Aquoeus extracts of rosmarinus officinalis, urtica diocia and soybean exert different effects on adenosine deaminase activity in cancerous and non cancerous human gastric and colon tissues

Zahide Esra Durak1*, Suleyman Buber2, Hilmi Kocaoglu3, Bahadir Oztuerk4
1Ordu University, Central Research Laboratory, ORDU, (TURKEY)
2Ankara University, Medical Faculty, Biochemistry Department, Ankara, (TURKEY)
3Ankara University, Medical Faculty, Surgical Oncology Department, Ankara, (TURKEY)
4Selcuk University Medical Faculty, Department of Biochemistry, Konya, (TURKEY)
E-mail : zaesrad@hotmail.com

ABSTRACT

This study aimed to investigate effects of aquoeus extracts of rosmarinus officinalis, urtica diocia and soybean on adenosine deaminase (ADA) activity in cancerous and non cancerous gastric and colon tissues. In method, cancerous and non cancerous human gastric and colon tissues removed by surgical operations were studied. In the samples, adenosine deaminase activities were measured with and without plant extract incubated for 1 h.

As a result, it has been observed that rosemary extract inhibits ADA enzyme in cancerous and non cancerous gastric tissues, but not in colon tissues, and urtica extract inhibits the enzyme only in cancerous gastric tissue. On contrast, soybean extract activates ADA enzyme in colon tissues significantly.

Inhibition of ADA enzyme might play a part in the proposed anti-cancer properties of rosemary and urtica diocia. However, the finding of ADA activation in colon tissues by soybean extract is a new one which needs further verification.

INTRODUCTION

Cancer is the major problem for all people in the world. The scientists have long been looking to natural remedies for the treatment of cancer because of side effects of chemotherapy and radiation therapy. In this regard, it has been observed that treatment of some types of cancers with plant sources may give rise to positive results.

There are many factors for cancer occurrence and development in humans. Most of the cancers are caused by environmental factors,[1] and of these, 30–40% of cancers are directly linked to the diet.[1] While many dietary recommendations have been proposed to reduce the risk of cancer, unfortunately few have significant supporting scientific evidence.[2]

Adenosine deaminase (ADA) is an enzyme (EC 3.5.4.4) involved in purine metabolism. It is needed for the breakdown of adenosine and for the turnover of nucleic acids. ADA is present virtually in all mammalian cells, and it is thought that its primary function in human beings is related to the immune system.[3] However, the full physiological role of ADA is not completely understood.[4] ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance.[3,5] It has also been proposed
that ADA, in addition to its role in adenosine breakdown, stimulates release of excitatory amino acids, and it is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins\textsuperscript{[3,4]}.

Some ADA inhibitors have been used for chemotherapeutic purposes in some types of cancers. From a scientific perspective of view, use of ADA inhibitors has helped much in understanding the mechanism of action of adenosine metabolites and analogs. ADA inhibitors have also led to the understanding of the regulatory processes associated with immunodeficiency characterized by a lack of ADA, and of maturation of the immune response\textsuperscript{[6]}. One of them, pentostatin (Nipent) is a nucleoside analog having potential to inhibit adenosine deaminase enzyme. Inhibition of ADA blocks the deamination reactions in the purine salvage pathway, result of which is the inhibition of ribonucleotide reductase. As a result, this process depletes the nucleotide pool and limits DNA synthesis\textsuperscript{[7]}.

*Rosmarinus officinalis* (Rosemary) contains a number of potentially biologically active compounds, including antioxidants carnosic acid and rosmarinic acid. Other chemical compounds include camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol and rosmanol. Rosemary antioxidants levels are found to be closely related to\textsuperscript{[9,19]}, some of which indicate a promising effect in controlling cancer development. This food has been shown to have significant antiproliferation activities against a variety of human cancer cell lines including breast, leukemia, prostate, lung and liver\textsuperscript{[11,12]}.

Urtica dioica, is a perennial plant growing in temperate and tropical wasteland areas around the world. The plant has been widely used for cancer treatment by people around the world for centuries. In the first century, Greek physicians Dioscorides and Galen reported that the leaf of uralica had diuretic and laxative properties and was useful for asthma, pleurisy and spleen illnesses. In fact, it is a herb that has a long tradition of use as an adjuvant remedy in the treatment of arthritis in Germany. Nettle leaf extract contains active compounds that reduce TNF-\(\alpha\) and other inflammatory cytokines\textsuperscript{[13,14]}. It has been demonstrated that nettle leaf lowers TNF-\(\alpha\) levels by potently inhibiting the genetic transcription factor that activates TNF-\(\alpha\) and IL-1B in the synovial tissue that lines the joint\textsuperscript{[15]}. Nettle root extracts have been studied in human clinical trials as a treatment for symptoms of benign prostatic hyperplasia (BPH). These extracts have been shown to help relieve symptoms compared to placebo both by themselves\textsuperscript{[16]} and when combined with other herbal medicines\textsuperscript{[17]}.

