Anticarcinogenic Activity of Allylmercapto-
captopril Against Aflatoxin-B1 Induced Liver 
Carcinoma in Rats

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ABSTRACT

Aim: The present study was aimed to investigate the cellular and mo-
lecular mechanisms of protective effects of allylmercaptocaptopril 
(AMC) against liver carcinoma induced by Aflatoxin B1, a potent in-
ducer of liver cancer.

Method: In this study we determined the protective effect of AMC 
on liver tissue, as well as on enzymatic liver functions by estimating 
glycolytic enzymes like hexokinase, phosphoisomerase and aldolase, 
gluconeogenic enzymes like glucose-6-phosphatase and fructose 1,6 
biphosphatase. Determination of total protein, DNA and RNA content 
also made to elucidate its action.

Result: Aflatoxin B1 treatment to rats resulted in significantly el-
evated levels of glycolytic enzymes like hexokinase, phosphoglucosi-
merase and aldolase and along with significant decrease in serum 
total protein, gluconeogenic enzymes and DNA and RNA content when 
compared to the control rats. The administration of AMC to the he-
patocellular carcinoma bearing rats resulted in restoration of most 
of enzymatic liver functions and also total protein content, DNA and 
RNA content.

Conclusion: Allylmercaptocaptopril has an ability to modulate the 
function of glycolytic and gluconeogenic enzymes, DNA and RNA syn-
thesis in hepatocellular carcinoma which proved its anticarcinogenic 
activity.

Key words: Hepatocellular carcinoma, aflatoxin B1, allylmercapto-
captopril, glycolytic enzymes, gluconeogenic enzymes

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant neoplasm of hepatocytes and constitutes more than 80% of primary malignant liver neoplasms in the world (1). Given that the burden of chronic liver disease is expected to rise owing to increasing rates of alcoholism, hepatitis B and C prevalence and obesity-related fatty liver disease and chemicals like aflatoxin, CCl4, nitrosodiethylamine, etc (2). Cancer of the liver is the sixth most common type of cancer worldwide, with 625,000 cases recorded. Globally, liver cancer accounts for 5.6% of all cancers in humans with more cases diagnosed in males (where it accounts for 7.5% of all cancers) than females (3.5% of all cancers) (3).

Aflatoxin is a toxin produced by Aspergillus flavus and A. Parasiticus. The different types of aflatoxins produced were designated as follows due to their blue and yellow-green autofluorescence B1, B2, and G1, G2. Aflatoxin B1 is the most potent. The International Cancer Institute identified aflatoxin, CCl4, nitrosodiethylamine, etc (2). Cancer of the liver is the sixth most common type of cancer worldwide, with 625,000 cases recorded. Globally, liver cancer accounts for 5.6% of all cancers in humans with more cases diagnosed in males (where it accounts for 7.5% of all cancers) than females (3.5% of all cancers) (3).

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Allylmercaptocaptopril was synthesized by previous reported method by Miron et al (8), with some major modifications. First, Allicin (diallyl disulfide oxide, Fig. 1) was synthesized by a modified method of Stoll and Seebeck (7). Allylmercaptocaptopril (AMC) was synthesized by the addition of captopril solution (1 mmol in 7.5 ml water, pH 5.5) to an aqueous solution of 0.55 mmol allicin. The reaction was monitored by HPLC analysis until captopril was no longer detected. The reaction mixture was then acidified by hydrochloric acid and extracted by ether to remove non-reacted allicin. The water phase was extracted with ethyl acetate. The organic phase was dried by Na2SO4, filtered and evaporated, re-dissolved in ethanol, and dried by speed vacuum concentrator. The reaction product was analyzed by HPLC and its structure confirmed by IR.
NMR and mass spectrometry. IR (KBr) spectra were recorded on a Perkin Elmer FTIR spectrometer (vmax in cm⁻¹) and 1H NMR spectra were recorded in CDCl₃ on a Bruker 500 MHz Avance spectrometer using TMS as internal reference (Chemical shift in δ ppm). Mass spectra were recorded using Agilent HP5937 spectrometer. The purity of synthesized compound was checked by HPLC. The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-visible detector. The data acquisition was performed by Spincotech 1.7 software. The system was equipped with reverse phase column Gemini C18 (150 mm × 4.6 mm i.d., 5 μm) (Phenomenex, Torrance, USA). The mobile consisted of 60% methanol in water containing 0.1% formic acid at a flow rate of 0.8 mL/min. The retention time was found to be 11.6 min. Spectral data (1H NMR, IR and mass) of the synthesized compound was in full agreement with the proposed structure. IR (KBr) v cm⁻¹: 3459 (OH), 2984 (C-H), 1728 (C=O), 939 (S≡S). 1H NMR (DMSO) δ (ppm): 10.02 (s, 1H, OH), 5.02 (t, 2H, SCH₂), 1.24 (m, 3H, CH₃). MS: m/z 289 (M⁺). The reaction between captopril and allicin to form allylmercaptocaptopril is illustrated in Figure 1.

