Rosmarinic Acid–Human Pharmacokinetics and Health Benefits

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ABSTRACT

Rosmarinic acid is a phenolic compound commonly found in the Lamiaceae (Labiateae) plant species. It is considered responsible for a wide spectrum of biological and pharmacological activities of plants containing this compound. The aim of the current review is to present the fate of rosmarinic acid inside the human body, explained through pharmacokinetic steps and to briefly present the health benefits of RA. Pharmacokinetics was at first studied in animal models, but several studies were conducted in humans as well. This compound can be applied topically, pulmonary, intranasally, and *via* intravenous infusion. However, peroral application is the main route of entry into the human body. Presumably, it is mainly metabolized by the gut microflora, providing simple, more easily absorbed phenolic units. Inside the body, the rosmarinic acid molecule undergoes structural changes, as well as conjugation reactions. Renal excretion represents the main path of elimination. Previously conducted studies reported no serious adverse effects of herbal remedies containing RA, as well as their positive effects on human health. In addition to *in vitro* studies, clinical investigations suggested its benefits in dermatological, allergic, and osteoarthritic disorders, as well as for improving cognitive performance and in metabolic syndrome treatment. Future studies should investigate the kinetics during long-term application in patients who would have potential benefits from RA usage. Pharmaceutical formulations designed to prevent the fast metabolism of RA and allow its penetration into other compartments of the human body are also interesting topics for future research.

Introduction

Rosmarinic acid (RA) is a compound that can be found in various plants. Originally isolated and named after rosemary (*Rosmarinus officinalis*, Lamiaceae), RA is also found in numerous other plants classified in the subfamily Nepetoideae, family Lamiaceae, such as sage, mint, thyme, lemon balm, basil, oregano, and others [1, 2]. The presence of RA is not reserved solely for Lamiaceae, as it can be found in other plants, primarily in the representatives of the Boraginaceae family but also in many others (e.g., Apiaceae, Araliaceae, Cucurbitaceae, Rubiaceae, Plantaginaceae [1], Polygonaceae [3], as well as in some hornworts, ferns and sea grasses [1, 2]).

The compound represents a phenolic secondary biomolecule, an ester of caffeic acid and (R)-(+)-3-(3,4-dihydroxyphenyl)lactic acid originating from amino acids L-phenylalanine and L-tyrosine, respectively (▶ **Fig. 1**) [4].

Although the structure of the compound was not known until 1958, early researchers suggested the presence of a compound responsible for the beneficial effects of some herbs [2].The aim of this review is to represent the fate of RA in the human body, explained through the pharmacokinetic steps, and to briefly present some of the health benefits of RA.

RA in Herbal Material

Chemical profiling studies often detect RA in extracts obtained from various plants. The mediums used for obtaining these extracts vary widely, although it appears that alcoholic extracts are the most frequent; extraction is usually performed by cold macer-

ation or heat-assisted techniques, while the extraction time ranges from 10 min to 30 days. Further analysis of the extracts is commonly carried out by the means of HPLC coupled with various detectors [5,6]. Researchers found that the content of RA varies between the species of the same genus, subspecies of the same species, samples collected in different seasons of the year, etc. [5]. However, the amount of RA can be used as a parameter for estimating herbal drug quality (e.g., lemon balm [*Melissa officinalis,* Lamiaceae] and rosemary leaves should contain ≥ 4% and ≥ 3% of total hydroxycinnamic acids expressed as RA, respectively) [7, 8]. **Table 1S** in Supporting information presents data on medicinal plants reported to contain RA. As the data suggests, the majority of the reported medicinal plants (especially those of the Nepetoideae subfamily of Lamiaceae family) represent a valid herbal source of this compound. However, no guarantee of high amount of RA can be given, as the reported amount of RA varied widely in some of the investigated species, and in some cases, even the absence of RA was reported. Thus, the first step in investigating herbal material containing RA should be the precise quantification of the compound before any type of *in vitro* or clinical investigation is conducted.

