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Low-Molecular Weight Metabolites from Polyphenols as Effectors for Attenuating Neuroinflammation

Diogo Carregosa,[†,‡] Rafael Carecho,[†,§] Inês Figueira,[‡,§] and Cláudia N Santos*[,†,‡,§]

[†]CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

[‡]iBET, Instituto de Biologia Experimental e Tecnológica, Avenida da República, Apartado 12, 2781-901 Oeiras, Portugal

[§]Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

ABSTRACT: Age-associated pathophysiological changes such as neurodegenerative diseases are multifactorial conditions with increasing incidence and no existing cure. The possibility of altering the progression and development of these multifactorial diseases through diet is an attractive approach with increasing supporting data. Epidemiological and clinical studies have highlighted the health potential of diets rich in fruits and vegetables. Such food sources are rich in (poly)phenols, natural compounds increasingly associated with health benefits, having the potential to prevent or retard the development of various diseases. However, absorption and the blood concentration of (poly)phenols is very low when compared with their corresponding (poly)phenolic metabolites. Therefore, these serum-bioavailable metabolites are much more promising candidates to overcome cellular barriers and reach target tissues, such as the brain. Bearing this in mind, it will be reviewed that the molecular mechanisms underlying (poly)phenolic metabolites effects, range from 0.1 to <50 μM and their role on neuroinflammation, a central hallmark in neurodegenerative diseases.

KEYWORDS: *neurodegenerative diseases, microglia, flavonoid, microbiota, brain*

1. NEURODEGENERATIVE DISORDERS AND DIETARY (POLY)PHENOLS

Neurodegenerative diseases (NDs) are chronic and progressive neurological syndromes, a consequence of nervous system dysfunction that ultimately leads to neuronal cell failure. These disorders are incapacitating and are characterized by impaired mental and movement function.[1] These diseases present a deeply complex pathophysiology prompting atrophy of structures of the central or peripheral nervous system and can be caused by hereditary or sporadic conditions.[2] However, we still do not have available disease-modifying therapies to delay or reverse disease progression, and their pharmacotherapy strategies are only dealing with symptomatic relief. Overall, NDs represent a current burden in the developed world which is projected to accelerate over time. These factors make the discovery of novel therapies to delay and prevent the development of these diseases an urgent but yet an unmet need.

A huge number of diseases are known to affect the nervous system, with most of them considered heterogeneous and multifactorial disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), brain cancer, degenerative nerve diseases, encephalitis, epilepsy, among others. They develop alterations in different brain regions and have different etiologies. The aggregation of misfolded proteins is one of the most common pathological processes, shared in AD, PD, HD, and others, commonly denominated as protein conformational disorders.[2] Besides the determinant role of protein aggregation, several other factors are common across major NDs. A new paradigm has begun to emerge to develop interventions targeting common mechanisms associated with aging to delay the onset of more than one age-related disease at the same time.[3] Increased oxidative stress, protein carbonylation, lipid peroxidation, DNA damage, mitochondrial dysfunction, vascular dysfunction, endoplasmic reticulum (ER) stress, unfolded protein response (UPR) dysregulation, and imbalanced proteostasis and neuroinflammation are common ND hallmarks which may require multitargeted interventions to tackle disease progression.

Neuroinflammation appears as one of the key processes involved in the major NDs and is a double-edged sword, being not only essential for recovery from several conditions but also playing detrimental roles contributing to disease progression. This process is controlled by microglia cells, the innate immune cells of the central nervous system (CNS), which can be stimulated to protect or act as a self-propelling mechanism of progressive neurodegeneration (Figure 1).[4,5] The primary role of microglia cells is to maintain the normal functions of the CNS by continuous surveillance of their surrounding microenvironment by extending and retracting their highly motile processes, maintaining homeostasis and neuronal integrity. Under detrimental stimuli, microglia cells are activated, losing their surveillance phenotype and their ramified morphology which is converted to an amoeboid-like structure that responds accordingly to the nature of the stimuli (Figure 1). Under moderate or transient damage, microglia cells

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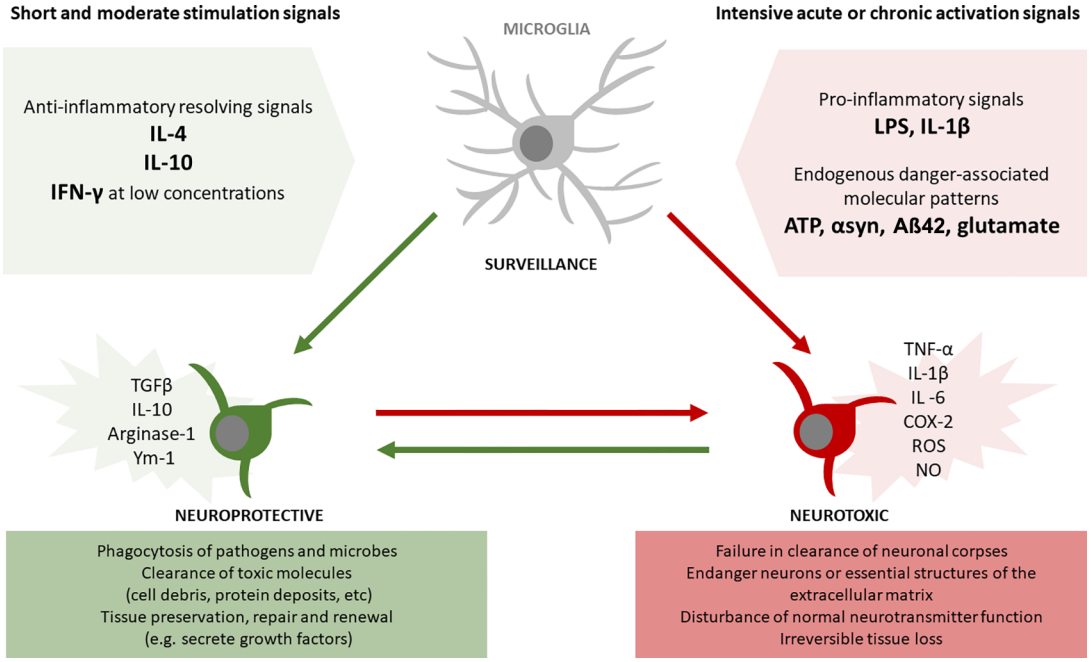

Figure 1. Microglia cell phenotypes at resting surveillance state (gray ramified) and upon activation by signals to the neuroprotective (green amoeboid) and neurotoxic (red amoeboid) phenotypes. IL, interleukin; IFN-γ, interferon gamma; LPS, lipopolysaccharides; αsyn, alpha-synuclein; Aβ42, amyloid beta 42; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; COX, cyclooxygenase; ROS, reactive oxygen species.

behave as protective cells, i.e., play immune resolving, anti-inflammatory functions and support cell renewal by secreting trophic factors (Figure 1). In contrast, under intensive acute or chronic activation, usually associated with NDs, microglia cells become neurotoxic and secrete an array of pro-inflammatory cytokines and reactive oxygen and nitrogen species (ROS, NOS) that may potentially impair neuronal activity (Figure 1).[5] The neurotoxic phenotype of microglia cells blocks their ability to circumvent inflammatory damage, feeding instead a vicious cycle of toxicity. Activation of microglia cells by low concentrations of cytokines, such as interferon-γ (IFN-γ), interleukin (IL)-4, or IL-10, supports differentiation and provides neuroprotection by regulating the levels of both insulin-like growth factor 1 (IGF-1) and tumor necrosis factor-α (TNF-α). However, stimulation with either toxic protein aggregates, e.g., amyloid β 42 (Aβ 42) and α-synuclein (αsyn), hallmarks of AD and PD, respectively, glutamate or lipopolysaccharide (LPS, only used in vitro) exacerbates the inflammatory response. Whenever facing strong "toxic" stimuli, short-lived microglia cells can potentially manage the neurotoxic factors, supporting regeneration and secreting neurotrophic factors. Nevertheless, when upon a chronic stimulus, microglia cells develop this neurotoxic phenotype. Different markers have been associated with the neurotoxic phenotype [e.g., TNF-α, IL-1β, IL-6, prostaglandins (COX-2), ROS, and NO] and with the neuroprotective phenotype [e.g., IL-10, transforming growth factor-β (TGF-β), enzymes able to inhibit ROS production (e.g., arginase-1), proteins that maintain the extracellular matrix (Ym-1) or perform phagocytic removal of toxic protein aggregates and of cellular debris] (Figure 1).[5,6] The main signaling cascade behind the activation of microglia cells involves Nuclear Factor kappa B (NF-κB) through a mitogen-activated protein kinase (MAPK) signal transduction pathway.[7] MAPK is a common signaling hub to transduce extracellular signals to the nucleus in microglia cells. Major MAPK cascades involved in gene expression alterations are ERK1/2, Jun N-terminal Kinase (JNK), and p38.[7,8]

