellbeing is underappreciated but some interventions that may improve olfactory function are simple and may provide other benefits.

Behavioural modification; It is well recognised that increases in physical activity promote increased neurogenesis and immature neuron survival in both the hippocampus and sub-ventricular system olfactory system but not in the olfactory bulb itself. A number of different mechanisms have been proposed to mediate the interactions between exercise and adult neurogenesis including brain derived neurotrophic factor, insulin-like growth factor 1 (IGF1), vascular endothelial growth factor (VEGF) and the Wnt signalling pathway. Neurogenesis induced by exercise is also thought to function as one of the experience driven mechanisms that promote neural plasticity. For obvious reasons conclusive attempts to link exercise, neurogenesis and increases in olfactory function in elderly patients are problematic but human epidemiological based studies have demonstrated that exercise does reduce olfactory decline in elderly patients. The olfactory system expressing high levels of plasticity is responsive to its environment. Olfactory environmental enrichment is also associated with increases in neurogenesis, improvements in short-term odour memory and olfactory discrimination. This work has been undertaken in rodents and the practical extrapolation of this approach to elderly humans is unclear. Olfactory function is also decreased in the obese, a phenotype that is likely to interact and be associated with ageing and low activity levels. Hippocampal neurogenesis is thought to be reduced in depression which may contribute to the development of deficits in olfactory function. One effect of antidepressants is that they increase neurogenesis and this is probably contributed too by reductions in stress that occur with an increase in mood. Treating depression in the elderly by alternative non-pharmaceutical means including cognitive behavioural therapies may also contribute to improvements in olfactory function. Pharmacological treatment; a range of pharmacological interventions may induce gains in olfactory function, these range from dietary supplementation to more traditional pharmacological treatments. The consumption of omega-3 fatty acids increases olfactory function, an effect that may be associated...
Mechanisms and potential treatments for declining olfactory function and neurogenesis in the ageing brain

with increased neurogenesis \(^{108}\). A range of other dietary supplements may also promote neurogenesis which as an advantageous side effect may improve olfactory function, these include curcumin \(^{109}\), flavonoids \(^{110,111}\), vitamin A \(^{112}\) and caffeine \(^{113}\).

Olfactory function and neurogenesis is also reduced in depression and it is noteworthy that anti-depressives are thought to work in part by increasing neurogenesis in the dentate gyrus \(^{22-25}\). \(^{103-105}\). However it is thought that they also induce increases in olfactory neurogenesis and olfactory function in rodents \(^{104}\), this is likely to be an effect replicated in humans as recovery from depression is thought to result in gains of olfactory function. \(^{105}\). Other drugs can also induce olfactory neurogenesis and differentiation, these include valproic acid and other histone deacetylase inhibitors used to treat epilepsy \(^{114}\). \(^{115}\). Two other non-pharmacological interventions for depression also increase neurogenesis and increase olfactory function, electroconvulsive therapy and transcranial magnetic stimulation \(^{116-119}\).

CONCLUSIONS

The neural systems supporting olfactory function are complex and are unusual in that they rely on the continuous neurogenesis throughout life. This occurs at four separate sites, the olfactory epithelium \(^{38-43}\), endogenously in the olfactory bulb \(^{61}\), the sub-ventricular zone \(^{60,66}\) and the dentate gyrus \(^{82,83}\). Olfactory function declines with advancing age and this is thought to reflect a reduction in this process \(^{1-14}\). \(^{44-47}\). A range of neurological disorders common in the elderly are also associated with reductions in neurogenesis and a decline in olfactory function may therefore be a surrogate marker or a harbinger of a developing neurological condition \(^{22-35}\). Olfaction is an underappreciated sensory modality in humans but declines in olfactory function are predictive of mortality in the elderly, whilst declines in the more appreciated senses of vision and auditory function are not \(^{17-21}\). This may reflect the reliance of olfaction on neurogenesis, a decline in which may be a robust surrogate marker of declining neurological function. Declines in olfactory function also lead to a reduction in the quality of life including the reduction of “flavour” or “palatability”, which in turn may be reflective of this decline in neurogenesis \(^{10-16}\). There are a number of potential mechanisms by which olfactory function may be improved in the elderly and given olfaction’s reliance on neurogenesis it is unsurprising that many of these are thought to work by improving this function. Olfaction is an underappreciated sensory modality and its role in the maintenance of a high quality of life and as a potential harbinger of mortality in the elderly is an underexplored topic that warrants further investigation. In particular improvement of neurogenesis in the elderly by a variety of approaches may yield improvements in a range of cognitive functions in parallel with olfaction including mood.

References

35 Weiler E. Postnatal development of the rat vomeronasal organ. Chem Senses 30 (Suppl 10) i27-i28.
changes of the regenerative mode in the mouse peripheral olfactory system following olfactotoxic drug methimazole-induced damage. J Comp Neurol 2011;519:2154-74.


66 Seib DR, Martin-Villalba A. Neurogenesis in the normal ageing hippocampus; a mini review. Gerontology 2015;61:327-35.


90 Trejo JL, Llorens-Martin M, Torres-Alemán I. The effects of exercise on spatial learning and anxiety-like behavior are


109 Escanilla O, Mandairon N, Linster C. Odor-reward learning and enrichment have similar effects on odor perception. Physiol Behav 2008;94:621-6.
From redox proteomics to clinical practice: search for therapeutic targets

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Alzheimer disease (AD) is the most common form of dementia in the elderly population, characterized by a gradual deterioration of memory and other cognitive functions. The major pathological characteristics of AD brains are the presence of senile plaques, made of amyloid β-peptide (Aβ), neurofibrillary tangles, composed of hyperphosphorylated tau protein, and neuronal loss. Among putative mechanisms responsible of neurodegeneration, several studies demonstrated the role of oxidative stress as an important factor contributing to the initiation and progression of AD. If from one side disruption of redox balance and increased production of free radicals are likely to be related to mitochondria dysfunction and/or aberrant accumulation of misfolded proteins, on the other side the abnormal accumulation of Aβ and tau proteins appears to promote the redox imbalance. In addition, evidence has suggested that oxidative stress may augment the production and aggregation of Aβ and facilitate the phosphorylation and polymerization of tau, thus forming a vicious cycle that promotes the initiation and progression of AD. Taken together, these findings suggest that therapeutic strategies aimed at preventing/reducing oxidative stress-mediated damage may be effective for the treatment of AD and other neurodegenerative disorders.

Key words: Alzheimer disease, Protein oxidation, Antioxidant, Protein aggregation, Chaperones

ALZHEIMER DISEASE AND OXIDATIVE STRESS: HYPOTHESIS OF NEURODEGENERATION

Alzheimer disease (AD) is the most common neurodegenerative disorders that affect middle- to old-aged individuals, with an incidence rate that increases almost exponentially with increasing age until 85 years of age. Sporadic AD accounts for approximately 95% of all AD cases and is caused by multiple etiological factors, such as gender, brain injury, education, vascular disease, the presence of the apoE4 gene, among others. The majority of AD cases are sporadic and present considerable heterogeneity in terms of risk factor profiles and neuropathological features. AD may be classified into mainly three stages of progression characterized by gradual increase of AD hallmarks starting from preclinical AD (PCAD), to amnestic mild cognitive impairment (MCI) and early AD (EAD). The core clinical features of AD include gradual and progressive decline in memory, executive function, and ability to perform daily activities. However, there is variability among individuals in age of onset, family history, and the appearance of noncognitive symptoms such as behavioral or motor abnormalities. Rates of disease progression and survival also vary considerably among different individuals. Pathologically AD is characterized by the deposition of senile plaques (SPs), neurofibrillary tangles (NFTs), decreased synaptic density and brain atrophy particularly in the hippocampus, amygdala and frontal cortex, that correlate with cognitive and memory deficits. SPs are composed by amyloid β-peptide (Aβ), comprising 39-43 amino acids formed by proteolytic cleavage of amyloid precursor protein (APP), a type I trans-membrane protein, by β-secretase and γ-secretase. Though for a long
time senile plaques have been considered as the primary pathogenic element of AD; recent evidence supports the notion that plaques may be an extra-cellular storage site for cells to deposit excess Aβ, and suggests that smaller aggregate form of Aβ(1-42) oligomers are the main neurotoxic species. Accordingly, experimental data have shown that plaques do not correlate with cognitive dysfunction in AD, but soluble oligomers do. NFTs are composed by tau, a microtubule-associated protein, that once hyperphosphorylated, is increasingly prone to form insoluble aggregates thus loosing its affinity for microtubules. These pathological lesions have been proposed to be the causative factors, and mechanism-based therapies have been developed to target both SP and NFTs. However, with a more comprehensive understanding of the molecular mechanisms involved in the neurodegenerative process, several other pathogenic factors have emerged, including excitotoxicity, calcium impairment, mitochondrial dysfunction, neuroinflammation and oxidative stress. All these mechanisms coexist and likely act in concert affecting each other at multiple levels.

Several data indicate that a dysregulation of redox homeostasis strongly participates in the early stage of AD, activating diverse cellular signaling pathways that trigger the initial toxic events. Among these, increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is observed in AD brain. As well, increased levels of oxidative stress (OS) markers of proteins, lipids, carbohydrates and nucleic acids have been detected in a large number of studies from AD brain and peripheral systems. In parallel, the levels of antioxidant enzymes were found to be decreased in different brain regions from AD patients. Accordingly, age-related memory impairment correlates with a decrease in brain and plasma antioxidants defense mechanisms. One of the most powerful antioxidant is glutathione (GSH), which is responsible for the endogenous redox potential in cells (GSH/GSSG). The role of OS in AD has been extensively investigated and several studies showed elevated levels of OS markers in post-mortem brain from AD patients and its early phases, MCI and early AD. Moreover, in AD and MCI brains, the increased oxidative damage to lipids and proteins coupled with reduced GSH and antioxidant enzyme activities have been shown to be localized to the synapses and correlate with the severity of the disease, suggesting an involvement of OS in synaptic loss. Importantly, many studies show elevation of OS already in MCI, which is proposed as an intermediate state between normal ageing and dementia, indicating that the oxidative damage in AD precedes the onset of the disease. These results suggest that OS may be one of the earliest alterations that occur during the initiation and development of AD.

Since the development of sophisticated proteomics platforms, large-scale studies of protein composition have been performed to study the mechanisms of disease pathogenesis, to characterize novel drug targets and to discover potential diagnostic and prognostic biomarkers. With relevance to the OS hypothesis of neurodegeneration, growing attention is given to analyze oxidative post-translational modifications (PTMs) as it has been demonstrated to alter protein function and to be linked to disease pathology. Investigations of oxidative PTMs, that occur in AD and other neurodegenerative disorders, have been performed with success using focused redox proteomics techniques supporting the potential impact of this approach on the study of neurological disorder and for the identification of therapeutic targets. This review discusses the most relevant redox-proteomics findings obtained in brain and blood from AD and mild cognitive impairment (MCI) patients with the aim to identify putative therapeutic targets. In addition, we discuss therapeutic strategies involving antioxidants and anti-aggregating compounds that may have the potential to prevent/slow the onset and progression of AD.

OXIDIZED PROTEINS SIGNATURE IN BRAIN AND PERIPHERY: POSSIBLE THERAPEUTIC TARGETS

OS is a common feature of several neurodegenerative diseases and redox proteomics approach has the power to identify pathology-specific alterations in different biological samples. A comprehensive redox proteomics analysis of different brain regions from AD, MCI subjects and, with some limitations, from subjects with early AD and preclinical AD is currently available. In parallel, the redox proteomics analysis of blood-based biofluids from AD and MCI has been performed. From these studies, it emerges that protein oxidation highly correlates with the clinical features, pathology and biochemistry of AD. Among the most relevant findings coming from brain tissue studies, it is important to underline that oxidative damage targets proteins involved in energy metabolism, antioxidant response, protein degradation, excitotoxicity, neuronal structure and mitochondrial functions. Oxidative-mediated dysfunctions of these proteins are likely involved in neurodegeneration at various stages of the disorder. By comparing the results obtained by different subjects, from early to moderate and to severe AD, it has been possible to identify some common targets of protein oxidative modification among different
phases of the disease. This approach allows identifying molecular events involved in the prodromal phase of AD, which will eventually participate to the chronic accumulation of cellular deficits ultimately culminating in the loss of cognitive functions.

