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Effects of an Antioxidant Extract on Adenosine Deaminase Activities in Cancerous Human Liver Tissues

[1]A. Avci, [2]M. Kaçmaz, [3]M. Kavutcu, [4]E. Göçmen and [1]İ. Durak
[1]Department of Biochemistry, School of Medicine, Ankara University, Ankara, Turkey
[2]Department of Biochemistry, Faculty of Medical, Kırıkkale University, Kırıkkale, Turkey
[3]Department of Biochemistry, Faculty of Medical, Gazi University, Ankara, Turkey
[4]General Surgery Clinics, Numune Hospital, Ankara, Turkey

Abstract: Effects of an antioxidant extract consisting mainly of garlic (*Allium sativum*) and red clover (*Trifolium pratense*) on adenosine deaminase (ADA) activities were investigated in cancerous and non cancerous human liver tissues and, results were compared with those of fludarabine. Ten cancerous and 10 non cancerous adjacent liver tissues were obtained from patients with metastatic type liver cancer by surgical operations. Kinetic analyses were carried out to establish V_{max} and K_m values of the reaction catalyzed by ADA under normal and inhibitor conditions. ADA activity was found lower in the cancerous tissues compared with non cancerous ones. Antioxidant extract created significant inhibitions on the ADA activities both in cancerous and non cancerous tissues. Inhibition percents were relatively higher in the cancerous tissues as compared with non cancerous ones. Furthermore, we have observed that inhibition percents created by the extract are higher than those of fludarabine at the concentrations studied. Results suggest that antioxidant extract exerts significant inhibition on the ADA activity in cancerous and non-cancerous human liver tissues. This might be rational basis for the use of these herbs in the alternative cancer therapy in the folk medicine.

Key words: Cancer, adenosine deaminase, antioxidant, garlic, red clover

INTRODUCTION

Adenosine deaminase is an important enzyme in the degradation of adenine nucleotides[1]. It is accepted as a key enzyme in purine metabolism and DNA turn over and thus, in the cancer process[2,3]. In several studies, tissue ADA activities were found increased, decreased or unchanged depending on types of tissue and cells studied[4-8].

Fludarabine (fludarabine phosphate) is a chemopreventive used in thetherapy of several types of cancers[9,10]. Its chemotherapeutic potential is known to be mainly resulted from its inhibitory activity on the ADA enzyme[10-12].

Epidemiological evidence has revealed that garlic consumption has played a significant role in the reduction of deaths caused by malignant diseases[13,14]. For example, it has been reported that the mortality rate among patients with gastric cancer was significantly lower in regions of China with higher garlic consumption[13]. Similar beneficial effects of garlic were observed in cases of gastric cancer in Italy[15]. It has been found that garlic consumption has led to decreased incidence of colorectal cancer among Japanese[16]. Alliin and one of its metabolites, allicin, were found to show tumor inhibiting effects[17,18]. Since then, although a number of studies have been made on the antitumor and cytotoxic actions of garlic and its organosulfure constituents[19-24], precise mechanism(s) on the anticarcinogenic action of garlic has not been clarified yet.

Similar to the garlic, red clover has long been used in the alternative cancer therapy among people. It is accepted to be one of the world's oldest and most common natural cancer remedies[25]. Although its anticancer compounds (isoflavones like genistein) may make it an effective anticancer food[26,27], the scientific study on red clover is still new and further researches are needed to clarify its possible role and action mechanism in the cancer treatment.

Corresponding Author: Dr. İlker Durak, Professor, Ankara Üniversitesi Tıp Fakültesi,
Biyokimya Anabilim Dalı (Dekanlık Binası), 06100 Sihhiye, Ankara, Turkey
Tel: +90 312 3116457 Fax: +90 312 3106370 E-mail: durak@medicine.ankara.edu.tr

MATERIALS AND METHODS

In the present study, 10 cancerous and 10 non cancerous adjacent liver tissues were obtained from 10 patients with metastatic type liver cancer originating from colon. After removed by surgical operation, tissues were washed with deionized water and stored at -30°C for about a month. Before the activity measurement, tissues were cleaned from the fatty part, homogenised in pH 7.2, 50 mM phosphate buffer and centrifuged at 10 000 rpm for 30 min. Upper clear layer was removed and used in the assays. ADA activity and protein amount were measured as described, respectively[28,29]. Kinetic analyses were carried out by incubating samples (100 μL) with extracts (10 μL) and with fludarabine (10 μL from -12.5 mg fludarabine phosphate/mL deionised water-solution).

Antioxidant extract was prepared by incubating 100 g fresh garlic and 10 g dry red clover in 500 mL water-alcohol-olive oil solution (100/10/1 v/v/v, respectively) for 10 days (SARMEX^{R}). After homogenisation, it was centrifuged at 10 000 rpm for 30 min and upper clear layer was removed to be used in the kinetic assays.

RESULTS AND DISCUSSION

ADA activity was lower in the cancerous liver tissue as compared with non cancerous adjacent one (Table 1). K_m value was also lower in the cancerous tissue, which was an indication for high affinity of the enzyme of the cancerous tissue against its substrate, namely adenosine.

