Research Article

Journal of
Experimental Stroke and
Translational Medicine

Pharmacological properties of rutin and its potential uses for Alzheimer's disease

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Received date: January 13, 2021
Accepted date: February 02, 2021
Published date: February 10, 2020

Abstract:

Rutin, a flavonoid with a wide range of biological activities, has a long history of use in nutritional supplements owing to its action against oxidative stress, inflammation, and hyperglycemia. Because of its pharmacological properties such as antioxidant, antiapoptosis, antiinflammation, rutin is proposed to treat Alzheimer's disease (AD). AD is a complex, multi-factorial neurodegenerative disease, and is characterized by neuronal atrophy of brain tissue. One of the pathological hallmarks of AD is the aggregation of soluble β amyloid (Aβ) into fibrillary deposits. Aβ aggregation induces neurotoxicity, oxidative stress and neuro-inflammation. In this review, we discussed the preclinical evidence on the antioxidant, antiapoptosis and anti-inflammatory proprieties of rutin, and the application of rutin in AD preclinical models. Rutin, delivered via oral and intraperitoneal routes, has been shown to functionally modify the cognitive and behavioural symptoms of AD in vivo due to its ability to cross the blood-brain barrier and act as both an antioxidant and an anti-inflammatory agent in the brain. Rutin attenuates oxidative stress, decreases the production of nitric oxide (NO) and proinflammatory cytokine and inhibits Aβ aggregation and cytotoxicity. Further studies to improve its bioavailability and investigations into its protective activities in AD would provide a concrete foundation for the use of rutin in clinical trials.

Keywords: rutin; AD; Aβ; antioxidant; antiapoptosis; antiinflammation

Introduction

Flavonoids, a group of natural substances with diverse phenolic structures, are found in fruits, vegetables, roots, grains, bark, flowers, stems, wine and tea [1]. The most common native flavonoid is rutin, which is found in a wide variety of plants (>70 plant species) and plant-based products [2, 3]. The nonmenclature of rutin varies in the literature and it may be referred to as rutoside, quercetin-3-O-rutinoside, vitamin P and sophorin. The etymology of the rutin classification has been linked to the Latin name for the rue plants Ruta graveolens, which can be dated back to the 19th century when rutin was first isolated. The content of rutin is the highest in leaves of rue plants (86.0 mg/g dw) followed by flowers of buckwheat (53.5 mg/g dw), flowers of pansy (33.5 mg/g dw), leaves of buckwheat (20.0 mg/g dw), and flowers of rose (10.0 mg/g dw) [4]. Buckwheat has been cultivated as a source of rutin for herbal drug preparation in the United States since the mid-20th century and nowadays buckwheat plants Fagopyrum are considered to be a major dietary source of rutin.

Chemically, rutin, 2-(3,4-dihyd-roxyphenyl)-5,7-dihydroxy-3-[β-L-rhamnopyranosyl-(16)—β-D-glucopyranosyloxy]-4H-chromen-4-1, is a glycoside comprising flavonolic aglycone quercetin alongside with disaccharide rutinose [Fig.1]. It appears as an odourless yellow crystalline powder that

is practically insoluble in H2O and partially soluble in alcohol [5]. Since living organisms are unable to synthesise rutin, ingestion of dietary sources of rutin is the major mechanism by which rutin is obtained in the body [6].

The IUPAC name of rutin (rutoside, quercetin-3-O-rutinoside) is 2-(3,4-dihyd-roxyphenyl)-5,7-dihydroxy-3-[β-L-rhamnopyranosyl-(1 6)—β-D-glucopyranosyloxy]-4H-chromen-4-1), and it is classified as a polyphenolic flavonoid.

Rutin has several pharmacological and biological effects, such as anti-inflammatory, antioxidant, antihypertensive, antiapoptotic, antiautophagic and neuroprotective activities [7]. It has various protective effects in vitro as well as in vivo against oxidative stress and lipid peroxidation [8]. It attenuates oxidative stress, decreases in vitro production of nitric oxide (NO) and proinflammatory cytokines, inhibits amyloid β (Aβ) aggregation and cytotoxicity [9]. Additionally, this bioflavonoid may have the potential to protect the kidneys against ischemia-reperfusion-induced injury [10], ameliorate oxidative stress and preserve hepatic and renal function after exposure to cadmium and ethanol [11], alleviate cisplatin-induced nephrotoxicity in rat, and attenuate gentamicin-induced renal damage via decreasing oxidative stress, inflammation, autophagy, and apoptosis in rodents [12]. It has been proposed that rutin can be used for treatment of Alzheimer's disease (AD) [13, 14].

