Effect of Garlic and Red Clover extracts on adenosine deaminase enzyme activities in cancerous and non-cancerous human liver tissues

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Abstract

Possible effects of aqueous garlic (*Allium sativum* Linn.) and red clover (*Trifolium pratense* Linn.) extracts on adenosine deaminase (ADA) activities were investigated in cancerous and non-cancerous human liver tissues and results were compared with those of Fludarabine, a known ADA inhibitor. 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 Vmax and Km values of the reaction catalyzed by ADA in the presence or absence of inhibitor. ADA activity was found to be lower in the cancerous tissues compared with non-cancerous tissues. Aqueous garlic and red clover extracts induced significant inhibition ADA activity in both the tissues. Inhibition percentage was relatively higher in the cancerous tissues as compared with non-cancerous tissues. Furthermore, we observed that inhibition percentage induced by garlic and red clover extracts were higher than those of Fludarabine at the concentrations studied.

Keywords: Liver Cancer, Adenosine deaminase, ADA, Garlic, *Allium sativum*, Red clover, *Trifolium pratense*.

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Introduction

Adenosine deaminase (ADA) is an important enzyme in the degradation of adenine nucleotides1. It is known as a key enzyme in purine metabolism and DNA turnover and thus, in the cancer process2–3. In several studies, tissue ADA activities have been found increased, decreased or unchanged depending on the types of tissues studied4–8. Fludarabine phosphate is a chemo-preventive agent used in the therapy of several types of cancers9, 10. Its chemotherapeutic potential is known to be mainly resulted from its inhibitory activity on the ADA enzyme10–12.

Epidemiological evidence has revealed that garlic (*Allium sativum* Linn.), consumption has played a significant role in the reduction of deaths caused by malignant diseases13, 14. The mortality rate among patients with gastric cancer was significantly lower in regions of China with higher garlic consumption13. Similar beneficial effects of garlic in cases of gastric cancer were also observed in Italy15. It was also observed that garlic consumption led to decreased incidence of colorectal cancer among Japanese16. Alliin and one of its metabolites, allicin, were found to show tumour inhibitory effects 17, 18. Since then, although a number of studies have been made on the anti-tumour and cytotoxic actions of garlic and its organosulfur constituents19–24, precise mechanisms of its anticarcinogenic action have not been clarified yet.

Similar to the garlic, red clover (*Trifolium pratense* Linn.) 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\textsuperscript{25}. Although its constituent anticancer compounds (isoflavones like genistein) may make it an effective anticancer food\textsuperscript{26, 27}, the scientific study on red clover is still new and further research is needed to clarify its possible role and mechanism of action in the cancer treatment.

**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 removal by surgical operation, tissues were washed with deionized water and stored at, \(-30^\circ\text{C}\) for about a month. Before the enzyme activity measurement, tissues were cleaned from the fatty part, homogenized in \(pH\) 7.2, 50 mM phosphate buffer and centrifuged at 10,000 rpm for 30 min and upper clear layer was removed to be used in the kinetic assays. Red clover extract was prepared similarly by incubating 30 g dry red clover in 500 ml water for 10 days. It was then prepared for the assays as for garlic.

Student’s t-test was used in the statistical analyses of the results. \(P\) values lower than 0.05 were judged as significant.

**Results**

Results are given in Table 1. As seen from the table, ADA activity was lower in the cancerous liver tissue as compared with non-cancerous adjacent one. \(K_m\) 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.

Garlic, red clover extracts and Fludarabine all exerted significant inhibitions on the ADA activity but inhibition percentages were higher for garlic and red clover extracts compared with Fludarabine at the concentrations studied. Inhibition percentages in the non-cancerous tissue were 79\% for garlic, 58\% for red clover and 35\% for Fludarabine. The same inhibition percentages in the cancerous tissue were however 86\% for garlic, 66\% for red clover and 24\% for Fludarabine. \(K_m\) values calculated in presence of inhibitor were lower than those calculated without inhibitors, namely extracts and Fludarabine. Kinetic analyses showed mixed type inhibitions (lowered \(V_{max}\) and \(K_m\) values) for all the inhibitors studied.