Several epidemiological studies from the last decades illustrate that diets rich in fruits and vegetables can have beneficial effects in human health,[9,10] preventing degenerative disorders and cognitive decline.[11,12] Dietary phenolics are described to modulate different aspects of synaptic plasticity, e.g., memory and/or learning improvement in both animals and humans,[10,13,14] albeit their mechanism of action on CNS remains poorly understood.[15] Their long-term supplementation in animal models suggests they can activate neuronal receptors, interact with signaling pathways (e.g., MAPK), and control the expression of specific genes.[16]

In fact, emerging evidence indicates that adherence to the Mediterranean diet, rich in polyphenols, is associated with a reduction of markers of inflammation. Therefore, the impact of (poly)phenol metabolites present in the Mediterranean diet on the innate immune system cannot be overlooked. Recently, a number of studies have described the contribution of this diet to the decrease of several markers of systemic inflammation which can have knock-on effects on the neuroinflammation status of the brain.[17] Another example is the effects of increased consumption of berries on a large prospective cohort of older women, establishing a relation with a reduced gradual progression of cognitive decline.[18] Moreover, it was shown that consumption of high levels of anthocyanin is associated with a reduced risk of PD development.[19] Various studies also show significant cognitive benefits of phenolics consumption in humans.[20−22] In fact, diet supplementation with wild blueberry was proven to improve cognitive function in older adults.[23] A recent study have shown that chronic blueberry supplementation improved brain perfusion, task-related activation, and cognitive function in healthy older adults, suggesting that supplementation with an anthocyanin-rich concentrate can improve brain activation in areas associated with cognitive

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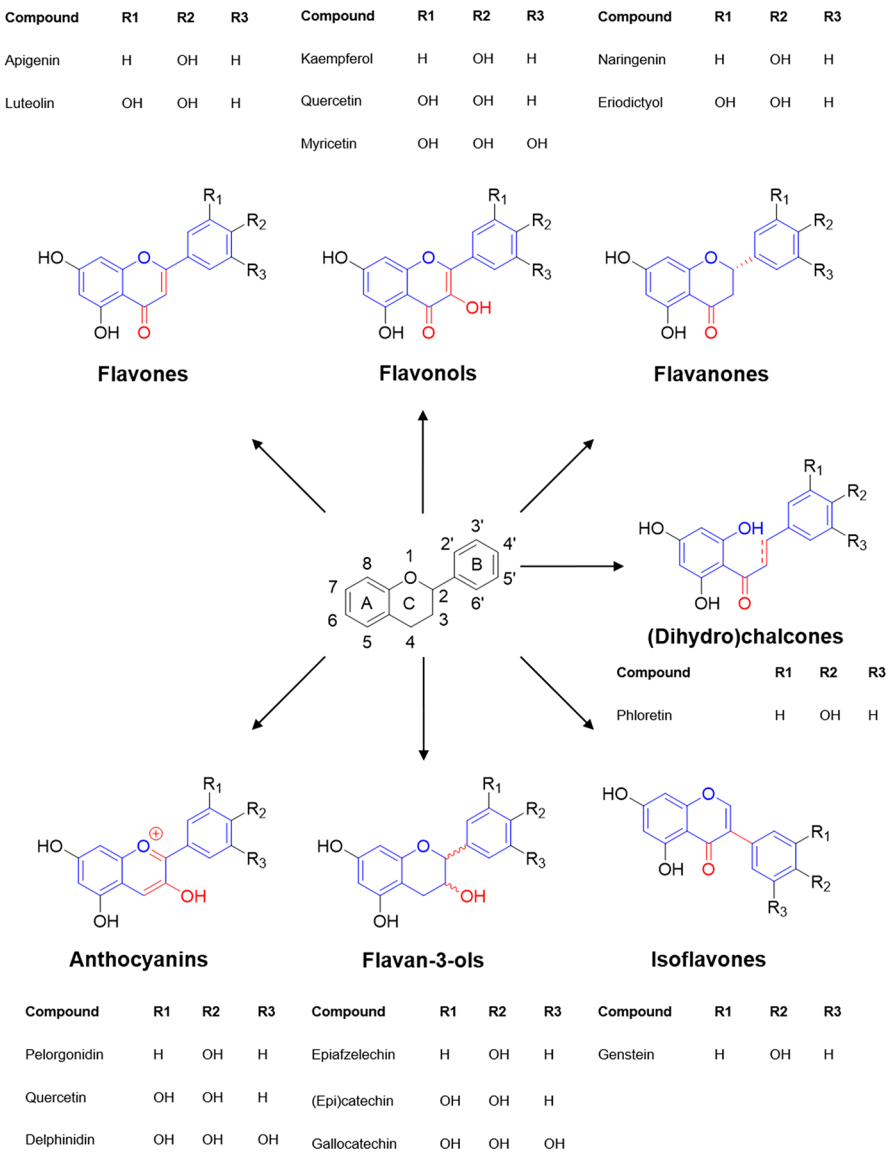

Figure 2. Different flavonoid classes with nutritional relevance. Flavonoid classes are represented as their 5,7-dihydroxyflavonoid derivatives. Red labeled bonds and chemical groups represent the major chemical characteristics of the flavonoid class.

function.[24] Moreover, in a double-blind, placebo controlled trial, the addition of easily achievable quantities of blueberry to the diets of older adults improved some aspects of cognition.[25] The results shown in these studies are supporting the idea that diets rich in (poly)phenols may have a positive impact on neurodegenerative diseases.

2. (POLY)PHENOL METABOLITES AND THEIR BRAIN PERMEABILITY

Research accumulating recently has suggested the huge potential of (poly)phenols toward health benefits.[26] Still for a profound understanding of (poly)phenols' effects on human health and their underlying mechanisms of action, their absorption, distribution, metabolism, and excretion in the human digestive tract must be considered and the ensuing circulating metabolites evaluated for their potential.

The term (poly)phenols represent a class of compounds possessing multiple aromatic rings and at least one hydroxyl group.[27] Nevertheless, this definition was not consensual since a multitude of authors referred to the metabolic monomeric units as polyphenolic. To avoid confusion, the term polyphenols was recently updated to (poly)phenols in order to engulf a wider range of structurally and metabolic related phenolics.[28,29] Inside the (poly)phenol family, several branches are present, namely, flavonoids, lignans, tannins, stilbenes, ellagic acid, phenolic acids, and many others. Among these, flavonoids and phenolic acids represent the most biologically relevant class of compounds, due to their significant presence in dietary products and their ability to reach circulation.

Flavonoids are composed of three rings, A, B, and C (Figure 2). Depending upon the chemical groups present on the C ring or the position of the B ring, flavonoids can be classified into different classes: flavones, flavonols, flavan-3-ols, isoflavones, flavanones, and anthocyanins. Chalcones and dihydrochalcones although not having a C ring are also classified by many as flavonoids, due to their similar backbone. All of the above-mentioned classes represent the high percentage of flavonoids in dietary products including tea, citrus fruit, berries, red wine, apples, and legumes.[30] These flavonoids mainly occur naturally in fruit and vegetables as glycosides, with the exception for

