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An overview of the neuroprotective potential of rosmarinic acid and its association with nanotechnology-based delivery systems: A novel approach to treating neurodegenerative disorders

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ABSTRACT

Neurodegenerative disorders (ND) are characterized by slow and progressive neuronal dysfunction induced by the degeneration of neuronal cells in the central nervous system (CNS). Recently, the neuroprotective effects of natural compounds with anti-inflammatory and antioxidant activities has been clearly demonstrated. This appears to be an attractive therapeutic approach for ND, particularly regarding the use of polyphenols. In this review, we present an overview of the neuroprotective potential of rosmarinic acid (RA) and discuss the use of nanotechnology as a novel approach to treating ND. RA presents a variety of biological important activities, i.e. the modulation of pro-inflammatory cytokine expression, prevention of neurodegeneration and damage reduction. However, its poor bioavailability represents a limitation in terms of pharmacodynamics. In this sense, nanotechnology-based carriers could allow for the administration of higher but still safe amounts of RA, aiming for CNS delivery. Nasal administration could be a pleasant route for delivery to the CNS, as this represents a direct route to the CNS. With these advantages, RA-loaded nanotechnology-based therapy through the nasal route could be promising approach for the treatment of ND.

1. Introduction

Neurodegenerative disorders (ND) such as amyotrophic lateral sclerosis, Alzheimer's, Huntington's, and Parkinson's diseases are characterized by slow and progressive neuronal dysfunction induced by the degeneration of neuronal cells in the central nervous system (CNS). The main clinical manifestations of neuronal damage are memory and cognitive impairment, locomotor difficulty, and emotional and/or behavioral problems (Barnham et al., 2004; Liu et al., 2017; Pérez-Hernández et al., 2016; Solanki et al., 2016).

The pathogenesis of ND is considered complex and multifactorial, involving a combination of genetic and non-genetic factors; the latter is associated with most ND cases. Among the non-genetic factors, alterations in the immune-inflammatory system and oxidative stress have been highlighted to play key roles in ND development and/or progression (Andersen, 2004; Bhat et al., 2015; Ramassamy, 2006; Wang et al., 2006).

Inflammation is a protective response to cell injury or infection, intended to remove or inactivate potentially damaging agents and prevent their destructive effects. However, when the inflammatory process persists uncontrollably (chronic inflammation), it can lead to the destruction of normal tissue. This condition is especially relevant in ND due to the prolonged progression of these diseases, in which chronic neuronal damage and protein aggregates are associated with constant activation of the microglial immune-inflammatory system (Cunningham et al., 2009; Fischer and Maier, 2015; Lull and Block, 2010; Wang et al., 2006; Wyss-coray and Mucke, 2002).

Oxidative stress is the result of imbalanced oxygen metabolism and consequent high intracellular non-physiological quantities of reactive oxygen species (ROS). It is highly associated with neuronal damage in several disorders, including ND (Andersen, 2004; Barnham et al., 2004; Bhat et al., 2015; Casetta et al., 2005; Liu et al., 2017; Uttara et al., 2009).

In addition to the complex etiology of ND pathogenesis, therapeutic strategies are limited by the difficulty in reaching the desired site of action, and thus fail to prevent disease progression. Currently, few

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available treatments prevent temporary ND progression and improve patient symptoms. In this context, the role of neuroprotective therapies has garnered considerable interest, with the aim of improving the management of these diseases (Bhullar and Rupasinghe, 2013; Pérez-Hernández et al., 2016; Solanki et al., 2016; Sood et al., 2014).

Recently, the neuroprotective effects of natural compounds that show anti-inflammatory and antioxidant activities has been clearly demonstrated; this appears to be an attractive therapeutic approach for ND. Among the natural products, polyphenols have attracted considerable attention as many studies have demonstrated the neuroprotective effect of a diet rich in phenolic compounds (Albarracin et al., 2012; Casetta et al., 2005; Kelsey et al., 2010; Koppula et al., 2012; Losada-Barreiro and Bravo-Díaz, 2017; Ramassamy, 2006; Solanki et al., 2016). In this review, we provide an overview of the *in vitro* and in *vivo* evidence supporting the neuroprotective potential of rosmarinic acid (RA), considering that this compound is a widely studied polyphenol (Amoah et al., 2016; Habtemariam, 2018). We also discuss the potential of nanotechnology-based delivery systems as a promising approach for ND treatment.

2. Rosmarinic acid (RA)

RA is a polyphenolic compound, an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Fig. 1) (Petersen and Simmonds, 2003). It was first isolated from rosemary *(Rosmarinus officinalis* L.) and described by Scarpati and Oriente (1958). The presence of RA has been reported in more than 30 families and 50 species of medicinal plants, particularly the Boraginaceae family and Nepetoideae sub-family of the Lamiaceae family and the species *Mentha* spp., *Melissa officinalis*, *Perilla frutescens, Salvia officinalis,* and *Thymus vulgaris* (Kim et al., 2015; Petersen, 2013; Petersen et al., 2009).

Various biological and pharmacological properties have been reported for RA in the literature. Among them, we can highlight some biological activities such as antidepressant (Takeda et al., 2002), anti-inflammatory (Rahbardar et al., 2017; Rocha et al., 2015), anti-melanogenic (Ding et al., 2010), antioxidant (Fadel et al., 2011; Tepe et al., 2007), antitumor (Osakabe et al., 2004b), antiviral (Swarup et al., 2007), antiangiogenic (Huang and Zheng, 2006), photoprotective (Psotova et al., 2006; Sánchez-campillo et al., 2009), and neuroprotective (Khamse et al., 2015; Lee et al., 2016).

Taking into account the wide distribution of RA, its easy isolation from the plant kingdom, and its well-documented biological properties, RA has arisen as a natural molecule of interest, particularly in the current context of research into new neuroprotective therapies (Bulgakov et al., 2012; Khojasteh et al., 2014).

2.1. RA anti-inflammatory activity

The potential of natural molecules to reduce or suppress an inflammatory response has received increased attention in recent years, considering the association between inflammatory processes and multiple diseases, including ND (Cunningham et al., 2009; Perry, 2004; Wyss-coray and Mucke, 2002). There have been many studies performed *in vitro*, *ex vivo,* and *in vivo* demonstrating the anti-inflammatory activity of RA. The most important are summarized in Table 1 to better describe the roles of RA in inflammatory processes.

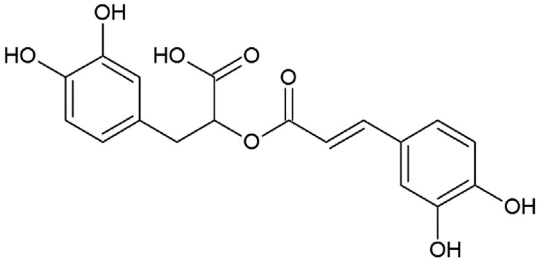

Fig. 1. Chemical structure of rosmarinic acid (RA).