Among others, redox proteomics data on proteins related to energy metabolism suggest that the impairment of ATP synthesis is a crucial event driving the neurodegenerative process. Indeed, ATP, the cell's energy currency, is extremely important at nerve terminals for normal neurotransmission and decreased ATP levels may lead to loss of synapses and synaptic function, both of which can affect propagation of action potentials and contribute to memory loss.

Interestingly, chaperones such as heat shock proteins (HSPs) use energy from ATP to help misfolded proteins to refold properly. Several HSPs have been found to be oxidatively-modified in AD including HSP90, HSP60 and HSP27 that were also found to be aberrantly induced in MCI compared with age-matched controls. Defective repairing systems may exacerbate protein misfolding and aggregation processes overloading proteasome removal, a condition known to occur in AD.

Though the study of post-mortem brain allowed to identify the molecular mechanisms of neurodegeneration, it should be taken into consideration that i) collection of post-mortem brain is difficult and allows to analyze limited sample size; ii) brain tissue cannot be used for early diagnosis of cognitive decline; iii) the analysis of early asymptomatic disease stage is needed to fully understand all the intrinsic and extrinsic factors involved in AD onset and progression. Thus, in recent years growing studies have been focused to establish a direct link between tissue specific damage and systemic alteration, as well as to identify biochemical markers of brain dysfunction that can be measured in body fluids such as cerebrospinal fluid (CSF), plasma and urine. Diagnostic criteria for AD and MCI are actually based on clinical features allowing only a "probabilistic" diagnosis and exclusion of other types of dementia. This low specificity represents a major limit for the therapeutic management of AD patients and for testing the efficacy of disease-modifying drugs. So far, CSF biomarkers have been focused on the amyloid cascade hypothesis of disease-modifying drugs. So far, CSF biomarkers that can improve the diagnostic and prognostic accuracy of current leading CSF biomarkers. CSF samples from aMCI, AD and control individuals were analyzed using redox proteomics to identify the specific oxidatively modified proteins in AD and MCI compared with controls. We found that the majority of carbonylated proteins identified by mass spectrometry are present early in the progression of AD, i.e., oxidatively modified CSF proteins were already present in MCI compared with controls and remain oxidized in AD, thus suggesting that dysfunction of selected proteins initiate many years before severe dementia is diagnosed.

In parallel, we also investigated the involvement of immune system in AD. Our method allows recognition of natural occurring antibodies by the identification of brain antigen targeted by human IgGs. Collected findings reveal that the alterations of autoantibodies profile both in CSF and serum correlate with disease staging and progression. However, we did not find a strong overlap between CSF and serum suggesting the existence of different immunogenic events. Interestingly, CSF autoantibodies recognized, among others, key players of energy metabolic pathway, including glycolysis and TCA cycle, found oxidatively modified in AD brain studies. These data suggest a potential casual sequence between oxidative damage at brain level, autoantibodies presence in CSF and reduced energy metabolism of AD patients.

Only few clinical two-dimensional electrophoresis-based studies focusing on protein oxidation in AD blood and CSF are currently available. The lack of data is probably due to the difficulty of analysing complex samples, such as biological fluid, with a wide variability among patients.

Proteomics approaches and targeted multi-analyte studies of CSF have been performed and lead to the identification of many proteins that are elevated or reduced in AD compared to cognitively normal controls. A recent study from our group applied targeted proteomics approach to discover putative CSF biomarkers that can improve the diagnostic and prognostic accuracy of current leading CSF biomarkers. CSF samples from aMCI, AD and control individuals were analyzed using redox proteomics to identify the specific oxidatively modified proteins in AD and MCI compared with controls. We found that the majority of carbonylated proteins identified by mass spectrometry are present early in the progression of AD, i.e., oxidatively modified CSF proteins were already present in MCI compared with controls and remain oxidized in AD, thus suggesting that dysfunction of selected proteins initiate many years before severe dementia is diagnosed. In parallel, we also investigated the involvement of immune system in AD. Our method allows recognition of natural occurring antibodies by the identification of brain antigen targeted by human IgGs. Collected findings reveal that the alterations of autoantibodies profile both in CSF and serum correlate with disease staging and progression. However, we did not find a strong overlap between CSF and serum suggesting the existence of different immunogenic events. Interestingly, CSF autoantibodies recognized, among others, key players of energy metabolic pathway, including glycolysis and TCA cycle, found oxidatively modified in AD brain studies. These data suggest a potential casual sequence between oxidative damage at brain level, autoantibodies presence in CSF and reduced energy metabolism of AD patients.

Compared with CSF, blood samples were the object of several studies. Thereafter, as expected, most of the proteomics studies on plasma/serum samples show the oxidation of protein involved in the inflammatory response. However, taking into account the technical and practical limitations in performing proteomics analysis in blood samples, it has been recently demonstrated that haptoglobin (Hp) β chain, among other proteins, was both down regulated and increased oxidative stress and subsequent damage to protein represent a further potential marker of AD development/progression.
events. Interestingly, in addition to its well-established inflammatory role, Hp has a specific capacity to inhibit aggregation/precipitation of a wide variety of proteins induced by different stress conditions. Indeed, when incubated with Aβ peptide Hp inhibits the formation of fibrils. The same considerations can also be applied to another chaperone protein found to be oxidized in human plasma from AD patients, alpha-2 macroglobulin. Redox proteomics data on plasma, when compared to studies on AD brain samples, show a parallelism for the oxidation of protein known as molecular chaperones, extracellular in one case and intracellular in the other. The oxidative modification of proteins with similar function but different compartmentalization suggests that the alteration of chaperone proteins may represent a common feature of both central and peripheral damage in AD. In addition, it was demonstrated that the impairment of the heme degradation pathways, due to oxidative modifications of the main components, heme-oxygenase and biliverdin reductase-A (HO-1/BVR-A), occurs in post-mortem brain from MCI and AD patients as well as in plasma samples. Therefore, HO-1/BVR-A system status in plasma could reproduce the on-going pathology at brain level, suggesting that the analysis of HO-1/BVR-A system in blood-related biofluids may reflect the “oxidative index” of the brain.

**PHARMACOLOGICAL PROSPECTIVE: ANTIOXIDANTS AND ANTI-AGGREGATING COMPOUNDS**

Collectively, results obtained by redox proteomics studies in the brain, blood and CSF from AD patients suggest therapeutic strategies based on antioxidant supplementation and compounds acting on aggregation mechanisms of Aβ and Tau may have the potential to prevent/slow AD neuropathology (Fig. 1). Intriguingly, OS itself seems to influence aggregation processes and “amyloidosis”.

Antioxidant therapy, as one of the promising therapeutic strategies for AD, has been studied for years. Antioxidants comprise both exogenous (natural or synthetic) and endogenous compounds acting in different ways. The natural antioxidant system can be classified into two major groups: enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic or low-molecular-weight antioxidants (LMWAs). As a whole, antioxidants are able to block pro-oxidant enzymes, neutralize radicals, or chelate transition-metal ions that catalyze radical generation. In addition, some antioxidants exert their effects by inducing endogenous antioxidant defenses, up-regulating the expression of redox-sensitive transcriptional factors. Hence, redox homeostasis in cells is derived from a fine-tuning of numerous factors. Considering that OS mediates multiple cellular processes, a therapeutic strategy aimed to prevent/slow OS-induced modifications require molecule/s able to target not only a single mechanism, such as the case of ligand/receptor interaction, but able to act at the crossroad of multiple pathways. Further, the variety of sources and sites of production of free radicals implicates an even higher heterogeneity in the antioxidant response. Moreover, it is important to underline that there is an extensive crosstalk between OS and other key toxic AD events, which amplify the complexity of these phenomena. Nevertheless, OS has recently been proposed to be a common, key element capable of articulating the divergent nature of different pathogenic mechanisms of AD. So far, several antioxidant compounds have been tested as neuroprotectants. Among the most well characterized exogenous compounds, vitamin E (α-tocopherol), vitamin C, and β-carotene are chain-breaking antioxidants that decrease free radical-mediated damage in neuronal cells. For example, vitamin E has been shown to attenuate Aβ-induced toxicity and improve cognitive performance in rodents. Sano and colleagues showed that treatment with α-tocopherol in patients with moderate AD was able to reduce neuronal damage and slow the progression of AD. Further, vitamin E was shown to suppress brain lipid peroxidation and reduce Aβ levels and senile plaque deposition in Tg2576 mice, if administered early prior to the appearance of the pathological hallmarks of AD. However, if vitamin E supplementation was started at a later time point when amyloid plaques deposition is already occurred, no protective effect on the amylodotic phenotype of these animals despite a reduction in brain oxidative stress was observed. As well, the levels of carboxyls and 8-OHdG were reduced after α-tocopherol administration in transgenic mice overexpressing human tau protein.

Vitamin C is a water-soluble antioxidant that inhibits lipid peroxidation and is a major defence against free radicals in the blood. Bagi et al. have shown that chronic vitamin C treatment is able to decrease high levels of isoprostanes, common markers of oxidative damage to cellular lipids, enhance NO bioavailability, restore the regulation of shear stress in arterioles, and normalize systemic blood pressure in methionine diet-induced hyperhomocysteinemia rats. Further, vitamin C reduces α-tocopheroxyl radicals in membranes and LDL to regenerate α-tocopherol and possibly inhibits α-tocopheroxyl radical-mediated propagation. Carotenoid is another lipid-soluble antioxidant that may reduce lipid peroxidation and improve antioxidant status. The most known and studied carotenoid is the β-carotene that is a potent antioxidant able to quench
singlet oxygen rapidly. Taken together, vitamin C, vitamin E, and carotenoids have shown to synergistically interact against lipid peroxidation.

Other promising antioxidants that have some potential therapeutic value in the treatment of neurodegenerative diseases are the mitochondrial-targeted antioxidants such as α-lipoic acid (LA), coenzyme Q10, NADH, Mito Q, Szeto Schiller (SS) peptide, and GSH. Mitochondrial dysfunction has been well demonstrated in many neurodegenerative diseases and mitochondrial fragmentation, altered mitochondrial distribution and also structurally and functional damage of mitochondria has been shown to be involved in the pathogenesis of AD.

LA is the coenzyme of mitochondrial pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, and is able to recycle other antioxidants such as vitamins C and E and glutathione and increase the production of acetylcholine or to act as a chelator of redox-active metals. It has been reported that chronic administration of the LA decreased lipid peroxidation but not Aβ load within the brains of both control and AD mice models, and improved Morris water maze performance in the Tg2576 mouse model but was ineffective at altering cognition in the Y-maze test. Similarly, Farr Sa et al. showed that chronic administration of LA to SAMP8 mice (a model of accelerated ageing) was able to reverse memory impairment and brain OS.

Although promising results have been obtained both by in vitro studies and animal models, no significant neuroprotective effects with antioxidant supplementation have been observed in clinical trials. So far, it is not completely clear whether nutrient therapy is an effective treatment of AD. Lloret et al. showed that vitamin E supplementation was not able to lower plasma OS for half of the AD patients and results from others have suggested that increased intake, either by diet or supplementation, of carotenes or vitamins C and E did not decrease the risk of developing AD (reviewed in). Taken together, these results indicate that antioxidant therapies have been successful in preclinical studies in animal models of AD but little benefit in clinical trials can be achieved. Moreover, Morris et al. reported that higher intake of foods rich in vitamin E may modestly reduce long-term risk of dementia and AD only among individuals without the APOEε4 allele, while dietary intakes of vitamin C, β-carotene, and flavonoids were not associated with dementia risk.

