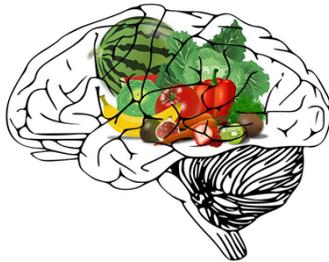




Workshop

Protective / preventive role of bioactive food components in human health

Nutraceuticals in neuroprotection



Cristina Angeloni

Department for Life Quality Studies
Alma Mater Studiorum-University of
Bologna (Italy)

Novisad, 13th December 2016

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI



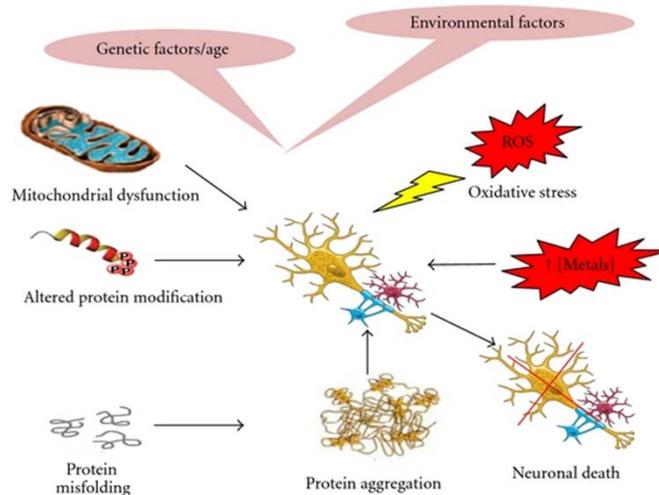
What is neurodegenerative disease?

- Neurodegenerative disease indicates a range of conditions which primarily affect the neurons.
- **Neurons** are the building blocks of the nervous system and normally **don't reproduce or replace themselves**.
- Neurodegenerative diseases are characterized by a progressive degeneration and / or death of neurons. This causes problems with movement (called ataxias), or mental functioning (called dementias).

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Common features of neurodegeneration



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

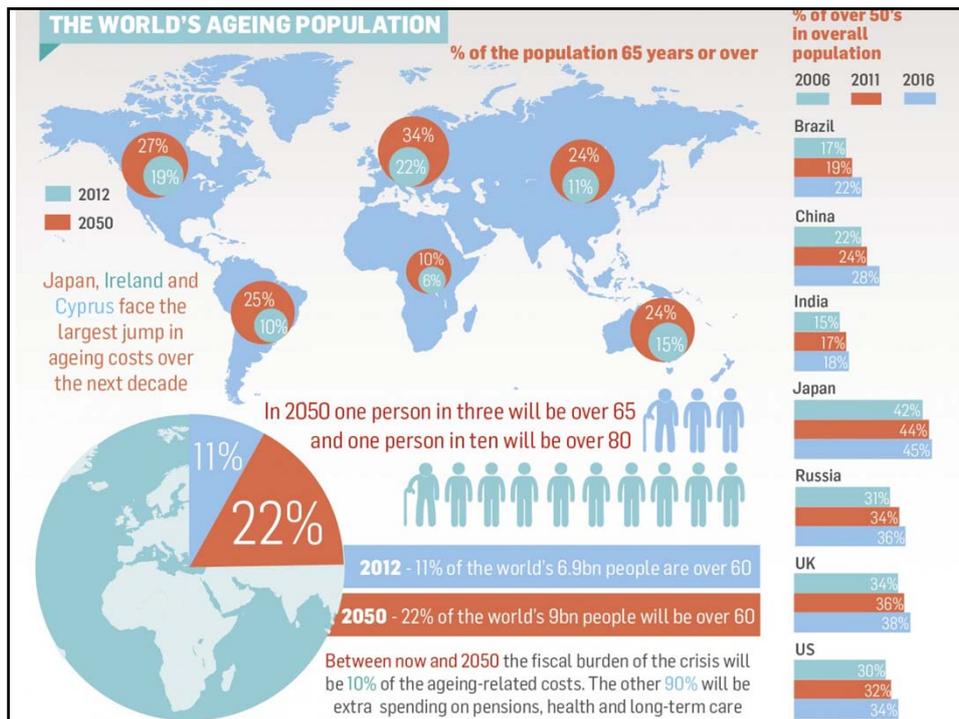


Aging and neurodegeneration

- ✓ Aging is the major risk factor for the majority of neurodegenerative diseases
- ✓ Dementia is a worldwide public health issue, currently affecting 35 million people, with numbers expected to reach 115 million by 2050
- ✓ Prevalence of Parkinson's disease is roughly estimated to be 0.3% of the general population and 1% in those aged more than 60 years

Corbett A. et al Front Biosci . 2011; 7:184-188.
Blin P. et al. Eur J Neurol. 2015;22:464-7