The first *in vitro* and *ex vivo* study was performed by Naito et al. (2003), and showed RA anti-inflammatory activity in an atherosclerosis inflammatory response induced by oxidized low-density lipoprotein (oxLDL). The authors demonstrated the inhibitory effect of RA on monocyte-endothelial interactions and on low density lipoprotein (LDL) oxidation associated with the potent free radical scavenging effect of RA. Additionally, an anti-atherosclerotic *ex vivo* effect of RA in apolipoprotein E-deficient mice was demonstrated and associated with an inhibitory effect of RA on plasma lipid peroxide production (Naito et al., 2003).

The anti-inflammatory activity of RA was also demonstrated *in vivo* in mice through topical application in skin papilloma (Osakabe et al., 2004b) and oral administration in hepatocellular carcinoma (Cao et al., 2016). In these studies, an anti-carcinogenic effect of RA was observed, and tumour suppression was related to the regulation of proinflammatory cytokine secretion, particularly tumour necrosis factor alpha (TNFα), interleukins 1-beta (IL-1β) and 6 (IL-6), as well as the inhibition of cellular adhesion and angiogenesis, which were associated with its antioxidant activity. Osakabe et al. provided further evidence of the anti-inflammatory activity and effectiveness of RA for seasonal allergic rhinoconjunctivitis in a randomized, double blind, age-matched, and placebo-controlled clinical trial (Osakabe et al., 2004a).

The suppression of inflammation induced by lipopolysaccharide (LPS) *in vitro* in human gingival fibroblasts and in bone marrow-derived dendritic cells, as well as *in vivo* in mice has also also reported for RA (Chu et al., 2012; Kim et al., 2008; Osakabe et al., 2002; Zdařilová et al., 2009). The findings of these studies demonstrated and confirmed the ability of RA to modulate proinflammatory cytokines (TNFα, IL-1β and IL-6), inflammatory chemokines (monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1alpha (MIP-1α)), and antioxidant mechanisms. In addition, studies performed by Chu et al. (2012) and Kim et al. (2008) demonstrated the suppression of intracellular ERK (extracellular ligand-regulated kinase) signalling by RA, also observed *in vitro* by Scheckel et al. (2008) and later by Liang et al. (2016) in an *in vivo* model of asthma.

Lembo et al. (2014) demonstrated the ability of RA to prevent damage induced by ultraviolet-B (UVB) radiation in a human keratinocyte cell line (HaCaT cells). They also confirmed the reduction of pro-inflammatory mediators (TNFα, IL-6, IL-8, MCP-1) and enhancement in protective interleukin 10 (IL-10). In the meanwhile, Ku et al. (2013) showed that RA also downregulates endothelial protein C receptor (EPCR) *in vitro* (in human endothelial cells) and *in vivo* (in a model of septicemia induced by cecal ligation and puncture). This study showed that RA treatment inhibits phorbol-12-myristate 13-acetate (PMA), TNFα, and induced EPCR release by the suppression of TNF-α converting enzyme (TACE) expression.

Rocha et al. (2015) used the carrageenan-induced paw edema model of local inflammation, hepatic ischemia-reperfusion, and thermal injury model of systemic inflammation in rats to evaluate the anti-inflammatory activity of RA administered orally or intravenously. The authors reported a reduction of about 60% in paw edema in RA-treated animals compared to control. The results were attributed to the anti-inflammatory activity of RA associated with a reduction in pro-inflammatory cytokines (TNFα, IL-1β, and IL-6). In a hepatic ischemia-reperfusion model, RA significantly decreased transaminase (AST and ALT) and LDH serum concentrations. In a thermal injury model, RA reduced multiorgan dysfunction markers by modulating matrix metalloproteinase-9 (MMP9) and factor nuclear kappa B (NF-κB), also observed *in vitro* by Lee et al. (2008) and Kim et al. (2008).

Recently, Rahbardar et al. (2017) used a neuropathic pain murine model to evaluate the intraperitoneal administration of RA in the neuro-inflammatory environment. In this study, the authors observed the ability of RA to modulate neuro-inflammation through the reduction of all inflammatory and oxidative markers studied (including

Table 1
In vitro, ex vivo, and *in vivo* studies of rosmarinic acid (RA) anti-inflammatory potential found in the literature.