AD is the most common neurodegenerative disease and the most common type of dementia. AD patients experience cognitive and mental deficits such as memory loss and confusion in addition to personality and intellect disorders. AD is the sixth leading cause of death in the United States, accounting for 3.6% of all deaths in 2014. In the United States alone, approximately 6.08 million Americans had either clinical AD or mild cognitive impairment due to AD in 2017. Approximately 44 million people in the world today suffer from dementia and AD accounts for about 60% of all dementia cases. Globally, dementia is the second largest cause of disability for individuals aged 70 years and older, and the seventh leading cause of death. There are nearly 10 million new cases every year; a number that is projected to grow to 82 million by 2030 and 152 million by 2050.

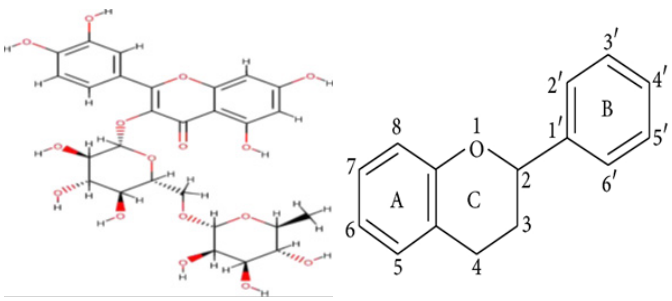

Figure 1: Chemical structure of rutin.

The current treatment strategy for AD is not curative but aims to maintain cognitive function, manage behavioural symptoms and slow symptom progression. Cholinesterase inhibitors may be prescribed for symptom management in mild to moderate AD. N-methyl D-aspartate (NMDA) antagonists can be prescribed to manage symptoms in moderate to severe AD. Since NMDA antagonists have a different mechanism of action to cholinesterase inhibitors, both treatments can be prescribed concomitantly. The impact of AD on patients, families, carers and wider society coupled with the limitations in current pharmacotherapy, which offer symptomatic improvement only, highlights a need for investment into novel drug targets. Emerging research has identified flavonoids as a unique class of therapeutic molecules with potential efficacy for AD. Rutin, a naturally occurring flavonoid, has been found to exhibit multiple properties (e.g. antioxidant, anti-inflammatory and cytoprotective functions) with clinical potential for the prevention and treatment of AD [15] and will be the focus of this review.

Pharmacological properties of rutin

Rutin as an antioxidant

Reactive oxygen species (ROS) are formed when cells exposed to oxygen generate oxygen free radicals. Endogenous free radicals are synthesized due to inflammation, mental stress, infection, ischemia, immune cell activation, excessive exercise, cancer, and aging while exogenous free radicals are formed due to air and water pollution, radiation, alcohol, heavy or transition metals, cigarette smoke, cooking, and industrial solvents [16, 17]. Mitochondria are the main source of endogenous ROS, but ROS can also occur in other organelles [18]. Examples of ROS include free radicals (superoxide, O–2), hydroxyl radicals (OH), and non-radicals (hydrogen peroxide, H2O2). OH has been recognized as the most reactive form of ROS and is thought to be primarily liable for the toxic effects of ROS. And O–2 is proposed to play a vital role in ROS production [19]. The defense mechanisms of small-molecule antioxidants and antioxidant enzymes have been found to decrease cellular ROS [20]. Superoxide dismutase (SOD) reduce O–2 into the more stable molecule, H2O2. H2O2 may generate OH, which is a highly reactive hydroxyl radical and can be reduced by catalase (CAT), glutathione peroxidase (GPX), and other peroxidases to

H2O and O2. The cellular antioxidant glutathione (GSH) is involved in two types of reactions. First, GSH in its reduced form non-enzymatically reacts with O–2 and OH for the elimination of ROS. GSH serves as the electron donor for the reduction of peroxides in the GPX reaction. When GSH reacts with ROS, it is oxidized and forms glutathione disulfide (GSSG) (the last product of GPX reactions). GSH can then be restored from GSSG by the reaction with glutathione reductase through the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to GSSG [21]. Several studies have indicated that GSH is involved in inhibiting DNA damage and apoptotic cell death after oxidative stress. Therefore, cellular antioxidants and antioxidant enzymes work together to inhibit ROS accumulation in the cell. Dysregulation of their functions is an indication of altered oxidative states, which may result in cell death.