Figure 1. Putative therapeutic strategies to prevent/slow AD neuropathology. Antioxidant supplementation may reduce OS-induced damage in the brain and in the periphery. As well, targeting aggregation mechanism of pathological proteins, Aβ and Tau, has been shown to be a promising approach for the treatment of AD and also for other neurodegenerative diseases caused by protein misfolding.
In the effort to understand the reasons of such failure, the first aspect to be considered is the design of trials that set up the treatment when clinical diagnosis of dementia is already significant \(^6^0\) \(^6^1\). Another major limit when administrating antioxidants is their poor bioavailability and low permeability across the blood-brain barrier, that needs the development of new delivery systems, such as those based on nanoparticles \(^6^2\). Future studies should be re-directed to consider such critical issues.

As discussed above, findings from AD brain and blood suggest that impairment of protein quality control, including chaperones, may significantly contribute to the onset and progression of AD and it may be considered a promising therapeutic target. Accordingly, molecular chaperones and chemical and pharmacological chaperones have been found to be effective in preventing misfolding of different disease-causing proteins. Chaperones are highly specific and can distinguish between the native and non-native states of targeted proteins. However, how they discriminate between correctly and incorrectly folded proteins and how they selectively retain and target the latter for degradation has not been clarified \(^6^3\). Proteins that fail to achieve their native state, either as a consequence of amino acid mutation or because of an error in the folding process, are recognized as misfolded and therefore targeted to degradation – protein ‘quality control’ (QC) system. For this reason, the QC system, including molecular chaperones, the ubiquitin proteasome system and autophagy, plays a critical role in cell function and survival.

In almost all protein-misfolding disorders, an error in folding occurs because of a mutation in the polypeptide or, in a few cases, unknown reasons. The formation of oligomers and aggregates occurs in the cell when a critical concentration of misfolded protein is reached. Protein aggregation involves the self-assembly of proteins into large \(\beta\)-sheet rich complexes. This process could result from aberrant protein folding and lead to “amyloidosis”, a condition characterized by deposits of protein aggregates known as amyloids in different tissues. Intriguingly, OS contributes to protein misfolding through a double mechanism: oxidants induce PTMs on protein structure that increased the propensity to form aggregates; further free radicals damage directly members of the QC thus causing the reduced ability to remove misfolded proteins \(^6^4\) \(^6^5\) \(^6^6\). These effects result in impairment the entire catabolic system of pathogenic proteins such as A\(\beta\), tau and alpha-synuclein among others.

Thus, disease-modifying strategies currently being pursued for AD mainly focus on amyloid \(\beta\) and Tau, especially in the aggregated state. In particular, much effort has been expended in the last decade on developing small molecules that have the ability to inhibiting A\(\beta\) aggregation. However, to date, no compounds have been successful and entered into clinical use. This failure has been explained because the inhibition of A\(\beta\) aggregation requires blocking interactions between A\(\beta\) monomers. Indeed, A\(\beta\)42 is an intrinsically disordered peptide \(^6^7\) that self-assembles into fibrillar aggregates as observed in the brain from AD subjects \(^6^8\). The failure of therapeutic strategies based on small compounds that can interfere with self-aggregation is caused by the incomplete knowledge of the mechanisms generating toxic species and how potential drugs are able to interfere with the aggregation pathway of A\(\beta\)42. In addition, it is increasingly evident that prefibrillar oligomeric species, rather than mature amyloid fibrils and plaques, represent the main pathogenic agents in AD and other neurodegenerative conditions \(^6^9\).

As the strategy of inhibiting A\(\beta\) aggregation has increasingly gained success, a number of inhibitors have been developed and the structure-activity relationships of potent inhibitors have been described \(^7^0\) \(^7^1\). These studies revealed that typical A\(\beta\) aggregation inhibitors such as Congo red (CR), chrysamine G (CG) and curcumin share a similar chemical scaffold. These molecules contain two aromatic groups or inositol groups (with a suitable substituted group) separated by a backbone of the appropriate length \(^7^1\). It is likely that the two terminal groups interact with A\(\beta\) protein residues to provide the binding affinity, whereas the linker facilitates binding of inhibitors to specific subregions.

A significant step-forward has been recently achieved by Vendruscolo’s group which demonstrated that bexarotene, an anticancer drug approved, selectively targets the primary nucleation step in A\(\beta\)42 aggregation, delays the formation of toxic species in neuroblastoma cells, and completely suppresses A\(\beta\)42 deposition in a C. elegans model of A\(\beta\)42-mediated toxicity \(^6^9\).

**CONCLUDING REMARKS**

In summary, evidence has demonstrated that OS is inseparably linked with several major pathological processes in AD including A\(\beta\)-induced neurotoxicity, tau pathology, mitochondria dysfunction, and metal dyshomeostasis. Redox proteomics studies performed by our group and others demonstrated that oxidative damage to selected proteins contributes to the onset and progression of AD by impairing the function of proteins involved in energy metabolism, QC, synaptic function, antioxidant response among others. However, if from one side free radical-mediated damage is a well-established marker of neurodegeneration, it is not the only toxic mechanism. If antioxidant efficacy
exist other mechanisms have to be targeted to modify AD progression. The search for antioxidants compound that present additional pharmacological effects are thought to offer a good possibility for prevention of AD. Removal of ROS or prevention of their formation may delay the onset or slow down the progression of AD through multiple mechanisms. The rationale is that single molecules, endowed with antioxidant properties and able to act at different steps in the neurodegenerative process, can produce additional neuroprotective effects against AD.

Another promising strategy is based on the development of small compounds able to inhibit Aβ and Tau aggregation. A large number of neurodegenerative diseases in humans result from protein misfolding and aggregation. A nascent polypeptide chain can become misfolded due to a specific gene mutation, as it occurs in almost all familial form of neurodegenerative diseases, or a matured native protein can also achieve a misfolded conformation inside the cell. These aggregated/misfolded proteins become neurotoxic because of the impairment of the protein QC. Thus, therapy should be directed to inhibit and/or reverse conformational changes in the protein molecules responsible. To achieve this goal, it is mandatory to understand the molecular details of the inhibition processes. Vey recent and enthusiastic findings have demonstrated the beneficial effects of bexarotene on Aβ aggregation. This strategy can also be translated to other neurodegenerative diseases.

References

damage. Patients: potential pathogenic role and link to oxidative profile in matching CSF and serum from AD and aMCI.


Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 1999;52:1555-62.


Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 1999;52:1555-62.


Lloret A, Badia MC, Mora NJ, et al. Vitamin E paradox in Alzheimer’s disease: it does not prevent loss of


Extending lifespan through autophagy stimulation: a future perspective

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Ageing is a natural process characterized by the gradual decline of physiological functions. In the last decades, human lifespan has considerably increased. Consequently, population ageing and the resulting increase of people affected by age-related diseases, is emerging as a major social and economic challenge in developed countries. This scenario has led to an exponential growth of research projects in the field of ageing, with the aim of identifying amenable drug targets and pharmacological interventions to extend human healthy lifespan. Extensive evidence in literature suggests that the dysfunction of autophagy, a highly conserved pathway involved in maintaining cellular homeostasis, is part of the ageing process with roles in the pathobiology of age-related diseases. Moreover, accumulating experimental data from invertebrate and vertebrate animal models demonstrate that intervening to increase lifespan also induces autophagy, suggesting that stimulating such cellular process may represent an effective strategy to increase longevity. Here, we reviewed the literature on autophagy in ageing and age-related diseases, also discussing the perspective of behavioral and pharmacological interventions that may increase healthy lifespan through autophagy stimulation.

Key words: Autophagy, Ageing, Lifespan

INTRODUCTION

Ageing, an intrinsic feature of life, is characterized by the gradual loss of capacity of organs, tissues and cells to maintain the functional and structural integrity upon perturbation by endogenous and exogenous insults. In the last decades, increased medical progresses and better living conditions have led to a dramatic lifespan increase of both developing and rich regions. Currently, ageing populations represent a global phenomenon, which is emerging as one of the major socioeconomic burden of the last century. As stated by the current European Commission Ageing Report 1, the demographic trends projected over the long term reveal that Europe is ‘turning increasingly grey’ in the coming decades. The projections show large and sustained increases in lifespan. In the EU, life expectancy at birth for males is expected to increase by 7.1 years over the long period, reaching 84.8 in 2060. For females, it is projected to increase by 6.0 years, reaching 89.1 in 2060. However, since the ageing process involves functional decline and increased risk of chronic diseases, the increase of life expectancy cannot straightforwardly translate into an equivalent increase in healthy life expectancy 2. Now the progressive increase in longevity, if not accompanied by a reduction of the prevalence of chronic disabling diseases, would lead to an increased demand of assistance/support for older people and economic burden on health-care systems. This scenario has led to a growing interest among the scientific community. Researchers in the field of the biology and genetics of ageing have rushed on such a cogent topic in order to develop safe interventions to further slowdown the ageing process increasing healthy lifespan 3,4.

From a molecular point of view, ageing is an absolute example of complexity characterized by the accumulation of cellular damage promoting disease mechanisms and ultimately death. Results from studies on molecular mechanisms of ageing and age-related diseases evidenced that the age-associated cellular damage largely...
results from the alteration of only a few evolutionarily conserved genetic and biochemical pathways. In particular, the main causes impaired cellular homeostasis associated to the ageing process are: genomic instability, changes in nutrient sensing, mitochondrial dysfunction, loss of proteostasis and autophagy efficiency. It is noteworthy that the molecular mechanisms underlying ageing-associated defects are interconnected and affect the same cellular processes responsible for most of the age-related diseases such as cancer, cardiovascular and neurodegenerative disorders. Therefore, efforts aimed at identifying pharmacological interventions to slow down the molecular progress of ageing may also contribute to delay or prevent many chronic diseases. In this context, there is a general consensus that dietary, behavior and pharmacological interventions which modulate intracellular signaling pathways involved in response to nutrient availability, oxidative stress and the overall cellular protection may delay ageing and improve healthy lifespan. In particular, recent findings indicate that conserved signaling pathways, such as the insulin/insulin-like growth factor 1 (IGF1), mammalian target of rapamycin complex 1 (mTORC1), and the AMP-dependent protein kinase (AMPK) pathway converge on autophagy to extend lifespan. On this basis, modulation of such pathway may be of great relevance to slow down ageing and delay or prevent age-related diseases though autophagy induction.

Here we focus on the role of autophagy on ageing and age-related disorders and reviewed the literature on anti-ageing interventions affecting this pathway.

**AUTOPHAGY**

Autophagy is an evolutionarily conserved process with an essential role in the maintenance of cellular and tissue homeostasis. The primary function of autophagy consists in degrading long-lived proteins, damaged or excess organelles or portions of cytoplasm for recycling. Autophagy contributes to cellular homeostasis also by its activation under stress conditions, such as nutrient and growth factor deprivation, oxidative damage, hypoxia or anoxia, ER stress, invasion of pathogens. Moreover, as discussed in more detail below, data from experimental studies in invertebrates and vertebrates have consistently shown a tight link between autophagy and longevity. Noteworthy, the efficiency of the autophagy pathway has been extensively reported to decrease with age and age-related diseases. In mammals, three main types of autophagy have been described based on the mechanism and type of cargo delivered to the lysosome: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).

Macroautophagy consists of a non-selective sequestration of cytoplasmic damaged organelles and proteins, followed by their vesicular transport to lysosomes. The process involves the formation, maturation, trafficking and subsequent degradation of double-membrane structures known as autophagosomes. Although macroautophagy is generally considered to be a non-selective process, accumulating evidence has clearly shown that it can also specifically target damaged or superfluous organelles. Depending on the cargo, different forms of selective autophagy have been described including mitophagy (mitochondria), pexophagy (peroxisomes), ER-phagy (ER), xenophagy (pathogens), and others. In microautophagy, cytosolic material is internalized for degradation in single-membrane vesicles that form through invaginations in the surface of lysosomes or late endosomes. In mammalian cells, this process has been recently shown to take place at the surface of the late endosomes (endosomal microautophagy) and to utilize the machinery required for the biogenesis of multivesicular bodies. In CMA, targeted proteins are translocated across the lysosomal membrane as a complex with chaperone proteins recognized by the lysosomal-associated membrane protein 2A (LAMP-2A) receptor, which induces their unfolding and degradation. CMA selectively degrades soluble proteins containing the specific amino acid sequence KFERQ. This peptide is recognized by cytosolic chaperone proteins belonging to the HSC-70 family. The balance between these three processes is fundamental for the autophagy regulation of cellular homeostasis.