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Alzheimer's disease

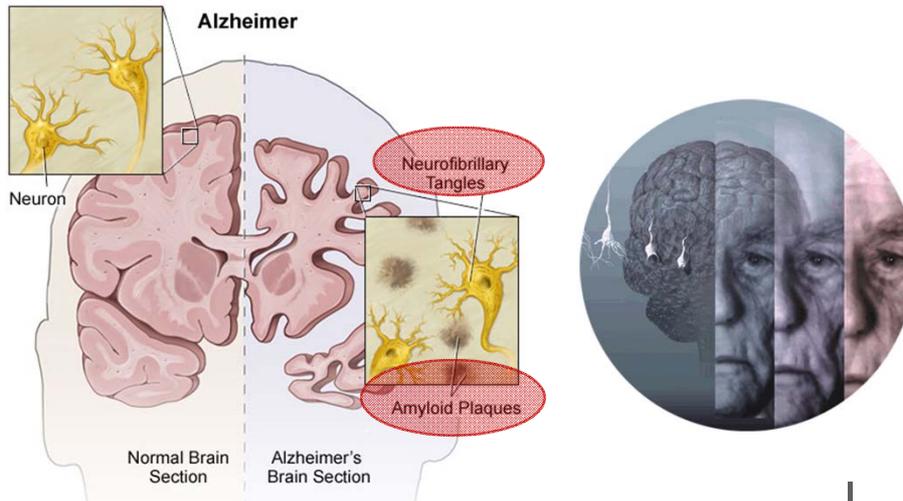
Is a progressive neurodegenerative disorder characterized by deposition of

- amyloid plaques
- neurofibrillary tangles,
- microglial and astroglial activation,

finally, leading to neuronal dysfunction and death.



Alzheimer's disease



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Pharmacological treatments

- Current treatments primarily focus on enhancement of cholinergic transmission.
- **These treatments are only symptomatic, and no disease modifying drug is available for AD patients**
- With failure of so many anti-amyloid trials, new targets are emerging such as inflammation, glucose metabolism, oxidant and carbonyl stress

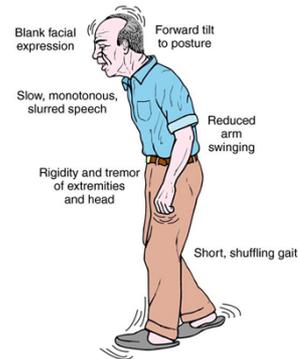


ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Parkinson's disease

- Parkinson's disease (PD) is a movement disorder currently affecting around 5–6 million.
- In 2030, about 10 million or more are expected to be afflicted with PD.
- The disease is characterized by motoric deficits including resting tremor, bradykinesia (slowness in movements), postural instability and rigidity



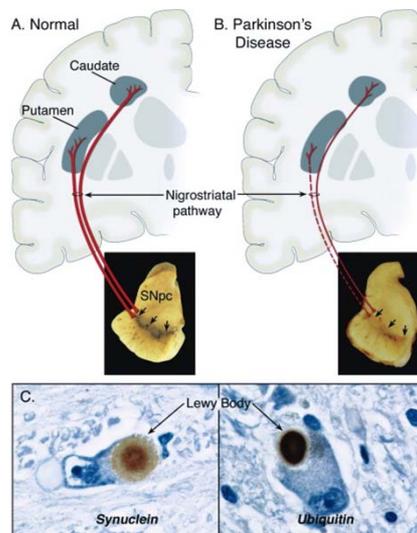
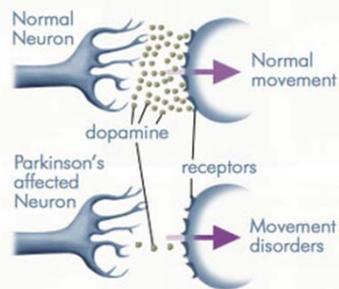
ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Parkinson disease

Depletion of striatal dopamine

Dopamine levels in a normal and a Parkinson's affected neuron.



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



PD and oxidative stress

- Dopamine is inactivated by the monoamine oxidase enzyme (MAO), a reaction that yields significant amounts of hydrogen peroxide that must be continuously detoxified by intracellular antioxidants.
- Oxidative and nitrative stress occurring in substantia nigra is prominent features of this disease
- The source of nitrogen species is related to alterations in iNOS activity. The origin of oxygen radicals is increased iron levels, alterations in antioxidant mechanisms, and mitochondrial dysfunction.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Current therapy

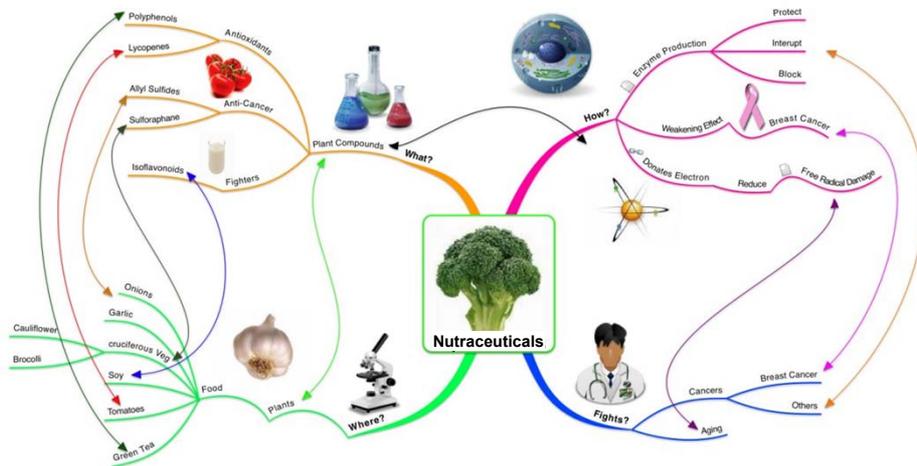
- At present, therapeutic strategies for the PD patient remain largely symptomatic
- Existing pharmacological treatments come with undesirable side effects.
- Pharmacological replacement of dopamine with L-Dopa remains the gold standard for PD treatment