Urtica dioica is the most frequently used herb in cancer therapy. Both roots and leaves of this plant were used traditionally\textsuperscript{[18]}. In a study, it has been observed that adenosine deaminase (ADA) activity in prostate tissue is inhibited by aqueous extract of Urtica dioica. ADA inhibition by Urtica dioica extract has been suggested as one of the mechanisms in the observed beneficial effect of Urtica dioica in prostate cancer\textsuperscript{[18]}.

Soybean is one of the few plants that provides a complete protein as it contains all eight amino acids essential for human health\textsuperscript{[19]}. There is much evidence suggesting that compounds present in soybeans can prevent cancer in many different organ systems. The evidence for specific soybean-derived compounds having a suppressive effect on carcinogenesis in animal model systems is limited, however. There is evidence that some products derived from soybean suppress carcinogenesis in vivo: a protease inhibitor, the Bowman-Birk inhibitor, inositol hexaphosphate (phytic acid) and the sterol beta-sitosterol. Other compounds that may be able to suppress carcinogenesis in animals are the soybean isoflavones. Soybean compounds reported to have other types of anticarcinogenic activity include soybean trypsin inhibitor, saponins and genistein. There is much evidence to suggest that diets containing large amounts of soybean products are associated with overall low cancer mortality rates, particularly for cancers of the colon, breast and prostate. It is believed that supplementation of human diets with certain soybean products may markedly reduce human cancer mortality rates\textsuperscript{[20]}.

As discussed above briefly, all of these plants deserve further studies with regard to the properties of cancer prevention and therapy.

**MATERIALS AND METHODS**

Twenty two cancerous gastric tissues and 22 non cancerous adjacent gastric tissues were obtained from patients with gastric cancer by surgical operation. Eleven cancer and 11 non cancer colon tissues were similarly obtained from patients with colon cancer. Tissues were
first cleaned by saline solution and stored at -80 °C until analysis. In the analysis process, they were first homogenized in saline solution (20 %, w/v). After homogenization, samples were centrifuged at 5000 rpm for 30 min to remove debris and to obtain clear supernatant fraction. Analyses were performed in this fraction\[21\].

The extracts were prepared by soaking plants into the distilled water at the concentration of 10 % (w/v) and waiting for 24 h at room temperature by continuously rotating. After the debris was removed, supernatants were centrifuged at 10,000 rpm for 20 min and upper clear part was removed to be used in the assays.

Protein concentrations of the tissues were measured by Lowry method\[22\] and ADA activity was measured by the method of Guisti\[23\]. ADA activity measurements were performed with and without plant extract for 1 h. Statistical evaluations were made by using Wilcoxon test and values lower than 0.05 were evaluated significant.

RESULTS

Results are shown in the TABLE 1. As seen from the table, rosemary extract inhibits ADA enzyme in cancerous and noncancerous gastric tissue but not in colon tissue. Urtica extract inhibits the enzyme only in cancerous gastric tissue. On contrast, soybean extract activates ADA enzyme in colon tissue.

<table>
<thead>
<tr>
<th>TABLE 1 : Effects of rosemary leaf, soy bean and urtica dioica extracts on ADA activities in gastric and colon tissues with and without cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>A-</td>
</tr>
<tr>
<td>B-</td>
</tr>
<tr>
<td>C-</td>
</tr>
<tr>
<td>D-</td>
</tr>
</tbody>
</table>

Statistical evaluation (Wilcoxon test)

A-B: 0.031 0.048 ns ns
A-C: ns ns 0.05 0.012
A-D: 0.039 ns ns ns

A : ADA activity without extract; B : ADA activity with rosemary leaf extract; C : ADA activity with soy bean extract; D : ADA activity with urtica dioica extract; p < 0.05 value was evaluated significant; n.s: Non significant

DISCUSSION

Nutritional foods are important sources for the treatment of some types of cancers, leading to the development of potential novel agents\[21-23\]. Several of the molecules available from foods have been shown to exert anticancer effects on cancer cells. These effects have been observed through in vitro and in vivo animal studies\[24-26\].

Rosemary (Rosmarinus officinalis L.) extract possesses antitumor properties against tumor cells from several organs. In a study, it has been observed that rosemary extract modulates estrogen and epidermal growth factor receptors in breast cancer cell lines\[27\]. Another study indicates that a standardized rosemary extract can disrupt the endoplasmic reticulum machinery to decrease the viability of prostate cancer cells and promote degradation of the androgen receptor. Two human prostate cancer cell lines, 22Rv1 and LNCaP, and prostate epithelial cells procured from two different patients undergoing radical prostatectomy were treated with standardized rosemary extract and evaluated by flow cytometry, MTT, BrdU, Western blot and fluorescent microscopy. A significant modulation of endoplasmic reticulum stress proteins was observed in cancer cells while normal prostate epithelial cells did not undergo endoplasmic reticulum stress. This biphasic response suggests that rosemary extract may preferentially target cancer cells as opposed to normal cells\[28\].