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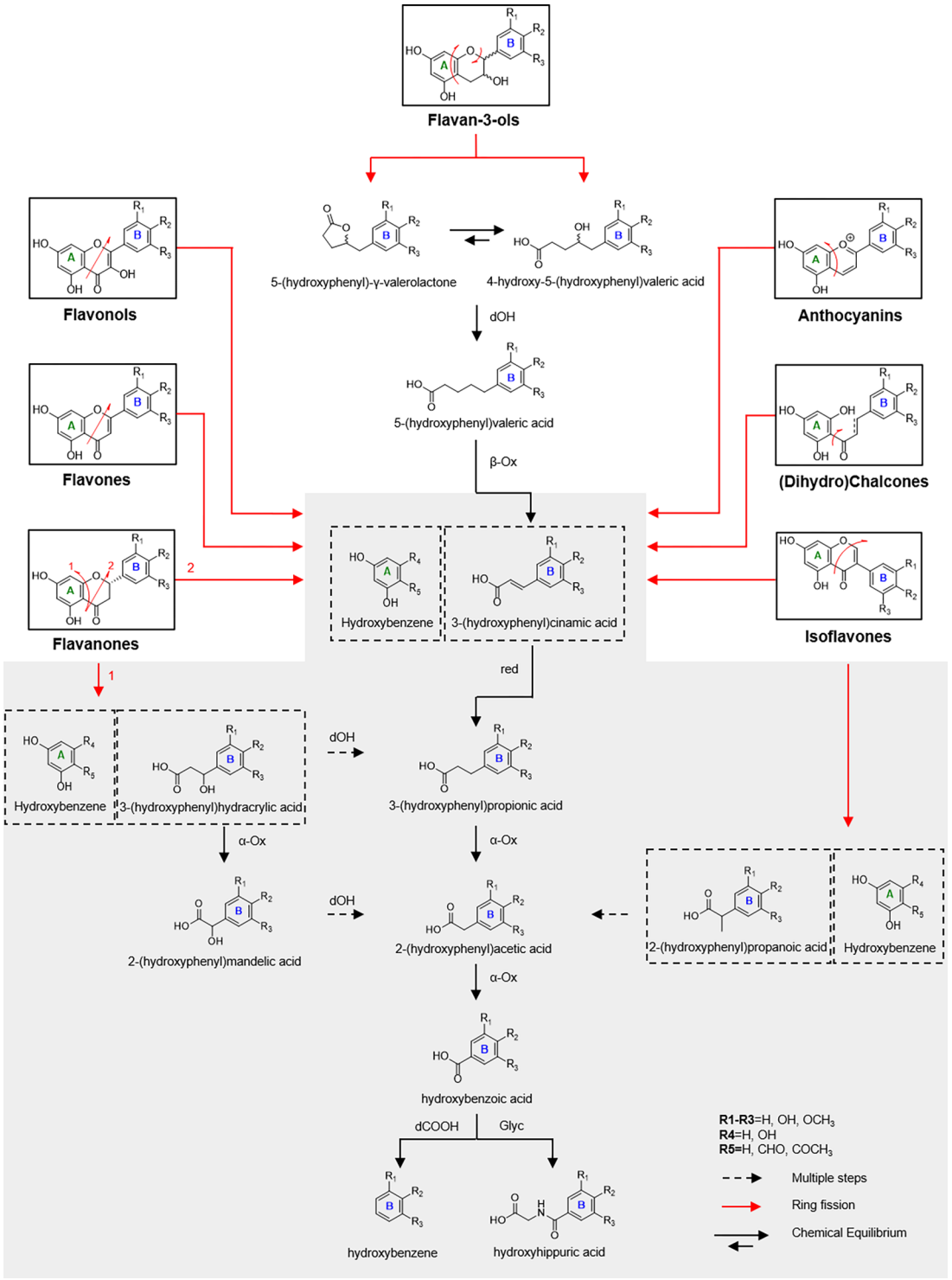

Figure 3. Conversion pathway demonstrating the metabolism of the most representative flavonoids in dietary products (black boxes) into low-molecular weight phenolic metabolites. Red arrows inside the black boxes indicate the possible microbiota mediated ring fission sites for each flavonoid. Red arrows emerging from the black boxes indicate ring fission metabolites. Compounds inside dotted black boxes represent the general structure of the low molecular weight phenolic metabolites subjected to further metabolism. Further metabolic reactions are represented by the black arrows reinforcing the convergence into more low-molecular weight phenolic metabolites with the potential to accumulate in higher concentrations than the remaining upstream compounds, like hydroxybenenes and (hydroxy)hippuric acids. Part of these metabolic reactions seem to be shared between both microbial and hepatic enzymes. Note that these metabolites can undergo phases I and II metabolism into sulfate and glucuronide conjugates. α-Ox, alpha oxidation; β-Ox, beta oxidation; dOH, dehydroxylation; dCOOH, decarboxylation; red, reduction; Glyc, glycination.

flavan-3-ols that can be found conjugated with gallic acid such is the case of (epi)catechin gallate.[31] Other flavonoids like dihydroflavonols, flavan-3,4-diols, coumarins, aurones, and neoflavonols also represent compounds with interesting

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Table 1. Low-Molecular Weight Phenolic Metabolites Resulting from Dietary Sources Rich in (Poly)Phenolic Content, Mainly Flavonoids"

IUPAC name	Common name	Detected	MW	Reference
Benezene diols and triols (C6)				
1,2-dihydroxybenzene	catechol	urine	110.11	100
2-hydroxyphenyl hydrogen sulfate	catechol-*O*-sulfate	plasma	190.17	45
2-methoxyphenol	guaiacol	plasma	124.14	101
2-methoxyphenyl hydrogen sulfate	guaiacol-*O*-sulfate	urine	204.20	102
1,3-dihydroxybenzene	resorcinol	plasma	110.11	103
3-hydroxyphenyl hydrogen sulfate	resorcinol-*O*-sulfate	plasma	190.17	104
1,2,3-trihydroxybenzene	pyrogallol	plasma	126.11	105
2,3- or 2,6-dihydroxyphenyl hydrogen sulfate [2]	pyrogallol-*O*-sulfate	plasma	206.17	45
(2S,3S,4S,5R,6S)-6-(2,3-or 2,6-dihydroxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid [2]	pyrogallol-1-*O*-glucuronide	urine	302.24	102
1-methoxy-2,3-dihydroxybenzene	1-methylpyrogallol	urine	140.14	106
2-hydroxy-6- or 3-methoxyphenyl hydrogen sulfate [2]	1-methylpyrogallol-*O*-sulfate	plasma	220.20	45
2-methoxy-1,3-dihydroxybenzene	2-methylpyrogallol	urine	140.14	106
3-hydroxy-2-methoxyphenyl hydrogen sulfate	2-methylpyrogallol-1-*O*-sulfate	plasma	220.20	45
3,5-dihydroxyphenyl hydrogen sulfate	phloroglucinol-*O*-sulfate	plasma	206.17	107
1,3,5-trimethoxybenzene	trimethoxyphloroglucinol	plasma	168.19	100
4-methyl-1,2-dihydroxybenzene	4-methylcatechol	plasma	124.14	108
2-hydroxy-4- or 5-methylphenyl hydrogen sulfate [2]	4-methylcatechol-*O*-sulfate	plasma	204.20	109
Benzaldehydes (C6-C1)				
4-hydroxybenzaldehyde	-	plasma	122.12	110
3,4-dihydroxybenzaldehyde	protocatechaldehyde	plasma	138.12	110
2,4,6-trihydroxybenzaldehyde	phloroglucinaldehyde	plasma	154.12	110
4-hydroxy-3-methoxybenzaldehyde	vanillin	plasma	152.15	100
Benzoic acids (C6-C1)				
Benzoic acid	-	plasma	122.12	110
2-hydroxybenzoic acid	salicylic acid	plasma	138.12	111
3-hydroxybenzoic acid	-	plasma	138.12	110
4-hydroxybenzoic acid	-	plasma	138.12	110
(2S,3S,4S,5R,6S)-6-(4-carboxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	benzoic acid-4-*O*-glucuronide	plasma	314.25	110
3,4-dihydroxybenzoic acid	protocatechuic acid	plasma	154.12	110
4-hydroxy-3- or 3-hydroxy-4- (sulfooxy)benzoic acid [2]	protocatechuic acid-*O*-sulfate	plasma	234.18	110
(2S,3S,4S,5R,6S)-6-(5-or 4-carboxy-2-hydroxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid [2]	protocatechuic acid-*O*-glucuronide	plasma	330.25	110
3,4-dimethoxybenzoic acid	veratric acid	plasma	182.18	108
3-hydroxy-4-methoxybenzoic acid	isovanillic acid	plasma	168.15	110
4-methoxy-3-(sulfooxy)benzoic acid	isovanilic acid-3-*O*-sulfate	plasma	248.21	110
(2S,3S,4S,5R,6S)-6-(5-carboxy-2-methoxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	isovanilic acid-3-*O*-glucuronide	plasma	344.27	110
3-methoxy-4-hydroxybenzoic acid	vanillic acid	plasma	168.15	110
3-methoxy-4-(sulfooxy)benzoic acid	vanillic acid-4-*O*-sulfate	plasma	248.21	110
(2S,3S,4S,5R,6S)-6-(4-carboxy-2-methoxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	vanilic acid-4-*O*-glucuronide	plasma	344.27	110
2,4-dihydroxybenzoic acid	-	plasma	154.12	112