**THE MACROAUTOPHAGY MACHINERY**

Macroautophagy (hereafter called autophagy) is the most prevalent form of autophagy. The autophagy process proceeds through (i) cargo recognition and assembly of an isolation membranes called phagophores; (ii) cargo sequestration into double membrane vesicles called autophagosomes/amphisomes; (iii) autophagosome/amphisome-lysosome fusion leading to the formation of autolysosome, and iv) cargo degradation by lysosomal hydrolytic enzymes (Fig. 1). To date, more than 35 autophagy-related genes (Atg) that are essential for autophagosome biogenesis have been identified in yeast. Many of these genes have orthologs in higher eukaryotes.

**NUCLEATION, ELONGATION AND CLOSURE**

The autophagic process begins with the assembly of an isolation membrane, the phagophore. This structure is formed by the recruitment of lipids and proteins from different pre-formed organelles such as the endoplasmic
The autophagic process consists of a non-selective sequestration of damaged organelles, proteins and lipids, which are delivered to lysosomes for degradation and recycling. Autophagy pathway starts with the activation of the ULK complex, which in turn activates the Beclin1-PI3K complex giving rise to the recruitment of ATG proteins. The process proceeds through the assembly of an isolation membranes called phagophore and the recognition of cargo. Phagophore undergoes multiple elongation events that culminate with cargo sequestration, originating double membrane vesicles called autophagosome/ampiphosome. The latter fuses with the lysosome, leading to the formation of the autolysosome, where cargo degradation by lysosomal hydrolytic enzymes occurs.

**Figure 1.** The macroautophagy pathway.

The autophagic process consists of a non-selective sequestration of damaged organelles, proteins and lipids, which are delivered to lysosomes for degradation and recycling. Autophagy pathway starts with the activation of the ULK complex, which in turn activates the Beclin1-PI3K complex giving rise to the recruitment of ATG proteins. The process proceeds through the assembly of an isolation membranes called phagophore and the recognition of cargo. Phagophore undergoes multiple elongation events that culminate with cargo sequestration, originating double membrane vesicles called autophagosome/ampiphosome. The latter fuses with the lysosome, leading to the formation of the autolysosome, where cargo degradation by lysosomal hydrolytic enzymes occurs.
derived from the C-terminal cleavage of LC3 by Atg4, to phosphatidylethanolamine to form the autophagosome membrane-bound lipidated LC3-I, i.e. LC3-II \(^{32}\). LC3-II promotes the elongation and closure of the autophagosomal membrane and is essential for cargo selection, because of its ability to bind to the scaffolding protein p62 \(^{33}\). After sealing of the autophagosomal membrane, the Atg12–Atg5–Atg16L1 complex is released from the newly formed autophagosome, whereas LC3-II remains bound to the autophagosome membrane until this fuses with lysosomes \(^{34}\).

**Maturation, Fusion and Degradation**

Autophagosome maturation consists of its fusion with lysosome to form the “autolysosome”), where the autophagosomal content is degraded by lysosomal acid hydrolases. As the autophagosome formation occurs at random sites in the cytoplasm, the autophagosome must travel to the endocytic system and fuse with late endosomes/multivesicular bodies to generate an amphisome, which finally fuses with lysosome, or alternatively it may fuse directly with lysosomes to form an autolysosome. Microtubules and the dynein motor complex are directly implicated in autophagosomal transport and fusion with lysosome \(^{35}\). The fusion step is under the control of proteins involved in intracellular membrane trafficking such as Rab GTPases, SNAREs and membrane-tethering complexes. Among GT-Pases, Rab7 regulates the trafficking of cargos along microtubules and participates to the fusion step with lysosomes \(^{36}\), while Rab11 has been shown to promote late endosome-autophagosome fusion \(^{37}\). SNAREs are membrane-anchored proteins localized on opposing membrane compartments that interact with each other to bring the opposing lipid bilayers together and allow their fusion to occur. While the majority of SNARE proteins are localized to endosomes and synaptic vesicles, a recent study has identified Syntaxin 17 as a SNARE specifically related to autophagy \(^{38}\). Membrane-tethers are thought to facilitate the docking and fusion process by bridging the opposing membranes and/or stimulating SNARE complex formation \(^{39}\). Within the autolysosome, the sequestered cargo is degraded and released into the cytoplasm for recycling. In addition to the autophagy machinery, proper lysosomal function is also essential for efficient fusion events and cargo degradation, as underlined by the evidence that lysosomal storage disorders are characterized by autophagy failure \(^{40}\).

**Autophagy Regulation**

Autophagy maintains cellular homeostasis under both normal and stress conditions. Autophagy is active at basal levels in most cell types where plays a housekeeping role in maintaining the integrity of intracellular organelles and proteins. Moreover, autophagy is induced during energy or nutrient deprivation in order to recycle intracellular components, restore the energy or nutrient deficiency and promote cellular survival. Consequently, cells have developed control mechanisms that tightly modulate autophagic activity in response to diverse environmental cues (Fig. 2). A major role in autophagy regulation is played by the mTORC1 \(^{13}\). mTORC1 functions to integrate a wide range of intra- and extracellular signals such as insulin, growth factors, mitogens, energy, and amino acids level to control protein synthesis, metabolism and promote cellular growth \(^{41}\). The core components of mTORC1 are the serine/threonine kinase mTOR (target of rapamycin), the scaffolding subunit Raptor (regulatory associated protein of mTOR), the kinase inhibitors DEPTOR (DEP domain containing mTOR-interacting protein), PRAS40 (proline-rich Akt substrate of 40 kDa) and mLST8 (mTOR associated protein) \(^{42}\). Under basal conditions, the mTORC1 complex associates with the ULK1/Atg13/FIP200 complex via a direct interaction between Raptor and ULK1 and inhibits autophagy through the phosphorylation and inactivation of ULK1 and Atg13 \(^{43}\). When cellular energy is depleted by nutrient deprivation, the mTORC1-dependent phosphorylation sites in ULK1 are rapidly dephosphorylated. Then, ULK1 phosphorylates itself, Atg13 and FIP200 leading to the assembly of the ULK1/Atg13/FIP200 complex, and to the induction of autophagy \(^{44}\). Alternatively, ULK1 is phosphorylated and activated by AMPK \(^{14}\), a kinase activated during nutrient deprivation and low energy charge. Activated AMPK induces autophagy by phosphorylating ULK1 at residues distinct from those phosphorylated by mTOR \(^{45}\). Therefore, the coordinated phosphorylation of ULK1 by mTORC1 and AMPK possibly controls autophagic flux in response to metabolic requirements. Furthermore, AMPK also activates autophagy by directly inhibiting mTORC1 through phosphorylation and activation of tuberous sclerosis complex 2 (TSC2) and Raptor \(^{46,47}\).

Besides, recent studies demonstrated that the transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, enhances autophagy by positively controlling the expression of lysosomal and Atg genes \(^{48}\). TFEB activity and nuclear translocation depend on its phosphorylation status. Under basal conditions, mTORC1 phosphorylates TFEB which is retained into cytosol. Cellular conditions such as stress, starvation and low energy inhibit mTORC1 and induce TFEB dephosphorylation and nuclear translocation. In the nucleus, TFEB activates the transcription of its target genes leading to lysosomal biogenesis and autophagy pathway activation \(^{49}\).
Accumulating findings indicate that different signaling pathways and environmental factors may converge on both mTOR and autophagy to regulate the lifespan of many species. For instance, the insulin/IGF1 hormonal system, which activates mTORC1 through the insulin receptor/phosphoinositide 3-kinase/AKT signaling pathway, has been shown to accelerate aging and increase mortality in many organisms. According to this, evidences have been reported that deletion of AKT/PKB prolongs life in Saccharomyces cerevisiae (S. cerevisiae), Caenorhabditis elegans (C. elegans), and Drosophila melanogaster (D. melanogaster) while inactivation of mTOR, which is upregulated by the PI3K/AKT/PKB cascade, extends lifespan in yeast, flies, worms and mice. Activation of autophagy by caloric restriction, which increases healthy lifespan in many organism including humans, is mediated by the inhibition of the insulin/IGF-1 signaling pathway leading to mTOR activity inhibition. Epigenetic factors may also affect aging through autophagy modulation. Manipulation of enzymes regulating the acetylation status of chromatin (sirtuins, histone acetyltransferases, histone deacetylases) has been reported to influence lifespan in many organism models. In particular, recent data demonstrate that the Sirtuin-type chromatin remodeling factors implicated in aging regulation may require autophagy for their lifespan extension effect in both invertebrates and vertebrates. Activated SIRT1, a NAD+-dependent protein deacetylases which overexpression has an anti-aging

Figure 2. Signaling pathways and stress responses converging on autophagy to regulate lifespan. Insulin/IGF-1 and TORC1 pathways inhibition, or SIRT1 and AMPK pathways activation increase lifespan through autophagy induction in a wide variety of species. Under basal conditions mTORC1 inhibits autophagy by associating with the ULK1/Atg13/FIP200 complex and inhibiting it. Under nutrient deprivation or low energy, different signaling pathways inactivate TOR kinase activity, thus inducing autophagy through the release and activation of the ULK1/Atg13/FIP200 complex. The interventions targeting different pathways which contribute to aging regulation by autophagy stimulation and result in improved health and enhanced lifespan, are shown. Green arrows: activating inputs; red bars: inhibitory interactions; light blue boxes: anti-aging interventions stimulating autophagy through activation/suppression of different signaling pathways which regulate longevity.
effect in mice, induces autophagy by transcriptional activation of some autophagy genes through deacetylation of chromatin proteins at several autophagy-related loci. Analogously, inhibition of histone acetylases strongly induces autophagy. These evidences indicate that protein acetylation at chromatin level may play a general role in the regulation of the autophagy pathway.

ROLE OF AUTOPHagy IN AGEING AND AGE-RELATED DISEASES

As mentioned above, numerous evidences have been reported indicating that autophagy efficiency decreases with age in almost all organisms. Besides, the progressive decrease of autophagy activity is considered one of the causes of the functional decline of biological systems during ageing supporting a link between autophagy and ageing process. Early findings suggesting a relationship between autophagy and lifespan come from studies on model organisms. For instance, decreased lifespan has been observed in short-lived yeast mutants with defective autophagy and studies on the nematode and the fruit fly indicate that protein acetylation at chromatin level may strongly induce autophagy. These evidences related loci.