Several mechanisms have been found to be responsible for antioxidant activities of rutin in both in vitro and in vivo models. It has been revealed that its chemical structure can scavenge ROS directly. Rutin is also alleged to increase the production of GSH, and upregulate cellular oxidative defense systems by increasing expression of various antioxidant enzymes such as CAT and SOD [22]. Additionally, rutin inhibits xanthine oxidase, which is involved in ROS generation [23]. It also scavenge ROS by donating hydrogen atoms to superoxide anions, peroxy radicals and hydroxyl radicals [24]. Research revealed that rutin effectively reduced the level of malondialdehyde (MDA), increasing CAT, GPX, SOD, GSH and nuclear factor erythroid 2–related factor 2 (Nrf2) levels in colistin-induced neurotoxicity [25]. Enhanced activity of xanthine and NADPH oxidase (NOX), which are the primary cellular enzymes responsible for the generation of superoxide radicals, is inhibited by rutin. Due to its polyphenolic structure, rutin can scavenge free radicals and chelate transition metal ions, which participate in Fenton reactions to generate reactive hydroxyl radicals [26, 27]. The main functional groups in the rutin molecule are the hydroxyl groups at positions 5 and 7 of the A ring, as well as the double bond in the C ring of the quercetin-polyphenolic component, which are responsible for its antioxidant activity [28]. Research has shown that under pathological conditions such as rheumatoid arthritis or cancer, rutin could inhibit the overproduction of oxygen radicals by neutrophils [29]. Furthermore, rutin has been found to facilitate the degradation of peroxides, including lipid peroxides, by regulating the level of GSH and effectively protected phospholipids from peroxidation. Several in vivo studies revealed that rutin treatment significantly attenuates decrease in the levels and activities of GSH and GSH-dependent enzymes (GSH-Px and GSSG-R) in rats [30]. In addition, rutin-facilitated regulation of the redox balance in fibroblasts and prevented decrease in nonenzymatic antioxidants, including vitamins E and C, after UV irradiation [31]. Rutin significantly reduced the cisplatin-induced oxidative stress by inhibiting lipid peroxidation and increasing antioxidant activity [32]. Research revealed that rutin could directly scavenge free radicals by chelating metal iron ions [33, 34]. Collectively, rutin reduces ROS production, NOX activity and oxidative products like MDA, thiobarbituric acid reactive substance, as well as increases antioxidant status such as SOD, GSH, GPX and CAT [35, 36 37, 38, 39, 40 &41].

Rutin as an antiapoptotic

Cell proliferation and elimination are essential in the maintainence of homeostasis of the physiological processes of an organism [42]. The unwanted cells are removed during the processes of pathogenesis, metamorphosis, embryogenesis as well as tissue turnover. The mechanisms of apoptosis are highly multifaceted and sophisticated, involving an energy-dependent cascade of molecular events [Figure 2]. There are two linked apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway, where molecules in one pathway can influence the other [43]. There is an additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell. The perforin/granzyme pathway can induce apoptosis via granzyme B or granzyme A. The extrinsic, intrinsic, and granzyme B pathways converge on the same terminal, or execution pathway, which starts with the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. Caspase-dependent apoptotic cell death occurs due to inactivation of survival pathways, like PI3K (phosphatidylinositol 3-kinase) /Akt (protein kinase B) pathway [44, 45]. Bcl-2 (B-cell lymphoma 2) inhibits intrinsic apoptosis by binding to the proapoptotic proteins Bax and Bcl-2 homologous antagonist/killer (Bak) [46]. The granzyme A pathway activates a parallel, caspase-independent cell death pathway via single stranded DNA damage [47].

Schematic representation of apoptotic events. There are two main pathways of apoptosis; the extrinsic and intrinsic pathways, as well as a perforin/granzyme pathway. Each requires specific triggering signals to initiate an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase (8, 9, and 10) leading to caspase-3 activation and initiating the execution

pathway. However, granzyme A works in a caspase-independent fashion. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.

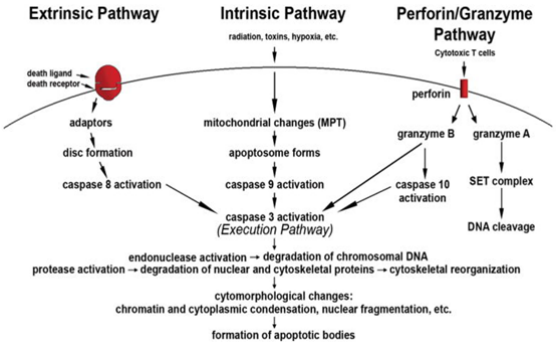

Figure 2: Schematic representation of apoptotic events..

