BIOACTIVITY AND POTENTIAL HEALTH BENEFITS OF ROSMARINIC ACID

Minireview

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Abstract

Substantial attention has been given to primary cancer prevention in daily life. Secondary metabolites of plants in the form of functional foods or nutraceuticals are known as preventive agents for a variety of diseases, including cancer. Rosmarinic acid (RA) is a naturally occurring polyphenolic compound, which contributes to the beneficial and health-promoting effects of herbs, spices and medicinal plants. There is a growing interest in the elucidation of the biological roles of this compound. Presented minireview summarizes the selected biological activities of RA with attention to its antitumor and chemopreventive activity. RA has been shown to act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of tumor cell differentiation. RA effectively inhibits angiogenesis, invasion of tumor cells and metastasis. With regard to toxicity, no obvious side effects of RA have been observed in studies to date. RA could be therefore used as chemopreventive/chemoprotective agent in clinical praxis. However, further studies are needed to explore the full potential of this compound.

Keywords: rosmarinic acid, cancer, chemoprevention, antitumor activity, adjuvant therapy

1 Introduction

Nutrition, particularly intake of vegetables and certain plant components, has been reported to have a major role in cancer risk reduction. Recently, there has been a growing research interest in rosemary (*Rosmarinus officinalis*), a common household plant grown in many parts of the world. It is used for flavouring food, a beverage drink, as well as in cosmetics and folk medicine. The main active compounds of rosemary include caffeic acid, rosmarinic acid (RA), ursolic acid, carnosic acid, and carnosol [1].

RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid derived from hydroxycinnamic acid (Fig. 1) [2, 3]. This water-soluble polyphenolic compound is a widely occurring natural compound found in the plant kingdom with interesting biological activities [2, 4]. Rosemary and other plants containing RA have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease, cataract, cancer and poor sperm motility [5]. RA contributes to the antioxidant activity of plants used in the cosmetic industry, such as *Rosmarinus officinalis* and *Sanicula europaea* [4]. It is therefore commercialized via cosmetic or food complement preparations with daily doses and day or week-long intake. This substance is such of interest that biotechnological production by plant *in vitro* cultures has been proposed [2, 6].

2 Occurence

RA was first isolated from *Rosmarinus officinalis* (Lamiaceae) by Scarpati and Oriente [7], but it is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae [6]. It was identified as one of the active compounds of several medicinal plants

such as perilla (*Perilla frutescens L.*), rosemary (*Rosmarinus officinalis L.*), sage (*Salvia officinalis L.*), mint (*Mentha arvense L.*), basil (*Ocimum basilicum L.*), thyme (*Thymus vulgaris L.*), *Melissa officinalis*, *Symphytum officinale* and *Heliotropium foertherianum* [1-3, 5, 8-11]. Not all members of the Lamiaceae, however, contain RA. The occurrence is mainly restricted to the subfamily Nepetoideae [3].

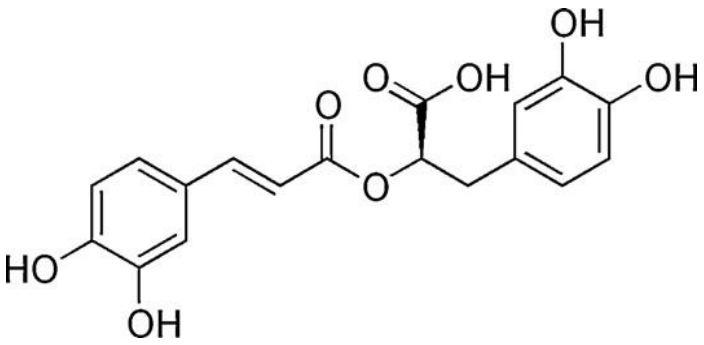

Fig.1 *Chemical structure of rosmarinic acid*

However, RA is also found in species of other higher plant families and in some fern and hornwort species and in monocotyledonous plants like the sea grass family Zosteraceae, the related Potamogetonaceae as well as the Cannaceae [3, 4].