Routes of Administration

As previously stated, RA is a compound that can be found in some of the most frequently used spices and aromatic representatives of the Lamiaceae family; thus, the peroral intake of RA is of special interest. These plants can be ingested in dried, often pulverized form that is added to food or used as spices. Aside from this, many medicinal plants of this kind are consumed as tea infusions [9, 10]. This compound can also be applied topically, which is consistent with the traditional use of lemon balm as a topical antiviral agent [7, 11, 12]. The application of proper pharmaceutical formulations intended to provide local effects could find its place in the treatment of various dermatological conditions. Some of them have already been attempted. A niosomal gel with 3% RA was tested in a simulated acne study performed on mice. Potent antibacterial and anti-inflammatory effects were demonstrated through the reduction of bacterial (*Propionibacterium acnes)* multiplication rate and inflammation rate. Additionally, the niosomal gel was demonstrated to be more effective than RA in the forms of solution or plain gel [13]. It is also suggested that topical application could find its place in the prevention of skin aging [14, 15], as *ex vivo* studies of ethosomes and liposomes containing RA showed an inhibitory effect against elastase and collagenase, the enzymes responsible for the loss of skin elasticity [15]. Intranasal application of solid lipid nanoparticles containing a 12 mg dose of pure RA gave promising results in rats as a noninvasive route of administration for potential targeted treatment of central nervous system diseases such as Huntington's disease [16]. Another study in rats demonstrated the possibility of a pulmonary application. An extract of Danshen (biological source *Salvia miltiorrhiza*), traditional Chinese medicine, containing RA was applied using microspray, and it was demonstrated that this type of application resulted in rapid absorption. Pulmonary application is of great significance in various diseases affecting the respiratory system, especially in conditions that require an immediate drug effect; thus, the possi-
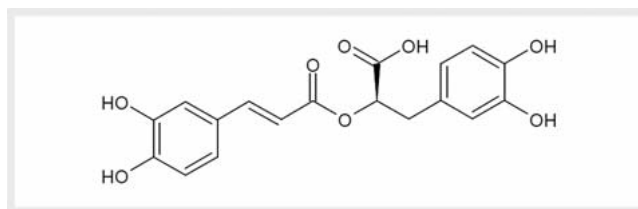
▶ **Fig. 1** Structure of rosmarinic acid.

bility of RA application in such a manner requires further investigations [17]. Ocular application of the drug is of importance in various eye diseases. An *in vitro* test performed on cell lines of the human retina and human cornea showed that the application of chitosan-based nanoparticles containing RA or organic-cultivated sage (*Salvia officinalis*) and savory (*Satureja montana*) extracts rich in RA exhibits promising potential and requires further research [18]. Another research study investigated an intravenous application of compounds isolated from Danshen-RA, lithospermic acid and lithospermic acid B. A mixture of these compounds was dissolved in a hypertonic solution of glucose and applied in the form of slow intravenous infusion. The study confirmed that this type of application is possible, effective, and safe, which is consistent with its use in traditional Chinese medicine for certain cardiac and pulmonary events [19].

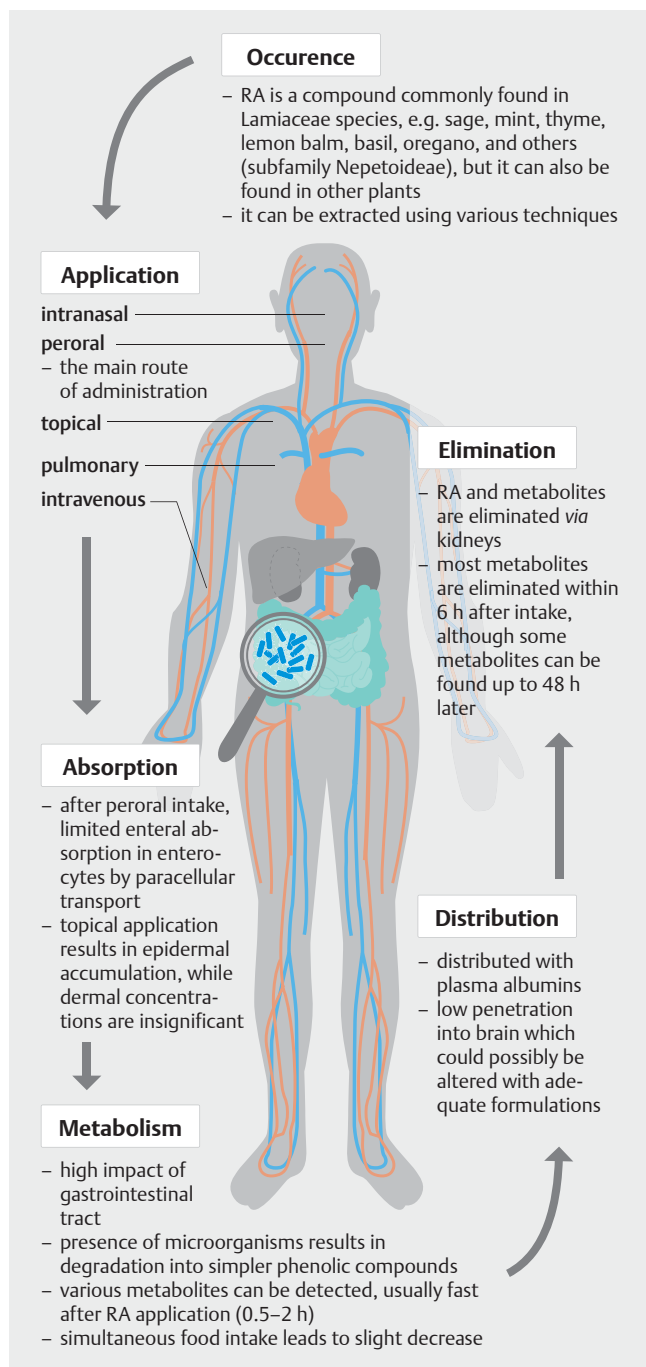## Investigation of RA's Pharmacokinetics