**Discussion**

Garlic\textsuperscript{30, 31} and to a lesser degree red clover\textsuperscript{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\textsuperscript{15, 16, 33}. Some organosulfur compounds like alliin, allicin and s-allyl cysteine have been accepted to play major role in this protective function\textsuperscript{34-38}. Despite all these findings, no exact mechanism(s) and active component(s) in the garlic extract have been clarified yet.

Red clover has also long been used to treat cancer and acoustic tumours 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 as an alternative to surgery and radiation therapy\textsuperscript{25, 26, 32, 39}. Red clover contains high amounts of isoflavone compounds such as genistein\textsuperscript{26}. Several researchers have shown that these isoflavones may help to prevent cancer\textsuperscript{26, 27}. In a study, it has been demonstrated that isoflavone derivatives inhibit the cell growth of stomach cancer lines \textit{in vitro}\textsuperscript{26} 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 has been found to inhibit carcinogen activation in cells in culture medium\textsuperscript{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 leukaemia and non-Hodgkin’s lymphoma. Inhibition of the ADA enzyme by purine nucleoside analogs such as Fludarabine is the rational basis for use of these analogs in the cancer therapy.

In the context 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 percentages of garlic and red clover extracts 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 such study aiming to investigate possible effects of these herbal 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.

Although there are several hypothetical explanations on how garlic and red clover extracts play part in the cancer preventive events, none has given satisfactory explanation yet. For example, it has been reported that organosulfur components might inhibit several tumour progressions in experimental animals. However, mechanism(s) of action 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 and, another isoflavone namely, biochanin A can inhibit tumour growth in stomach cancer cell lines. However, mechanism(s) of action of these compounds have neither been documented yet. Lowered Vmax and Km values, which are indications of high affinity of the enzyme against its substrate under inhibition conditions, reveal mixed type inhibition mechanisms for all the substances used in the present study.

### Table 1: Vmax (mIU/mg) and Km values (μM) of adenosine deaminase (ADA) enzyme activities in cancerous and non-cancerous (control) human liver tissues measured in conditions with and without inhibitor (Mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n=10)</th>
<th>Cancer (n=10)</th>
<th>Student’s t-test Control vs Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vmax</td>
<td>Km</td>
<td>Vmax</td>
</tr>
<tr>
<td>A (without inhibitor)</td>
<td>3.99±1.33</td>
<td>145±46</td>
<td>3.08±1.24</td>
</tr>
<tr>
<td>B (with Garlic)</td>
<td>0.84±0.40</td>
<td>50±22</td>
<td>0.44±0.28</td>
</tr>
<tr>
<td>C (with Red clover)</td>
<td>1.68±0.86</td>
<td>80±25</td>
<td>1.07±0.60</td>
</tr>
<tr>
<td>D (with Fludarabine)</td>
<td>2.61±1.52</td>
<td>100±26</td>
<td>2.36±1.10</td>
</tr>
</tbody>
</table>

**Student’s t-test**

| A vs B       | P<0.005 | P<0.005 | P<0.005 | P<0.005 | -      | -      |
| A vs C       | P<0.01  | P<0.005 | P<0.005 | P<0.005 | -      | -      |
| A vs D       | P<0.05  | P<0.01  | P<0.05  | P<0.005 | -      | -      |
| B vs C       | P<0.05  | P<0.05  | P<0.01  | n.s.    | -      | -      |
| B vs D       | P<0.005 | P<0.01  | P<0.005 | P<0.05  | -      | -      |
| C vs D       | P<0.05  | P<0.05  | P<0.005 | P<0.05  | -      | -      |
**Research Article**

**Conclusion**

The results suggest that the constituents of aqueous garlic and red clover extracts both exert higher inhibitions on the ADA activity and the inhibition percentage induced by the extracts are higher than Fludarabine in the cancerous tissues as compared with those of non-cancerous tissues. This might be the rational basis for the use of these herbs in the alternative cancer therapy in the folk medicine.

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