



Aqueous Extract from Rhubarb Plant Inhibits Adenosine Deaminase Activity in Cancerous and Non Cancerous Human Gastric and Colon Tissues

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Abstract

Aim: Investigation of possible effects of rhubarb extract on adenosine deaminase (ADA) activity in cancerous and non cancerous human gastric and colon tissues to obtain information about possible mechanism of anti cancer action of rhubarb.

Materials and methods: Cancerous and non cancerous human gastric and colon tissues removed from patients by surgical operations were used in the studies. The extracts were prepared in distilled water. Before and after treatment with the extracts, ADA activities in the tissue homogenates were measured.

Results: ADA activity was found to be higher in gastric tissue compared with colon tissue, but no differences were found between ADA activities of cancerous and non cancerous tissues. It was found that rhubarb extract significantly inhibited ADA activity both in cancerous and non cancerous gastric and colon tissues.

Conclusion: Our results suggest that aqueous extract from rhubarb inhibits ADA activities in both gastric and colon tissues significantly. It is suggested that in addition to other proposed mechanisms, accumulated adenosine due to the inhibition of ADA enzyme might also play part in the anticancer properties of the rhubarb.

Keywords: Adenosine Deaminase; Rhubarb; Cancer.

Introduction

Cancer is one of the major problems for all people in the world. Side effects of chemotherapy and radiation therapy have been known obviously. Since the therapies at present do not result in successful results for some types of cancers, the scientists have long been looking to natural remedies for the treatment of cancer. In this regard, plant-derived compounds were evaluated first because of the findings that use of some plants in some rural populations to treat some types of cancers give rise to positive results. In fact, plants have a long history in the treatment of cancer (1) and studies related to anti-cancer agents from plant sources started in the 1950s with the discovery and development of some alkaloids (2, 3).

Rhubarb, as one of these plants, has been used as a traditional medicine since ancient times and today it is still used in various herbal preparations. Anthraquinones are important group of bioactive components found in rhubarb. Emodin is one of the most effective anthraquinones found in rhubarb, and it is found to be capable of inhibiting cellular proliferation, induction of apoptosis, and prevention of metastasis. These capabilities are supposed to occur through multiple signaling pathways. Aloe-emodin is another major anthraquinone in rhubarb having anti-tumor properties. Both of these anthraquinones can induce cell cycle arrest through increasing the p53 expression level and together with upregulation of p21(4).

Cytotoxic(5), cytostatic (6, 7) and antitumor effects of rhubarb have been reported in cancer cells in mice (8) and in vitro. It possesses anti-proliferative activity, inducing cell cycle arrest in cancer cell lines(6). Anthraquinone extracts from rhubarb plant have been shown to induce cytotoxicity in cancer cell lines (5) and tumor necrosis in mice(8). The polyphenols in rhubarb selectively kill or prevent the growth of cancer cells. It can be used for the cases where cancers have proven resistant to classical treatments(4, 9,10).

Adenosine deaminase (ADA) is an enzyme (EC3.5.4.4) involved in purine metabolism. It is needed for the breakdown of adenosine and for the turnover of nucleic acids in tissues. 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(11). However, the full physiological role of ADA is not completely understood (12). ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance(11-13). It has also been proposed that ADA, in addition to its role in adenosine breakdown, stimulates release of excitatory amino acids and is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins(11, 12).

As discussed briefly above, although some mechanisms are supposed for the action of rhubarb in the cancer process, it is obvious that in addition to known mechanisms, there should be some others unknown in detail yet. Therefore, we think that further studies are needed. As to the subject, investigation of the effects of aqueous extract from rhubarb on ADA activity in cancerous and non cancerous human tissues might give useful results since ADA is a key enzyme in purine nucleotide metabolism, thereby in cancer process.

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 homogenised in saline solution(20 %, w/v). After homogenisation, homogenates were centrifuged at 5000 rpm for 30 min to remove debris and to obtain clear supernatant fraction. Analyses were performed in this fraction(14).

The extracts were prepared by soaking ground powder (rhubarb) 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, plant 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(15) and ADA activity was measured by the method of Guisti et al.(16).

Statistical evaluations were made by using Wilcoxon test and values lower than 0.05 evaluated significant.

Results

Results were shown in the table 1. As seen from the table, rhubarb extract significantly inhibits ADA enzyme activity both in cancerous and non cancerous gastric and colon tissues. Furthermore, ADA enzyme activity was found to be higher in gastric tissue compared with colon tissue. There were however no differences between ADA enzyme activities of cancerous and non cancerous tissues for both tissues as well.