There is growing evidence suggesting that autophagy plays a protective role by removing damaged macromolecules and organelles. For instance, under starvation, ROS regulate autophagy through the activation of the conserved autophagy protein ATG4. In particular, the essential autophagy protein ATG4 is a Cys-dependent protease directly targeted by mitochondrial ROS under nutrient deprivation. The redox-dependent inactivation of ATG4 leads to increased autophagosome formation by inhibition of LC3 de-lipidation. Although the precise link between ROS and autophagy is not completely understood, collectively it seems conceivable that ROS induce autophagy to reduce oxidative damage. It is well known that the ageing process is also accompanied by increased oxidative stress, which are able to oxidize many biological molecules. Therefore, the oxidative damage is a cumulative phenomenon during the entire life of an organism and autophagy plays a protective role by removing damaged macromolecules and organelles. On this regard, accumulating evidences suggest the existence of an intriguing crosstalk between ROS production and autophagy regulation and numerous studies indicate that several molecular players are involved in the complex interplay between ROS and autophagy. For instance, under starvation, ROS regulates autophagy through the activation of the conserved regulatory protein AMPK, a positive regulator of autophagy. Oxidative stress can also activate the tumor suppressor protein p53 that is able to trigger autophagy through its transcriptional activity. Conversely, it has been reported that the cytoplasmic form of p53 inhibits autophagy through the p53 inducible protein TIGAR and ROS also activate redox-sensitive proteases that are involved in autophagy. In particular, the essential autophagy protein ATG4 is a Cys-dependent protease directly targeted by mitochondrial ROS under nutrient deprivation. The redox-dependent inactivation of ATG4 leads to increased autophagosome formation by inhibition of LC3 de-lipidation. Although the precise link between ROS and autophagy is not completely understood, collectively it seems conceivable that ROS induce autophagy to reduce oxidative damage. It is well known that the ageing process is also accompanied by increased oxidative stress. So it appears that oxidative stress may contribute to ageing not only directly but also indirectly, by increasing the request of autophagic degradation of damaged material. In conclusion, the decline in autophagy function with ageing results in the accumulation of damaged substances and organelles leading to cellular dysfunction and, ultimately, death. On the other hand, numerous genetic studies reveal that the overexpression of autophagy essential genes promotes lifespan extension.
and improves health span in multiple model organisms confirming the tight connection between autophagy, lifespan and ageing.\textsuperscript{85,87}

The loss of autophagy efficiency with ageing is an important factor contributing to several age-related disorders including neurodegeneration, cancer, infection, cardiovascular dysfunction and muscle atrophy.\textsuperscript{3,88} For example, many studies support that autophagy failure contributes to many late-onset human neurodegenerative diseases. One of the common pathological features of these pathologies is the accumulation of cytosolic aggregate-prone proteins, such as mutant tau, \(\alpha\)-synuclein and mutant huntingtin which are distinctive features of Alzheimer, Parkinson and Huntington diseases, respectively.\textsuperscript{99,100} The autophagy impairment reduces cellular clearance of the soluble forms of aggregate-prone proteins, leading to increasing aggregation and cell toxicity.\textsuperscript{97-99} Importantly, it has been demonstrated that autophagy induction prevents the age-dependent accumulation of damage in neurons and reduces toxicity in several organism models.\textsuperscript{93-95} Although many evidence supports a connection between autophagy and different types of cancer, the exact role of autophagy in tumorigenesis is still controversial.\textsuperscript{96} A strong support indicating that autophagy may act as a tumor-suppressor pathway comes from the observations that essential autophagic genes are mutated in human cancers suggesting that impairment of autophagic machinery might contribute to tumorigenesis.\textsuperscript{97-99} As autophagy protects cells from the insults caused by the accumulation of unfolded, dysfunctional, aggregated proteins, increased ROS level and DNA damage, the cellular damages derived from autophagic deficit may favor cancer occurrence in certain cells.\textsuperscript{100} On this regard, several findings suggest that a number of autophagy genes also have properties of onco-suppressors and loss of Beclin1 increases tumor cell proliferation.\textsuperscript{99} On the other hand, the pro-survival function of autophagy could allow the mutant cells to survive in conditions of hypoxia and nutrient deprivation, as those occurring in the core of solid tumors. Indeed, it has been found that malignant cells maintain autophagic activity, albeit at low levels, to promote tumor survival under metabolic stress conditions.\textsuperscript{104,105} Besides, it has been reported that autophagy is necessary for the maintenance of the energetic requirements of cancer cells.\textsuperscript{106,107} In conclusion, functional autophagy appears to be essential in preventing tumorigenesis, but its pro-survival role under stressing condition may support malignant cells to allow tumor growth and development. Therefore, autophagy upregulation may have beneficial effects as prophylactic treatment, while reducing autophagy may be of benefit in existing tumors.

Autophagy is also an important defense mechanism against infections. By sequestering and degrading intracellular-invading bacterial pathogens, autophagic degradation takes part to the innate immune response against a number of microbial pathogens that invade eukaryotic cells.\textsuperscript{108,109} For instance, it has been reported that Group A Streptococcus is internalized into autophagosome-like structures and degraded in an Atg5-dependent manner.\textsuperscript{110} A number of other pathogens, like Toxoplasma gondii, Listeria monocytogenes, Salmonella enterica, or Rickettsia conorii, have been found to be targets of autophagic degradation.\textsuperscript{108,111} Therefore, failure of autophagy pathway may represent an advantage for bacterial pathogens, leading to an increased susceptibility to infectious agents in the elderly.

**AUTOPHAGY STIMULATION PROMOTES LIFESPAN EXTENSION**

The ageing process is mainly characterized by the lifelong accumulation of cellular damage. Therefore, enhancing or preserving the activity of mechanisms eliminating cellular damage may be useful to decrease not only the rate of damage accumulation, but may also slow down degenerative processes which occur with ageing. On the other hand, it is well documented that autophagy, which has a crucial role on clearing cellular damage, decrease with age in diverse organisms ranging from yeast to mammals. On this basis, restoration of normal autophagic function may represent a useful therapeutic strategy aimed at increase lifespan and preventing, or at least delaying, age-related disorders. On this regard, many studies indicate that genetic, dietary or pharmacological interventions extending lifespan in many model organisms also induce autophagy. On the contrary, numerous interventions extending lifespan require autophagy for their longevity-promoting effects (Fig. 2). Among this, the beneficial effects of caloric restriction (CR), i.e. the reduction of total calorie intake by 20-50% without malnutrition or the diminished intake of specific dietary components, have been extensively documented.\textsuperscript{113,114} Data from experimental studies in invertebrates and rodents have consistently shown that reduced food intake, avoiding malnutrition, play major roles in promoting health and longevity.\textsuperscript{53,115} In addition, numerous CR clinical studies in humans have given very encouraging results raising the possibility to consider CR for long-term clinical trials focused on healthspan.\textsuperscript{116} For instance, evidence has been reported indicating that a CR of around 15% may be most favorable against mortality during ageing.\textsuperscript{117} Moreover, long-term CR with adequate intake of nutrients results in several metabolic adaptations that reduce the risk of developing type 2 diabetes, hypertension,
cardiovascular disease, neurodegeneration and cancer. The effect of CR on autophagy induction is mainly mediated by the inhibition of the insulin/IGF1 signaling pathway that ultimately leads to inhibition of mTOR activity and promote survival during ageing. In S. cerevisiae, starvation causes down-regulation of the TOR-S6K and Ras-adenylate cyclase-PKA pathways, and induces the activation of stress resistance transcription factors regulating many protective and metabolic genes. Analogously, in yeast, flies, worms, and mammals, fasting reduces circulating IGF-1 and leads to down-regulation of PI3K-AKT, mTOR and PKA pathways and the activation of multiple transcription factors related to stress resistance and survival. The anti-ageing effects of CR have been functionally linked to autophagy also through the activation of both the energy sensors Sirtuin1 (SIRT1) and AMPK. In particular, it has been reported that SIRT1, which is linked to autophagy also through the activation of both AMPK activation may extend lifespan in worms and mice and may also induce autophagy. These observations indicate that the lifespan extension mediated by CR is related to autophagy activation and suggest a strong correlation between CR and autophagy. Apart from CR, several pharmacological interventions with both clinically approved compounds and natural compounds have been reported to delay ageing through activation of the autophagic machinery in diverse species from yeasts, flies, nematodes up to mice. Interestingly, autophagy is often activated in association with mTOR pathway inhibition and inhibitors of this pathway are widely used as inducers of autophagy. Rapamycin, a direct inhibitor of the mTOR kinase, has already been approved for uses as anticancer agent, antifungal antibiotic and immunosuppressant. Rapamycin treatment has been shown to extend lifespan as well as healthspan in different animal models of ageing. Importantly, it has been demonstrated that the lifespan-extending effect of rapamycin is strictly dependent on autophagy induction in yeast, nematodes and flies. In addition to enhancing longevity, it has been reported that rapamycin treatments clear aggregate-prone proteins in cell and animal models of many age-related neurodegenerative diseases such as Alzheimer, Parkinson and Huntington diseases through autophagy induction. Although inhibition of mTOR activity clearly has beneficial effects during ageing, long-term administration of rapamycin and rapamycin derivatives required in chronic disorders may have a range of undesirable side effects including increased infections, reduced male fertility, hyperlipidemia, insulin resistance, and diabetes mellitus. To date, the effectiveness of rapamycin and rapalogs to slow ageing in humans remains to be determined, but the results obtained so far clearly indicate that it is possible to slow down ageing and delay the onset or progression of age-related diseases laying the basis for encouraging future developments. Other convincing evidence that induction of autophagy leads to extension of lifespan come from different studies using natural compounds such as spermidine and resveratrol. Spermidine, which is a naturally occurring ubiquitous polyamine, is among the most effective inducers of autophagy. In yeast, spermidine acts as an inhibitor of histone acetylases and affects the transcription of several genes, some of which are involved in the autophagic degradation machinery. It has been reported that exogenous supply of spermidine promotes longevity via induction of autophagy in yeast, flies and worms. Likewise, nutrient supplementation with food containing spermidine increases longevity and reduced age-related pathology in mice. On the other hand, the inhibition of autophagy by genetic manipulation abolishes the beneficial effects of spermidine on lifespan in both flies and worms, indicating a strong correlation between the autophagy induction and the pro-survival effect mediated by spermidine. Because of spermidine proved to be non-toxic in mice and human studies, it should be considered as potential intervention strategy promoting healthspan on human.

Resveratrol, a polyphenol found in grape berry skin, red wine and other plants, has been reported to have positive effects on lifespan in a range of organisms including mice. It is an anti-oxidant natural compound with anti-inflammatory, anti-cancer and antiviral properties. Resveratrol is also a potent inducer of autophagy. Findings have been reported indicating that the promoting lifespan effect of resveratrol requires autophagy and inhibition of autophagy pathway by genetic or pharmacological manipulation abolishes these beneficial effects. The autophagy stimulation induced by resveratrol is mediated by direct activation of SIRT1, a member of sirtuin deacetylases. Based on the observation that resveratrol activates SIRT1, which is a deacetylase, and spermidine inhibits acetylases, it has been suggested that these two compounds mediate longevity through convergent pathways. A proteomic study on human colon carcinoma cells revealed that they induce convergent acetylpoteine modifications that control the autophagic network. Another polyphenol compound with antioxidant and anti-inflammatory activities similar to those of resveratrol is oligonol. A recent study demonstrated that oligonol, analogously to resveratrol, may act as an anti-ageing molecule by inducing autophagy via up-regulation of SIRT1 gene expression and the AMPK pathway.
This finding further supports that Sirtuins may represent attractive drug targets to promote healthy ageing. The discovery of natural sirtuin-activating compounds (STACs) with beneficial effects on heathspan prompted the production of more potent and bioavailable synthetic SIRT1 activators \(^{151}\). These compounds, as well as resveratrol, mimic the beneficial effects of CR and have also shown promising results in treating age-related diseases such as cancer, type 2 diabetes, inflammation, cardiovascular disease and neurodegeneration in different animal models \(^{151-162}\). Resveratrol and synthetic STACS have also been extensively tested in humans with conflicting reports of their efficacies \(^{163-165}\). Interestingly, a recent study demonstrated a non-linear dose response for the protective effects of resveratrol in humans and mice \(^{166}\). This finding may be useful to clarify the contradictory results so far obtained with STACs in human contributing to understand the actual efficacy of these compounds as anti-ageing agent in human. In the last years, efforts to identify safer autophagy promoting drugs have led to the identification of new small molecules able to activate autophagy without interfere with the mTOR pathway \(^{167}\).

Among these, riLmenidine, a well-tolerated United States Food and Drug Administration-approved anti-hypertensive drug, has been reported to induce autophagy in mice and in primary neuronal culture and also attenuate Huntington’s disease symptoms in a mouse model of the disease \(^{168}\). Therefore, riLmenidine may be considered for the treatment of Huntington’s disease and related disorders that commonly occur with ageing.