The initial investigations of RA's pharmacokinetics were conducted on animal models, primarily in rats and mice. These data gave starting points for human studies, such as for the main metabolites, pharmacokinetic parameters, and routes of elimination, although it was later discovered that the pharmacokinetics of RA in animals and humans is somewhat different [20]. The data given in further text originate from human studies, unless otherwise stated. The scheme of human RA pharmacokinetics is presented in ▶ **Fig. 2**, and the explanation of the specific segments is provided in further text.

Absorption

Lipinski's rule ("the rule of 5") is often used to estimate whether a compound is "drug-like" and a potential candidate for peroral application [21]. The *in silico* calculated values of the parameters determined according to this rule are given in ▶ **Table 1** [22].

Since the compound does not violate a single rule, promising bioavailability is expected after peroral application. According to these *in silico* calculations, the absorption of RA is 59.14% [22]. However, *in vitro*, as well as *in vivo* investigations of RA absorption, suggest that its intestinal permeability is much lower, approximately 1% of the applied dose [23, 24], and that the absorption of the compound occurs *via* enterocytes by paracellular transport in tight junctions [25].

Absorption through the skin is also investigated as RA-rich extracts can be found in topical formulations. An *ex vivo* study conducted on intact human skin has shown that RA accumulates in

Occurence

– RA is a compound commonly found in Lamiaceae species, e.g. sage, mint, thyme, lemon balm, basil, oregano, and others (subfamily Nepetoideae), but it can also be found in other plants
– it can be extracted using various techniques

Application

intranasal
peroral
– the main route of administration
topical
pulmonary
intravenous

Elimination

– RA and metabolites are eliminated *via* kidneys
– most metabolites are eliminated within 6 h after intake, although some metabolites can be found up to 48 h later

Absorption

– after peroral intake, limited enteral absorption in enterocytes by paracellular transport
– topical application results in epidermal accumulation, while dermal concentrations are insignificant

Distribution

– distributed with plasma albumins
– low penetration into brain which could possibly be altered with adequate formulations

Metabolism

– high impact of gastrointestinal tract
– presence of microorganisms results in degradation into simpler phenolic compounds
– various metabolites can be detected, usually fast after RA application (0.5–2 h)
– simultaneous food intake leads to slight decrease

▶ **Fig. 2** A scheme of human pharmacokinetics of rosmarinic acid. Source: Christine Lackner

the epidermis, while the penetration into the hydrophilic dermis can practically be disregarded. Higher amounts of RA were detected when olive oil was used in the formulation, possibly due to the presence of natural penetration enhancers [26].

▶ **Table 1** The rule of Lipinski and values calculated for rosmarinic acid.

Lipinski's rule	Calculation for rosmarinic acid
molecular weight ≤ 500 Daltons	360.32
log P (logarithm of octanol-water partition coefficient) ≤ 5	1.626
hydrogen bond donors ≤ 5	5
hydrogen bond acceptors ≤ 10	8
number of violations for rosmarinic acid	0

Distribution

Since RA is relatively insoluble in water [27], it can be expected that the compound is distributed throughout the circulatory system by plasma proteins. An *in vitro* study of the interaction between RA and the human serum albumin revealed that RA is probably carried by these proteins. A moderately strong bond is formed through hydrophobic interactions between these proteins and RA [28, 29]. Studies conducted on human volunteers lack data on the body distribution of RA; however, some data from animal studies are available. The topical application of RA in rats resulted in distribution into blood, skin, bones, and muscles; after intravenous application, the compound was detected also in soft tissues of internal organs such as the spleen, liver, lungs, and heart [20]. Similarly, after intraperitoneal application in mice, the highest amount of RA was found in the kidneys, followed by the lungs, spleen, and liver [30]. Another interesting aspect of RA's distribution is the concentration achieved in the brain. The data suggest that the hematoencephalic barrier effectively prevents penetration into the central nervous system [23, 30]. As previously suggested, a targeted approach (intranasally) using a specific pharmaceutical formulation results not only in passing the blood-brain barrier and achieving higher concentrations in the brain but also in reducing the amount of RA that is distributed to be metabolized [16]. Some of the concentrations/amount of RA detected in animal tissues are presented in ▶ **Table 2**.