Table 1: Adenosine Deaminase (ADA) Enzyme Activities in the Tissues with and Without the Rhubarb Extract. (ADA Enzyme Activity in IU/Mg Protein; Mean±SD)

	ADA activity without extract	ADA activity with extract
Non-cancerous colon tissue	5.53±3.32	4.35±3.64*
Cancerous colon tissue	5.45±2.32	4.33±2.02*
Non-cancerous gastric tissue	11.19±9.57	7.51±5.85*
Cancerous gastric tissue	11.45±8.57	4.63±2.97*, #

* p<0.05 as compared to the same tissue without extract; # p<0.05 as compared to non-cancerous gastric tissue with extract according to Wilcoxon test

Discussion

Plants are the important sources of effective conventional drugs for the treatment of some types of cancers, leading to the development of potential novel agents(17, 18, 19). One of them used for this purpose is rhubarb, which is known to have some functional constituents with anti cancer potential. Several of the molecules available from rhubarb root have potential anticancer effects on cancer cells. These effects have been observed in vitro and in vivo animal studies. The general anti-inflammatory and antioxidant activities of anthraquinones and tannins have been known for a long time for cancer-fighting roles. In addition, anthraquinones have specific tumor cell killing capabilities. The most highly concentrated anthraquinone in rhubarb root, emodin, prevents cell-cycle progression and induces cell death in cancer cells. Emodin has also been thought to prevent cancer metastasis, spreading of cancer to different organs (20-23). Another anthraquinone in rhubarb root, rhein, inhibits glucose uptake in cancer cells and alters membranes of cancer cells to induce death. Anthraquinones also can reduce radiation-induced lung damage while improving lung function in patients with lung cancer. While rhubarb has been reported to have cancer-fighting abilities as an individual herb, it is also a component of Essiac tea, a popular alternative cancer treatment drink prepared along with sheep sorrel, burdock root, and slippery elm bark(24).

In a previous study, it has been demonstrated that that emodin exerts an antiproliferative effect, likely through apoptosis induction-related mechanism(s), which is reproducible in various human pancreatic cancer cell lines. In another study, it has been shown that the angiogenic properties of rhubarb root may partly account for its use in inflammatory diseases. The anthraquinones with hydrophilic structure played a substantial role in inhibiting angiogenesis. The value of an angiogenic model is further supported(9, 25). In fact, plant derived products as a source of cellular growth inhibitory phytochemicals on cancer cell lines have been reported previously (26, 27).

Therefore, it seems valuable to investigate possible effects of rhubarb extract on some critical components having function in the living cells in the body. In this regard, ADA seems of importance since it is a key enzyme in the purine metabolism, inhibition of which may give selective advantage to combat with cancer. Therefore, investigation of possible effects of some plants including rhubarb may give some useful information about their anticancer potential mechanisms. From a scientific perspective, the use of ADA inhibitors has helped enormously in understanding the mechanism of action of adenosine metabolites and analogs whose catabolism was heretofore neglected with respect to their specificities of action. ADA inhibitors have also enabled us to understand the regulatory processes associated with immunodeficiencies characterized by a lack of ADA, and to further understand the maturation of the immune response (28). Of them, pentostatin is a nucleoside analog that inhibits the activity of the enzyme adenosine deaminase. Inhibition of adenosine deaminase blocks the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine in the purine salvage pathway. This accumulation of metabolites inhibits ribonucleotid reductase, which depletes the nucleotide pool and limits DNA synthesis (29).

Looking at our results, it seems that ADA activity is higher in gastric tissue compared with colon tissue, but there are no differences between ADA activities of cancerous and non cancerous tissues for both tissues. In some previous studies, higher ADA activities were reported in several types of cancer tissues (13, 30-32). However, we found no differences between ADA activities of the control and cancer tissues, which might be resulted from the fact that all of these patients had received intense chemotherapy before surgical operation. These kinds of therapies might decrease ADA enzyme activity in cancerous tissue as well.

Our results however suggest that components of aqueous extract from rhubarb inhibit ADA activities in both gastric and colon tissues significantly. Inhibition degrees in ADA activity are almost the same in the cancerous (malign) and non cancerous adjacent tissues (benign). It seems quite possible that in addition to other proposed mechanisms, accumulated adenosine due to the inhibition of ADA enzyme might also play an important function in the anti-cancer properties of rhubarb through inhibition of ribonucleotid reductase and depletion of nucleotide pool for new DNA synthesis.

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