**CONCLUSIONS**

Ageing is a natural multifactorial process characterized by the gradual accumulation of cellular damage culminating in impaired function, increased susceptibility to develop diseases and ultimately death. Accumulating evidence indicates that ageing can be delayed in animal models by genetic and small molecule interventions, raising the possibility that anti-ageing therapies are an effective opportunity also in humans. Autophagy, an evolutionarily conserved process, promotes the elimination of dysfunctional organelles, protein aggregates and intracellular pathogens in order to maintain cellular homeostasis in both normal and stress conditions. Many findings have clearly established the molecular and mechanistic relationship existing between autophagy and ageing. On this regard, studies carried out in different organisms indicated that interventions aimed at increasing lifespan and healthspan also stimulate autophagy and vice versa. For instance, enhanced longevity can be achieved by CR, mTORC1 complex inhibition and sirtuin-activating compounds. All of these interventions act through autophagy stimulation. Therefore, autophagy represents a potential target to slow down the ageing process and prevent/delay age-related diseases. However, most of the compounds described so far as effective anti-ageing agents target multiple longevity signaling pathways upstream of autophagy and their long-term administration may have undesired side effects, such as alteration of cell growth and homeostasis. Further studies should focus on the precise nature of regulatory interactions between longevity pathways and the autophagy machinery, in order to develop safer and more specific anti-ageing drugs. One limitation of testing new compounds with anti-ageing effects in mammals is the lack of suitable in vivo models to explore long-term effects of drugs on the ageing process. Although mice are the main mammalian model in ageing studies, they are not suited for large-scale unbiased screening of new drugs due to macroscopic differences with humans in terms of metabolic and gene regulation frames. Alternatively, since most of the pathways modulating the ageing process in mammals are highly conserved and have homologies with short-living organisms, yeast, flies, and worms, these organisms have been extensively used as pilot experimental models to test candidate anti-ageing drugs, the identification of which may accelerate the discovery of treatments that extend the lifespan/healthspan in other species, potentially including humans. The results obtained so far on intervention strategies aimed at stimulate autophagy have laid the basis for encouraging future developments. The ultimate goal is not only to increase longevity, but also to prevent or delay pathogenic mechanisms of age-related diseases.

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**References**

Extending lifespan through autophagy stimulation: a future perspective

24 Arias E, Cuervo AM.


19 Yang Z, Klionsky DJ.

18 Cuervo AM.


12 Oldham S, Hafen E.

11 Martinez-Lopez N, Athonvarangkul D, Singh R.


8 Houtkooper RH, Pirinen E, Auwerx J.

7 Inoki K, Kim J, Guan KL.


Li L, Chen Y, Gibson SB. Starvation-induced autophagy is...


Extending lifespan through autophagy stimulation: a future perspective


162 Graff J, Kahn M, Samiei A. A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration. J Neurosci 2013;33:8951-60.


Osteoarthritis (OA) is the most common degenerative joint disease; it represents a major public health problem and is ranked among the top 10 causes of disability worldwide, especially in elderly subjects. Although the main characteristic of OA is the progressive degeneration and loss of joint cartilage, it is now commonly accepted that all the articular components are involved, as the structural alterations observed in OA include sub-chondral bone changes, osteophyte formation, variable degrees of synovial inflammation, degeneration of ligaments and hypertrophy of the joint capsule. There are currently no treatments that delay or halt OA progression; in general, therapeutic agents that modulate the cellular activities in individual joint tissues such as bone, cartilage or synovium have proven to be effective in arresting or slowing the progression of joint pathology in animal models of OA. Particularly, bisphosphonates may determine some positive structural and symptomatic effects in the treatment of OA through different mechanisms, including their ability to modify osteoclast and osteoblast metabolism in the sub-chondral bone and to inhibit the synovial inflammatory changes.

Key words: Bisphosphonates, Osteoblast, Osteoclast, Osteoarthritis, Inflammation

BACKGROUND

Osteoarthritis (OA) is the most common degenerative joint disease and represents a major public health problem. OA is ranked among the top 10 causes of disability worldwide, especially in elderly subjects. Major features of OA include chronic pain, joint instability, stiffness and radiographic joint space narrowing. Although the main characteristic of OA is the progressive degeneration and loss of joint cartilage, it is now commonly accepted that all the articular components are involved, as the structural alterations observed in OA include sub-chondral bone changes, osteophyte formation, variable degrees of inflammation of the synovium, degeneration of ligaments and hypertrophy of the joint capsule. The sub-chondral bone undergoes structural changes that affect the overlying articular cartilage, such as increased thickness of the cortical plate, changes of mass, architecture and mineralization of the bone, development of bone cysts and growth of osteophytes at the joint margins. Nevertheless, the exact relationship between the changes in the sub-chondral bone and other osteoarthritic events has yet to be fully elucidated and controversy exists regarding the timing and sequence of the pathological changes. The mechanisms responsible for the pathogenesis of sub-chondral bone changes in OA are still unclear, but many clinical and experimental evidences suggest that both osteoblasts and osteoclasts can be involved. It has been clearly demonstrated that osteoblast-like cells isolated from sub-chondral bone of osteoarthritic patients have abnormal metabolic activity. Decreased bone mineral content and decreased number of trabeculae in sub-chondral bone structure in the early OA stages have been observed by magnetic resonance imaging and high-turnover type bone metabolism derangement has been considered as a possible cause of OA. An increasing number of experimental and clinical data suggests that the impairment of sub-chondral bone is crucial to the development and progression of OA. Many studies support the hypothesis that the skeletal adaptations can antedate detectable alterations in the structural integrity of the articular cartilage. Despite the

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Bisphosphonates and osteoarthritis

Bisphosphonates and osteoarthritis are anti-resorptive agents that inhibit the recruitment (BPs) could become a disease-modifying therapy. BPs modulate bone remodelling, such as Bisphosphonates (BPs) could become a disease-modifying therapy. BPs are anti-resorptive agents that inhibit the recruitment and maturation of osteoclast precursors and the activity of mature osteoclasts in the bone. These drugs have been used for decades in clinical practice for the treatment of bone diseases characterised by an increase of bone remodelling processes, in particular post-menopausal and glucocorticoid induced osteoporosis, Paget’s disease, multiple myeloma and bone metastases. BPs have more recently been proposed as potential drugs able to modify the natural history of OA by preventing the loss of structural integrity in the sub-chondral bone compartment.

EXPERIMENTAL DATA AND ANIMAL MODELS

It has been shown that BPs prevent the development of bone alterations in animal models of OA and exert a chondroprotective effect, but their direct effects on chondrocytes function are not clearly known. Besides their inhibitory effects on the maturation and activation of the osteoclasts, BPs also influence osteoblasts in vitro, by affecting their differentiation, proliferation, migration and cytokine expression. It results in either stimulatory or inhibitory effects, depending on the dosage and the kind of BPs used. Nevertheless, very few studies have assessed the ability of BPs to modify in vitro the metabolic activity of the sub-chondral osteoblasts in OA joints. The treatment with alendronate of human osteoblasts obtained from OA patients led to a significant increase in the level of expressed RANKL mRNA and RANKL/osteoprotegerin mRNA ratio, due to an increased expression of RANKL without any effect on osteoprotegerin mRNA expression or osteoprotegerin secretion. Nevertheless, other data showed that BPs did not stimulate the synthesis or the expression of any proteins in the sub-chondral osteoblasts. Moreover, high doses of BPs may inhibit the osteocalcin synthesis in these cells.

Other experimental studies evaluating the effectiveness of bone anti-resorptive agents for OA have shown promising results on animal models. Some data support the hypothesis that BPs may reduce the progression of osteophyte formation and the sub-chondral bone resorption.

The preventive use of alendronate has been investigated in a rat model of severe OA, in which decreased trabecular bone volume fraction (BV/TV), development of sub-chondral sclerosis of the tibia and progressive loss of cartilage were induced by the combination of moderate exercise, over a 6 weeks period, together with weekly intra-articular injections of papain in the knee during the first 3 weeks. A mild increase of trabecular thickness and an improved preservation of both BV/TV and cartilage extracellular matrix compared to the control group were observed in the rats treated with alendronate 12 weeks after the beginning of OA induction. Nonetheless, alendronate had no effect in reducing the sub-chondral bone sclerosis during OA progression. Furthermore, alendronate significantly decreased bone remodeling, reduced osteophytosis, protected the cartilage extra-cellular matrix from degradation, improved the content of glycosaminoglycans in the cartilage and reduced the synovial macrophage activation.

Other studies on different animal models of OA showed that alendronate markedly reduced cartilage lesions and delayed cartilage degeneration, showing a chondroprotective effect. In animals treated with alendronate, the formation of osteophytes was suppressed and fewer microscopic alterations of the cartilage were shown in comparison to the placebo group, in which various degrees of fibrosis, cracks, cell loss and multicular chondrocyte clusters were observed. Furthermore, alendronate reduced cartilage neoangiogenesis and the expression of matrix metallo-proteinase 13 (MMP-13), interleukin-1β (IL-1β), vascular endothelial growth factor (VEGF), RANKL and markers of cartilage degradation, such as serum cartilage oligomeric matrix protein (COMP), urinary C-telopeptide of type II collagen (CTX-II) and type-X collagen. On the other hand, the Bone Morphogenetic Protein 2 (BMP-2) expression increased after treatment with BPs. In the sub-chondral bone, alendronate induced a significant increase of hystomorphometric parameters of bone formation (bone volume fraction, trabecular bone thickness, trabecular number) with a reduction of markers of bone resorption (trabecular separation).

In a rat model of OA induced by glycolysis inhibitor, the intra-articular administration of zoledronic acid significantly reduced pain and attenuated or prevented the degeneration of bone and cartilage. In a rat model of knee OA induced by anterior cruciate ligament transection, animals treated with systemic high dose of zoledronic acid showed milder macroscopic ulcerations of cartilage, lesser cartilage softening and fibrillation,
without complete disorganization, when compared to rats treated with placebo; the microscopic morphology of the articular cartilage was better in the zoledronic acid treated rats, with only a partial disorganization of the matrix and with presence of proliferating chondrocytes, indicating on-going cartilage repair and regeneration process. Hypocellularity was the prevalent finding in the placebo group. In rats treated with intra-articular zoledronic acid, the histological cartilage score was higher compared to placebo and indicated that the progression of synovitis (in terms of inflammation and necrosis) was significantly lower. In the same experimental animal model of OA, pamidronate prevented or even reversed cartilage damage, inducing an increase of chondrocytes and extra cellular matrix, reducing fibrosis of cartilage surface.

**CLINICAL STUDIES**

In controlled clinical trials and in open label clinical trials, BPs showed to be effective in the treatment of pain and in the treatment of impaired function and radiographic joint progression in OA patients. Two large randomised controlled studies have been performed to assess the efficacy of risedronate in patients with knee joint OA. In a 1 year prospective, double-blind, placebo-controlled study (BRISK), a total of 285 patients with mild to moderate knee joint OA were randomized to receive once-weekly risedronate (5 mg or 15 mg) or placebo. Besides the reduction of markers of cartilage degradation and bone resorption, a weekly dose of 15 mg risedronate significantly improved the global assessment score, reduced the walking aids and determined a significant improvement in the Western Ontario and McMaster University (WOMAC) pain score after 12 months of treatment when compared to the placebo group. No evident effects were achieved in the group of patients treated with 5 mg weekly risedronate. No significant differences were found in the intervention groups concerning the radiographic joint space narrowing. Only 8% of the OA patients in the placebo group had detectable progression of the disease.

These results were not confirmed by two parallel multicentre randomized, double-blind, placebo-controlled phase III studies performed in North America and Europe (KOSTAR). Placebo or risedronate in different doses (5 mg/day, 35 mg/week or 50 mg/week) were given during 2 years to a total of 2483 randomised patients. In both the studies, no significant differences in the mean change from baseline in pain scores and on patient global assessment score (WOMAC) between treatment groups were found. Similarly, no significant difference in radiographic progression was reported. Only 13% of the OA patients receiving placebo showed a significant radiographic progression over the 2 years follow-up. It can be speculated that this large clinical trial was underpowered based on the low rate of OA progression in the placebo group.