In plants, RA is supposed to act as a preformed constitutively accumulated defense compound. Chemical synthesis of RA was long sought after and was finally achieved in 1991 by Albrecht. Since then a number of chemical syntheses of RA and its derivatives, e.g. the methyl ester, different stereoisomers or the less hydroxylated isorinic acid, have been described [3, 4].

3 Bioavailability

RA is well absorbed from gastrointestinal tract and from the skin [5]. Experiments in rats demonstrated that RA applied topically to skin was absorbed percutaneously and became distributed in skin, blood, bone and muscle, while intravenously administered RA was distributed in various tissues such as lung, spleen, heart and liver [11].

RA is rapidly eliminated from human and rat blood circulation after oral administration ($t_{1/2}$ = 9 min) and is metabolized predominantly to caffeic acid, coumaric acid and ferulic acid [2]. It was reported that most of the orally administered RA was excreted in urine as degraded forms such as *m*-hydroxyphenylpropionic acid and *m*-coumaric acid. Similar studies in humans found that after intake of perilla extract, 1-O-(2,4,5-trimethoxycinnamoyl)-β-glucuronide was excreted in the urine, and that this compound was derived from RA contained in the extract. These results suggest that RA was absorbed and metabolized as conjugated and/or methylated forms, and that the majority of RA absorbed was degraded into conjugated and/or methylated forms of caffeic acid, ferulic acid and m-coumaric acid before being excreted gradually in the urine [11].

RA exhibits very low toxicity [2, 4]. Moreover, no symptoms of rosemary toxicity have been reported in the literature [1].

4 Biological activities of rosmarinic acid

RA has shown several interesting biological activities, such as antiinflammatory, antiviral, antibacterial, antiangiogenic, antimutagen, antidepressant, antiallergenic, antineurodegenerative, HIV-1 inhibitory and antioxidant effects [2, 4, 12-16]. These effects include enhancement of superoxide and hydroxyl scavenging, inhibition of both low-density lipoprotein and oil oxidation, suppression of arachidonate metabolism formation and inhibition of haemolysis and hyaluronidase and β-hexosaminidase activity [11]. RA is also known to reduce hypotension, to possess hepatoprotective, cytoprotective, neuroprotective, gastroprotective or antiacetylcholinesterase properties [2, 4, 12-14, 17-20]. RA increases the production of prostaglandin E2 and reduces the production of leukotriene B4 in human polymorphonuclear leucocytes, and inhibits the complement system [5]. The antiinflammatory properties are thought to be based on the inhibition of lipoxygenases and cyclooxygenases and the interference of RA with the complement cascade [4].

5 Chemopreventive activity

RA is considered to possess cancer chemopreventive properties. It has been shown, that RA is able to inhibit the development of cancer in preclinical models but data are conflicting [21].

DNA damage induced by oxidative and alkylating agents contributes to carcinogenesis, leading to possible mutations if replication proceeds without proper repair. However, some alkylating agents are used in cancer therapy due to their ability to induce DNA damage and subsequently apoptosis of tumor cells [8].

Oral administration of RA prevented the formation of skin tumors during DMBA-induced mouse skin carcinogenesis. Also, oral administration of RA brought back the status of phase I and phase II detoxication agents, lipid peroxidation byproducts, antioxidants and apoptotic markers (p53, Bcl-2, caspase-3 and caspase-9) in DMBA treated mice [22]. The results of another study suggest that RA suppresses oral carcinogenesis by stimulating the activities of detoxification enzymes, improves the status of lipid peroxidation and antioxidants, and downregulates the expression of p53 and bcl-2 during DMBA-induced oral carcinogenesis [23].

Protective effects of extracts of three Salvia species containing RA - *Salvia officinalis* (SO), *Salvia fruticosa* (SF), and *Salvia lavandulifolia* (SL), against DNA damage induced by oxidative and alkylating agents were also determined. SO and SF protected against oxidative DNA damage in human colon carcinoma cells HCT15. SO and SL decreased DNA damage induced by MNU in another human colon carcinoma cell line CO115 [8].