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Nutraceuticals



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Bioavailability of nutraceuticals

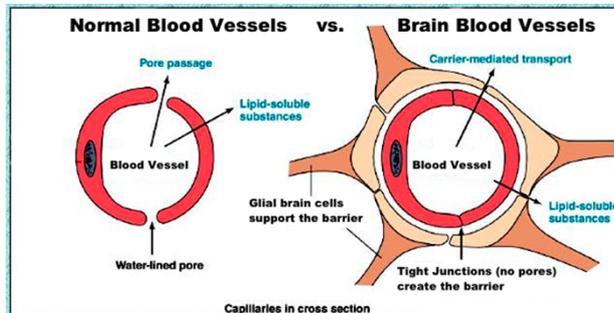
- The bioavailability of nutraceuticals is largely diversified from one compound to another, due to their chemical structure. Their **absorption rate, metabolism, and biological activities** are affected by their composition, but also depend on the direct interaction with other dietary components, such as proteins, carbohydrates, fiber, fat and alcohol.
- In order to reach the brain, orally ingested nutraceuticals must **cross two barriers**, i.e. the **enterocytes in the intestine** and the **blood–brain barrier**.



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



The blood brain barrier



It is a membrane that prevents the uncontrolled leakage of substances from the blood into the brain.

It is a complex, dynamic, adaptable interface that controls the exchange of substances between the CNS and the blood

- This complexity in barrier function explains much of the difficulty in developing drugs that can cross the BBB, but on the other hand it offers unique and diverse opportunities for drug development.

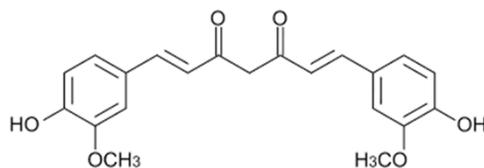
ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Curcumin



Curcumin, a diarylheptanoid polyphenol isolated from the rhizomes of *Curcuma longa* L. is a food spice used extensively in the Indian cuisine and Ayurvedic medicine.



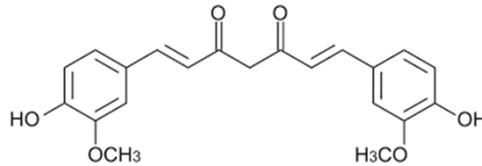
Curcumin has been reported to have multiple pharmacological activities and to be effective against a wide variety of diseases due to its anti-carcinogenic, hepatoprotective, thrombosuppressive, cardioprotective, anti-arthritis, anti-infectious and neuroprotective properties.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Curcumin

- The antioxidant potential of curcumin derives from its capacity to scavenge free radicals due to its unique structure that can donate H-atoms or transfer electrons from two phenolic sites.
- As this phenolic compound is more soluble in lipophilic conditions, it performs the ROS scavenging activity most effectively within the polar cytoplasm.
- Curcumin could bind to the redox-active metals iron and copper



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Curcumin bioavailability

- Limitations for the use of curcumin in nutraceutical interventions is its **limited bioavailability** which is mainly due to:
 - poor aqueous solubility,
 - poor membrane permeability,
 - degradation and rapid metabolism (biotransformation of curcumin to curcumin O-glucuronide and curcumin O-sulfate, which are catalyzed by UDP-glucuronyltransferase and sulfotransferases in enterohepatic organ, contribute to major absorption barrier

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



How to increase curcumin bioavailability

- Physically redesigned curcumin preparations using nanoparticles, liposomes or inclusion complexes have been developed
- Piperin has been added to curcumin preparation, piperin reduces the expression of hepatic UDP-glucuronyltransferase and sulfotransferases
- A recent paper demonstrated that pre-treating with piperin prior to curcumin administration rather than increasing dosage of piperin is much more effective
- Duration of piperin pre-treatment should be within 0.5 to 6 hours

Zeng et al., *Biopharm Drug Dispos.* 2016 Nov 23

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Curcumin effects against AD

- Curcumin decreases A β levels through inhibition of A β oligomer formation, along with decreasing oxidative stress and inflammation in mice (*Fang et al., Bioorg. Med. Chem. Lett.* 2014; 24:40-43).
- Curcumin nutraceutical intervention attenuated oxidative injury, microgliosis, spatial memory deficits, postsynaptic loss, and A β deposits in a model of AD in rats.
(*Frautschy et al. Neurobiology of aging.* 2001;22:993-1005)