In a study with carnosol which is an active constituent of rosemary, it has been reported to possess anti-inflammatory and anticancer activities. However, the molecular mechanisms underlying the anticancer effects of carnosol remain poorly understood. It has been found that carnosol significantly reduced the viability of human colon cancer (HCT116) cells in a concentration- and time-dependent manner. Treatment of cells with carnosol induced apoptosis, which was associated with activation of caspase-9 and -3 and the cleavage of poly-(ADP-ribose) polymerase (PARP)\[29\].

Our results show that rosemary extract can significantly inhibit ADA enzyme in cancerous and noncancerous gastric tissues. This finding is of significance because of the fact that inhibition of adenosine deaminase blocks the deamination of adenosine to inosine, and deoxyadenosine to deoxynosine in the purine salvage
pathway. This accumulation of metabolites inhibits ribonucleotide reductase, which depletes the nucleotide pool and limits DNA synthesis[7].

In a study, anti-proliferative activity of urtica dioica extract on the human breast cancer cell line (MCF-7) and fibroblasts isolated from foreskin tissue was evaluated using MTT assay. Mechanisms leading to apoptosis were also investigated at the molecular level by measuring the amount of anti and pro-apoptotic proteins and at the cellular level by studying DNA fragmentation and annexin V staining by flow cytometry. The aqueous extract of Urtica dioica showed antioxidant and antiproliferative effects. The anti proliferative activity was found to be associated with an increase of apoptosis as demonstrated by DNA fragmentation. Study findings warrant further research on Urtica dioica as a potential chemotherapeutic agent for breast cancer[30].

A study investigated the hepatoprotective, nephroprotective, and antioxidant activity of Urtica dioica L methanolic extract (UDME) against cisplatin (CP) toxicity in Erhlich ascites tumor (EAT)-bearing mice. In this investigation, levels of serum hepatic enzymes, renal function markers, and oxidant/antioxidant parameters of liver tissue were measured. Mice were inoculated with EAT on day 0 and treated with nothing else for 24 hours. After a single dose of CP administration on day 1, the extract was given at the different doses daily during 6 days. Almost all doses of UDME performed a significant (P < 0.05) preventive role against CP toxicity. This suggests that UDME has a protective capacity and antioxidant activity against CP toxicity in EAT-bearing mice, probably by promoting antioxidative defense systems[31].

Our results show that urtica dioica extract inhibits ADA enzyme only in malign gastric tissue. This finding may be also of importance like action of rosemary leave extract on ADA enzyme relating to gastric cancer treatment.

In a soy food study, it has been reported that dietary soy consumption can lower the risk for breast cancer. Current human and animal data provide evidence for several anticancer properties of soy and its isoflavones. Although the specific quantities and constituents responsible for the observed anti-cancer effects have not been elucidated, it appears that soy isoflavones do not function as an estrogen, but rather exhibit anti-estrogenic properties. However, their metabolism differs between humans and animals and therefore the outcomes of animal studies may not be applicable to humans. The majority of breast cancer cases are hormone-receptor-positive; therefore, soy isoflavones should be considered as a potential anti-cancer therapeutic agent and warrant further investigation[31].

A study was conducted to examine the association between soy isoflavones consumption and risk of breast cancer incidence or recurrence. Soy isoflavones consumption was inversely associated with risk of breast cancer incidence. However, the protective effect of soy was only observed among studies conducted in Asian populations but not in Western populations. Soy isoflavones intake was also inversely associated with risk of breast cancer recurrence. Stratified analyses suggested that menopausal status may be an important effect modifier in these associations. They failed to identify a dose-response relationship between total isoflavones intake and risk of breast cancer incidence. This study suggests soy isoflavones intake is associated with a significant reduced risk of breast cancer incidence in Asian populations, but not in Western populations[32].

In our study, we have however observed that soybean extract significantly activates ADA enzyme in cancerous and non cancerous colon tissues. As far as we know, these results are first ones showing activating effects of soybean extract on ADA enzyme. For the time being, what is the importance of this finding is not clear for us and needs further studies.

Studies in patients with breast, colorectal, or prostate cancer show that the influence of dietary factors on survival remains to be determined. Adiposity and a lack of physical activity, however, appear to influence cancer outcome negatively[33].

To sum up, rosemary leaf and urtica dioica extracts inhibit ADA enzyme in cancerous gastric tissues significantly but does not affect the enzyme in colon tissue. It seems quite possible that accumulated adenosine due to the inhibition of ADA enzyme might play an important function in the anti-cancer properties of rosemary and urtica dioica leaves, possibly through inhibition of ribonucleotide reductase and depletion of nucleotide pool for new DNA synthesis. However, soybean extract activates ADA enzyme in colon tissues, the signifi-
cance of which is not known by us at the moment. Therefore, further researches including cell culture and animal studies are needed to obtain more information about the subject.

REFERENCES