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Table 1. continued

IUPAC name	Common name	Detected	MW	Reference
Benzoic acids (C6-C1)				
2,5-dihydroxybenzoic acid	-	plasma	154.12	41
2,6-dihydroxybenzoic acid	-	plasma	154.12	41
3,5-dihydroxybenzoic acid	-	plasma	154.12	41
3,4,5-trihydroxybenzoic acid	gallic acid	plasma	170.12	110
3-methoxy-4,5-dihydroxybenzoic acid	3-methylgallic acid	urine	184.15	100
4-methoxy-3,5-dihydroxybenzoic acid	4-methylgallic acid	plasma	184.15	110
(2S,3S,4S,5R,6S)-6-(5-carboxy-3-hydroxy-2-methoxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	4-methylgallic acid-3-*O*-sulfate	plasma	360.27	45
3,5-dimethoxy-4-hydroxybenzoic acid	syringic acid	plasma	198.17	110
Ethyl 3,4,5-trihydroxybenzoate	ethyl gallate	plasma	198.17	112
Phenylacetic acids (C6-C2)				
phenylacetic acid	-	plasma	136.15	100
2-(2-Methoxyphenyl)acetic acid	-	urine	166.18	100
2-(3-hydroxyphenyl)acetic acid	-	urine	152.15	96
2-(4-hydroxyphenyl)acetic acid	-	plasma	152.15	110
2-(3,4-dihydroxyphenyl)acetic acid	homocaffeic acid	plasma	168.15	110
2-(4-hydroxy-3-methoxyphenyl)acetic acid	homovanillic acid	urine	182.18	96
Mandelic acids (C6-C2) *2-hydroxyl*				
2-hydroxy-2-phenylacetic acid	mandelic acid	urine	152.15	113
2-hydroxy-2-(4-hydroxyphenyl)acetic acid	4-hydroxy-mandelic acid	urine	168.15	96
2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)acetic acid	vanilmandelic acid	urine	198.18	96
Hippuric acids (C6-C2-N)				
benzoylglycine	hippuric acid	plasma	179.18	110,114
3-hydroxybenzoyl glycine	3-hydroxyhippuric acid	plasma	195.17	115
4-hydroxybenzoyl glycine	4-hydroxyhippuric acid	urine	195.17	96
Cinnamic acids (C6-C3)[1] *unsaturated*				
3-(3,4-dihydroxyphenyl)propenoic acid	caffeic acid	plasma	180.16	110
3-(3-hydroxy-4-methoxyphenyl)propenoic acid	isoferulic acid	plasma	194.19	116
3-(3-methoxy-4-hydroxyphenyl)propenoic acid	ferulic acid	plasma	194.19	110
3-(3-hydroxyphenyl)propenoic acid	*m*-coumaric acid	urine	164.16	117
3-(4-hydroxyphenyl)propenoic acid	*p*-coumaric acid	urine	164.16	100
3-(5-hydroxyphenyl)propenoic acid	*o*-coumaric	urine	164.16	100
3-(4-hydroxy-3,5-dimethoxyphenyl)propenoic acid	*sinapic acid*	urine	224.21	118
3-(phenyl)prop-2-enoic acid	cinnamic acid	plasma	148.16	100
Phenylpropionic acids (C6-C3)				
3-(3-hydroxyphenyl)propionic acid	3-hydroxydihydrocinnamic acid	urine	166.18	115
3-(4-hydroxyphenyl)propionic acid	4-hydroxydihydrocinnamic acid (phloretic acid)	urine	166.18	100
3-(3,4-dihydroxyphenyl)propionic acid	dihydrocaffeic acid	urine	182.18	96
3-(3-metoxy-4-hydroxyphenyl)propionic acid	dihydroferulic acid	urine	196.20	96
3-(3-hydroxy-4-metoxyphenyl)propionic acid	dihydroisoferulic acid	urine	169.20	119
Phenylhydracrylic acid (C6-C3)				
3-(3-Hydroxyphenyl)-3-hydroxypropionic acid	3-(3'-Hydroxyphenyl)hydracrylic acid	urine	182.18	120

[a]The compounds mentioned in this class are representing the (E) isomer. [b]This compound have more than one structural isomer.

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properties and biological activities. Yet their lower presence in dietary products makes them less important from a nutritional perspective.

Results from intervention studies have shown that flavonoids are subjected to a series of metabolic events, resulting in a huge variety of low-molecular weight phenolic metabolites that reach circulation at considerably higher concentrations than their parent compounds.[32]

Even though the full mechanism behind the metabolism of (poly)phenols into low molecular weight phenolic metabolites has not been fully unveiled, some consensus has been established. Phenolic metabolites resulting from digestive and hepatic activity usually differ from their parent compounds, which are found in fruits and vegetables.[10,33] Absorption events are accompanied by multiple metabolic reactions, which occur in the small and large intestines, in the liver, and in cells, resulting in a wide variety of derivatives (e.g., sulfated, methylated, and glucuronidated). Upon ingestion, flavonoid aglycones can be absorbed in the small intestine due to the action of glycosidases such as lactase phloridzin hydrolase (LPH) in the brush border of the small intestine epithelial cells or cytosolic β-glucosidase (CBG) within epithelial cells.[34] (Poly)phenol conjugates with sugar moieties that are resistant to the action of LPH/CBG and that are not absorbed in the small intestine to any degree reach the colon.[34] In the colon, the remaining conjugates are cleaved and the resulting aglycones undergo ring fission by the action of host microbiota producing several low-molecular weight phenolic metabolites.[35] Ring fission sites are dependent on the structure of phenolic parent compounds (Figure 3). For example, ring fission of flavan-3-ols like catechin, epicatechin, and epigallocatechin produce 5-(hydroxyphenyl)valeric acid and 5-(hydroxyphenyl)-γ-valerolactones, which are not produced by any other flavonoid classes. Furthermore, only ring fission of flavanones produce 3-hydroxypropionic acid (3-(hydroxyphenyl)hydracrylic acid), while isoflavones produce 2-(hydroxyphenyl)propanoic acid. Another exclusive class of metabolites are urolithins that are the result of ellagic acid metabolism. The production of urolithin A, B, or no urolithin production is dependent on the host microbiota composition, which can divide the host into metabotypes A, B, and 0.[36] Also, urolithins, unlike other low-molecular weight phenolic metabolites, are not further metabolized into smaller compounds but rather excreted intact in urine as phase II conjugates.[37]

Ring fission of most flavonoids happens on the C ring leading to the formation of 3-(hydroxyphenyl)cinnammic acids, such as ferulic acid and caffeic acid, from the B ring and hydroxybenzenes or benzaldehydes, such as phloroglucinol or phloroglucinaldehyde, and from the A ring.[38] The 2,3 double bonds of the B ring derived phenolic compounds can be reduced leading to the formation of the saturated 3-(hydroxyphenyl)propionic acids, such as dihydroferulic and dihydrocaffeic acid.[38] Of note, the mechanism of formation of 3-(hydroxyphenyl)propionic acid from flavanones is not still fully elucidated and could be produced by 3-(hydroxyphenyl)-cinnamic acid reduction or dihydroxylation of 3-(hydroxyphenyl)hydracrylic acid.

Further oxidation of 3-(hydroxyphenyl)propionic acids can produce 2-(hydroxyphenyl)acetic acid. Several authors suggest that 3-(hydroxyphenyl)hydracrylic acid can be dehydroxylated to 2-(hydroxyphenyl)mandelic acid that can also generate 2-(hydroxyphenyl)acetic acid, although this route is not so well characterized. The same is valid for the conversion of 2-(hydroxyphenyl)propanoic acid originating from isoflavones into 2-(hydroxyphenyl)acetic acid. Moreover 2-(hydroxyphenyl)acetic acid can be oxidized into hydroxybenzoic acids, such as protocatechuic acid, gallic acid, and syringic acid. These components can be further converted into two different sets of metabolites, hippuric acids through glycination and hydroxybenzenes, such as catechol and pyrogallol, through decarboxylation.