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



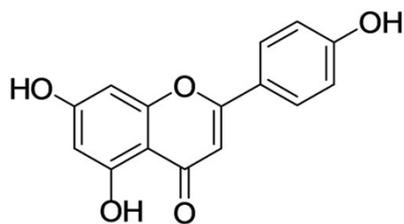
Curcumin effects against PD

- In a mice model of PD, three weeks of curcumin treatment significantly improved behavioral alterations, oxidative damage and mitochondrial enzyme complex activities (*Khatri and Juvekar Pharmacol Biochem Behav. 2016;150-151:39-47*)
- Curcumin protected against 6-OHDA-induced neural impairments in rats, improving memory abilities, elevating levels of SOD and GSH-Px, and reducing concentration of malonaldehyde (MDA). In addition, dopamine levels were increased in the SN and nerve growth factor expressions was up-regulated. (*Song et al., Pathol Res Pract 2016;212:247-51*)

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Apigenin



- Apigenin is a flavonoid abundant in the flowers of the chamomile plant and also found in other sources such as celery, parsley, peppermint.
- Apigenin crosses the BBB.
- The half-life of apigenin in plasma was calculated to be 12 h in healthy humans

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Apigenin neuroprotective activity

- Apigenin has shown protection against A β induced toxicity in rat cerebral microvascular endothelial cells (Zhao et al., *Mol. Cancer* 2011;10:104).
- Apigenin has shown potent anti-oxidant and antiapoptotic properties, by protecting rat neuronal cells subjected to oxygen and glucose deprivation/reperfusion injury (Guo et al., *Neurochem. Res.* 2014;39: 2197-2210).



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Apigenin animal studies

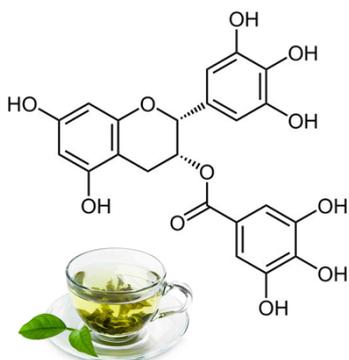
- Transgenic AD mice fed with apigenin (40 mg/kg) for 3 months have shown improvements in memory and learning deficits, and a reduction of fibrillar amyloid deposits (Zhao et al., *Molecules* 2013;18: 9949-9965)
- Cognitive enhancing effects have also been reported for apigenin in young male rats, showing apigenin improved memory in the [passive avoidance task](#) (Popovic et al., *J. Psychopharmacol.* 2014 28, 498-501)



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Epigallocatechin-3-gallate from tea



Unfermented teas are derived from the steamed and dried leaves of the *Camellia sinensis* plant. Polyphenols from green tea have been shown to be powerful hydrogen-donating antioxidants, free radical scavengers of ROS and RNS in vitro.

Of the four major tea catechins in fresh tea leaves, epigallocatechin-3-gallate is the major constituent (~60%), followed by epigallocatechin, epicatechin and epicatechin-3-gallate (ECG).

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Bioavailability

- Oral administration of EGCG at a dose of 100 mg/kg led to a C_{max} of 1.52 ± 0.1 mM.
- EGCG concentrations in the brain were much lower, as a dose of 50 mg/kg only leads to tissue concentrations of approximately 5 ng EGCG/g tissue.
- After absorption, EGCG is modified by sulfation, glucuronidation, and methylation before hepatic metabolism.
- Serum half-life in mice and humans was calculated to be around 4-5 h

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



In vivo effect of EGCG

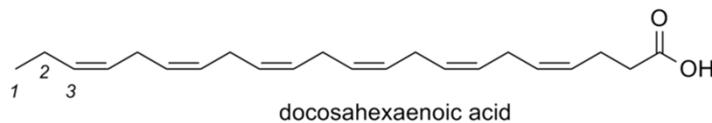
- AD mice treated with 20 mg/kg EGCG via intra-peritoneal injections for 60 days have shown decreased A β levels, via promotion of the α -secretase pathway (*Rezai-Zadeh et al., J. Neurosci. 2005; 25, 8807-8814*).
- Oral administration of the EGCG (50 mg/kg daily), has shown improved behavioral manifestations and cognitive impairments in AD mice (*Walker et al., J. Alzheimer's Dis. 2015; 44: 561- 572*).
- EGCG has also been reported to prevent neuronal cell death caused by A β in AD mice (*Levites et al., FASEB J. 2003;17: 952-954*).