Metabolism

Impact of the gastrointestinal tract

The environment of the human gastrointestinal tract (GIT) can greatly affect the content of ingested phenolic compounds [25]. Throughout the GIT, pH values vary greatly. The highly acidic gastric environment can lead up to ≥ 50 % reduction of RA's concentration; in contrast, alkaline intestinal digestion was demonstrated to have a lower impact. Pure RA was found to be relatively stable in various pH mediums, while the extracts containing RA showed a reduction of RA content [31, 32], up to 99 % in the case of thyme [31]. An *in vitro* study comparing 2 formulations, divided powder and hard gelatine capsules, demonstrated that capsules should be the preferred form of intake for oregano (*Origanum vul-*

▶ **Table 2** Amount/concentration of rosmarinic acid (RA) detected in organs of laboratory rats after compound application.

Animal model	Route of administration	Dose of RA	Detected amount/concentration in some of organs	Reference
rat	intranasal	12 mg (in a form of solid lipid nanoparticles)	brain: 5.69 µg	[16]
	intravenous	Extract of *Salvia milthiorrhiza* containing RA was applied in dose of 56 mg/kg (concentration of RA in extract was not determined).	heart: 149.91 ng/mL liver: 175.61 ng/mL spleen: 68.46 ng/mL lung: 198.04 ng/mL kidney: 9353.9 ng/mL brain: 5.12 ng/mL	[70]
	intravenous	Extract of *Salvia milthiorrhiza* containing ≥ 10.1 % RA was applied in dose of 60 mg/kg.	heart: 1.08 µg/g liver: 0.29 µg/g spleen: 0.07 µg/g lung: 3 µg/g kidney: 43.65 µg/g brain: 0.06 µg/g	[71]

gare), as they protect the present RA from the process of gastro-intestinal digestion [33].

The gut microbiota represents an important organ with a significant role in the metabolism of nutrients, drugs, and other xenobiotics [34]. In general, a major part (up to 95%) of the ingested complex phenolic compounds cannot be absorbed in the intestine. However, these compounds are further metabolised by the intestinal microflora [20, 25]. In most of the cases, complex phenolic compounds are at first degraded into simpler units (e.g., phenolic acids), in order to be absorbed in the digestive tract [35]. Similarly, the degradation of RA into 2 phenolic acids would allow their further absorption [20, 36], and it was demonstrated *in vitro* in Caco-2 cell culture that microbial esterases were responsible for cleaving the bond of the RA molecule rather than the esterase present in the mucosa of the intestinal tract [36].

Some of the studies investigated the effects of gut microbes on the RA content. In one study, an *in vitro* process of colonic fermentation of a rosemary extract containing RA by using microorganisms from rat feces was performed. It was demonstrated that 14% of the RA content is lost through this process [32]. In contrast, another study stated that an even higher percentage of RA (> 90%) can be hydrolyzed *in vitro* by probiotic *Lactobacillus* strains in optimal conditions [37]. Finally, a study using human fecal samples for *in vitro* fermentation of phenolic compounds present in thyme (*Thymus vulgaris*) was performed. A fast decline in RA concentration had occurred during the first 6 h of fermentation, and it was reduced to trace levels after 12 h. The same research also tested the amount of RA present in feces of human volunteers consuming thyme-enriched olive oil over a period of 3 wk. This analysis showed no RA presence, thus confirming a high level of degradation in the gastrointestinal tract [38].

Metabolic pathways

Studies conducted in rats provided starting data about metabolites that are likely to appear in plasma and urine [39–41]. Later research has shown that metabolites detected in human subjects are predominantly the same, although the main metabolic pathways are quantitatively somewhat different. Some of the *in vitro* obtained data in Caco-2 and Hep G2 cell cultures, as models of the small intestine and liver respectively, demonstrated that rosemary extracts containing RA, as well as the pure compound, can be partially absorbed and metabolized in the previously mentioned cells [42]. The metabolites detected in this study [42], as well as in the study testing the metabolism of RA in human liver microsomes [43], pointed out the most probable metabolites of RA, which were also confirmed in studies on human volunteers. However, the metabolites are most likely the result of both microbial and hepato-intestinal enzymatic reactions.

Shortly after peroral or intravenous application, RA is detected in blood in its free form (▶ **Table 3**).