Treatment	Model	Main result	Mechanism	References
[RA] 1–50 μM	*In vitro* model of atherosclerosis inflammatory response induced by oxLDL	RA anti-inflammatory activity associate to inhibitory effect on adhesion molecule expression induced by oxLDL	Inhibitory effect on monocyte-endothelial interaction RA and LDL oxidation, associated to free radical scavenging activity	Naito et al. (2003)
[RA] 1.52 $mg.kg^{-1}$ (oral treatment for 6 w, 1 administration/day)	*Ex vivo* model of atherosclerosis in apolipoprotein E-deficient mice (4-w)	RA reduced aortic atherosclerotic lesion areas in mice	Inhibitory effect on plasma lipid peroxide levels	Naito et al. (2003)
[RA] 1–40 μM (treatment for 14 h)	*In vitro* model of TNF-α-induced upregulation of CCL11 and CCR3 in human dermal fibroblasts	RA attenuated TNF- α -induced expression of CCL11 and CCR3	Suppressor effect on IKK-β activity in NF-kB activation signalling	Lee et al. (2006)
[RA] 0.1–200 μM (treatment for 24 h or pre-treatment 1–2 h prior induction with LPS)	*In vitro* model of LPS-Induced production of MCP-1 and MIP-1α via the MAPK pathway in bone-marrow derived dendritic cells	RA inhibited LPS-induced up-regulation in bone-marrow derived dendritic cells	Reduction of expressions of MCP-1 and MIP-1α and inhibition of LPS-induced activation of MAPK and the nuclear translocation of NF-κB	Kim et al. (2008)
[RA] 5–20 $\mu Mol.L^{-1}$ (pre-treatment for 1 h and co-treatment)	*In vitro* model of COX2 expression in colon HT-29 and breast MCF-7 cancer cells and non-malignant breast epithelial MCF10A	RA prevented COX-2 activation by AP-1-inducing agents in both cancer and non-malignant mammary epithelial cells	Reduction of COX-2 expression and AP-1 activation also antagonized ERK1/2 activation	Scheckel et al. (2008)
[RA] 1 $\mu g\ mL^{-1}$ (treatment for 4–24 h)	*In vitro* model of oxidative stress and inflammation induced by LPS in human gingival fibroblasts	RA suppressed LPS-induced biological changes in gingival fibroblast	Reduction of ROS production, intracellular GSH depletion and lipid peroxidation, regulation of pro-inflammatory cytokines (TNFα, IL-1β, IL-6), suppressed expression of iNOS	Zdařilová et al. (2009)
[RA] 0.1–2 μM	*In vitro* model of PMA-, TNF-α, and IL1b-induced EPCR shedding in human endothelial cells	RA decreased EPCR release	Inhibitory effect in PMA-, TNFα, IL induced EPCR shedding by suppression of TACE expression	Ku et al. (2013)
[RA] 2.7 μM (treatment for 4 and 24 h)	*In vitro* model of UVB-induced cytokine/chemokine gene expression in HaCaT cells	RA prevent UVB-induced damage	Reduction in pro-inflammatory mediators (TNFα, IL-6, IL-8, MCP-1) and enhancement of the protective IL-10	Lembo et al. (2014)
[RA] 150 $mg.kg^{-1}$ (oral - treatment concurrently with LPS injection)	*In vivo* model of liver injury induced by LPS in D-GalN-sensitized (Male BALB/c mice 7-9-w)	RA liver protection through reduction of plasma aspartate aminotransferase levels	Scavenging or reducing effect on superoxide or peroxynitirite	Osakabe et al. (2002)
[RA] 1.35 $mg.kg^{-1}$ (topical - pre-treatment for 30 min, 1 administration)	*In vivo* model of skin papilloma (Male BALB/c mice 7-9-w)	RA anti-carcinogenic effect via inhibition of the inflammatory response and scavenging of reactive oxygen radicals	Inhibitory effect on adhesion molecule, chemokine and eicosanoid synthesis, and on oxidative DNA injury	Osakabe et al. (2004b)
[RA] 5, 10 and 20 $mg.kg^{-1}$ (intraperitoneal - pre-treatment 1 h prior LPS administration)	*In vivo* model of acute lung Injury induced by LPS (Male BALB/c mice weighing 18–20 g)	RA potent anti-inflammatory effect in *in vivo* models of lung injury induced by LPS	Reduction in pro-inflammatory cytokines (TNFα, IL-1β, IL-6) and suppression of intracellular signalling ERK	Chu et al. (2012)
[RA] 1.4 μg per mice (treatment 12 and 50 h after sepsis induction)	*In vivo* model of septicemia induced by CLP (Male C57BL/6 mice weighing 18–20 g)	RA decreased EPCR release	Inhibitory effect in PMA-, TNFα, and induced EPCR release by suppression of TACE expression	Ku et al. (2013)
[RA] 0, 25 and 50 $m.kg^{-1}$ (oral or intravenous - pre-treatment for 30 min)	*In vivo* model of local inflammation (carrageenan in-induced paw edema) and systemic inflammation (hepatic ischemia–reperfusion and thermal injury) (Male Wistar rats weighing 100–380 g)	RA significantly decreased paw edema and systemic inflammation	Reduction in pro-inflammatory cytokines (TNFα, IL-1β and IL-6), AST, ALT and LDH in serum concentration.; modulation of NF-κB and MMP9	Rocha et al. (2015)
[RA] 75, 150 and 300 $mg.kg^{-1}$ (intragastric - treatment for 10 days, 1 administration/day)	*In vivo* model of hepatocellular carcinoma (H22-xenografts) (Male SPF Kunming mice weighing 18–22 g)	RA suppressed the tumour growth in H22-xenografts model with fewer toxic effects	Inhibitory effect on pro-inflammatory cytokines (TNFα, IL-1β, IL-6) and angiogenic factors, as well as suppression of NF-kB p65	Cao et al. (2016)
[RA] 5, 10 and 20 $mg.kg^{-1}$ (intraperitoneal - pre-treatment 1 h prior ovalbumin administration for 4 days)	*In vivo* model of allergic asthma induced by ovalbumin (Female BALB/c mice weighing 18–20 g)	RA decreased the number of inflammatory cells and mucus hypersecretion in the airway	Suppressor effect on ERK and activation of NF-kB p38	Liang et al. (2016)
[RA] 40 $mg.kg^{-1}$ (intraperitoneal - treatment concurrently with surgery, once a day for 14 days)	*In vivo* model of neuropathic pain (Male Wistar rats weighing 230–280 g)	RA modulated neuro-inflammation	Reduction in iNOS, COX2, IL-1β, PGE2, and MMP2	Rahbardar et al. (2017)

[RA]: rosmarinic acid concentration; oxLDL: oxidized low-density lipoprotein; LDL: low density lipoprotein; TNFα: tumour necrosis factor alpha; CCR3: complement component 3; CCL11: motif chemokine ligand 11; MCP-1: monocyte chemoattractant protein-1; MIP-1α: macrophage inflammatory protein-1α; MAPK: mitogen activated protein kinases; COX2: cyclooxygenase 2; HT-29: human colon adenocarcinoma cell line; MCF-7: human breast adenocarcinoma cell line; MCF10: human breast epithelial cell line; PMA: phorbol-12-myristate-13-acetate; IL: interleukin; EPCR: endothelial cell protein C receptor; HaCaT: immortalized nontumorigenic human epidermal cell line; LPS: lipopolysaccharides; D-GalN: D-galactosamine; CLP: cecal ligaion punture; H22: interferon-gama; AP-1: activator protein-1; ERK: extracellular-regulated kinase; GSH: glutathione; iNOS: inducible nitric oxide synthase; ROS: reactive oxygen species; TACE: transarterial chemoembolization; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NF-kB: nuclear factor kappa B; MMP9: metalloproteinase 9; LDH: lactate dehydrogenase; PGE: prostaglandin E; IKKβ: IkB kinase.

Table 2
Main *in vitro* and *in vivo* studies of rosmarinic acid (RA) antioxidant potential found in the literature.