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Docosahexaenoic acid



- Docosahexaenoic acid is an essential omega-3 (n-3) polyunsaturated fatty acid (PUFA) that is found abundantly in marine fish.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Docosahexaenoic acid

- DHA is known to be the most important n-3 PUFA in the brain, accounting for roughly 15% of total fatty acids in grey matter, where it is enriched at synapses
- Epidemiological data indicate that a low dietary intake of n-3 PUFA is a risk factor for AD
- People who ingest higher levels of DHA are less likely to develop cognition defects and AD



DHA bioavailability

- As free acids, the fish oil fatty acids are well absorbed (>95%).
- As triacylglycerols, eicosapentaenoic acid and docosahexaenoic acid were absorbed only 68% and 57%
- DHA has a half-life of 2.5 years
- Omega-3-fatty acids can easily penetrate the BBB
- Ideal daily dose would be between 180 and 2000 mg



Cellular studies on DHA

- DHA has **anti-inflammatory effects**. It downregulates the production of proinflammatory cytokines such as IL1 β , IL6 and TNF- α , and inhibit NF- κ B transcriptional activity (*Mullen et al., J. Nutr. Biochem 2010; 21: 444:450*).
- DHA provided cortical neurons in vitro a higher level of resistance to the cytotoxic effects induced by soluble A β oligomers (*Florent et al., J. Neurochem. 2006; 96: 385-395*).
- DHA decreased A β secretion from aging human neuronal cells (*Lukiw et al., J. Clin. Investig. 2005; 115: 2774-2783*).

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Human studies on DHA

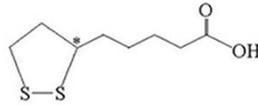
- 174 subjects with mild to moderate AD were fed with n-3 or a placebo for 6 months. In the n-3 treated group a significant decrease in Mini-Mental State Examination (MMSE) decline rate has been observed when compared to the placebo group. (*Freund-Levi et al., Archives Neurol. 2006; 63: 1402-1408*).
- A study on 23 patients with mild cognitive impairment for 24 weeks using n-3 fatty acid (1.8 g/day) and placebo (olive oil) exhibited improvement in Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) compared to the placebo group (*Chiu et al., Prog. Neuro Psychopharmacol. Biol. Psychiatry 2008; 32: 1538-1544*).

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



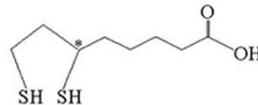
Lipoic acid

α -Lipoic Acid



Oxidized

α -Dihydrolipoic Acid
(DHLA)



Reduced

α -lipoic acid (LA) is chemically synthesized, but can be considered a natural compound as it is a naturally occurring precursor of an essential cofactor for mitochondrial enzymes, including pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



LA bioavailability

- LA is a low molecular weight compound which easily penetrates the BBB
- After crossing the BBB, LA is absorbed into cells and tissues, and it is then reduced to dihydrolipoate acting as a potent antioxidant
- LA has a limited bioavailability ($29.1 \pm 10.3\%$) due to high first-pass metabolism.
- In humans, plasma maximum concentration (C_{max}) values of 1.15 mg/mL mg have been observed for LA at a dose of 1000 mg

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Lipoic Acid and neurodegeneration

- Lipoic acid increases acetylcholine production by activation of choline acetyltransferase and increases glucose uptake
- Lipoic acid chelates redox-active transition metals, inhibiting the formation of hydroxyl radicals and ROS, increasing the levels of reduced glutathione.
- Lipoic acid can scavenge lipid peroxidation products

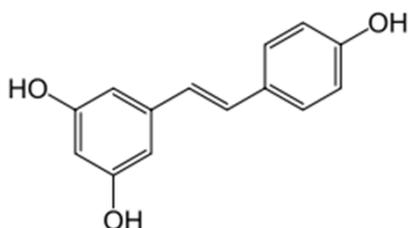


Lipoic acid and neurodegeneration

- In vitro studies have shown that lipoic acid protects cultured hippocampal neurons from A β induced neurotoxicity (*Zhang and Frei, FASEB J. 2001; 15: 2423-2432*)
- Lipoic acid inhibits the formation of A β fibrils (*Ono et al., Biochem. Biophysical Res. 2006; 341:1046-1052.*)
- Lipoic acid can also activate the Nrf2-ARE pathway and act as a glutathione (GSH)-boosting drug, increasing GSH levels in cultured astroglia by up to 1.4 fold (*Steele et al., Redox Biol. 2013; 1:441-445*).



Resveratrol



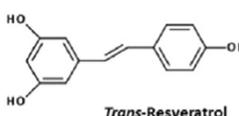
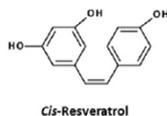
- Resveratrol (3, 5, 4' trihydroxystilbene) is a polyphenolic phytoalexin, produced in response to damage in grapevines and legumes, such as peanuts and tea

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Resveratrol

- Resveratrol exists in two geometric isomers. Cis-resveratrol is less common and very unstable, while trans-resveratrol is biologically more active than cis-isomer



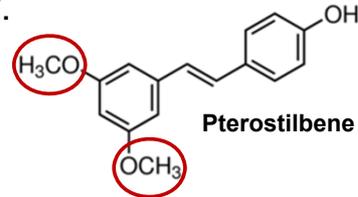
- Resveratrol demonstrates an unusually strong ability to remove free radicals thanks to the presence of three groups - OH in positions 3, 4 and 5, aromatic rings and a double bond in the molecule.
- Removing hydroxyl groups, or replacing them with -OCH₃ groups, results in a loss of the antioxidant properties of the compound .