As seen in ▶ **Table 2**, the concurrent consumption of food and RA leads to a slight decrease of RA concentration in plasma and prolongs the time necessary for achieving the maximum concentration. The compound can also be detected in the form of a sulphate, glucuronide, and/or sulphoglucuronide [44]. An *in vitro* study in human liver microsomes suggested that glucuronidation of RA is the main metabolic pathway [43]. When comparing the 2 doses (250 and 500 mg of RA), it was evident that an increase of dosage was followed by an increase of free RA concentration, while the concentration of conjugated forms increased insignificantly. All of this suggests that conjugational processes are characterized by saturation kinetics [45].

Simultaneously with the previously mentioned processes, a conjugation reaction of RA methoxylation also occurs, so it is possible to detect methyl conjugate (met-RA) in blood. This metabolic reaction is slower, so this compound's maximum concentration is achieved 2 h after ingestion (0.65 ± 0.07 µmol/L, after a dose of 200 mg of RA) [44]. Interestingly, a study following a 30-day intake of spearmint (*Mentha spicata*) extract rich in RA detected met-RA glucuronide as the main marker metabolite of RA's subchronic consumption [46].

The metabolism of the caffeic acid segment of RA structure was further studied, revealing several metabolites resulting from various reactions. Ferulic acid was detected in blood [45]. This

► **Table 3** Concentrations of rosmarinic acid, determined in human studies.

The route of application	Number of participants	Dose of RA	Maximum of detected RA concentration (C_{max}) in plasma	Time of C_{max} determination (T_{max})	Reference
Peroral	6 male healthy volunteers	200 mg	1.15 ± 0.28 µmol/L	0.5 h	[44]
	6 healthy volunteers	250 mg (fasted)	72.22 ± 12.01 nmol/L	1 h	[45]
		500 mg (fasted)	162.20 ± 40.20 nmol/L	1 h	[45]
		500 mg (fed)	142.20 ± 45.20 nmol/L	3 h	[45]
Intravenous	6 male and 6 female healthy volunteers	≥ 4.5 % RA in 100 mg of mixture[a]	174 ± 61 ng/mL	0.47 h	[19]
		≥ 4.5 % RA in 200 mg of mixture[a]	308 ± 77 ng/mL	0.54 h	[19]

[a] Intravenously applied mixture contained ≥ 87.7 % of lithospermic acid B, ≥ 4.5 % of rosmarinic acid and ≥ 0.9 % lithospermic acid

compound is a product of RA catabolism *via* caffeic acid, while further sulphatation and methoxylation of the hydroxyl group may occur [44]. Similar to the conjugation of RA, this reaction is fast (detection 0.5 h after ingestion of 200 mg of RA showed 0.36 ± 0.17 µmol/L) [45]. Another catabolic product was detected in human plasma: 2,4,5-trimetoxycinnamic acid. This compound is also metabolized through caffeic acid from RA, but it undergoes reactions of hydroxylation, dihydroxylation, and methoxylation [40]. Also, the metabolism of the caffeic acid segment in human subjects can lead to the formation of *m*-coumaric acid and its conjugates [44]. An *in vitro* study also presumed that 2-(3′,4′-dihydroxyphenyl)propionic acid is an important intermediary compound [38]. Specifically, the first metabolic pathway leads to the formation of phenylacetic acid (as detected in an *in vitro* study of RA fermentation with human fecal samples) [38], while the second leads to the formation of protocatechuic acid (as detected in human subjects consuming *Origanum onites* extract containing RA) [47]. The latter compound can also be further metabolized into 2 compounds, *p*-hydroxybenzoic acid or vanillic acid (and its sulphate conjugate) [46,47].

The complete metabolism of RA is presented in ► **Fig. 3**.

Elimination

Initial investigations conducted in rats had detected an increased concentration of RA in kidneys, suggesting renal elimination; some metabolites were also found in the rats' bile [20,48]. After peroral application in humans, free and conjugated forms of RA and met-RA were detected in urine of healthy volunteers. Caffeic, ferulic, 2,4,5-trimetoxycinnamic acid, *m*-coumaric acid, vanillic and *p*-hydroxybenzoic acid, all in free and conjugated forms were also found, but in lower concentrations [40,44,47]. In the same manner as the metabolism, the elimination is also rapid. Specifically, the majority of the metabolites are eliminated within 6 h after intake, while it is rather hard to detect traces of other metabolites after 24–48 h [44]. The previously mentioned research describing intravenous application is also consistent with this data. Namely, a steady state is achieved with continuous infusion, but after the infusion termination, a fast decrease of the concentra-

tions of RA and its metabolites in blood and their increase in urine is recorded [19].