Furtado RA et al [14] investigated the ability of RA to prevent chemically induced chromosome breakage or loss and primary DNA damage using the micronucleus and comet assays with Chinese hamster lung cells V79, respectively. The chemotherapeutic agent doxorubicin (DXR) was used as the DNA-damaging agent. The cultures were treated with different concentrations of RA alone or in combination with DXR. Their results showed that RA exerted no genotoxic effect, but significantly reduced the frequency of micronuclei and the extent of DNA damage induced by DXR. The supposed mechanism of action was based on the antioxidant activity of RA.

The hypermethylation of DNA is a key epigenetic mechanism for the silencing of many genes, including those for cell cycle regulation, inflammatory and stress response, DNA repair and apoptosis. Hypermethylation of certain genes, particularly tumor suppressor genes, is known to be associated with the inactivation of various pathways involved in tumorigenesis. The reactivation of hypermethylated genes by the inhibition of specific DNA

methyltransferases (DNMTs) became a promising area in cancer therapy and chemoprevention. For chemoprevention purposes, the chemical components of edible fruits and vegetables are particularly useful, and several reports have been published in recent years, indicating that phytochemicals may reactivate genes silenced by aberrant methylation [24]. A long-term exposure to these chemicals in diet might potentially lead to an effect which can be sufficient for chemoprevention. RA has also been shown to inhibit the DNMT activity for example in nuclear extracts from breast cancer cell line MCF7 [25].

6 Antiproliferative activity

Rosemary extract (RE) has been shown to have significant antiproliferation activities against a variety of human cancer cell lines including breast, leukemia, prostate, lung and liver, which could be potentially useful for increasing the efficacy of conventional chemotherapeutic agents [9, 18, 26].

RE has significant antiproliferation activity on human ovarian cancer cells A2780 and its CDDP resistant daughter cell line A2780CP70. RE enhanced the antiproliferation effect with CDDP on both A2780 and A2780CP70 cells. Supposed molecular mechanism is the inhibition of the proliferation of ovarian cancer cell lines by affecting the cell cycle at multiple phases. It induces apoptosis by modifying the expression of multiple genes regulating apoptosis, and holds potential as an adjunct to cancer chemotherapy [9].

RA has been also shown to inhibit proliferation and induce apoptosis in activated hepatic stellate cells HSC-T6. Flow cytometric analyses and transmission electron microscope observations revealed that HSC-T6 treated with RA underwent apoptosis in a time dependent manner and displayed typical apoptotic features in the cells. The phosphorylation in signal transducer and activator of transcription protein-3 (STAT3), which regulates cell survival, proliferation and differentiation in a variety of tissues was markedly decreased and correlated with downregulation of CyclinD1 and B cell lymphoma/leukemia-2 (Bcl-2) [18].

RA was shown to inhibit the proliferation and induce apoptosis of Jurkat T cells but the mechanism of action of RA in apoptosis remains elusive. RA inhibited Jurkat cell proliferation by altering the expression of cyclins and cyclin-dependent kinase inhibitors and induced apoptosis most likely acting through the mitochondrial pathway and possessed no antioxidant properties. RA inhibited the proliferation of Jurkat cells in a dose-dependent manner by suppressing the expression of cyclin D3 and p21(Cip1/Waf1) and up-regulating p27(Kip1). RA induced apoptosis of Jurkat cells in a dose-dependent manner and failed to protect them from hydrogen peroxide (H_2O_2)-mediated apoptosis. Induction of apoptosis by RA correlated with suppression of Bcl-2 but not of Bak or PUMA. Overexpression of Bcl-2 protected Jurkat cells from both H_2O_2- and RA-induced apoptosis by altering the ratio of anti- to pro-apoptotic members of the Bcl-2 family [27].