Antioxidant extract and fludarabine both exerted significant inhibitions on the ADA activity but, inhibition percents were higher for antioxidant extract compared with fludarabine at the concentrations studied. Inhibition percents in the non cancerous tissue were 85% for antioxidant extract and 35% for fludarabine. The same inhibitions in the cancerous tissue were however 92% for the extract and 24% for fludarabine. K_m values calculated under inhibitor conditions were lower than those calculated without the extract and fludarabine.

Kinetic analyses showed mixed type inhibitions (Lowered V_{max} and K_m values for the inhibitors studied).

Garlic[30,31] and of a lesser degree red clover[32] are perhaps most widely quoted herbs with therapeutic potentials. In addition to several diseases like atherosclerosis, both have long been used as folk medicines in cancer therapy. Several epidemiological studies have revealed that garlic consumption is associated with reduced mortality and morbidity[15,16,33]. Some organosulfur compounds like alliin, allicin, s-allyl cysteine etc. have been accepted to play major role in this protective function[34-37]. Despite all these findings, no exact mechanism(s) and active component(s) in the garlic extract have not been clarified.

Red clover has also long been used to treat cancer and acoustic tumors among people. The use of red clover as an anticancer agent can be traced back to the 1940s when herbalist Harry Hoxey was promoting the herbs an alternative to surgery and radiation therapy[25,26,32]. Red clover contains high amounts of isoflavone compounds such as genistei[26]. Several researchers have shown that these isoflavones may help to prevent cancer[27]. In a study, it has been demonstrated that isoflavone derivatives inhibit the cell growth of stomach cancer lines *in vitro*[27] and supposed that this might occur through activation of a signal transduction pathway for apoptosis. In another, biochanin A, one of the isoflavones in red clover was found to inhibit carcinogen activation in cells in culture medium[27]. However, the precise mechanism of action and responsible constituents for proposed benefits of red clover in the cancer process is unknown.

Fludarabine has been established to exert significant inhibition to the metabolic conversions of purines to their active triphosphates by ADA. In particular, it exhibits substantial activity against lymphoid malignancies such as chronic lymphocytic leukemia and non-Hodgkin's lymphoma[4]. Inhibition of ADA by purine nucleoside analogs such as fludarabine is the rational basis for use of these analogs in the cancer therapy.

Table 1: Mean±SD of adenosine deaminase (ADA) enzyme activities (V_{max} = mIU mg^{-1} protein) and K_m values (μM) in cancerous and non-cancerous human liver tissues measured in normal and inhibition conditions

	Control (n=10)		Cancer (n=10)		Student's t-test [Control vs. Cancer]	
Groups	V_{max} (mIU mg^{-1})	K_m (μM)	V_{max} (mIU mg^{-1})	K_m (μM)	V_{max}	K_m
A (without inhibitor)	3.99±1.33	145±46	3.08±1.24	120±35	p<0.050	p<0.05
B (with extract)	0.78±0.54	45±30	0.38±0.26	52±28	p<0.025	NS
C (with fludarabine)	2.61±1.52	100±26	2.36±1.10	70±20	NS	p<0.05
Student's t-test						
A vs. B	p<0.0005	p<0.0005	p<0.0005	p<0.0005	-	
A vs. C	p<0.05	p<0.01	p<0.05	p<0.005	-	
B vs. C	p<0.005	p<0.01	p<0.0005	p<0.05	-	

Under the lights of these explanations, how can our results be evaluated? We think that lowered ADA activity might be an attempt to slow rapid growth of cancer cells in the cancerous tissues. As seen from the Table 1, inhibition percents of the antioxidant extract in the cancerous and non-cancerous tissues are substantially higher than that of fludarabine at the concentrations studied. This finding may be one of the rational bases for the use of both herbs in the cancer therapy in the folk medicine. As far as we know, there is no study aiming to investigate possible effects of this kind of extracts on ADA activity in cancerous tissues. However, only in one study, it has been established that ADA is inhibited in aortic endothelial cells by garlic extract and suggested to contribute to the hypotensive activity and vessel protective effects of garlic[38]. Although there are several hypothetical explanations on how garlic and red clover extracts play part n the cancer preventive events, none has given satisfactory explanation yet. For example, it has been reported that organosulphur components might inhibit several tumor progressions in experimental animals[34,36,37]. However, action mechanisms of these compounds have not been clarified. Similarly, as to the anticancer potential of red clover, it has been reported that, an isoflavone from red clover can inhibit carcinogen activation in cells[27] and another isoflavone namely, biochanin A can inhibit tumor growth in stomach cancer cell lines[26]. However, activity mechanism of these compounds have not been documented yet. Lowered V_{max} and K_m values which is an indication of high affinity of the enzyme against its substrate under inhibition conditions reveal mixed type inhibition mechanisms for the substances used in the present study.

Results suggest that the constituents of the antioxidant extract cause significant inhibitions on ADA activity. It might be one of the rational basis for the use of these herbs in the alternative cancer therapy in folk medicine.

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