Treatment	Model	Main result	Mechanism	References
[RA] 0.9–18 mg.L^{-1} (pre-treatment for 4 h)	*In vitro* model of UVA-induced changes in HaCaT cells	RA UVA photoprotective effect	Suppression of UVA-induced ROS production and CCR3 activation, decrease in lipid peroxidation and glutathione, and increase in ATP	Psotova et al. (2006)
[RA] 2.9 μg.mg^{-1}	*In vitro* DPPH free radical-scavenging and β-carotene/linoleic acid systems	RA antioxidant activity	Free radical-scavenging capacity and inhibition of linoleic acid oxidation	Tepe et al. (2007)
[RA] 0.25–100 μM	*In situ* model of lipoperoxidation in liposomes of 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC)	RA spontaneously penetrates membranes to inhibit lipid peroxidation	Spontaneously insertion in lipid membranes, with a higher affinity for unsaturated lipids	Fadel et al. (2011)
[RA] 0.25 mM	*In vitro* lipid oxidation system	RA antioxidant effect against lipid oxidation	Antioxidation reaction occurs at the catechol position of its 2-oxyphenylpropanyl moiety during the first stage of lipid oxidation	Fujimoto and Masuda (2012)
[RA] 3–13.5 mM	*In vitro* model pro-oxidant (generation of H_2O_2 and free radicals by the action of peroxidase) and antioxidant (consumption of H_2O_2 and free radicals) activity	RA pro-oxidant and antioxidant activity	Production of H_2O_2 during its auto-oxidation and generation of free radicals (horseradish peroxidase substrate). Ability to eliminate H_2O_2 and sequestrate free radicals	Muñoz-Muños et al. (2013)
[RA] 0.1–0.5 mg.mL^{-1}	*In vitro* model of antioxidant (β-carotene bleaching, DPPH free radical scavenging, reducing power and chelating effect) and DNA damage protection	RA antioxidant potential and protective effects on pBR322 plasmid DNA against the mutagenic and toxic effects of UV and H_2O_2	Free-radical scavenging activity and DNA damage protection. Authors suggestion: Intercalation into DNA alter the DNA structural integrity, interrupt the production of oxidizing species	Sevgi et al. (2015)
[RA] 0.625, 1.25, 2.5, or 5 mM (pre-treatment 1 h prior to UVB exposure)	*In vitro* model of UVA-induced damage in HaCaT cells	RA cytoprotective activity against UVB radiation associated with ROS elimination	Increase of expression and activity of SOD, CAT, heme oxygenase-1, and Nrf2	Fernando et al. (2016)
[RA] 0–3 mM (treatment)	*In vitro* model of oxidative challenge elicited by t-BHP in human hepatoma HepG2 cells	RA cytoprotective activity against t-BHP cell damage	Free radical-scavenging activity	Adomako-bonsu et al. (2017)
[RA] 50–600 μM (treatment for 4 days)	*In vivo* model of RA exposure to *Caenorhabditis elegans*	RA extend *C. elegans* lifespan	Hormesis, antioxidative/pro-oxidative properties, modulation of genetic players all contribute to *C. elegans* life extension	Pietsch et al. (2011)
[RA] 10 mg.kg^{-1} (oral - treatment for 21 days, 2 w after diabetes induction)	*In vivo* model of lipid peroxidation in strepotozotocin -induced diabetic rats (Male Wistar rats weighing 200–250 g)	RA prevents lipid peroxidation	Increase in AChE activity, modulation of cholinergic neurotransmission and prevention of damage oxidative stress in brain	Mushtaq et al. (2014)
[RA] 10 mg.kg^{-1} (oral - treatment for 21 days, 2 w after diabetes induction)	*In vivo* model of lipid peroxidation in strepotozotocin -induced diabetic rats (Male Wistar rats weighing 200–250 g)	RA effectively reduced the oxidative stress induced by strepotozotocin	Normalized δ-ALA-D and other oxidative stress parameters in liver and kidney	Mushtaq et al. (2015)
[RA] 10/20 mg.kg^{-1} (oral - pre-treatment once a day during 7 days)	*In vivo* model of hepatotoxicity induced by carbon tetrachloride (Male and female Kunming mice weighing 18–22 g)	RA hepatic protective effect	Reduction in the serum levels of AST and ALT. Increase antioxidative properties (SOD, CAT, GSH-Px, and TBARS)	Yang et al. (2015)
[RA] 50, 100 and 200 mg.kg^{-1} (oral-treatment once a day for 30 days)	*In vivo* model of aging mice (Male Kunming mice weighing 47.56 ± 3.61 g)	RA prevents progression of oxidative stress-related aging processes	Radical-scavenging activity, alteration of antioxidant enzymes (SOD, CAT, and GSH-Px) and inhibition of lipid peroxidation	Zhang et al. (2015)
[RA] 100 mg.kg^{-1} (intraperitoneal - treatment for 24 h afte sepsis induction)	*In vivo* model of oxidative DNA damage induced by sepsis (Male Wistar rats weighing 200–300 g)	RA attenuates sepsis-induced oxidative damage	Reduction in the MDA and TNF-α levels, increase in GSH, SOD, and GSH-Px activities in rat liver and kidneys	Bacanli et al. (2016)

[RA]: rosmarinic acid concentration; Nrf-2: nuclear factor erythroid 2; δ-ALA-D: Delta-aminolevulinic acid dehydratase; SOD: superoxide dismutase; CAT: catalase; TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde AChE: acetylcholine; t-BHP: t-butyl hydroperoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TNFα: tumour necrosis factor α; CCR3: complement component 3; HaCaT: immortalized nontumorigenic human epidermal cell line; GSH: glutathione; ROS: reactive oxygen species; AST: aspartate aminotransferase; ALT: alanine aminotransferase; H_2O_2: hydrogen peroxide.

induced nitric oxide synthase (iNOS), cyclooxygenase-2 (COX2), IL-1β, prostaglandin E − 2 (PGE2), and matrix metalloproteinase 2 (MMP2) in treated animals. Additionally, the authors suggested RA as a potential candidate in treating different inflammatory neurological disorders.

2.2. RA antioxidant activity

The potential of natural molecules to inhibit the formation or eliminate free radicals has received increased interest in recent years, based on the relation that has been established between oxidative stress and many disorders, including the ND (Albarracin et al., 2012; Brewer, 2011; Yanishlieva et al., 2006). The free radical scavenger potential of RA is well-reported and has been described by several authors. The most important studies are summarized in Table 2 to better discuss the role of RA in oxidative stress (Lecomte et al., 2010; Pérez-Tortosa et al., 2012; Tepe et al., 2007; Zhu et al., 2014).

Compared to other phenolic acids and their derivatives, studies have shown that RA exhibits excellent antioxidant activity (RA > chlorogenic acid > caffeic acid > ferulic acid > coumaric acid), demonstrated by the β-carotene/linoleic acid co-oxidation, deoxyribose, DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing ability of plasma), DNA damage, TEAC (Trolox equivalent antioxidant capacity), and HClO (hypochlorous acid sequestration) assays (Sevgi et al., 2015; Soobrattee et al., 2005).

In general, antioxidant compounds work by eliminating free radicals by donating their own electrons to neutralize the adverse effects of ROS. For many years, polyphenols have been thought to act by protecting molecules from oxidative damage by eliminating free radicals. However, increasing evidence shows that this is a simplified view of its mechanism of action (Losada-Barreiro and Bravo-Díaz, 2017).

Regarding RA, it is known that the presence of two catechol groups in its structure (Fig. 1) is strongly associated with its antioxidant potential. Studies have shown that the antioxidant activity in the moiety related to 3,4-dihydroxyphenylactic acid is similar to the moiety of caffeic acid, suggesting that protons from one of the catechol groups may be removed to form intramolecular hydrogen bonds to stabilize the resulting radicals, although 3,4-dihydroxyphenylactic shows a great facility for donating protons (Cao et al., 2005; Fujimoto and Masuda, 2012). Conversely, other authors have reported that the moiety of caffeic acid is the first to undergo oxidation (Gil et al., 2013).