As expected, in both the North American and European groups, a dose-dependent decrease in the levels of markers of bone resorption and cartilage degradation (urinary CTX-II) with risedronate was observed within 6 months and continued through 24 months; interestingly, in a sub-analysis, it was demonstrated that in subjects with accelerated cartilage degradation at baseline, the biochemical response after 6 months of risedronate use was associated with a significant reduction in radiological progression compared to subjects with no response in CTX-II levels. The further analysis of the subgroup of patients with significant radiographic progression of joint space narrowing showed that, in comparison to the placebo group, the trabecular structure was better retained in patients treated with risedronate 15 mg/week and an improved trabecular number was found in the group of patients treated with 50 mg/week dose of risedronate over 2 years, with a preservation of the structural integrity of the subchondral bone.

A phase 2 randomized, partially blind clinical trial evaluated the efficacy of different dosages (0.5 mg or 1 mg or 2 mg once a week for 4 weeks; 1 mg twice a week for 2 weeks) of intra articular clodronate vs hyaluronic acid (20 mg once a week for 4 weeks) in patients with primary knee OA. No statistically significant differences in pain or mobility scores were reported during the initial five weeks of treatment between the groups. After adjusting for multiple comparisons and paracetamol use, the authors reported a significant reduction of pain in patients treated with 1 mg clodronate compared to the hyaluronic acid group.

Kawasaki et al. evaluated the additive effect to therapeutic exercise of risedronate 2.5 mg/day or glucosamine compared to exercise alone in patients with knee OA. After 18 months, a significant improvement in pain was found in all the treatment groups, but no significant differences between the groups concerning the functional outcomes, pain and joint space width were observed.

The effect on pain and bone marrow oedema (MRI) of a single intra-venous injection of zoledronic acid 5 mg was compared to placebo in patients with knee OA in a randomized controlled trial. Zoledronic acid induced a significant improvement of pain after six months of treatment, but not after three or twelve months. A reduction in total bone marrow oedema was also reported after 6 months in the zoledronic acid group.

An open randomized pilot trial evaluated the efficacy of intra-venous clodronate compared to
hydroxychloroquine for the treatment of erosive OA of the hands. A significant reduction of pain scores and a significant improvement of functional scores and patient’s global assessment were found in patients treated with clodronate at 12 months, whereas hydroxychloroquine resulted to be ineffective.43

One clinical trial, performed on a small case-series, assessed the use of alendronate in symptomatic hip OA. Patients were randomly assigned to receive alendronate 35 mg/weekly and calcium lactate 600 mg/daily or calcium lactate alone for 2 years. Alendronate induced a significant reduction of pain at 12 months, whereas the control group showed worsening of pain; nevertheless, the prevention of OA radiographic progression was not observed neither in alendronate treated patients, nor in placebo group44.

The effect of clodronate was assessed in a double-blind, randomized placebo-controlled trial in which patients with symptomatic knee OA were randomized to receive intra-articular injection of 2 mg clodronate or placebo for 4 weeks. Clodronate treatment significantly reduced pain scores and improved the Lequesne functional index, which was associated to a reduced analgesic consumption compared to placebo 45.

A randomized, double-blind, placebo-controlled study assessing the efficacy of i.v. neridronate in controlling pain in patients with acute painful knee OA showed that intra-venous neridronate 100 mg daily for 10 days significantly reduced pain and improved quality of life compared to placebo; further, the bone marrow lesions evaluated by MRI showed a significant decrease only in the neridronate group.46

In a cross sectional study the effects of alendronate and other anti-resorptive drugs (oestrogens and raloxifene) on the structural features of knee OA in elderly women, assessed by magnetic resonance imaging and radiography, and on the severity of symptoms, were compared.47 No significant association between overall use of anti-resorptive drugs and the presence of knee pain and radiographic changes of OA of the knee were found. Nevertheless, alendronate, but not oestrogens, was associated with less severe knee pain. Whilst there was no statistically significant difference between the intervention groups in WOMAC pain score, a statistically improved scores in the alendronate group compared to the no-treatment group was observed. Both alendronate and oestrogens were associated with significantly fewer sub-chondral bone attrition and bone marrow abnormalities of the knee, as assessed by MRI, suggesting a potential structural effect on knee OA.

The Fracture Intervention Trial (FIT) is a large randomised study that evaluated the anti-fracture effect of alendronate versus placebo over 4 years in post-menopausal women. A subgroup of 200 participants in this study was randomly selected for further radiographic analysis focusing on features of spinal OA, in order to examine the effects of alendronate on the progression of spinal disc degeneration compared to placebo group. The adjusted mean change in osteophyte score in the alendronate treatment group was lower compared to the placebo group. Nevertheless, the spinal radiographic changes were subtle, with minor clinical relevance, as acknowledged by the investigators. The adjusted mean changes in disc-space narrowing were also lower in the alendronate group than the placebo group, but the difference did not reach a statistical significance 49.

In the analysis of data from the Osteoarthritis Initiative (OAI) the effects of potential benefits of BPs over a number of years were evaluated. The investigators aimed to examine the effect of long-term (up to 4 years) BPs use on OA symptom and structural outcomes in people selected from the OAI cohort, a multicentre population-based observational cohort study of knee OA. Patients with clinical OA in early stages with poor prognosis were included and grouped in those not using bisphosphonates and those who used the drugs for other purposes (treatment of osteoporosis or Paget’s disease). Differences between users and non-users in knee pain severity scores, WOMAC score and radiographic joint-space narrowing at each annual time point were assessed. A significant decrease of the numerical scale ratings of pain was observed after 2 and 3 years, but the effect had declined by year 4. Nonetheless, the WOMAC pain scores in BPs users were not significantly lower than the non-users. Only a trend towards less joint-space narrowing by year 4 was observed in patients using bisphosphonates compared with those not using the drugs 50.

Overall, the available data from clinical studies, showed that BPs are effective in terms of reduction of joint pain and stiffness and in improving function, although their effect on OA structural changes and progression are controversial 51.

CONCLUSIONS

BPs may determine some positive structural and symptomatic effects in the treatment of OA through different mechanisms. BPs may affect osteoclast and osteoblast metabolism in the sub-chondral bone, reducing the high bone turnover mediated by osteoclasts and by inducing an anabolic effect on osteoblasts. Further, BPs may benefit the OA joints by inhibiting the synovial inflammation, which is associated with both symptoms and structural damage progression 52 53. Even if many studies showed a positive effect of various BPs
on different clinical and structural findings of OA, the real effectiveness of these drugs as disease modifying therapy is not clearly established. A number of methodological weaknesses, such as the heterogeneity of patients, the different stages of disease of recruited patients, the small-size of case-series, and the lack of evaluation of bone and cartilage lesions by using standardized scoring system limit the scientific value of the mentioned studies. Nevertheless, further clinical studies could confirm the usefulness of BPs for treatment of OA, considering the well-known pain-relieving anti inflammatory effects of these drugs and the key role played by subchondral bone in the pathogenesis of OA.

References


Sensible prescribing for older adults: illustrated cases

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Older adults are especially vulnerable to adverse drug events leading to emergency hospitalisations. Clinicians should aim to ensure the maximum benefit from medications and few adverse effects by avoiding excessive, inappropriate or inadequate prescribing. Sensible prescribing for older people requires knowledge of guideline based therapy, principles of safe prescribing, drug interactions and checking patient compliance in taking medications correctly. Real case examples are provided here to allow application of these principles and to highlight several important points in sensible prescribing for older people.

Key words: Geriatrics, Drug information, Medication safety, Pharmacy education

INTRODUCTION

Older adults are especially vulnerable to adverse drug events leading to emergency hospitalisations. Clinicians should aim to ensure the maximum benefit from medications and few adverse effects by avoiding excessive, inappropriate or inadequate prescribing. Several resources are available to assist with this endeavor. The World Health Organization has published a practical manual to provide general guidelines to good prescribing. STOPP criteria and Beers criteria offer a list of potentially inappropriate medicines, which are to be avoided in older people. Practical steps are available for de-prescribing medicines in patients who experience polypharmacy.

Pharmacists play an important role in teamwork with clinicians to ensure high quality prescribing and should be aware of these guidelines. However, while the literature provides the science, these will never replace clinical experience in applying the art of sensible prescribing. Real case examples are provided here to allow application of these principles and to highlight several important points in sensible prescribing for older people.

CASE 1

BACKGROUND
73 year old female admitted 3 weeks prior for sepsis from left leg cellulitis and gangrene. She has a background of peripheral vascular disease with right below knee amputation, ischaemic cardiac disease and congestive cardiac failure with stents inserted two and four years prior, hypertension, hyperlipidaemia and type 2 diabetes. She was diagnosed with septic shock requiring admission to intensive care unit for inotropic support and intubation and left below knee amputation before she improved and was transferred to the wards. She was subsequently fully dependent on all activities of daily living including feeding.

PROGRESS
Her inpatient stay was also complicated by left arm cellulitis from a cannula site, aspiration pneumonia, sacral pressure ulcer and an acute haemoglobin drop requiring transfusion with gastroscopy confirming erosive gastritis. She was referred for Geriatric Medicine input for ongoing management and discharge planning. On review, she complained of stump pain. She was reluctant to sit up due to dizziness. She was tearful during
the conversation. Clinically, she was malnourished and dehydrated with dry mucous membranes and low jugular venous pressure. Blood pressure 120/70 mm Hg. Cardiorespiratory examination was normal. The left arm cellulitis area, bilateral lower limb stumps and pressure ulcer were clean. Serum potassium was 5.3 mmol/L and creatinine 60 mmol/L.

**Medication List**

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dose, Frequency, Route</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>10 mg OD PO</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>300 mg OD PO</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>75 mg OD PO</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>Isosorbide mononitrate</td>
<td>60 mg OD PO</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.125 mg OD PO</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Furosemide</td>
<td>60 mg BD IV</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>25 mg OD PO</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>60 mg OD PO</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Ceftazidime (day 10)</td>
<td>1000 mg BD IV</td>
<td>Aspiration pneumonia</td>
</tr>
<tr>
<td>Metronidazole (day 10)</td>
<td>500 mg TDS IV</td>
<td>Aspiration pneumonia</td>
</tr>
<tr>
<td>Cloxacillin (day 7)</td>
<td>1000 mg Q6H IV</td>
<td>Upper limb cellulitis</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>20 mg OD PO</td>
<td>Gastritis</td>
</tr>
<tr>
<td>Sodium chloride 0.9%</td>
<td>2.5 mL QID NEB</td>
<td>Respiratory secretions</td>
</tr>
</tbody>
</table>

OD: once daily; BD: twice daily; TDS: three times daily; QID: four times daily; PO: oral; IV: intravenous; NEB: nebulisers

**Medication Changes**

As she was clinically dehydrated with possible symptomatic postural hypotension, amlodipine, irbesartan, furosemide and spironolactone were ceased with regular fluid balance review. Due to her gastrointestinal bleed, clopidogrel was withheld and Omeprazole increased to 40 mg daily. All three antibiotics were stopped as the courses were completed. Saline nebulisers were discontinued and Physiotherapy was requested to assist with clearing respiratory secretions.

Regular Paracetamol and Morphine as required was given for the stump pain. As there was a possible neuropathic component and depression, she was commenced on Amitriptyline. She had a nasogastric tube for high protein supplementation for malnutrition and to facilitate healing of the pressure area.

**Discharge Medications**

Paracetamol 1 g three times daily.
Amitriptyline 20 mg at night.
Omeprazole 40 mg once daily.
Carvedilol 12.5 mg twice daily.

**Learning Points**

During sepsis, vasodilation may cause hypotension requiring significant fluid resuscitation and possibly inotropic support. Anti-hypertensives and diuretics should be withheld until recovery (unless diuretics are required for pulmonary oedema). The clinical fluid status should be assessed daily and gradually adjusted or recommenced after recovery from the acute phase.