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Bioavailability

- Resveratrol is poorly bioavailable to the human body.
- Enhanced absorption with methylated analogs (pterostilbene) and piperine supplements
- A dose of 25 mg leads to a plasma concentration of the free form ranged from 1 to 5 ng/mL
- Neuroprotective effects in the CNS suggest that it crosses the BBB



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



In vivo AD studies on resveratrol

- Resveratrol in rats reduced A β -induced neuronal loss and memory impairment through a reduction of iNOS expression and lipid peroxidation and an increase in the expression of heme *oxygenase-1* (Huang *et al.*, *PLoS One* 2011; 6: e29102).
- Dietary ingestion (300 mg/kg/day) of resveratrol to AD mice showed a large reduction of plaque formation in the medial cortex, striatum and hypothalamus (Karuppagounder *et al.*, *Neurochem. Int.* 2009; 54: 111-118).
- Very few clinical studies on resveratrol have been reported.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



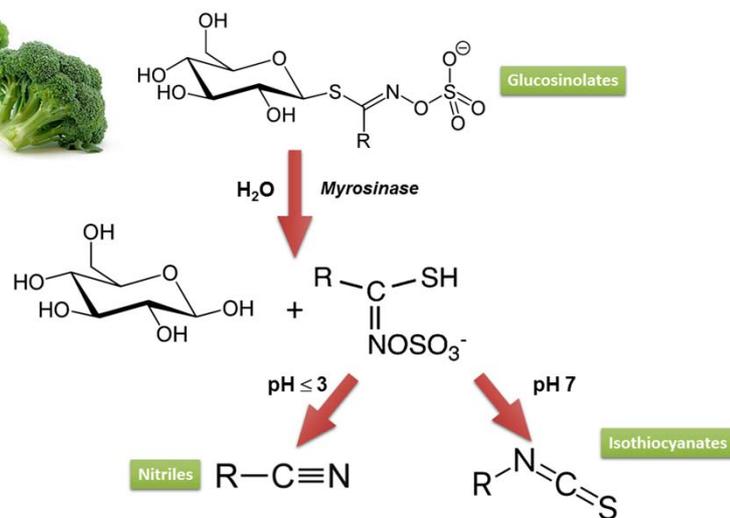
PD studies on resveratrol

- RV exerts neuroprotective effects on dopaminergic neurons probably related to antioxidant properties of the drug (*Alvira et al., Neuroscience, 2007; 147:746–756*)
- RV inducing activation and expression of SIRT1 protects against pathological α -synuclein aggregation. SIRT1 can deacetylate and activate heat shock factor 1 (HSF1), which affects transcription of molecular chaperons including heat shock proteins 70 (hsp70). Hsp70 regulate homeostasis of cellular proteins decreasing the formation of abnormal protein aggregates (*Westerheide et al., Science 2009; 323:1063–1066*)

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Sulforaphane



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Bioavailability of sulforaphane

- Absorption of sulforaphane depends on plant myrosinase residual activity at gut level and on the gut microbiota thioglucosidase activity.
- Sulforaphane is a lipophilic compound characterized by low molecular weight, so it can diffuse through the plasma membrane into the enterocytes.
- Once into the cell, sulforaphane is quickly conjugated to glutathione by the cytosolic enzyme glutathione-S-transferase, driving its passive absorption into the cell.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



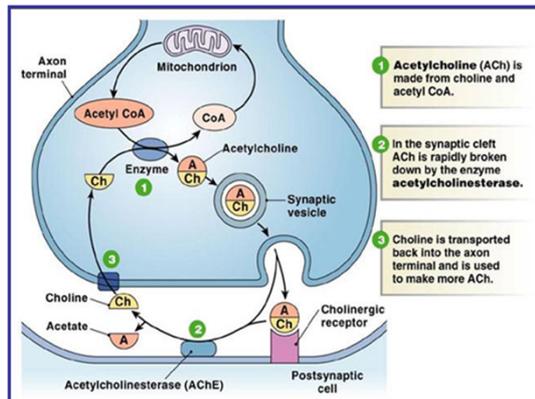
Sulforaphane in AD

- In mice, a 6 days SF treatment ameliorated cognitive dysfunction induced by A β exposure. In particular, SF improved spatial working memory and contextual memory (*Kim et al., Amyloid 2013; 20: 7-12*)
- One hypothesized mechanism by which **SF** could counteract A β peptide-induced toxicity in the brain is its **direct reaction with A β peptides**. SF binds covalently and specifically at position 16 and 28 to the free NH₂ group of N-terminal aspartic acid and the ϵ -amino group of lysine and reduces the inclination of A β to aggregate (*Yasuhara et al., Brain Res 2007;1133: 49-52*).