In vitro and *in vivo* studies have demonstrated different pathways of RA antioxidant activity, including increased antioxidant enzyme expression (catalase (CAT), glutathione peroxidase (GPx) and reductase, superoxide dismutase (SOD), nitric oxide synthase), reduced DNA damage, decreased amounts of oxygen and nitrogen reactive species, and lower levels of lipoperoxidation markers (Bacanli et al., 2016; Fernando et al., 2016; Mushtaq et al., 2015, 2014; Psotova et al., 2006); the latter is being widely studied and is associated with the spontaneous insertion of RA into lipid layers (Fadel et al., 2011).

Another mechanism of action associated with RA antioxidant activity is related to the regulation of erythroid nuclear factor expression (Nrf-2), which may result in an increase in antioxidant status. Nrf-2 is responsible for regulating the basal and post-induction expression of several antioxidant enzymes, such as CAT, GPx, and SOD (Domitrović et al., 2013; Fernando et al., 2016; Shang et al., 2017).

2.3. RA neuroprotective potential

As stated in the previous sections, the anti-inflammatory and antioxidant activities of RA have been well-established in several experimental models. The search for evidence for the neuroprotective effect of RA has been the focus of several *in vitro* and *in vivo* studies, presented in Table 3.

Table 3 shows that most of the studies correlate neuroprotective activity with the antioxidant activity of RA. *In vitro* studies involving a neurotoxicity model induced by hydrogen peroxide on dopaminergic neuronal cells (SH-SY5Y), glioblastoma cells (A172 and C6), or rat neuroblastoma cells (N2A), demonstrated the protective potential of RA against neuronal/glial damage induced by oxidative stress. As its main protection mechanism, RA induced an intracellular decrease in ROS production and/or accumulation, lipid peroxidation, and apoptotic process (Costa et al., 2013; Ghaffari et al., 2014; Lee et al., 2016, 2008). The protective effect of RA was also demonstrated *in vitro* in a neurotoxicity model induced by 6-hydroxydopamine in dopaminergic cells (MES23.5) and was correlated with a reduction in mitochondrial function and ROS production (Ren et al., 2009).

Other *in vitro* models involving oxidative stress, excitotoxicity, ischemic injury, and ciguatoxin toxicity have also been reported for RA and reinforce its protective effect on neuronal cells, mainly related to its antioxidant activity (Braidy et al., 2014; Fallarini et al., 2009). A recent study performed by Andrade et al. (2016) involving *in vitro* models of antioxidant activity (cholinesterases (ChEs), monoamine oxidases (MAO-A and MAO-B) and catechol-O-methyl transferase (COMT)) as well as *in silico* approaches showed the multifunctional profile of RA with regard to targets related to neurodegeneration. The results suggest that RA may be explored as a model for the development of new antioxidant molecules possessing additional MAO and COMT inhibitory effects to be further investigated for ND treatment (Andrade et al., 2016).

In line with the *in vitro* studies mentioned above, *in vivo* studies involving models of Parkinson's disease, epilepsy, and Alzheimer's disease have also been performed and have confirmed the neuroprotective effect of RA (Coelho et al., 2015; Khamse et al., 2015; Wang et al., 2012).

Wang et al. (2012) confirmed the results obtained by Ren et al. (2009) in a hydroxydopamine-induced Parkinson's disease model. In this study, the neuroprotective effect of RA against nigrostriatal neurodegeneration was demonstrated after RA intragastric administration for 21 days, with a reduction in iron levels in the substantia *nigra pars* and regulation of Bcl-2/Bax gene (apoptosis regulator) expression as a mechanism of action.

Regarding studies involving epilepsy, the neuroprotection of neuronal cells was also observed after RA intraperitoneal administration and intragastric administration for 7 days. The results of RA neuroprotective effect in these studies were mainly associated with the reduction of free radicals, DNA damage, and lipoperoxidation (Coelho et al., 2015; Khamse et al., 2015).

A recently developed study by Gok et al. (2018) evaluated the neuroprotective effect of RA intracerebroventricular injection over a period of 14 days in an *in vivo* model of Alzheimer's disease induced by bilateral injection of Aβ42 peptide. The data showed and reinforced the neuroprotective effect of RA due to effects on the antioxidant-oxidant imbalance and cholinergic impairment, thus increasing cholinergic tonus, attenuating lipid peroxidation, and potentiating antioxidant defense (Gok et al., 2018).

Regarding the anti-inflammatory action of RA, an *in vivo* study demonstrated the neuroprotective effect of intraperitoneal administration of RA for 5 days on memory loss in a model of ischemia, correlated to a reduction in astrogliosis and improved expression of neurotrophic factors (Fonteles et al., 2016). Another *in vivo* study involving bone marrow lesions evaluated the neuroprotective effect of RA (intragastric administration for 7 days) in a model induced by laminectomy (Shang et al., 2017). The results demonstrated the neuroprotective effect of RA, possibly due to the regulation of pro-inflammatory cytokines (IL-6, IL-1b, TNF-α, and MCP-1) and associated with a reduction in ROS and modulated Nrf-2 expression.

As described above, most *in vivo* studies involving RA neuroprotection have been performed using intragastric administration. However, there have been several pharmacokinetic studies involving administered orally RA in the literature, demonstrating its poor oral bioavailability (Baba et al., 2004; Konishi et al., 2005; Nakazawa and Ohsawa, 1998; Shang et al., 2017; Wang et al., 2017; Yang et al., 2017).

Table 3
In vitro and *in vivo* studies of rosmarinic acid (RA) neuroprotection found in the literature.