Health Benefits and Efficacy

Numerous *in vitro* studies were conducted in order to show the beneficial effects of RA. Some of its confirmed activities include antioxidative, antibacterial (against both gram-positive and gram-negative bacteria), antiviral, astringent, analgesic, anti-inflammatory (mediated by the inhibition of lipoxygenase, cyclooxygenase, the complementary cascade and pro-inflammatory mediators), antihyperglycemic (mediated by the pancreatic amylase and glucosidase inhibition and by sensitizing glucose transporters), hepatoprotective (mediated by the inhibition of fibrosis, and scavenging of different reactive radical species), immunomodulatory (mediated by the stimulation of the cell immune system), anticancer (against various types of carcinoma, including colorectal, pancreatic, breast, lung, ovarian, melanoma, and several others), cardioprotective, neuroprotective properties, and many others [5,20,49–52]. Most of these studies are performed with a pure compound or with extracts containing RA. As previously pointed out, the quantification of RA applied in the studies is of great importance in order to evaluate the validity of the applied preparation. However, bearing in mind the previously presented pharmacokinetics of the compound (especially its limited peroral absorption, fast metabolism, and limited distribution to the central nervous system), large differences between the results obtained *in vitro* and the potential *in vivo* effects can be expected. This is primarily due to the presumed discrepancies between the tested concentrations in laboratory/preclinical settings and the presumably highly reduced concentration that would be detected at the site of the pharmacological effect.

Apart from preclinical investigations, several clinical studies tested RA-containing extracts and products in patients with different medical conditions in order to evaluate the "real" health benefits of RA. One study demonstrated positive co-therapeutic effects of spearmint tea containing a high percentage of RA in patients with knee osteoarthritis. The patients were consuming approximately 280 mg of RA daily during 16 wk, in the form of a tea
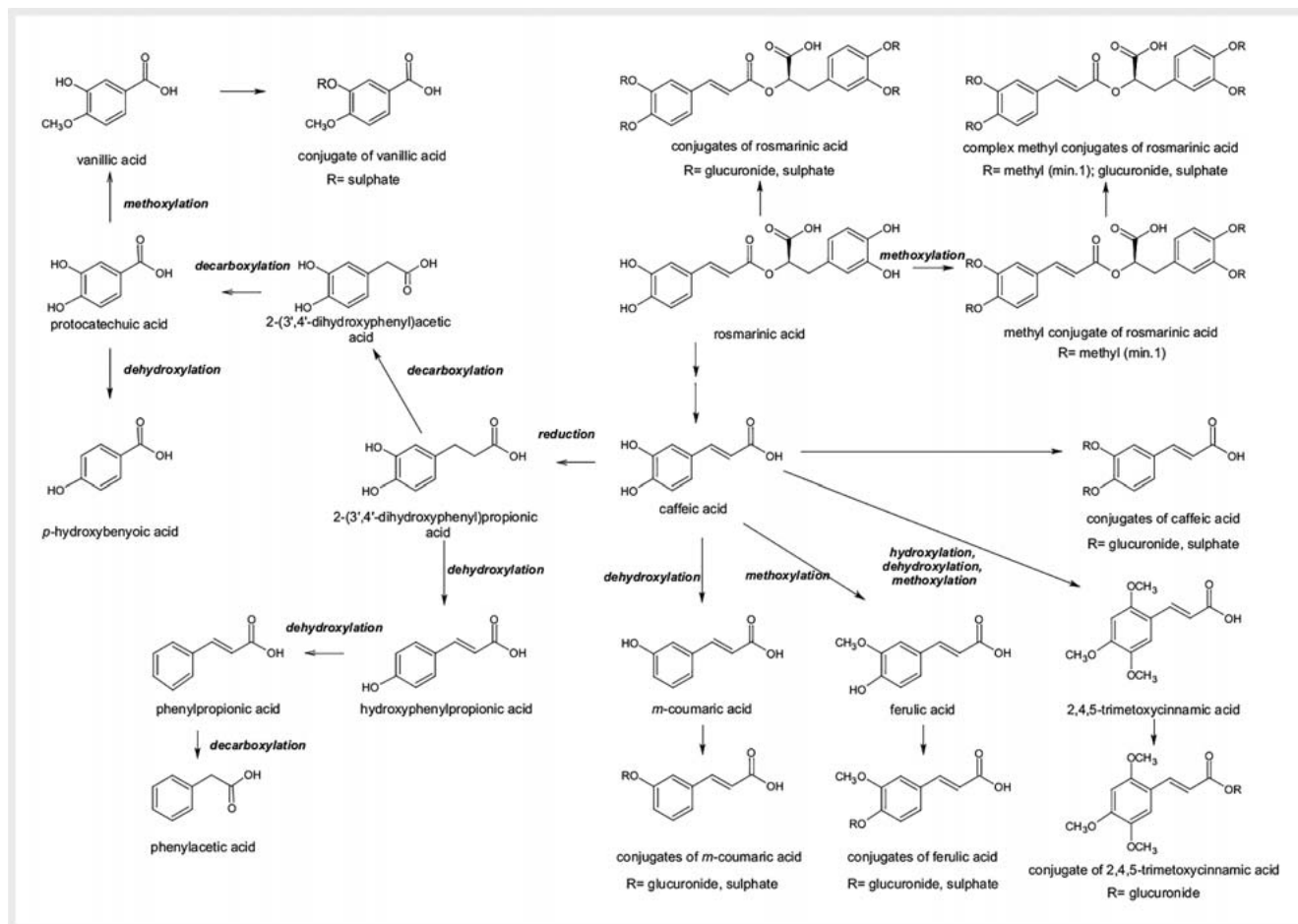
▶ **Fig. 3** Presumed metabolism of rosmarinic acid, based on metabolites detected in human studies.