Therefore, these low molecular weight phenolic metabolites are the culmination of multiple steps, involving both microbiota and human metabolism. The convergence of multiple flavonoid classes happens during several hours after ingestion. Flavonoid conjugates and their first metabolites like 3-(hydroxyphenyl)cinnamic acid appear in circulation in minutes to an hour after ingestion.[39] Meanwhile end-point metabolites like hippuric acid and hydroxybenzoics can be present up to 24 h after ingestion of edible fruits.

In any step of their catabolism, circulating phenolic metabolites can undergo phase II metabolism in the liver, leading to the formation of sulfate, glucuronide, and methylated conjugates through the action of sulfotransferases (SULTs), uridine-5′-diphosphate glucuronosyltransferases (UGTs), and catechol-*O*-methyltransferases (COMTs), respectively.[40] It is however still not fully understood if phase II conjugates can still be catabolized into smaller metabolites or if they are directly excreted in the urine without further modifications. Moreover, since both endogenous human and microbiota metabolism play important roles in producing low-molecular weight phenolic compounds in blood circulation, it is difficult to unravel the origin of one compound. For example, ferulic acid in blood could arise from direct ingestion of dietary conjugated ferulic acid, from ring fission of flavonoids, or from the action of COMT on caffeic acid.[41]

Overall, the correlation between parent compounds and the derived gut-phenolic metabolites is puzzling; many low-molecular weight phenolic metabolites can arise through the metabolism of a wide group of structurally diverse parent compounds (e.g., flavonols, flavan-3-ols, anthocyanins, chalcones, isoflavones, flavanones, and flavones) (Figure 3 and Table 1), whereas few are associated with an unique gut-phenolic metabolites (e.g., urolithins from ellagitannins).[42] For other (poly)phenolic components, data is still lacking (e.g., pyranoanthocyanins, coumarins, and other minor dietary components).[42] In general, the role of gut microbiota in the metabolism of (poly)phenols and the generation of low-molecular weight metabolites is rapidly consolidating and is of great importance.[43]

A very important aspect when considering the bioactivity of (poly)phenol metabolites is their circulating concentrations. Parent (poly)phenols or their phase II conjugates only reach the circulation in concentrations in the nanomolar range. On the other hand, low-molecular weight phenolic metabolites which can originate from different flavonoid classes (Figure 3) tend to be present in concentrations vastly superior when compared to the parent compounds. Czank et al. found that ingestion of 500 mg of ^{13}C cyanidin-3-glucoside provided 42-fold higher abundance of ^{13}C-labeled metabolites relative to ^{13}C cyanidin-3-glucoside at their respective maximum serum concentration.[44] The flavonoid conjugate was present in serum at a maximum concentration of 141 nM at 1.8 h. Meanwhile, 14 different metabolites were also detected, with the most abundant, hippuric acid, reaching a concentration of 1962 nM

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at 15.7 h.[44] Pimpão et al., in a human intervention study using 500 mL of a puree of berries, have found that hydroxybenzoic phase II metabolites reached concentrations of over 20 μM in some volunteers.[45] Moreover, hippuric acid has been found in circulation up to 24 h and reaching concentrations well above 25 μM.[46]

The ability of any dietary phenolic to directly influence the nervous system will be dictated by the ability of its ensuing phenolic metabolites to cross the blood-brain barrier (BBB).[35,47] The BBB is a very dynamic and complex interface, carrying the responsibility of protecting the CNS from toxic agents. It controls the molecular exchanges between the blood and the neuronal tissue, hence playing a key role in the control of the accessibility of nutrients and other compounds to the brain.[48] Evidence of BBB capacity to uptake some phenolic metabolites is growing in the literature, alongside studies consistent with potential activity of phenolic metabolites in the brain.[49,50] The majority of these studies that focused on oral administration of dietary phenolics, directly or their derived phenolic metabolites, have reported their capacity to reach the brain, e.g., (−)epicatechin,[51] hesperetin, naringenin,[52] quercetin,[53] and anthocyanins.[49,54] To date, despite the fact that gut-phenolic metabolites are in general accepted to comprise a considerable percentage of the total diversity of phenolic metabolites,[44,45] only a very limited number of studies have specifically focused on bioaccessibility of gut-phenolic metabolites to the brain (e.g., valerolactones and phenolic acids).[55] In a recent pharmacokinetic study in rats, upon intravenous administration, some gut-phenolic metabolites were observed to reach the brain.[50] Recent methods now being implemented, such as microdialysis sampling, give the ability to recover (poly)phenols in vivo. Using this system coupled with HPLC with chemiluminescence detection, Liang Wu et al. demonstrated the ability of (+)catechin and (−)epicatechin to reach the brain.[56] Using a similar system, Zhang et al. showed that nearly 30% of orally administered protocatechuic acid can be recovered in the rat brain.[57]

BBB permeation has also been ascertained by using in vitro assays with immortalized human microvascular endothelial cells such as hBMEC or hCMEC/D3 in monolayers in transwell inserts to test for apical and basal presence of the compounds. Faria et al. have shown that (+)catechin and (−)epicatechin are able to cross hCMEC/D3.[58] Hydroxycinammic acids such as ferulic acid have also been shown to cross these BBB models,[59] and the same was demonstrated for hydroxybenzenes and benzoic acid derivatives.[15] Transport through the BBB varies between 5 and 10% for the tested low-molecular weight polyphenol metabolites. Additionally, two of the gut-phenolic metabolites, namely, catechol-sulfate and pyrogallol-sulfate, lead to new end-point metabolites in human brain endothelial cells.[15]

Whether the effects induced by dietary phenolics on brain functions are mediated directly in the brain or involve other mechanisms triggered from cells in the periphery remains unclear. Some reports indicated that the effects of flavonoids and metabolites on the brain do not seem to be exclusively dependent on their blood-brain barrier permeation.[16] (Poly)-phenols have been shown to impact cardiovascular functions modulating blood pressure, vasodilation, and the presence of inflammatory markers in circulation that can directly affect the brain.[60,61] However, if phenolic metabolites do have a direct effect on the brain, then the low molecular weight phenolic metabolites may have the capacity to have a major contribution, due to their higher circulating concentrations. This review will be focused on the data available only for the direct effects of low-molecular weight phenolic metabolites (Table 1) in the context of the molecular mechanisms associated with neuroinflammation.

3. LOW-MOLECULAR WEIGHT (POLY)PHENOL METABOLITES AS EFFECTORS FOR ATTENUATING NEUROINFLAMMATION

For a full comprehension of dietary (poly)phenols effects on NDs, an unified view of in vitro, cellular, and animal model studies is required to reveal the complete panorama of their molecular targets and mechanisms. Associations between higher intakes of flavonoid-rich diets with lower levels of inflammatory biomarkers have been established.[62] However, inconsistent results on the preventive anti-inflammatory effects of flavonoid supplementation reinforce the necessity for more prospective randomized trials with larger sample sizes and under rigorous clinical conditions.[62] Moreover, while human trials are more reliable and the closest to the real scenario, they are time-consuming and costly and it is difficult to assess the direct effect of the (poly)phenols in trials related to neurodegenerative diseases. Animal and cellular models have provided unparalleled tools for compound screening assays to reveal their mechanisms of action. Despite of the importance of animal studies with isolated compounds, it does not reflect a nutritional scenario but, in most cases, a pharmacological perspective. Besides that, nutritional studies reveal overall effects and pathways affected but most of them do not identify the relevant metabolites that can comprise the true effectors against neurodegeneration acting in the brain, especially since their presence on the brain has been overlooked. *In vitro* assays are of most importance to reveal crucial interactions of (poly)phenols with other small molecules, proteins or even metals. They can prove an indispensable tool to get a comprehensive picture and tune our understanding *in vivo*. We will highlight and discuss our recent knowledge on cellular studies focused on the low molecular weight (poly)phenol metabolites (Table 1) to delineate their main molecular mechanisms of attenuation of neuroinflammation and identify their putative targets. Cellular models are starting points to reveal the molecular mechanisms of (poly)phenol metabolites and the role on cellular homeostasis when considered under experimental conditions close to physiological conditions. We will not address nutritional studies in animals for the reasons described above.