Treatment	Model	Main result	Neuroprotection mechanism	References
[RA] 14–56 μM (pre-treatment for 30 min)	*In vitro* model of H_2O_2-induced neurotoxicity in SH-SY5Y (human dopaminergic neuronal cell line)	RA protective effect against neurotoxicity induced by oxidative stress	Regulation of apoptotic process and molecule hemeoxygenase 1	Lee et al. (2008)
[RA] 1–100 $\mu M.L^{-1}$ (pre-treatment for 7 or 14 days)	*In vitro* model of oxidative stress, excitotoxicity and ischemia–reperfusion injury in SH-SY5Y and SK-N-BE (human neuroblastoma)	RA protective effect in all *in vitro* models of neuronal death tested	Prevention of oxidative stress, intracellular Ca^{2+} overload, and expression of the c-fos gene	Fallarini et al. (2009)
[RA] 0.01–0.1 $mM.L^{-1}$ (pre-treatment for 24 h)	*In vitro* model of 6-hydroxydopamine-induced neurotoxicity in MES23.5 (dopaminergic cell line)	RA protective effect against neurotoxicity induced by 6-hydroxydopamine	Reduction of mitochondrial dysfunction and ROS production	Ren et al. (2009)
[RA] 1 $nM.L^{-1}$ (pre-treatment for 30 min)	*In vitro* model of neurotoxin MPP + induced neurotoxicity in MES23.5 (dopaminergic cell line)	RA protective effect against neurotoxicity induced by neurotoxin MPP+	Improvement of mitochondrial dysfunction and reduction of apoptotic process	Du et al. (2010)
[RA] 83.3 $\mu M.L^{-1}$ (treatment with H_2O_2 and RA for 24 h)	*In vitro* model of H_2O_2-induced neurotoxicity in A172 (human astrocytes)	RA protective effect against neurotoxicity induced by oxidative stress	Reduction of accumulation of intracellular ROS	Costa et al. (2013)
[RA] 2.8–280 $\mu M.L^{-1}$ (pre-treatment for 1 h)	*In vitro* model of neurotoxicity in human primary neuronal cells induced by ciguatoxin	RA potential reduction in neurotoxicity induced by ciguatoxin	Reduction of neuronal excitability	Braidy et al. (2014)
[RA] 1–100 μM (pre-treatment for 12 h)	*In vitro* model of H_2O_2-induced neurotoxicity in N2A (mouse neuroblastoma)	RA neuroprotective effect against neurotoxicity induced by oxidative stress	Reduction of lactate dehydrogenase disruption, mitochondrial dysfunction and intracellular ROS	Ghaffari et al. (2014)
[RA] 0.5–5 mM (pre-treatment for 60 min)	*In vitro models* of antioxidant activity (ChEs, MAOs, COMT inhibition) and *in silico* approaches	RA showed *in vitro* multifunctional profile in targets related to neurodegeneration	Reduction in the levels of oxygen and nitrogen free radicals, and inhibition of lipid peroxidation. Inhibition of MAOs and COMT	Andrade et al. (2016)
[RA] 0.5–10 $\mu g.mL^{-1}$ (treatment with H_2O_2 for 24 h, after treatment with RA for 24 h)	*In vitro* model of H_2O_2-induced neurotoxicity in C6 glial cells	RA effect against neurotoxicity induced by oxidative stress	Reduction cell damage and lipid peroxidation through the regulation of mRNA and expression of iNOS and COX-2	Lee et al. (2016)
[RA] 20 $mg.kg^{-1}$ (intragastric - treatment for 21 days, 1 adm/day after Parkinson induction)	*In vivo* model of Parkinson's disease induced by 6-hydroxydopamine (Female Wistar rats weighing 200–220 g)	RA neuroprotective effect against degeneration of nigrostriatal dopaminergic system	Reduction of nigral levels of iron and regulation of the expression of Bcl-2/Bax gene.	Wang et al. (2012)
[RA] 1, 2 or 4 $mg.kg^{-1}$ (intraperitoneal - pre-treatment for 30 min, 1 adm)	*In vivo* model of epilepsy induced by pentylenetetrazole (Male CF1 mice weighing 30–40 g)	RA neuroprotective effect against oxidative and DNA damage	Reduction of free radicals and DNA damage	Coelho et al. (2015)
[RA] 10 $mg.kg^{-1}$ (intragastric - pre-treatment for 7 days, 1 adm/day)	*In vivo* model of temporal lobe epilepsy induced by kainic acid (Male Wistar rats weighing 250–300 g)	RA neuprotective effect preventing hippocampal neuronal loss	Reduction of free radicals and lipoperoxidation	Khamse et al. (2015)
[RA] 0,1, 1 ou 20 $mg.kg^{-1}$ (intraperitoneal - pre-treatment for 5 days, 1 adm/day)	*In vivo* model of memory deficits induced by permanent middle cerebral artery occlusion (Male Swiss mice weighing 25–30 g)	RA neuroprotective effect against memory deficits induced by cerebral ischemia	Reduction of astrogliosis and improvement of neurotrophic factors	Fonteles et al. (2016)
[RA] 20 $mg.kg^{-1}$ (intragastric - treatment for 7 days, 1 adm/day after spinal cord injury induction)	*In vivo* model of spinal cord injury induced by Laminectomy (Male Wistar rats weighing 250–275 g)	RA reverts effect of neurons apoptosis	Regulation of pro-inflammatory cytokines (IL-6, IL-1b, TNF-α, and MCP-1), reduction of ROS and modulation of expression of Nrf-2	Shang et al. (2017)
[RA] 50 $mg.kg^{-1}$ (intraperitoneal -treatment for 14 days, 1 adm/day after intracerebroventricular injection)	*In vivo* model of Alzheimer's disease induced by bilateral injection of Aβ42 peptide (Male Wistar rats weighing 250–300 g)	RA neuroprotective effect on antioxidant-oxidant imbalance and cholinergic damage	Increment cholinergic tone and suppression of oxidative stress via attenuating lipid peroxidation and potentiating antioxidant defence	Gok et al. (2018)

[RA]: rosmarinic acid concentration; Nrf-2: nuclear factor erythroid 2; δ-ALA-D: Delta-aminolevulinic acid dehydratase; SOD: superoxide dismutase; CAT: catalase; TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde AChE: acetylcholine; t-BHP: t-butyl hydroperoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TNFα: tumour necrosis factor α; CCR3: complement component 3; HaCaT: immortalized nontumorigenic human epidermal cell line; GSH: glutathione; ROS: reactive oxygen species; AST: aspartate aminotransferase; ALT: alanine aminotransferase; H_2O_2: hydrogen peroxide; MPP+: 1-methyl-4-phenylpyridinium ion; ChE: cholinesterase, MAO: monoamine oxidase; COMT: catechol-O-methyl transferase; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase-2; IL: interleukin; TNFα: tumour necrosis factor alpha; MCP-1: monocyte chemoattractant protein-1.

Among the factors described and related to poor RA bioavailability are its poor solubility in water and inefficient membrane permeability (Casanova et al., 2016; Medronho et al., 2014). RA, as an ionizable strong acid (pKa 2.9), has a pH-dependent distribution coefficient, which can affect its solubility in body fluids or its permeability (Danaf et al., 2016; Hu and Li, 2011; Yang et al., 2015). Furthermore, *in vitro* studies in Caco-2 cells have demonstrated the poor permeability of RA (Konishi and Kobayashi, 2005; Yang et al., 2015). Despite conflicting results, the stability of RA has also been reported as an important factor related to its poor bioavailability, mainly following oral administration, when passage through the gastrointestinal tract occurs (Bel-Rhlid et al., 2009; da Silva et al., 2014; Gayoso et al., 2016).

Considering its poor water solubility, inefficient permeability through biological barriers, high instability, and consequently low bioavailability, new technological approaches for the administration of RA have been studied. Some strategies involve RA association/incorporation in cyclodextrins (Aksamija et al., 2016; Çelik et al., 2011; Medronho et al., 2014), phospholipid complexes (Yang et al., 2015), solid lipid nanoparticles (Bhatt et al., 2014; Campos et al., 2014, 2015, Madureira et al., 2016b, 2016a, 2015), and chitosan micro/nanoparticles (Casanova et al., 2016; da Silva et al., 2016, 2014), most of them aiming to improve oral absorption, bioavailability, and therapeutic efficacy. Among the recently demonstrated approaches designed to circumvent these problems, the use of nanotechnology-based delivery systems has emerged as a promising strategy (Cho and Borgens, 2012).