Treatment with intravenous antibiotics should be reassessed after 48 hours. When culture results are available, the narrowest spectrum antibiotic should be used. Change to oral therapy should be considered unless there is a persistent focus of infection requiring ongoing intravenous therapy such as abscess, osteomyelitis or endocarditis.

Risk versus benefit of antiplatelet agents or anticoagulants must be considered during each clinical presentation. They should be withheld in the setting of an acute bleed. Pain assessment includes determining the type and severity of pain. Stump pain responds well to paracetamol and opiates, while phantom limb pain or neuropathic pain may be treated with tricyclic antidepressants or gabapentin. If the pain is quite severe, a step down approach may be preferred. It is important to plan follow-up analgesia prescriptions, especially for opiates so that requirements may be reassessed for down-titration once the injury has healed.

**Case 2**

**Background**

85 year old female was referred to Geriatric Medicine Clinic for intermittent abdominal pain and perianal discomfort. She was seen by a Renal Physician for chronic renal disease with creatinine stable at 120 mmol/L. She has background of diabetes, hyperlipidaemia and an unremarkable gastroscopy and colonoscopy for the abdominal pain. At the Renal Clinic review, the Nephrologist identified polypharmacy and discontinued Ferrous Fumarate (constipation), Ranitidine, Multivitamins and Gliclazide (random blood glucose 3 to 4 mmol/L).

**Progress**

On review, abdominal pain was described as intermittent cramps throughout the abdomen. This worsened when passing bowel motions which were usually hard and infrequent. However, there was watery stool up to three times daily during the previous two days. She was clinically dehydrated. Blood pressure was 90/60 mm Hg. Examination identified abdominal fullness in the descending colon without tenderness. Bowel sounds were present. She declined a rectal examination due to discomfort. Faecal impaction with overflow diarrhea from constipation was strongly suspected.
**Medication list**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Dose, frequency, route</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>80 mg BD PO</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Losartan</td>
<td>50 mg OD PO</td>
<td>Chronic renal disease</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>10 mg OD PO</td>
<td>Hyperlipidaemia</td>
</tr>
<tr>
<td>Folic acid</td>
<td>5 mg OD PO</td>
<td>Supplement</td>
</tr>
<tr>
<td>Vitamin B complex</td>
<td>1 tab OD PO</td>
<td>Supplement</td>
</tr>
</tbody>
</table>

**Private clinic**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Dose, frequency, route</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole</td>
<td>40 mg OD PO</td>
<td>Dyspepsia</td>
</tr>
<tr>
<td>Prucalopride</td>
<td>2 mg OD PO</td>
<td>Stimulate colon motility</td>
</tr>
<tr>
<td>Mebeverine</td>
<td>200 mg BD PO</td>
<td>Antispasmodic: abdominal pain</td>
</tr>
<tr>
<td>Proctosedyl ointment</td>
<td>15 mg BD TOP</td>
<td>Haemorrhoids</td>
</tr>
<tr>
<td>Soluble dietary fibre</td>
<td>1 tsp BD PO</td>
<td>Constipation</td>
</tr>
<tr>
<td>Vitamin B complex</td>
<td>1 tab OD PO</td>
<td>Supplement</td>
</tr>
</tbody>
</table>

OD: once daily; BD: twice daily; PO: oral; TOP: topical

**Medication changes**

Fortunately, all medications were brought in separated into two boxes (one from hospital and the other from private clinic). Duplication in vitamin B complex supplement was identified. She was continuing to take Gliclazide even after advice to discontinue it.

She was prescribed daily phosphate enemas for two days followed by Lactulose as required. Fibre was withheld initially as it may contribute to excessive gas and bloating, which may be additional discomfort in addition to faecal impaction. She may resume this with plenty of fluids once bowel motions were regular.

Prucalopride is a 5-HT4 receptor agonist which stimulates motility during chronic constipation. Mebeverine is an antispasmodic without anticholinergic side effects mainly used in irritable bowel syndrome to reduce abdominal cramps. The former will help with constipation but may exacerbate her cramps, while the latter may improve cramps but worsen constipation. Both were discontinued as they had antagonistic effects.

Losartan was withheld due to hypotension. On further discussion of risks versus benefits, she wanted to discontinue the Atorvastatin, vitamin B supplement and Folic acid.

At follow-up in two weeks, she had regular bowel motions and her abdominal pain resolved.

**Discharge medications**

Pantoprazole 40 mg at night.
Proctosedyl ointment 15 mg twice daily.
Soluble dietary fibre 1 tsp twice daily.

**Learning points**

It is useful to have patients bring in all their medications, including alternative therapies and supplements. This enabled us to identify that she has seen another clinician privately and is on additional medications not on public electronic records. Differences between medication prescriptions and what is taken at home may be identified as well.

Consider whether symptoms patients experience could be an adverse effect from a medication. Rather than start another drug for symptomatic treatment, the culprit drug should be ceased. Caution is required when patients are on similar drug classes or types, which increases the likelihood of adverse events. Similarly, there is a reduced benefit to patients with antagonists with an additional problem of polypharmacy and risk of other drug interactions.

Shared decision making is important to ensure alignment in goals of treatment between the health professional and the patient. This is a useful process particularly with polypharmacy and in elderly or frail patients where the goal of treatment is quality of life rather than longevity.

**CASE 3**

**Background**

85 year old female acutely admitted for reduced level of consciousness and hypoglycaemia. She has a background of Parkinson’s disease, ischaemic heart disease, hypertension, hyperlipidaemia and type 2 diabetes. She had multiple recurrent admissions for hypoglycaemia; presumed secondary to poor oral intake as diabetes medications were discontinued previously.

**Progress**

She clinically improved after paramedics commenced her on intravenous dextrose. Clinical examination was unremarkable.

**Medication list (according to clinical records)**

- Clopidogrel 75 mg once daily.
- Co-careldopa 125 mg three times daily.
- Omeprazole 20 mg daily.
- Senna 7.5 mg at night.
- Lactulose 20 mL once daily.
- Fleet enema 1 bottle as required.

Relatives were requested to bring in all her medications from home. Her medications are shown in the Figure 1.

**Medication changes**

All previously discontinued medications that the patient still had were discarded in hospital. For current medications, they had two boxes or bottles of each drug
returned. The main carer was educated regarding the medicines and a list was provided for their reference.

**Discharge Medications**
No changes to medication list.
Multiple medications from home removed.

**Learning Points**
It is useful to have all medicines from home brought in for drug reconciliation. There is a tendency to prescribe regular medications on discharge from hospital. For those with recurrent admissions, this risks accumulation of medications at home. They may also have picked up a new prescription before the admission. We should check whether the patient needs a prescription for regular medications before prescribing on discharge. Changes to regular medication should be highlighted to the patient, so they know which ones to discontinue when they return home.

**Conclusions**
These cases are real examples of problems encountered in clinical practice that detract from safe prescribing and provides learning points to use in subsequent patient encounters. Sensible prescribing for older people requires knowledge of guideline based therapy, principles of safe prescribing, drug interactions and checking patient compliance in taking medications correctly. It is hoped that these examples illustrate the principles that are required for sensible prescribing in older people.

**References**
Everybody knows that to treat the elderly is a tricky and hard job. There are many reasons, but the main variables are: the different pharmacokinetics (PK) and pharmacodynamics in comparison to the adults, polypharmacy, drug interactions, the commonness of adverse reactions, the presence of multimorbidities and comorbidities, and patient compliance. A drug’s PK changes with aging, primarily as a consequence of changes in body composition and in renal physiology, but also as a result of acute or chronic diseases, being male or female, and of treatment with other drugs; moreover, the gut is also able to affect drug destiny due to changes in the microbiota, in the diet, and to exposure to drugs that affect the microbiota itself. The changes in body composition (fat/muscle ratio) that are typically observed in the elderly have major effects on the water amount in the body and on the distribution volume of a drug. Similarly, changes in the function of the liver, which, together with the kidneys, plays a central role in a drug’s metabolism, conjugation and excretion/demolition are also typical of the aging process and affect PK. In addition, the effect of the genetic background, the expression of enzymes (such as cytochromes), the competition or inhibition/stimulation induced by different drugs, and the density of receptors also account for the observed pharmacodynamics of a drug in the older patient.

Finally, factors that may make the response to a drug even more hard to predict include the extremely diverse effects of a molecule, resulting from the activation of intertwined and complex post-receptor pathways. Despite extensive knowledge of drugs of common use, drug interactions frequently trigger unexpected adverse events, which are sometimes negligible, but sometimes very dangerous, devastating, with harmful consequences, leading to a patient’s hospitalization or even to his death. The presence in a patient of a “hyper-polypharmacy” (more than 10 drugs) brings the probability of an adverse event very high. In Ireland, old people taking more than 10 drugs have increased from 1.5% in 1997 to 21.9% in 2012 with most of these medications likely being prescribed on the basis of recent guidelines for chronic diseases. Finally, I mentioned the issue of compliance because this is another puzzling factor (how could you imagine the adherence to the therapy of your old patient?) that further complicate this problematic area. In this context, many studies show a very discouraging scenario: after six-to-twelve months from the beginning of a defined treatment, elderly patients will typically forget or voluntarily avoid the prescribed drug, and that become an even bigger problem if the patients is supposed to take several drugs. The inappropriateness of many medications is another common problem, which I shall not address here. Finally, these issues become further compounded, when a caregiver is involved. The problems and complaints of the latter add complexity to the patient management and may make our treatment choices more difficult.

Overall, it is clear that it is almost impossible to foresee with reasonable certainty the effect of a prescription and the adverse events it will cause. Besides these critical, but realistic, considerations, I’d like to recommend a brief, but stimulating, paper on this topic, that addresses the problem of the therapy prescription in elderly patients with dementia.

**Key words:** Polypharmacy, Dementia, Deprescribing
In a recent paper, Carole Parsons, an Irish clinical pharmacist, underlines that the appropriateness of drug prescriptions is frequently neglected in this group of patients and that very little information can be found in the literature to inform therapeutic decisions. Therefore, she hopes (and solicits) that more data will become available in the near future.

The patients diagnosed with dementia have a reduced life expectancy compared to patients who do not have a dementia. In addition, a diagnosis of dementia is typically associated with an advanced age, and a high multi- and co-morbidity. In such complicated patients, no validated criteria or indications, no studies evaluating the burden of the therapy or describing the best choices have been published, and only some warning or potential threats were reported.

The literature analysis performed by the Author showed that "considerable scope exists to improve the quality of prescribing for people with dementia" in term of mis-, over- and under-prescribing.

The Author reported that, surprisingly, 40-60% of the elderly affected by dementia are on anticholinergic medications, which are clearly contraindicated since they frequently cause a worsening in a patient’s cognitive functions.

The Author also showed that antipsychotic agents that are commonly prescribed to elderly are largely useless and that frequently physicians simply prescribe antipsychotics by habit or drift. Similarly, the use of antibiotics in the late stage of dementia is also controversial, either because of the negligible or not significant symptoms that are associated with common infections in the elderly, such as urinary tract infections, or because antibiotics do not modify the clinical trajectory of the infection itself and because the latter commonly relapses anyways.

On the other hand, pain killers are frequently under-prescribed in the elderly, an aspect that has been extensively studied in literature, in particular in cognitively impaired patients the treatment is even more neglected. Such habit usually reflects the fact that pain is under-detected in the elderly, which, in turn, is due to the inability of many patients to communicate properly. Notably studies also show that, although efficient and validated scales for detecting pain in not-communicating patients do exist, they are normally not used in routine clinical practice. Thus the study stressed that more attention should be paid to fighting pain in the elderly.

Treating elderly is really complicated and it requires the geriatrician to pay considerable attention to his patients. The study by Parsons warrants further investigations to answer the many open questions in this area. I said that treating elderly is tough, but treating and prescribing appropriately in patients with dementia is even tougher. Thus, it is mandatory to begin developing evidence-based guidelines to approach the problem, where the ultimate goal of this effort should obviously be improving patient care and quality of life, but also providing the caregiver with better information, and saving health systems’ budgets, which would otherwise be wasted in many useless or poorly prescribed medications.

References