Accumulating evidence highlights the molecular and cellular pathways modulated by parent (poly)phenols, which, though not always nutritionally relevant, can give us clues about the putative mode of action of their (poly)phenol metabolites in a nutritional context. Flavonoids have been shown to inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1 in microglial cells, suggesting close involvement in pathways such as NF-κB or MAPK.[63] In addition, there is strong evidence that blueberry (poly)phenols inhibit the production of NO, IL-1β, and TNF-α in activated microglia cells.[64] Another example of a parent compound with anti-inflammatory activity, however with poor bioavailability, is caffeic acid phenethyl ester (CAPE). Some studies highlight its potential to modulate NF-κB activation in microglia cell models stimulated with TNF-α and significantly attenuate TNF-α-induced Toll-like receptor (TLR) 2 expression.[65] However, CAPE has low solubility and poor bioavailability

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Table 2. Neuroprotective Evidence (in Vitro) for Some Bioavailable (Poly)Phenol Metabolites[77]

Class	(Poly)phenol metabolite	Cell model	Concentration (μM)	Incubation time	Anti-inflammatory evidences	Reference
Benzene diols and triols	Catechol	Mouse microglia cell line (BV-2) + LPS Mouse microglia cell line (BV-2) + LPS + rat neuroblastoma cells (B35)	18[1]	30 min pre-incubation before insult	↓ LPS-induced NO and TNF-α production ↓ expression of iNOS ↓ nuclear translocation of NF-κB ↓ IκB degradation *(except 4-methylcatechol)* ↓ p38 MAP kinase phosphorylation	77
	4-methylcatechol		16[1]			
	Pyrogallol-*O*-sulfate	Mouse microglia cell line (N9) + LPS	5	6 h pre-incubation before insult	↓ LPS-induced TNF-α production ↓ nuclear translocation of NF-κB ↓ IκB degradation	88
	1-O-methylpyrogallol-*O*-sulfate				↓ LPS-induced TNF-α production ↓ intracellular superoxide production ↓ CD40 production	
	4-Methylcatechol-*O*-sulfate				↓ LPS-induced NO ↓ intracellular superoxide production ↓ CD40 production	
Benzoic acids	Gallic acid	Mouse microglia cell line (BV-2)+ β-amyloid Mouse primary glial cells+ β-amyloid Conditioned media from BV-2 cells and primary microglial cells were transferred to Neuro-2A cells	5–50	12 h pre-incubation before insult	↓ iNOS expression levels ↓ COX-2 expression levels ↓ IL-1β expression levels ↓ TNFα expression levels	89
	Protocatechuic acid	Mouse microglia cell line (BV-2) + LPS	5-20	1 h pre-incubation before insult	↓ LPS-induced TNF-α production ↓ LPS-induced IL-6 production ↓ LPS-induced IL-1β production ↓ LPS-induced PGE_2 production ↓ TLR4 expression ↓ NF-κB activation ↓ MAPKs activation	87

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Table 2. continued

Class	(Poly)phenol metabolite	Cell model	Concentration (μM)	Incubation time	Anti-inflammatory evidences	Reference
Benzoic acids	4-*O*-methylgallic acid	Mouse microglia cell line (N9) + LPS	5	6 h pre-incubation before insult	↓ LPS-induced NO ↓ intracellular superoxide production ↓ CD40 production	15
	4-*O*-methylgallic acid-3-*O*-sulfate				↓ LPS-induced NO ↓ intracellular superoxide production	
	Vanillic acid 4-*O*-sulfate				↓ LPS-induced TNF-α production	
			1, 5 and 25	24 h co-incubation with insult	↓ LPS-induced NO ↓ LPS-induced iNOS ↓ LPS-induced COX-2 ↓ LPS-induced IL-1β ↓ LPS-induced IL-6	91
Cinnamic acids	Ferulic acid	Mouse microglia cell line (BV-2) + LPS	10–100	2 h pre-incubation before insult	↓ LPS-induced TLR4 levels ↓ LPS-induced TNF-α levels ↓ LPS-induced iNOS levels ↓ LPS-induced p-JNK levels ↓ LPS-induced NF-κB levels ↓ LPS-induced Bax levels	92
		Mouse microglia cell line (MG6) + LPS	10, 50 and 250	24 h co-incubation with insult	No effect on LPS-induced NO,TNF-α, and IL-6 levels *For only 50 and 250 μM of ferulic acid:* ↓ LPS-induced Indoleamine 2, 3-dioxygenase mRNA expression ↓ nuclear translocation of NF-κB ↓ MAPK p38 activation	93

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Table 2. continued

Class	(Poly)phenol metabolite	Cell model	Concentration (μM)	Incubation time	Anti-inflammatory evidences	Reference
Cinnamic acids	Cinnamic acid	Mouse microglia cell line (BV-2) + LPS	100-300	2 h pre-incubation before insult	↑ SOCS3 expression levels ↑ CREB activity ↓ LPS-induced TNF-α production ↓ LPS-induced iNOS production ↓ LPS-induced IL-1β production ↓ LPS-induced IL-6 production	94
Phenylacetic acids	2-(3,4-dihydroxyphenyl)acetic acid (3,4-DHPA) 2-(3-hydroxyphenyl)acetic acid (3HPA)	Human neuroblastoma cell line (SH-SY5Y) + SIN-1[2]	0.1, 1 and 10	18 h pre-incubation before insult	↓ MAPK p38 activation (3-HPA and 4HPP) ↓ ERK1/2 activation	97
Phenylpropionic acids	3-(4-Hydroxyphenyl)propionic acid (4HPP) 3-(3-Hydroxyphenyl)propionic acid (3HPP)					

[a]The concentration tested was 2 μg/mL, and it was converted to molar for comparison purposes. [b]3-Morpholinosydnonimine (SIN-1) is a peroxynitrite generator described as inducing phosphorylation of protein tyrosine residues in brain cells and has been used as a neuronal damage inductor. In the study, SIN-1-induced nitrosative stress in a human neuroblastoma cell line (SH-SY5Y) and was used as a model of neuroinflammation.

that limits in vivo efficacy. Consequently some nanotechnologies strategies are emerging to enhance bioavailability and improve the clinical use of CAPE.[66] Nevertheless, in a nutritional scenario, the current trend is to analyze the effect of defined (poly)phenol metabolites in specific cells/tissues, instead of those of their parent dietary phenolics, under near physiological concentrations and residence time. The potential to reduce LPS-induced TNF-α secretion in THP-1 monocytes was evaluated, demonstrating that some metabolites of flavonoids, individually and in combination, appear to present more anti-inflammatory effects than their parent compounds.[67] Importantly, the study of isolated polyphenol metabolites is often limited to their molecular mechanisms in neurodegeneration cell models and their specific biological effects in animal models, and such studies miss the synergetic effects of the diversity of (poly)phenol metabolites that coexist in serum. The overall diet complexity, the potential synergy that may occur in compounds derived from the metabolism cannot be fully depicted in studies using isolated pure compounds.[68] Studies with (poly)phenol-enriched fractions or with the bulk of digested/metabolized compounds may be useful to approach these interactions and depict the molecular effects of the overall metabolites in cells.[15,69,70] For instance, simulated gastro-intestinal digestion blackberry extracts revealed neuronal protection to oxidative insult that was not related to the levels of ROS and glutathione (GSH), suggesting a preconditioning effect by the induction of caspase activity.[69,71] Moreover, bioaccessible raspberry metabolites resulting from the in vitro digestion of raspberry extract significantly inhibited microglial pro-inflammatory activation by LPS through the inhibition of Iba1 expression, TNF-α release, and NO production.[72]

A plethora of mammalian cell models have been used to study the protective action of (poly)phenol metabolites toward NDs. Although there is some evidence mainly highlighting the ability of several circulating metabolites to reduce oxidative stress, lipid peroxidation, and cytotoxicity in neuronal models, few studies have explored their antineuroinflammatory properties.[73,74] On the other hand, more complex models have addressed effects of (poly)phenols in particular pathological processes associated with each disease as well as toxicity of specific disease proteins.[75,76] Cellular models to access the anti-inflammatory potential of (poly)phenols for neurodegeneration are in general based on using microglia cells and explore the therapeutic potential of metabolites to mitigate inflammation induced by LPS or TNF-α. Since microglia are the resident innate immune cells of the CNS, microglial cell lines such as N9 and BV-2 have been preferred. Although the evidence for modulatory effects of (poly)phenol metabolites in microglial cells is still very scarce, the ability of the low-molecular weight phenolic metabolites to reduce neuroinflammatory markers has been reported (Table 2) and in some cases the underlying involved pathways have been proposed.