beverage. The positive effect was primarily noted through pain reduction, as the patients significantly reduced the number of analgesics used for the symptomatic treatment of the underlying condition [53]. Another study using a spearmint extract with a high content of RA (900 mg of extract with ≥14.5% of RA, during 90 days) tested this herbal preparation as a potential nootropic herbal remedy, concluding that it effectively improves reactive agility in young, healthy individuals, which is potentially important in athletic performances requiring stationary and multi-directional activity [54]. The same herbal preparation of RA in 2 doses (600 mg or 900 mg) applied to the older population over the course of 90 days was shown to improve their cognitive performance (often affected by the aging process), as well as lead to sleep induction and general mood improvement, especially when applied in a higher dosage [55]. Another study tested 900 mg of spearmint extract containing 15% of RA during 30 days, also in an older population. The results suggested that cognitive performance, including attention, concentration, and planning was improved due to the RA supplementation [46]. Improved cognitive performance (better working memory results) and the anti-stress (anxiolytic) effects of RA-rich food (an ice-tea type of beverage and yoghurt containing 0.3 g or 0.6 g of lemon balm extract with >6% of RA) were confirmed in the young population [56]. A

cream containing 0.3% of RA was used in a 2-mo study in patients with moderate atopic dermatitis; it was shown that the RA cream successfully reduced several problems, including erythema, crusting, edema, and local pruritus [57]. Another beneficial effect is recorded in application in patients suffering from seasonal allergic rhinoconjunctivitis. The application of 200 mg of RA during 21 days resulted in a decrease of symptoms (itchy nose and eyes and watery eyes). The reduced number of neutrophils and eosinophils in nasal lavage fluid suggests that RA reduced inflammation mediated by these cells, presumably through the inhibition of IgE production [58, 59]. Some studies suggested a potential role of RA-containing extracts as co-therapeutics in metabolic syndrome therapy [60]. Lemon balm extract showed positive effects primarily expressed through hypolipemic activity. Namely, a reduction of serum triglycerides [61–63], cholesterol [61, 63], and biomarkers of oxidative stress [63], as well as an increase of high-density lipoproteins [63, 64] were recorded in patients with diabetes type 2 [62, 64], in patients with chronic stable angina [63], and in workers professionally exposed to agents triggering high levels of oxidative stress products [61]. All of the data suggest that potent *in vitro* antioxidant activity appears to be effective *in vivo*. Furthermore, some data from animal studies also point out that hypolipidemic activity is mediated through an increased synthesis of bile

acids, increased oxidation of fatty acids, decreased synthesis of new fatty acids, and decreased synthesis of cholesterol [65]. All of these studies suggest that RA and herbal extracts containing it possess high and diverse potential for application in various groups of patients, and that RA could find its place as an adjuvant to certain conventional drugs.

Safety

The previously mentioned studies examining human pharmacokinetics of RA were conducted using a single dose application in a small number of healthy volunteers [19, 44, 45]. At the same time, the safety of RA was investigated through physical examination, the appearance of adverse effects, the evaluation of biochemical blood parameters (blood count, biochemical parameters of liver and kidney function) [19, 45], and cardiac function (electrocardiogram) [19]. No side effects of single dose application in humans were detected.