Mitogen activated protein kinase (MAPK) is involved in signal transduction for apoptosis [48], and its levels indicate if the cell survives or dies, as they reveal cell damage [49]. The expression of MAPK is upregulated during the process of apoptosis in neurons and glial cells after spinal cord injury [50, 51]. Additionally, MAPK expression could facilitate the inflammatory response [52]. Inactive p38 MAPK is distributed mainly in the cytoplasm, and is translocated to the nucleus upon activation to regulate gene expression through the phosphorylation of transcription factors [53]. Extracellular stimuli, such as inflammatory cytokines, induce the phosphorylation and activation of p38 MAPK through a kinase cascade [54, 55]. Activated p38 MAPK induces the expression of enzymes, such as cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS), in addition to numerous inflammatory-related molecules, which facilitate the inflammatory response. It has been demonstrated that p38 MAPK expression in the spinal cord injury model was reduced by rutin thus indicating its protential to protect the cells of the spinal cord by lowering pro-apoptotic proteins expression [56]. Research revealed that rutin protects human dopaminergic cells against rotenone-induced injury by inhibiting the p38 MAPK signalling pathway [57]. Rutin also significantly protects fibroblasts from ultraviolet (UV)-induced apoptosis, particularly in response to UVA, through reduced caspase activation and cytochrome c release, as well as increased Bcl-2 expression. Additionally, it has been found that decrease of caspase-9 and caspase-3 activities are the key to the neuroprotective action of rutin on spinal cord cells. This observation was in line with previous reports indicating that rutin alleviated prion peptide-induced cell death by inhibiting caspase-3 activity in dopaminergic and hippocampal neurons [58, 59]. Furthermore, it has been revealed that rutin pretreatment significantly attenuates H2O2-induced apoptosis in HUVEC cells. Additionally, treatment with rutin reduced p53 expression, a protein involved in activation of DNA repair mechanisms and induction of apoptosis in response to DNA damage. Rutin administration has been found to attenuate ischaemic neural apoptosis by reducing p53 expression and lipid peroxidation in addition to upregulating endogenous antioxidant defence enzymatic activity [60]. These results are corroborated by a study conducted on the effect of rutin on sevoflurane- and propofol-induced neurotoxicity which concluded that rutin treatment was associated with a reduction in neuroapoptosis [61]. There is experimental evidence that rutin reduces apoptotic cells in ischaemic-reperfusion-induced apoptosis in both in vivo and in vitro models as well as in doxorubicin- and pirarubicin-induced cardiotoxicity by suppressing caspase-3, -7 and -9 protein expression [62, 63]. Caspase protein downregulation is correlation with an increase in Bcl-2 and a decrease in Bax protein expression [64, 65]. This suggests that rutin may prevent apoptosis via the Bcl-2 regulated apoptotic pathway, but the exact mechanisms underlying rutin's ability to modulate Bcl-2, Bax and caspase proteins are not yet fully elucidated.

Rutin as an anti inflammatory agent

Targeting neuroinflammation is a focus for AD therapy and rutin's anti-inflammatory properties may in part explain its efficacy in AD pathology. Rutin has anti-inflammatory properties, which are related to the inhibition of NFβB (nuclear factor kappa light chain enhancer of activated B cells), and NFβB-dependent pro-inflammatory cytokines [66]. In a carfilzomib-induced cardiotoxicity rat model, rutin pre-treatment using doses of 20 and 40 mg/kg caused a marked downregulation of NF-βB mRNA expression by increasing the expression of its inhibitory protein, IβB-β thus reducing the expression of numerous pro-inflammatory cytokines such as interleukin-6 (IL-6), C-reactive protein (CRP) and TNF-β [67]. Studies revealed that rutin suppresses phosphorylation of NFβB by inhibition of MAPK in lung tissue, in addition to decreasing the expression and cytoplasmic relocation of NFβB [68].