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Benzene diols and triols and their conjugates (Table 1) are abundant low-molecular weight flavonoid metabolites since they can appear in circulation either by ring fission from the A and B rings (Figure 3). Moreover, some are present in specific foods, e.g., catechol, guaiacol, pyrogallol, and 4-methylcatechol occur in coffee, beer, and cocoa.[30] A study from 2008 reported the beneficial effects of catechol and its conjugates (Figure 4,

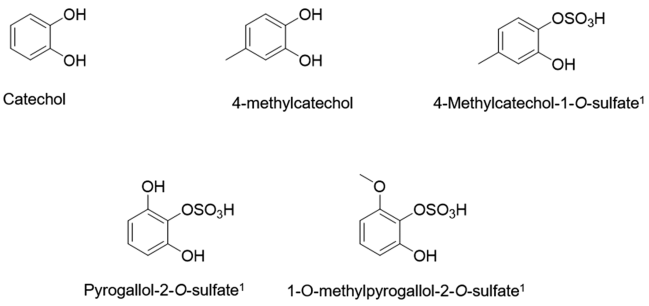

Figure 4. Benzene diols and triols evaluated as attenuators of neuroinflammation in microglia cells. [1]Other structural isomers exist for this molecule as shown in Table 1.

Table 2) that consistently decreased LPS-induced NO and TNF-α production in the BV-2 microglia cell line. Moreover, these compounds also inhibited the expression of inducible nitric oxide synthase (iNOS) as well as nuclear translocation of the p65 subunit of NF-κB, IκB degradation, and phosphorylation of p38 MAPK,[77] key players in inflammatory processes. Other substituted catechols (3-methylcatechol and 4-*tert*-butylcatechol) are equally effective, which opens the window to inspire further modifications for pharmacological applications. A more recent study, using a different microglia cell line (N9), showed that catechol and pyrogallol conjugates (both methyl and sulfates, Figure 4, Table 2) improved cellular responses to oxidative and inflammatory injuries via modulation of the NF-κB pathway.[15] In the same study, catechol-sulfate was ineffective to interfere with the key players of the NF-κB pathway, suggesting that the slight modification of sulfation of one hydroxyl, leaving only a single free hydroxyl, could alter its activity.[15] It will be interesting to have more data for further physiological conjugates.

A different view of the effect of benzene diols and triols on neuronal cells came from the 60s. Although pyrogallol is a bioavailable polyphenol metabolite which results from the consumption of polyphenol-rich meals, it also has toxic and pro-oxidant effects at higher concentrations. Pyrogallol was seen as a potent modulator of neurotransmitter levels in the brain.[78] The understanding of its brain penetration[79] and effects on monoamine oxidase and catechol-*O*-methyltransferase[80] highlighted the fact that pyrogallol is undoubtfully a brain bioavailable metabolite, appearing to be a versatile molecule with an evident modulatory potential in brain activity. Such potential led to the understanding of how pyrogallol can influence several fine-tuned mechanisms such as blood pressure,[81] dopamine levels,[82] and iron homeostasis and, consequently, cellular oxidative stress.[83] As such, pyrogallol was used as a neurotoxic agent at nonphysiological concentrations both in vitro and in vivo,[84,85] reviewed in ref 86. This duality of roles for pyrogallol may rely on the fact that at low concentrations, as it is detected circulating after consumption of polyphenol-rich meals, may be preconditioning the cells to a later insult. Pyrogallol is priming the cells to better respond to more hostile alterations in cell environment that may arise. It might be plausible that there are similar mechanisms for other low-molecular weight phenolic metabolites.

Benzoic acids conjugates were also studied as attenuators of neuroinflammation (Table 2). This is a very important class not only because the majority of flavonoids' metabolism converge to benzoic acid derivatives (Figure 3) but also because they are very well represented in diverse foods and therefore could be ingested directly (e.g., gallic acid and protocatechuic acid in beer, wine, berries, cereals, grape, apple, kiwi, olive oil, vinegar, chicory).[30,87,88] Mi-Jeong Kim and colleagues have demonstrated the neuroprotective effects of gallic acid (Figure 5, Table 2) using the BV-2 cell line and

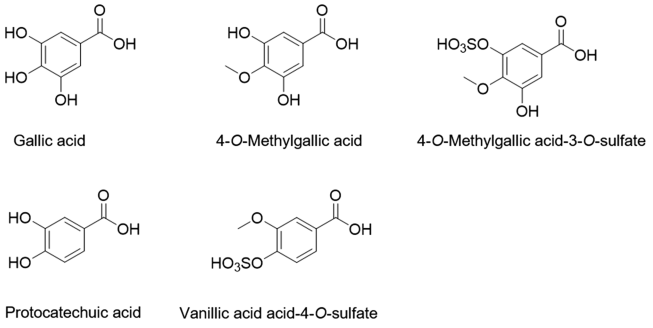

Figure 5. Benzoic acids evaluated as attenuators of neuroinflammation in microglia cells.

primary microglia cells prior to Aβ stimulation. Conditioned media was transferred to Neuro-2A cells, and in both situations, gallic acid prevented Aβ neurotoxicity by inhibiting the expression levels of iNOS, COX-2, IL-1β, and TNF-α.[89] Gallic acid conjugates (methyl and sulfate) (Figure 5, Table 2) also proved efficacy in reducing LPS-induced NO as well the levels of intracellular superoxide,[88] which suggests that these further metabolic alterations do not affect its ability to modulate the inflammatory markers. In addition, protocatechuic acid, that differs from gallic acid in only one hydroxyl (Figure 5, Table 2), has also presented potent anti-inflammatory effects in BV-2 LPS-stimulated microglia. Mechanistic assays have revealed its ability to inhibit the production of TNF-α, IL-6, IL-1β, and PGE_2. Protocatechuic acid also suppressed the activation of NF-κB and MAPKs, and it was also able to inhibit the TLR4 expression.[87]

Although there are many food sources of free and conjugated ferulic acid, its bioavailability depends on the form in which it is ingested as free ferulic acid has limited solubility in water and hence poor bioavailability. However, ferulic acid appears in circulation as a result of the metabolism of caffeic acid, chlorogenic acid, and anthocyanins.[30,90] Some studies have evaluated the ability of ferulic acid to attenuate inflammatory markers in microglia cells (Figure 6 Table

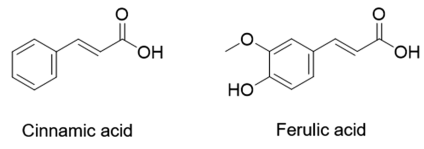

Figure 6. Cinnamic acids evaluated as attenuators of neuroinflammation in microglia cells.

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2).[91,92] While the trend observed for this compound was to reduce more than one pro-inflammatory mediators in microglia cells (Table 2), only the study of Byung-Wook Kim and colleagues had nutritional relevance, since they tested physiologically relevant levels of ferulic acid likely to reach the circulation.[91] In another microglia cell line, ferulic acid was ineffective in reducing LPS stimulated production of NO, TNF-α, and IL-6 and was only effective in other mediators at nonphysiological concentrations over 50 μM (Table 2).[93] Similarly, another study observed that ferulic acid attenuated the JNK/NF-κB inflammatory signaling pathway and apoptosis in LPS-induced BV-2 microglia cells at both 10 μM and 100 μM but then focused on assessing effects on the TLR4-mediated signaling pathway but only for the most effective concentration (100 μM).[92] Cinnamic acid (Figure 6, Table 2) is another example of an effective metabolite tested for reducing several pro-inflammatory markers although at levels (>100 μM) only suitable for pharmacological approaches.[94] The data gathered reveals ferulic acid potential for attenuating pro-inflammatory mediators in microglia. Although relevant metabolites, ferulic and isoferulic acid are not the major metabolites derived from coffee consumption,[95] as sulfates are the dominant conjugates over the glucuronides[95] Therefore, it will be very important to fill the gap in evaluating the activity of these circulating bioavailable conjugates as attenuators of neuroinflammation.