Other studies investigating the efficacy and health benefits of herbal extracts containing RA in general reported a lack of serious adverse effects [54–56, 58, 59]. Some of the recorded complaints in groups that had received RA included headaches, constipation, diarrhea [53], worsening of cystic acne, and an oily scalp [55], etc. However, it is important to point out that some complaints were also recorded in placebo groups, including dry mouth, itchy skin, and denture staining [53]. A study that tested a 2-mo application of an RA-containing cream reported no local adverse effects on the skin affected by atopic dermatitis [57].

Future Studies and Considerations

Current data lack information on several aspects of RA applications. As previously presented, available pharmacokinetic studies were conducted in a limited number of volunteers, and evaluated single-dose applications. The presented clinical trials suggest an effectiveness of RA-containing preparations after consumption over longer periods of time. Therefore, pharmacokinetic studies after chronic application of this secondary metabolite would possibly contribute to better understanding of metabolic pathways and the pharmacological potential and could explain the mechanism of activity. While one study gave some data on sub-chronic consumption of RA (30 days) [46], it can be expected that positive pharmacological effects would require a longer period of time. Establishing the longest safety period of RA's usage is also of interest.

Applied doses varied greatly in studies related to the application of RA-based preparations. From a potential therapeutic point of view, it would be useful to determine the adequate dose and dosage regimen for various conditions being treated. The extrapolation of data obtained in preclinical studies performed on rodents allowed the calculation of the upper tolerable daily intake of RA in humans of 500 mg [45]. Another aspect of interest would be to determine C_{trough}–the lowest concentration that a drug reaches before the next dose is administered–as only the adequate values of C_{trough} over a certain period of time will result in beneficial effects.

Secondly, current data suggest that modified pharmaceutical formulations are required for RA application. As peroral application results in limited absorption, fast metabolism, and fast elimination, it would be necessary to rationalize a formulation that would overcome these limiting factors. An adequate formulation is also needed in order to protect RA from being degraded by the gastrointestinal tract. This could possibly be achieved by using solid lipid nanoparticles [66, 67] or liposomes [68]. Other routes of administration would also require formulations improving delivery to targeted locations (e.g., brain or eye), where RA would be of potential benefit in patients [16, 18]. Previous studies demonstrated the importance of a vehicle used for topical drug formulation since inadequately chosen excipients could decrease the RA release from formulations [15, 26].

Finally, in order to determine the true benefits of RA use, studies should be performed in patients with different diseases and conditions, primarily with prominent pro-oxidant and pro-inflammatory mediators. The mentioned studies that confirmed the efficacy and health benefits reported positive pharmacological effects in patients primarily associated with antioxidant, antiallergic, and hypolipidemic activities [61, 63, 64, 69]. However, further studies are required in order to elucidate the potential mechanism(s) of these recorded activities.

Conclusion

RA is a compound with promising potential for further clinical studies. Additional data on its pharmacokinetics are necessary in order to elucidate its position in the prevention and co-therapy of various pathological conditions. Fast processes of its metabolism, distribution, and elimination, as well as its limited peroral absorption suggest that there is a need for adequate pharmaceutical formulation development that would allow targeted and extended activity.

Methodology

Bearing in mind that RA is a relatively well-known compound of natural origin, this review focused on the less investigated aspects of its pharmacokinetics in the human body and clinical effectiveness. The comprehensive search for relevant scientific articles was conducted through PubMed, Scopus, ScienceDirect, and Google Scholar. The search was conducted using a combination of various key words, including "rosmarinic acid", "Lamiaceae", "content", "pharmacokinetics", "application", "administration", "absorption", "distribution", "metabolism", "microbial", "fermentation", "elimination", "health benefit", "efficacy", "safety", "clinical trial", "human", etc. The retrieved articles were inspected for their content, and focus was given to research articles dealing with studies performed on human subjects. Pharmacokinetic studies on laboratory animals were originally excluded in case a certain topic lacked human data (e.g., as was the case with the Distribution section), data were supplemented from studies conducted on animals. Additionally, references of valid articles were inspected in order to retrieve additional articles. Only articles written in English were included. The search was performed from January to the end

of July 2020. No limitations were given for years of published articles used for this review.

Supporting Information

Table 1S contains data on medicinal herbs referred by European Medicines Agency to contain rosmarinic acid, and the data on content of the compound in extracts of these herbs, obtained by various procedures of extraction.

Contributors' Statement

Data collection: M. Hitl, N. Kladar, B. Božin. Design of the paper: M. Hitl, N. Gavarić, B. Božin. Statistical analysis: not applicable. Analysis and interpretation of the data: M. Hitl, N. Kladar, N. Gavarić. Drafting the manuscript: M. Hitl, N. Kladar, N. Gavarić, B. Božin. Critical revision of the manuscript: N. Kladar, N. Gavarić, B. Božin.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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