Rutin has been shown to exert anti-inflammatory effects in UVB-irradiated mouse skin by inhibiting COX-2 and iNOS expression via suppression of p38/MAPK [69]. Rutin regulates liver inflammation and fibrogenesis by regulating TLR4 and P2X7r [70]. In diabetic cardiomyopathic rats, rutin (50 mg/kg/d) introduced post-diabetically and administered orally for 24 days reduced the expression of TNF-β and CRP. Pre-

treatment with rutin at 100 mg/kg/day for 8 days in sepsis-induced cardiac injury in mice was found to attenuate TNF-β, IL-6 levels and cardiac inflammation. Furthermore, rutin may have the potential to inhibit proinflammatory TNF-β and IL-1β release from monocytes [71]. Rutin inhibited high mobility group box 1 (HMGB1) release, down-regulated HMGB1-dependent inflammatory responses in human endothelial cells, and inhibited HMGB1-mediated hyperpermeability and leukocyte migration in mice [72]. HMGB1 protein acts as a late mediator of severe vascular inflammatory conditions. Another study was carried out to determine the immunomodulatory effect of rutin, catechin, and hesperidin on macrophage function. This study showed that rutin, catechin, and hesperidin possessed immunomodulatory activity by promoting macrophage proliferation and suppressing lipopolysaccharide (LPS) stimulated TNF-β, IL-1β, IL-6 and NO levels in rat macrophages [73].

There is also evidence of rutin's anti-allergic inflammatory effects which may protect against allergic rhinitis. Rutin also demonstrates the ability to suppress chemokines (ICAM-1 and MIP-2), inflammatory cytokines and the activation of caspase-1. Topical administration of rutin inhibited serum histamine and mast cell infiltration into a mouse ear model of atopic dermatitis and allergic contact dermatitis (ACD). In addition, rutin suppressed ACD based on ear thickness and lymphocyte proliferation, inhibited serum IgG2a levels, and downregulated the expression of interferon INF-β, and IL-4, IL-5, IL-10, IL17 and TNF-β in an ACD ear model [74].

Alzheimer's disease (AD) pathology and amyloid β toxicity

AD can be characterised as either late-onset (sporadic) or early-onset (familial). Sporadic AD, which occurs in those aged 65 and over, is the most common subset of AD and accounts for 95% of AD cases. The remaining 5% of cases are classified as familial AD. Patients can exhibit symptoms of familial AD as early as 30 years of age and it is theorised that individuals who develop familial AD have inherited a dominant gene responsible for accelerating the disease [75]. Characterised as a progressive neurodegenerative disease, symptoms of AD gradually become more severe over time. It usually presents in three main stages. Early symptoms include forgetting recent events, forgetting the names of objects and regularly repeating questions or conversations. Symptoms occurring in the medial stage of the disease include increased confusion and disorientation, delusions and repetitive behaviour. As the disease progresses, the end stage symptoms include difficulty swallowing, considerable weight loss and difficulty moving.

AD has been characterised by neuronal atrophy of brain tissue. AD is associated with an abnormal accumulation and clearance of Aβ and tau proteins in the brain. The aggregation of soluble β amyloid into fibrillary deposits is the pathological hallmark of the disease. Aβ discovery and accumulation in brain resulted in the formulation of the "Amyloid Cascade Hypothesis" which states that Aβ deposition results in the formation of neurofibrillary tangles, neuronal cell death and dementia [76]. The amyloid cascade hypothesis proposes that Aβ accumulation in the brain is the first pathological event in AD [Figure 3] [77]. There are 2 main forms of Aβ deposited in the parenchyma: Aβ40 and Aβ42 which differ depending on whether the C terminus of Aβ ends at the 40th or 42nd amino acid. Aβ42 deposition predominates in AD patients; immunocytochemical probing revealed that all senile plaques in the AD cortex were Aβ42-positive whilst only one third were Aβ40-positive. There was a strong correlation between Aβ40 and mature plaques whereas diffuse plaques were positive for Aβ42 and negative for Aβ40 [78]. Aβ is a cleaved product of the Aβ precursor protein (APP) via proteolysis by β-secretase (BACE1) and β-secretase [79]. In AD, excessive accumulation of Aβ monomers results in their assembly into soluble, diffusible oligomers e.g. Aβ dimers which directly induce tau hyperphosphorylation and neurite degeneration [80]. When the oligomers reach a critical concentration, they form 2 major insoluble lesions: extracellular neuritic plaques (NPs) and intracellular neurofibrillary tangles (NFTs). NFTs are composed of hyperphosphorylated tau which disrupts microtubules and impairs axonal transport.