7 Angiogenesis

Angiogenesis plays a crucial role in the growth and metastasis of tumors and several chronic inflammatory diseases such as rheumatoid arthritis and proliferative diabetic retinopathy. It is a complex process that includes degradation of basement membrane, endothelial cell proliferation, migration and adhesion and tube formation. Inhibition of angiogenesis has been recognized as a promising therapeutic approach for the control of tumor growth and metastasis and chronic inflammatory diseases [16].

RA has been shown to inhibit several important steps of angiogenesis including proliferation, migration, adhesion and tube formation of human umbilical vein endothelial cells (HUVEC) in a concentration-dependent manner. RA also reduced intracellular reactive oxygen species (ROS) level, H_2O_2-dependent VEGF expression and IL-8 release of endothelial cells. These findings suggested that the antiangiogenic potential of RA might be

related to its antioxidative activity, which further resulted in the inhibition of ROS-associated VEGF expression and IL-8 release [16].

8 Multidrug resistance

Multidrug resistance (MDR) has been a major problem in cancer chemotherapy. Li FR. et al [1] studied the reversal effect and its potential mechanism of RA on SGC7901/Adr cells. Their results showed that RA could reverse the MDR of SGC7901/Adr cells, increase the intracellular accumulation of Adr and Rh123, and decrease the transcription of MDR1 gene and the expression of P-gp in SGC7901/Adr cells. Their results indicated that RA was a potential multidrug resistance-reversing agent [28].

In another study constituents in rosemary methanol extracts demonstrated inhibition of P-glycoprotein activity in MDR human breast cancer MCF-7 cells, and human cervical cancer KB-C2 cells [9].

9 Adjuvant therapy

In recent years, several studies have been directed toward developing an adjuvant therapy that reduces chemotherapy-induced negative side effects and enhances their therapeutic efficacy. DXR is a potent antitumor drug, but is unfortunately potently cardiotoxic. RA has been shown to effectively protect cardiac muscle cells from DXR-induced cell death. *In vitro*, DXR significantly decreased the viabilities of H9c2 cells accompanied by apoptotic features, such as a change in nuclear morphology and caspase protease activation. RA was found to markedly inhibit these apoptotic characteristics by reducing intracellular ROS generation, inhibiting JNK and ERK activation and by recovering the mitochondria membrane potential ($\Delta\psi$). In addition, RA reversed the downregulations of *GSH, SOD* and Bcl-2 induced by DXR [29].

Moreover, RA has been shown to be a candidate for a new therapeutic approach in bone metastasis from breast carcinoma. RA could inhibit the migration of MDA-MB-231BO human bone-homing breast cancer cells dose-dependently. Furthermore, in ST-2 murine bone marrow stromal cells cultured with RA there was shown a significant and dose-dependent increase in alkaline phosphatase activity, with the number and size of mineralized nodules increasing. RA may inhibit bone metastasis from breast carcinoma mainly via the pathway of the receptor activator of NF kappaB ligand (RANKL)/RANK/osteoprotegerin and by simultaneously suppressing the expression of interleukin-8 [30].

From a randomized controlled trial, foods enriched with bioactive compounds, including RE, were found to be a promising adjuvant therapy in advanced breast cancer patients. The mechanism underlying this effect was considered to be through lowering oxidative stress in the patients by RE [1].

10 Conclusion

Today we can see in biomedical disciplines a significant interest in natural substances such as RA. Study of this kind of molecules is very valuable because the molecular design is not performed prior to their synthesis but is determined after extraction of molecules. Therefore, it is possible to focus more on testing of their effect *in vivo* and *in vitro* than on the testing of stability and physicochemical parameters of syntetic molecular derivates. RA is a typical example of such structure of natural origin, generally called as natural compounds. With respect to its bioavailability and great chemopreventive and antiproliferative effects together with its great potential in adjuvant therapy and reversing of multidrug resistance RA is therefore a promising molecular structure. Further applied clinical research can determine the real extent of our expectations of RA use in therapeutic praxis.

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