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA

Sulforaphane in AD

In an AD mice model, SF significantly ameliorated the cholinergic system reactivity, increasing acetylcholine level



decreasing acetylcholinesterase activity, and increasing choline acetyltransferase expression in the hippocampus and frontal cortex of mice (Lee et al., *Pharmacol Res* 2014; 85: 23-32)

Sulforaphane in PD

- SF significantly counteracted nigrostriatal neurodegeneration in mice thanks to its ability to enhance the endogenous antioxidant defense system and to counteract neuroinflammation.
- In particular, **SF activated the Nrf2** mediated pathway inducing the over-expression of HO1 and NQO1 and reduced astrogliosis, microglia activation and the release of two proinflammatory cytokines such as IL-6 and TNF- α (Kazwa et al., *Antioxidants & redox signaling* 2011;14: 2347-2360)



Sulforaphane as an inducer of glutathione prevents oxidative stress-induced cell death in a dopaminergic-like neuroblastoma cell line

Andrea Tarozzi,* Fabiana Morroni,* Adriana Merlicco,* Silvana Hrelia,† Cristina Angeloni,† Giorgio Cantelli-Forti* and Patrizia Hrelia*

*Department of Pharmacology, Alma Mater Studiorum, University of Bologna, Bologna, Italy

†Department of Biochemistry 'G. Moruzzi', Alma Mater Studiorum, University of Bologna, Bologna, Italy

Abstract

The total GSH depletion observed in the substantia nigra (SN) appears to be responsible for subsequent oxidative stress (OS), mitochondrial dysfunction, and dopaminergic cell loss in patients with Parkinson's disease. A strategy to prevent the OS of dopaminergic cells in the SN may be the use of chemopreventive agents as inducers of endogenous GSH, antioxidant and phase 2 enzymes. In this study, we demonstrated that treatment of the dopaminergic-like neuroblastoma SH-SY5Y cell line with sulforaphane (SF), a cruciferous vegetables inducer, resulted in significant increases of total GSH level, NAD(P)H:quinone oxidoreductase-1, GSH-transferase and -reductase, but not GSH-peroxidase, catalase and superoxide dismutase activities. Further, the elevation of GSH levels, GSH-transferase and NAD(P)H:quinone oxidoreductase-1 activities was correlated to an increase of the resis-

tance of SH-SY5Y cells to toxicity induced by H₂O₂ or 6-hydroxydopamine (6-OHDA). The pre-treatment of SH-SY5Y cells with SF was also shown to prevent various apoptotic events (mitochondrial depolarization, caspase 9 and 3 activation and DNA fragmentation) and necrosis elicited by 6-OHDA. Further, the impairment of antioxidant capacity and reactive oxygen species formation at intracellular level after exposure to 6-OHDA was effectively counteracted by pre-treatment with SF. Last, both the cytoprotective and antioxidant effects of SF were abolished by the addition of buthionine sulfoximine supporting the main role of GSH in the neuroprotective effects displayed by SF. These findings show that SF may play a role in preventing Parkinson's disease.

Keywords: antioxidant activity, dopaminergic cells, glutathione, neuronal death, oxidative stress, sulforaphane. *J. Neurochem.* (2009) 111, 1161–1171.

RESEARCH ARTICLE

Sulforaphane protects cortical neurons against 5-S-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, Nrf-2 and the upregulation of detoxification enzymes

David Vauzour¹, Maria Buonfiglio², Giulia Corona¹, Joselita Chirafisi¹, Katerina Vafeiadou¹, Cristina Angeloni², Silvana Hrelia², Patrizia Hrelia³ and Jeremy P. E. Spencer¹

¹Molecular Nutrition Group, School of Chemistry, Food and Pharmacy, The University of Reading, Whiteknights, Reading, UK

²Department of Biochemistry 'G. Moruzzi', University of Bologna, Bologna, Italy

³Department of Pharmacology, University of Bologna, Bologna, Italy

The degeneration of dopaminergic neurons in the substantia nigra has been linked to the formation of the endogenous neurotoxin 5-S-cysteinyl-dopamine. Sulforaphane (SFN), an isothiocyanate derived from the corresponding precursor glucosinolate found in cruciferous vegetables has been observed to exert a range of biological activities in various cell populations. In this study, we show that SFN protects primary cortical neurons against 5-S-cysteinyl-dopamine induced neuronal injury. Pre-treatment of cortical neurons with SFN (0.01–1 μM) resulted in protection against 5-S-cysteinyl-dopamine-induced neurotoxicity, which peaked at 100 nM. This protection was observed to be mediated by the ability of SFN to modulate the extracellular signal-regulated kinase 1 and 2 and the activation of Kelch-like ECH-associated protein 1/NF-E2-related factor-2 leading to the increased expression and activity of glutathione-S-transferase (M1, M3 and M5), glutathione reductase, thioredoxin reductase and NAD(P)H oxidoreductase 1. These data suggest that SFN stimulates the NF-E2-related factor-2 pathway of antioxidant gene expression in neurons and may protect against neuronal injury relevant to the aetiology of Parkinson's disease.

Received: May 1, 2009
Revised: August 28, 2009
Accepted: October 1, 2009



Loss of proteostasis and neurodegeneration

- There is a strict association between loss of proteostasis in neurons and neurodegeneration.
- The impairment of the protein degradation system leads to an abnormal accumulation of toxic protein oligomers, which are considered the starting material for the development of neurodegenerative proteinopathy.
- The spectrum of CNS linked to proteinopathies is particularly broad and includes AD, PD, Lewy body dementia, Pick disease, frontotemporal dementia, Huntington's disease (HD), ALS and many others.