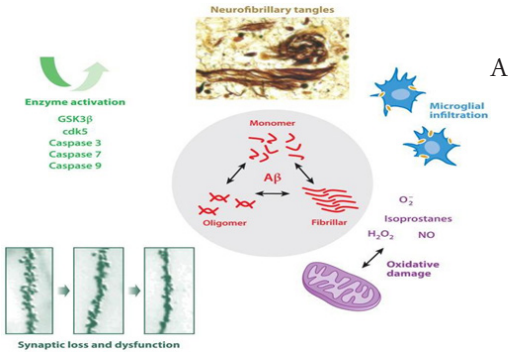

Figure 3: Amyloid β toxicity

aggregation induces neurotoxicity, oxidative stress and neuro-inflammation [81]. Previous research using tissue cultures demonstrates the toxic effects of Aβ fibrils to neurons as cell death occurred within 24 hrs of ββ exposure. The mechanism of cell death may be due to apoptosis triggered by the oxidative effects of Aβ [82]. There is also evidence of the differing relative toxicities of intracellular and extracellular Aβ; intracellular injection of Aβ42 results in neuronal death and intracellular ββ is seen in early AD [83]. Research revealed that the Aβ42 fragments are more

susceptible to aggregation than the more predominant but less active Aβ40 fragment, and an increase in the cerebrospinal fluid Aβ42:Aβ40 ratio is also associated with increased neurotoxicity [84].

Amyloid β toxicity. An equilibrium between several species of extracellular and intracellular Aβ, including monomeric, oligomeric, and fibrillar forms, causes toxicity through several mechanisms including microglial infiltration, the generation of reactive oxygen species, and synaptic damage. Neurofibrillary tangles are generated by Aβ-induced tau phosphorylation and cleavage. Enzymes activated directly by extracellular Aβ include GSK3β, Cdk5, and multiple caspases, which activate tau cleavage and phosphorylation among their many deleterious effects.

Metal ions are required in the brain for a number of important activities including the neuronal activity within the synapses and metalloproteins cellular processes [85]. However, there is evidence suggesting that metals in and around the amyloid plaques (e.g. copper (Cu), zinc (Zn) and iron (Fe)), play an significant role in the pathogenesis of AD [86]. Copper enhances amyloid precursor protein (APP) dimerization and increases the release of extracellular Aβ42 [87]. Both APP and Aβ have strong Cu-reductase activity, producing Cu+ from Cu2+ with hydrogen peroxide generated as by-product [88]. Aβ has some metal-binding sites on its first 15 amino acids, constituted by the histidines 6, 13, and 14 and the tyrosine residue at position 10, all of which have well-known and potent metal-binding sites, particularly for Cu2+ [89]. The ββ possesses the ability to reduce Cu2+ and Fe3+ towards a nearby affinity to the best metallic Cu+ and Fe2+, respectively. Molecular oxygen can react with reduced metals thus generating a superoxide anion, which in turn combines with two hydrogen atoms to form hydrogen peroxide that may later react with another reduced metallic ion and then forming the hydroxyl radical by the Fenton reaction. The Aβ in its radical form can extract protons from the neighbouring lipids or proteins, thus generating lipid peroxides and carbonyls, respectively [90]. There is experimental evidence supporting the theory that metals play a role in the toxicity of Aβ as their entire withdrawal from the reacting medium or the use deferoxamine resulted in significantly lowered ββ toxicity levels in cellular cultures [91, 92]. Increased expression of the divalent metal transporter 1 (DMT1) in the senile plaques of AD patients has been demonstrated, in APP/SS1 transgenic mice, and even in cellular lines overexpressing APP. It was suggested that such impairments in iron homeostasis could contribute to an increase in oxidative stress caused by Aβ [93].

Palop and Mucke (2010) theorised that Aβ reduces excitatory transmission across synapses by reducing glutamatergic synaptic transmission. Increased levels of Aβ is thought to inhibit neuronal excitability via a negative feedback loop. Loss in excitatory transmission over a prolonged period is thought to cause synaptic loss and a decline in neurological function. Aβ1–42 binds with a significantly greater affinity to the β7-nicotinic receptors than Aβ1–40. It is proposed that this differential binding may play a significant role in the internalisation and accumulation of Aβ in cholinergic neurons, a theory that is supported by experiments which have successfully blocked the internalisation and accumulation of Aβ1–42 using β7 receptor antagonists. Since Aβ1-42 predominantly accumulates in neurons that have β7-nicotinic receptors, it has been suggested that the presence of this receptor may be an underlying factor for the selective cellular toxicity shown by Aβ in the brain of AD patients [94]. Due to susceptibility to aggregation and potent neurotoxicity of amyloid fibrils in the brain, the strategy of inhibiting Aβ42 aggregation has long been considered a focus for effective disease modifying therapy for AD [95]. In healthy individuals, Aβ production and clearance is rapid, which is estimated at around ~7.6% and 8.3% respectively, of the total volume of Aβ produced per hour [96].