3. RA-loaded nanotechnology-based delivery systems

Natural products have shown potential as therapeutic agents against various ND, although their performance may be less impressive partly due to their low bioavailability. In this sense, the use of nanotechnology-based delivery systems for natural products could overcome these limitations and increase therapeutic responses (Watkins et al., 2015). There are many studies demonstrating the benefits of encapsulation, such as increase in bioavailability (Bonifacio et al., 2014; Siddiqui and Sanna, 2016), targeting to specific tissues (Watkins et al., 2015), and controlled release (Kumari et al., 2012). Other benefits include the protection of volatile essential oils (Ansari et al., 2012), as well as reduction of cytotoxicity (Musthaba et al., 2009). The most commonly used nanostructures for drug delivery are polymer nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, liposomes, and nanoemulsions (Fig. 2).

According to the literature, RA nanoencapsulation may improve solubility, stability, permeability across biological membranes, and bioavailability, crucial for RA therapeutic potential in ND. Overall, the possibility of targeting opens a prerogative for targets such as hard-to-reach tissues, like the brain.

The first studies involving the use of nanotechnology aiming to increase the oral bioavailability of RA were developed by Campos et al. (2014) and Madureira et al. (2015) involving solid lipid nanoparticles (SLN). Campos et al. (2014) optimized RA-loaded SLN (0.15 mg/mL) using experimental design. A high association efficiency of RA (close to 99.8%) was noted for optimized formulations composed of 0.5% Witepsol® and 1–2% Tween® 80. Similar results were also observed when nanoparticles were composed of carnauba wax (Madureira et al., 2015). The optimum conditions that led to the most stable SLN was 1–1.5% carnauba wax and 2% Tween® 80. Comparing the physicochemical characteristics of RA SLN developed in both studies, the use of Witepsol® led to lower particle size diameters, despite having similar values of polydispersity index, zeta potential, and RA association efficiency. Following up these studies, Madureira et al. (2016a, 2016b) evaluated RA-loaded SLN in gastrointestinal conditions and observed that both SLN maintained RA antioxidant activity and presented a controlled release profile, with greater release under intestinal conditions; moreover, these authors demonstrated its safety through *in vitro* and *in vivo* studies (Madureira et al., 2016a, 2016b). In another study, the incorporation of a RA-rich plant extracts (*Salvia officinalis* or *Satureja mount*) in SLN composed of Witepsol® or carnauba wax was performed. RA incorporation (80%) in SLN ranging from 300 nm to 400 nm was reported for both extracts. In this study, the SLN from plant extracts were also tested under gastrointestinal conditions; the controlled release of RA was demonstrated for both formulations, although greater stability was observed for the Witepsol®-based SLN (Campos et al., 2015).

The incorporation of RA into nanotechnology-based delivery systems may bypass the inconvenience of the poor oral bioavailability of this phenolic acid. However, another barrier to the performance of RA is related to its poor availability in the CNS, which is significantly reduced in view of its limited ability to cross the blood-brain barrier (BBB), also described for other polyphenols (Kuo and Rajesh, 2017; Li et al., 2018; Szwajgier et al., 2017). The BBB is located at the level of the cerebral microvasculature and is critical for the maintenance of CNS homeostasis. Although it restricts the entry of potentially neurotoxic substances into the brain, it also represents an important obstacle for the delivery of therapeutic agents directly to the CNS, essential for neuroprotective therapies (Lochhead and Thorne, 2012; Mistry et al., 2009). Studies have demonstrated and confirmed that only a small amount of RA can enter in intact form into the brain. However, its neuroprotective effect might be associated with its metabolites, which may more easily cross the BBB and also have therapeutic potential in ND (Li et al., 2018). Other studies suggest that phenolic acids can accumulate at nanomolar or micromolar concentrations, and even at this low concentration may be pharmacologically relevant (Szwajgier et al., 2017). Nevertheless, to overcome this limitation and increase RA bioavailability and activity in CNS, alternative approaches have been reported in the literature.

Kuo and Rajesh (2017) developed RA polyacrylamide-chitosan-poly (lactide-co-glycolide) nanoparticles (close to 140 nm) conjugated with cross-reacting material 197 and apolipoprotein E, and demonstrated BBB crossing aiming at Alzheimer's disease treatment. This approach rises as an alternative to RA delivery to the CNS.

On the other hand, nasal administration has emerged in recent years as a route of interest for the administration of compounds for delivery to the CNS, i.e. through the olfactory bulb, thereby circumventing the BBB (Costantino et al., 2007; Grassin-Delyle et al., 2012; Mackay-Sim and St John, 2011; Mujawar et al., 2014; Nakazawa and Ohsawa, 1998; Paun et al., 2010). In Fig. 3, a schematic diagram demonstrates the possible mechanisms of drug absorption through the nose. The main advantages of this route are the rapid onset of the therapeutic effect due to the large surface area of the nasal mucosa, the absence of first-pass metabolism and non-invasiveness, and more pleasant experience for the patient (Costantino et al., 2007; Grassin-Delyle et al., 2012; Mujawar et al., 2014; Nakazawa and Ohsawa, 1998; Paun et al., 2010).

The first study involving the use of nanotechnology and the nasal route for RA delivery in ND was performed by Bhatt et al. (2014). In this study, RA-loaded SLN were developed aiming at the treatment of Huntington's disease. SLN composed of glyceryl monostearate stabilized by a mixture of surfactants (soy lecithin, Tween® 80 and hydrogenated soy phosphatidylcholine) were prepared by means of a hot homogenization technique. The procedure lead to monodisperse SLN (polydispersity index < 0.29) exhibiting a particle size close to 150 nm and an RA association efficiency higher than 60%. *In vivo* studies performed in a murine Huntington's disease model demonstrated the ability of RA-loaded SLN to attenuate motor and locomotor deficits as well as striatal oxidative stress. Pharmacokinetic studies comparing the nasal and intravenous routes for SLN administration were also performed by the same research group and demonstrated a higher effective brain concentration when RA was administrated through the nasal route (Bhatt et al., 2014).