Phenylacetic acids and phenylpropionic acids metabolites found in blood after wine consumption,[96] such as 2-(3,4-dihydroxyphenyl)acetic acid, 2-(3-hydroxyphenyl)acetic acid, 3-(4-hydroxyphenyl)propionic acid, and 3-(3-hydroxyphenyl)-propionic acid (Figure 7, Table 2), have shown to be

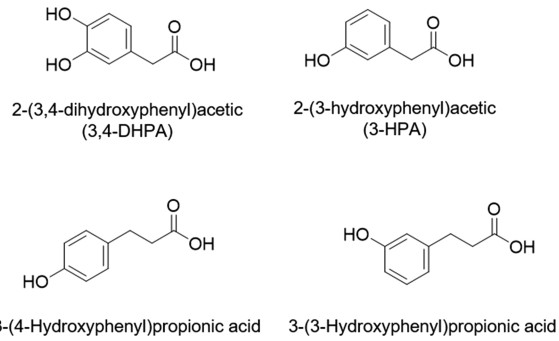

Figure 7. Phenylacetic acids and phenylpropionic acids evaluated as attenuators of neuroinflammation in microglia cells.

neuroprotective by significantly decreasing ERK 1/2 activation. Moreover 2-(3-hydroxyphenyl)acetic acid and 3-(4-hydroxyphenyl)propionic acid also reduce mitogen-activated protein kinase (MAPK) p38 and in SH-SY5Y cells modulate neuroinflammatory pathways normally triggered by nitrosative stress.[97]

Other studies evaluated the anti-inflammatory potential in microglia cells for other compounds similar to low molecular (poly)phenol metabolites. For example, phloroglucinol derivatives have potent anti-inflammatory effects and can effectively cross the blood-brain barrier.[98] Mechanistic studies have revealed neuroinflammation inhibition potential by targeting Src/phosphatase and tensin homologue deleted on chromosome 10 (PTEN)/Akt signaling.[98] Notably, these results were supported using two more complex cellular approaches as primary mixed mesencephalic neuronal/glial cultures and primary rat microglia cultures, both stimulated with LPS.[98]

In general, we may infer that the main molecular targets of the low-molecular weight (poly)phenol metabolites in stimulated microglia cells includes (Figure 8): (1) inhibiting the microglial activation of inflammatory cytokines, including TNF-α, IL-1β, IL-6; (2) inhibiting iNOS induction and subsequent nitric oxide (NO) production in response to glial activation; (3) inhibiting expression of COX-2 and resulting reduction of prostaglandins; and (4) downregulating activity of pro-inflammatory transcription factors such as NF-κB through modulation of glial and neuronal signaling pathways.

Overall, cellular studies taking into consideration (poly)-phenol metabolism and biological activity against neuro-degeneration, using physiologically relevant concentrations and relevant resident times, mark a mentality shift and the beginning of a new era in (poly)phenol health benefits research.

4. FINAL CONSIDERATIONS

Many studies corroborate the theory that (poly)phenol-rich supplements or foods have a positive impact preventing or attenuating neuroinflammation and therefore ameliorating NDs development and progression. A large amount of evidence has come from in vitro research using single flavonoids, typically aglycones, at supra-physiological concentrations which have given valuable clues for further studies with relevant circulating metabolites. In fact, increasing evidence suggests that metabolism of (poly)phenols may actually increase their biological activity. The anti-inflammatory effects of physiologically attainable (poly)phenol metabolites in healthy subjects is starting to be unveiled.

The sustained release of pro-inflammatory mediators that characterize neuroinflammation is harmful to microglia cells. Therefore, having compounds that can promote a shift in cells from inflammatory and neurotoxic phenotype to an anti-inflammatory and neuroprotective phenotype are crucial to efficiently repair the damage and restore the homeostasis, downregulating inflammation. The data gathered has identified the main molecular targets of the low-molecular weight (poly)phenol metabolites in stimulated microglia cells and highlighted their potential to attenuate neuroinflammation. These low-molecular weight (poly)phenol metabolites produced by gut microbiota are well absorbed in the intestine and persist in the plasma for a substantial time. Therefore, they could play a relevant role and alter our perspective of an effective prevention and cotreatment for NDs.

Still, the uncovering of the neuroprotective potential of (poly)phenol metabolites to attenuate neuroinflammation and their mode of action in neuronal cells is in its infancy. More studies to examine the diversity of the metabolites and evaluate how and when various types of conjugates (sulfates, glucuronides, and methylated) may alter their efficacy of the metabolites are needed. Moreover, metabolites have different pharmacokinetics and some of them may circulate simultaneously in specific time window, which increases the complexity of possible interactions and synergistic actions. Another layer of complexity needed to embrace the physiological environment where the metabolites act in vivo inside the brain is to use more biologically relevant cellular models. Current cell culture methodologies (2D) used to assess (poly)phenol metabolites biological activity are limited in their ability to reconstruct and mimic the cellular

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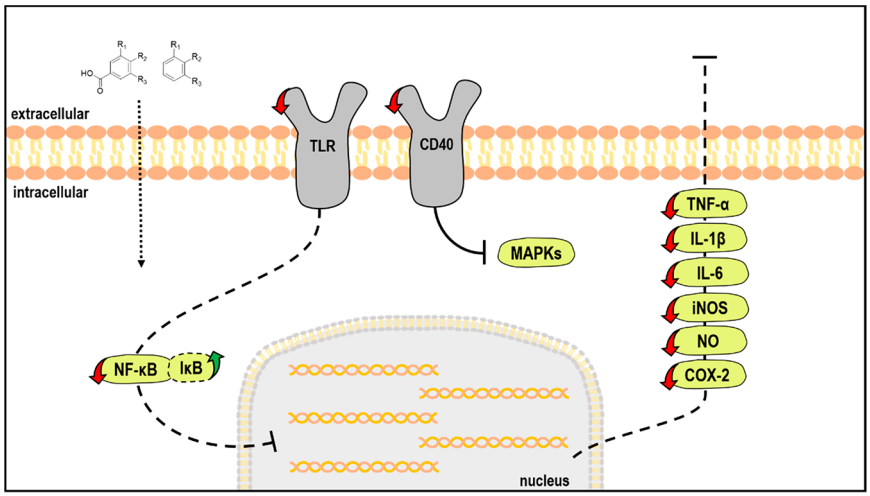

Figure 8. Main molecular targets of the low-molecular weight (poly)phenols metabolites in stimulated microglia cells. The pathways are the main entries highlighted in Table 2 as affected by the indicated physiological concentrations (also indicated in Table 2, ranging from 0.1 to <50 μM) of the metabolites in microglia cell systems.

environment that is present in vivo. In vitro models for preclinical research using stem cells, patient-specific induced pluripotent stem cells, and reprogrammed somatic cells from patients are already applied in disease modeling and drug discovery and may be applicable to test the brain benefits of (poly)phenol metabolites. Three-dimensional (3D) culture systems represent a more physiologically accurate model allowing more relevant cell−cell and cell−matrix interactions. The development of increasingly robust advanced model systems is a fast-growing field fed by the demands of the pharmaceutical industry. Some attempts have already evaluated cytoprotection by (poly)phenol metabolites in a 3D model containing neurons and astrocytes.[15,99] Approaching the missing link between (poly)phenol metabolites and neuroglia signaling and communication is crucial for tuning translation of how diet can modify age-related neurological diseases, like PD and AD.[15,99]

In the future, (poly)phenol research may accelerate the discovery of bioactive molecules with potential application in the prevention and therapeutics of neurodegenerative conditions. To design nutritional or pharmaceutical approaches using (poly)phenols, it is imperative to explore their benefits in animal models to examine their effects at the whole organism level. This is a promising area and will definitely provide knowledge of particular added value to postpone the development of NDs. Translation of these concepts could contribute to designing new dietary recommendations, and the established knowledge on bioactivity of phenolic metabolites can leverage future development of cost-effective nutraceutical/pharmaceutical therapies toward NDs. Both approaches may contribute to increase healthy life expectancy by delaying the onset of neurodegenerative diseases.

AUTHOR INFORMATION

Corresponding Author

*E-mail: claudia.nunes.santos@nms.unl.pt. Phone: +351 218 803 101. Fax: +351 218 851 920.

ORCID

Cláudia N Santos: 0000-0002-5809-1924

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Notes

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ABBREVIATIONS USED

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; Aβ, amyloid beta; CAPE, caffeic acid phenethyl ester; COX-2, cyclooxygenase 2; ER, endoplasmic reticulum; HD, Huntington disease; IFN-γ, interferon gamma; IGF1, insulin-like growth factor 1; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinases; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; ND, neurological disease; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD, Parkinson's disease; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TLR, toll like receptor; TNF-α, tumor necrosis factor alpha; UPR, unfolded protein response; αsyn, alpha synuclein

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