Aβ has been found in membranous intracellular structures such as the endoplasmic reticulum, the Golgi system, lysosomes, endosomes, and in the mitochondria's inner membrane or matrix [97]. Nevertheless, the origin of mitochondrial Aβ is uncertain. APP is believed to be located in the mitochondrial external membrane, but to date, only β-secretase enzymes have been identified in the inner mitochondrial membrane and enzymes with β secretase activity have not yet been found. An alternative explanation is that Aβ peptide is elaborated on a separate site and then moved inside the mitochondria . In the presence of calcium, Aβ can create transition pores in the mitochondrial membrane through which cytochrome C can be released and initiate, pro-apoptotic signalling pathways. Aβ can directly inhibit the generation of mitochondrial ATP, and affect the correct functioning of the β-subunit of ATP synthase [98]. Cellular exposure to Aβ generates an increase in intracellular calcium, which is associated with cell damage and cell death [99]. However, the mechanism by which this increase in intracellular calcium occurs is not well understood. A variety of Aβ-activated receptors and channels are thought to be involved, but it is also known that Aβ can directly interplay with the lipid components of the cell membrane, forming pores or ionic channels that help Ca2+ to enter into the cell. Pharmacological blockage of these pores or ionic channels was found to attenuate Ca2+ entering into the cell and neuronal damage. Without altering enzymatic mitochondrial machinery, Aβ administration in sub-toxic doses over a prolonged

period, inhibits the transportation of nuclear proteins to the mitochondria thus impairing mitochondrial membrane potential and the production of ROS [100]. The activation of enzymes such as NOX, xanthine oxidase, and A2 phospholipases (in both cytosolic and calcium-dependent forms) is involved in the mitochondrial dysfunction and the production of ROS mediated by Aβ. When such enzymes are pharmacologically blocked, ROS production and mitochondrial dysfunction by Aβ are significantly reduced.

Applications of rutin in AD

Effects of rutin on Aβ induced toxicity

Rutin can inhibit aggregation and cytotoxicity of Aβ inhibit damage of mitochondrial, decrease the production of ROS [101]. Several studies demonstrate that rutin can interfere with aggregation and toxicity of Aβ, inhibit oxidative stress induced by Aβ, reduce Aβ42 levels in mutant human APP overexpressing cells, and reduce senile plaques in the brain of APP transgenic mice [102, 103]. Research revealed that polyphenol compounds exhibit inhibitory effects on Aβ42 aggregation by binding hydrophobic β-sheet channels with their aromatic structure and simultaneously disturb the formation of Aβ hydrogen bonds through the action of hydroxyls as electron donors [104, 105, 106, & 107]. Rutin is composed chemically of an aromatic core with polyhydroxyl groups which may be responsible for its aforementioned mechanisms of action [108]. Rutin was found to reduce the Aβ42-induced cytotoxicity by interacting with Aβ to modify the structure of Aβ oligomers and inhibit their cytotoxicity. Rutin was found to decrease Aβ25–35 fibril formation and accumulation in in vitro, thereby inhibiting neurotoxicity, as well as decreasing Aβ plaque aggregation, NO production, pro-inflammatory cytokine production and oxidative stress in in vivo.

Neuroprotective effects of rutin

Rutin exerts its neuroprotective potentials by interacting with critical protein and lipid kinase signalling cascades (such as PI3K/Akt, protein kinase C and MAPK) in the brain which results in the inhibition of apoptosis triggered by Aβ and promotes neuronal survival and synaptic plasticity. It has beneficial effects on the vascular system leading to changes in cerebrovascular blood flow by angiogenesis and neurogenesis. Oral rutin administration may protect the CA3 region of the hippocampus in rats and have an impact on their behaviour, decreasing memory impairment due to trimethylin toxicity [109, 110, & 111]. Rutin attenuates age-related memory deficit in mice [112]. Rutin significantly attenuated memory deficit in AD transgenic mice, decreased oligomer Aβ level, increased SOD activity and GSH/GSSG ratio, reduced GSSG and MDA levels, down regulated microgliosis and astrocytosis, and decreased IL –Iβ and IL-6 levels in brain. Rutin improved memory and behaviour in open field, elevated plus and Y- mazes tests, possibly due to the reduction in neuroapoptosis in sevoflurane or propofol exposed neonatal mice. Rutin prevented cognitive deficits and morphological changes in the hippocampus; and attenuated lipid peroxidation, COX-2, GFAP, IL-8, iNOS and NFβB in a rat model of sporadic dementia [113]. It also prevented memory deficits and ameliorated oxidative stress, apoptosis and neurite growth in a rat model for cognitive dysfunction [114]. Rutin exerted an antidepressant-like effect, potentially facilitated by its NMDA receptor-mediated neuroprotective action on the hippocampus [115]. Rutin pre-treatment reduces infarct size and neurological deficits in rats following middle cerebral artery occlusion and protects the antioxidant content of enzymes in the brain [116, 117]. Rutin protected against neurodegenerative effects of prion accumulation by increasing the production of neurotrophic factors and inhibiting apoptotic pathway activation [118]. Rutin also has potential anticonvulsant and antioxidant activities against oxidative stress in kainic acid-induced seizures in mice [119].