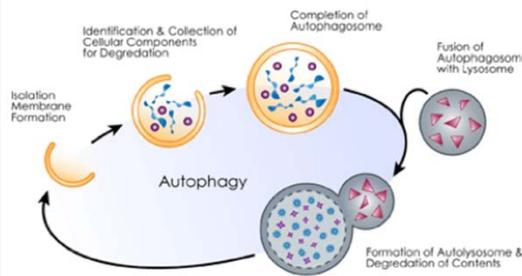
Degradation of unwanted proteins

- Unwanted proteins are safely eliminated via two major mechanisms:
 - **autophagic** degradation in the lysosome
 - by targeted breakdown in the **proteasome**.
- Even if autophagy seems to be a less selective process compared to the proteasome, in recent years, the role of autophagy impairment in neurodegenerative disease has been widely demonstrated.



Autophagyc degradation

Autophagic pathway involves the de novo synthesis of vesicles called autophagosomes, which can embed organelles, protein aggregates, and invading pathogens.



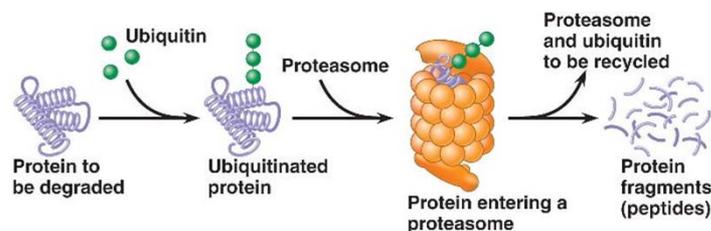
The autophagosomes fuse with endosomal compartments to form amphisomes before fusing with the lysosome, where their contents are degraded and the resulting metabolites are recycled back to the cytoplasm.

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Proteasomal degradation

- Proteasomal degradation in neurons is under the control of the peptide ubiquitin. Ubiquitin covalently binds to the target proteins in a process called conjugation, sealing the fate of proteins that are digested by the proteasome.



- The amino acids obtained by protein degradation are then reused by the cell.

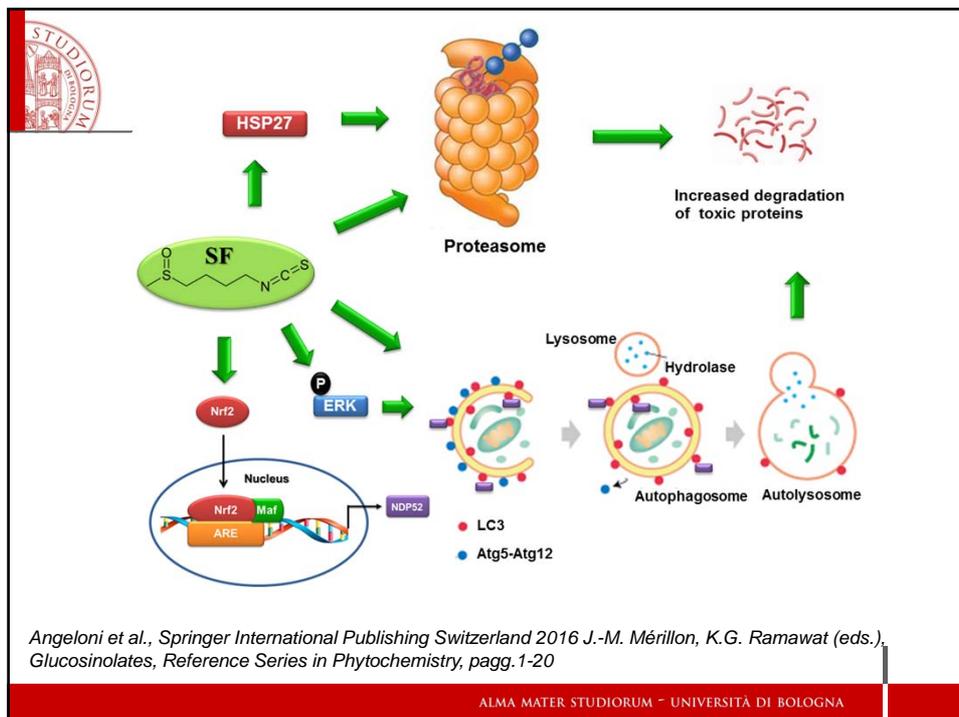
ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Sulforaphane and protein degradation

- **SF increases the expression** of the catalytic core subunits **of the proteasome**, leading to enhanced proteasome activity
- **SF induces autophagy** via extracellular signal-regulated kinase (ERK) activation, independent of Nrf2 activity in neuronal cells (Jo et al., *FEBS Lett* 2014; 588: 3081-3088).
- In vivo data obtained in mice, in the brain, SF is able to enhance autophagy increasing both microtubule-associated protein 1 light chain 3 (LC3)-I and LC3-II levels (Liu et al., *Neurochem* 2014; 129: 539-547)

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA



Summarizing

- Nutraceuticals show different activities against neurodegeneration
- Despite encouraging animal data, the percentage of positive human trials in AD and PD is surprisingly low
- The effect of treatment appears more effective when initiated early in the disease
- Careful prospective studies with these molecules in patients with neurodegeneration may be conducted for assessing the therapeutic benefits.