However, the main limitation for nasal absorption of active compounds is mucociliary clearance. The presence of cilia, i.e. small

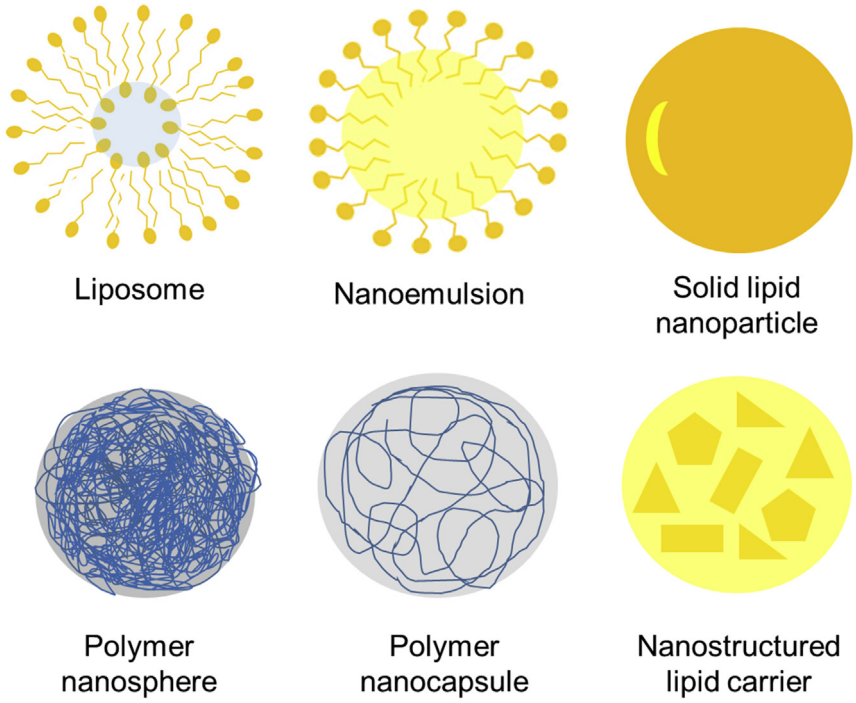

Fig. 2. Nanotechnology-based delivery systems for natural products.

projections on the surface of epithelial cells, promotes the movement of mucus to the nasopharynx, followed by the rapid removal and replacement of mucus (Grassin-Delyle et al., 2012; Ugwoke et al., 2005). Considering these limitations, some strategies have been assessed to improve the nasal absorption of drugs, such as the use of nanotechnology-based systems associated with mucoadhesive polymers to enhance drug penetration and increase residence time in the nasal mucosa (Casettari and Illum, 2014; Csaba et al., 2009; Eskandari et al., 2011; Kumar et al., 2008; Ong et al., 2014; Shinde et al., 2011; Ugwoke et al., 2005).

Our research group has been extensively studying lipid nanotechnology-based delivery systems, especially nanoemulsions, and has described the incorporation of active substances and water-insoluble plant extracts into these systems, aiming to increase their solubility and permeability through biological membranes (Argenta et al., 2014; Balestrin et al., 2016; Bruxel et al., 2012; Kelmann et al., 2008; Martini et al., 2007). However, due to the low viscosity of nanoemulsions, the association with mucoadhesive systems has been proposed to increase the direct absorption of natural compounds in the CNS following nasal delivery. Chitosan has been explored due to its interesting biological and physicochemical properties. It is a cationic polysaccharide derived from chitin with mucoadhesive properties related to the electrostatic interaction between the polymer and the sialic groups of mucin (Casettari and Illum, 2014; Fachel et al., 2018; Prego et al., 2006, 2005).

In this context, we recently developed and optimized chitosan-coated nanoemulsions (CNE) for RA nasal delivery, intended to be used as a potential neuroprotective therapy (Fachel et al., 2018). CNE

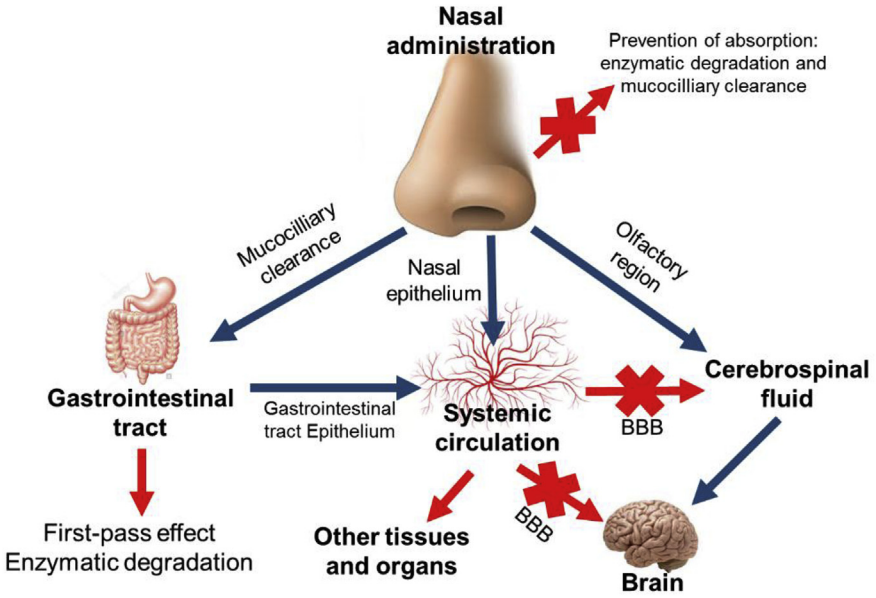

Fig. 3. Schematic diagram demonstrating the mechanisms of drug absorption through the nose.

containing RA (1 mg .mL^{-1}) with a mean size of 258.01 ± 3.82 nm, a polydispersity index of 0.272 ± 0.01, zeta potential of 44.98 ± 1.75, and an association efficiency over 89% was achieved with medium chain triglycerides and egg lecithin (lipid phase) and obtained through an oil-in-water spontaneous emulsification procedure and further coating with chitosan. In addition, RA CNE presented *in vitro* mucoadhesive potential, extended drug release, and long-lasting permeation with greater retention at the porcine nasal mucosa, associated with a good safety profile determined using the MRC-5 cell line (normal human lung fibroblasts). These findings show that this system may be a suitable carrier for RA nasal delivery for neuroprotective therapies.

4. Conclusions

RA presents a variety of biological important activities, especially anti-inflammatory and antioxidant, through the prevention of oxidative neuronal damage or the modulation of pro-inflammatory cytokines and other signalling molecules. As many ND are related to the excessive production of ROS, inflammatory processes, and neurodegenerative damage, RA plays a role in the prevention of neurodegeneration or even in damage reduction. However, the poor oral bioavailability of RA represents a limitation in terms of pharmacodynamic outcomes after administration. In this sense, the formulation of RA encapsulated or complexed with nanotechnology-based delivery systems could enhance its solubility, protect it from degradation, allow it access to difficult-to reach tissues, and consequently improve its bioavailability. These formulations could be polymer or lipid-based, as long as the development is optimized to load a high but safe amount of RA. Although this formulation could circumvent some important limitations, nasal delivery could also be a pleasant route for CNS delivery, as it provides direct access to the CNS. Besides the advantages, there is a lack of studies demonstrating these combined approaches, which could produce such interesting results. In conclusion, considering the neuroprotective potential of RA discussed here, the use of nanotechnology-based therapy associated with the nasal route could represent a novel approach to ND treatment.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2018.11.003.

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