Additionally, rutin is important in the promotion of neural crest cell survival in CNS. The neural crest is a progenitor comprising of neural and mesenchymal potentials. When applied to trunk neural crest cells, rutin augmented their viability without altering cell differentiation and proliferation, potentially due to the modulation of ERK2 and PI3K pathways [120]. It is also revealed that rutin has a sedative effect. Rutin, given via the intraperitoneal route caused a depressant action on the CNS, potentially medicated by the GABAA receptor [121].In a study on haloperidol-induced orofacial dyskinesia, rutin treatment reversed behavioural changes such as orofacial dyskinesia movements, stereotypic rearing, locomotor activity, and percent retention coupled with the restoration of biochemical and neurochemical parameters [122].

Inhibition of neuroinflammation by rutin

Neuroinflammation is a complex response to brain injury involving the activation of glia, release of inflammatory mediators, such as cytokines and chemokines, and generation of ROS [123]. Inflammatory responses in the brain are associated with increased levels of prostaglandins (PGs), particularly PGE2. Elevated PGE2 and inflammatory mediators are characteristic of the ageing brain. An increased state of neuroinflammation renders the aged brain more susceptible to the disruptive effects of both intrinsic and extrinsic factors such as infection, diseases toxicants, or stress.

AD pathology has been linked to microglial secretion of

proinflammatory cytokines, PGs, ROS and NOS thereby resulting in chronic stress, and if sustained over a prolonged period, even neuronal death [124, 125]. Neuroinflammation associated with AD can be inhibited by nonsteroidal anti-inflammatory drugs (NSAIDS) via maintaining Ca2+ homeostasis, targeting β-secretase, Rho-GTPases and PPAR [126]. The NSAIDs ibuprofen, indomethacin and flurbiprofen were found to decrease ββ(1-42) peptide toxicity in both in vivo and in vitro models through the inhibition of COX [128].

Rutin administration resulted in a reduction in neuroinflammation in rat model of AD and produced neuroprotective effects in dexamethasone-treated mice [128]. There is evidence that rutin decreases TNF-β and IL-1β generation in microglia in a rat model of spinal cord injury [129]. Rutin inhibited apoptosis by decreasing oxidative stress, Bax/Bcl-2, caspase-3 and -9 and c-Jun and p38 phosphorylation in Dopaminergic cells exposed to LPS [130]. Additional pharmacological mechanisms of rutin in the literature include the inhibition of Aβ aggregation and cytotoxicity, the prevention of mitochondrial damage, reduction in pro-inflammatory cytokine (TNF-β and IL-1) production, and an increase in the levels of CAT and SOD. Sodium rutin was found to attenuate neuroinflammation, enhance microglial-mediated Aβ clearance, ameliorate synaptic plasticity impairment and reverse spatial learning and memory deficits in two mouse models of AD [131].

Conclusion

Rutin is a flavonoid with distinguished pharmacological effects and promising therapeutic potential. The ability of rutin to exert its neuroprotective effects could be ascribed to its antioxidant as well as antiapoptotic and anti-inflammatory activities. Rutin can inhibit aggregation and cytotoxicity of Aβ; inhibit mitochondrial damage, decrease the production of ROS, MDA, GSSG, NO, iNOS and proinflammatory cytokines; increase SOD, GPx, CAT activity; and upregulate GSH. The ability of rutin to provide neuroprotection against pathological insult offers hope in its utilization and development as a safe and effective neurotherapeutic agent for AD. However, its low bioavailability owing to high metabolism, poor absorption, and rapid excretion makes its potential use as a therapeutic agent restricted. There is a paucity of clinical trial evidence exploring the efficacy of rutin in AD patients. The focus of future studies of rutin as a neuroprotective agent should be to improve its bioavailability, developing related molecules with greater gut and brain penetrability, a major barrier which has impeded the development of rutin-derived drugs.

Acknowledgement

This work was supported by a funding from the Petroleum Technology Development Fund,

Nigeria, and a funding from the Wellcome Trust (200633/z/16/z).

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