**Figure 1. Schematic illustration of the chemical reaction between captopril and allicin that forms allylmercaptocaptopril.**

Preparation of liver homogenate
At the end of the experimental period on the 15th day, the animals that were fasted overnight were killed by cervical decapitation. Blood was collected in heparinized tube. Plasma was separated by centrifugation at 3000 rpm for 20 min. Liver tissue was immediately excised from the animals, weighed and carcinomatous tissue was carefully dissected free of haemorrhagic, necrotic and non-tumour material. Ten percent homogenate was prepared in 0.1M, Tris HCl buffer (pH 7.4) using a Potter Elvehjem homogenizer.

Biochemical analysis
The protein content (9) and glycolytic enzymes like hexokinase (10), phosphoglucoisomerase (11), aldolase (12) and gluconeogenic enzymes like glucose-6phospha-
Anticarcinogenic activity of allylmercaptocaptopril

tase (13-14) and fructose-1,6-biphosphatase (15) were assayed. The nucleic acids from the tissues were extracted by the method of Schneider with trichloroacetic acid. RNA and DNA were estimated by the method of Burton and Rawal et al respectively (16-17).

Statistical analysis

All data were expressed as mean±SD. The groups were compared using one-way ANOVA followed by Turkey multiple comparison test.

RESULTS

Biochemical changes

The variations of DNA, RNA and protein content in different groups of animals were depicted in Table 1. An increase in the level of DNA and RNA was observed in case of group II animals in which hepatocellular carcinoma was untreated. In group III, the levels of both DNA and RNA were decreased to near normal levels in which the animals were treated with AMC (50mg/kg). In group IV there was no significant change in the DNA and RNA levels when compared to normal group. In contrast there was a steep fall in the protein content in group II animals when compared to group I. But in group III, which consists of drug treated animals the protein content was restored to its normal levels.

In group IV there is no observable change in protein content.

Table 2 and 3 shows the activities of glycolytic and gluconeogenic enzymes in different group of animals. There was an increase in the activity of hexokinase, phosphoglucoisomerase and aldolase in liver homogenate in group II animals when compared to normal group of rats. The drug treated animals has shown a decrease in the activities of glycolytic enzymes when compared to group II and were nearer to normal group, due to the drug treatment in group III. While in group IV animals there was no significant change in activity of glycolytic enzymes.

On the other hand there was a fall in the activity of gluconeogenic enzymes in group II animals when compared to normal group. But in case of drug treated animals group III these levels were elevated when compared to cancer induced rats and were approximately parallel to the normal group of rats. Thus, the deviation in the enzyme activities in cancer induced rats were counteracted by the drug treatment and were almost similar to the normal group of animals. In group IV the values were approximately nearer to normal group and there is no significant change.

DISCUSSION

It is commonly observed that cancer cells possess an abnormal pattern of energy metabolism when compared to normal cells. Studies on experimental rat hepatomas have shown that metabolic alterations occurring in the

Table 1. Effect of AMC on DNA, RNA and protein content in liver cancer induced rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA, mg/g</td>
<td>6.06±0.24*</td>
<td>8.71±0.31</td>
<td>5.76±0.17**</td>
<td>6.23±0.38***</td>
</tr>
<tr>
<td>RNA, mg/g</td>
<td>3.83±0.16*</td>
<td>5.13±0.52</td>
<td>4.02±0.47*</td>
<td>3.93±0.24***</td>
</tr>
<tr>
<td>Protein, mg/g</td>
<td>158.46±9.83*</td>
<td>89.33±8.47</td>
<td>150.37±9.01*</td>
<td>153.52±9.27***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D; n=5. Statistical significance: (*p<0.01 and NS-Not Significant). One way ANOVA followed by Tukey test.

Table 2. Effect of AMC on glycolytic enzymes in Aflatoxin B1 induced liver carcinoma in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>9.93±0.48*</td>
<td>15.02±1.53</td>
<td>10.56±0.84*</td>
<td>10.02±0.57***</td>
</tr>
<tr>
<td>Phospho-glucoisomerase</td>
<td>12.47±0.34*</td>
<td>16.39±1.87</td>
<td>11.18±0.81*</td>
<td>11.97±0.86**</td>
</tr>
<tr>
<td>Aldolase</td>
<td>11.56±0.29*</td>
<td>14.80±1.81</td>
<td>10.96±0.53*</td>
<td>11.01±0.72***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D; n=6. Statistical significance: (*p<0.01, and NS-Not Significant). One way ANOVA followed by Turkey test. Units are expressed as follows: Hexokinase- Nanomoles of glucose-6-phosphatase liberated; Phosphoglucoisomerase - Nanomoles of fructose liberated; Aldolase - Nanomoles of glyceraldehyde liberated.
Tumours are often accompanied by the changes in the activities of various enzymes, including key enzymes of carbohydrate metabolism (18). The present study depicts that there was an increase in the activities of glycolytic enzymes i.e. hexokinase, phosphoglucoisomerase and aldolase in cancer induced rats. This infers that the rate of glycolysis was enhanced during tumour growth (19). There exists a direct correlation between glycolytic activity and hexokinase in a variety of tumour cell lines (20).

The function of hexokinase is to metabolize glucose to glucose-6-phosphatase. It is present in liver and plays an important role in phosphorylation of glucose in glycolysis, glucose transport. Hence, the fast growing cells like tumours catabolise large amounts of glucose and its metabolic pathway is activated more in the direction of hexose monophosphate shunt rather than gluconeogenesis (21). The levels of hexokinase in carcinoma bearing animals group II greatly exceed than normal group of rats group I. Phosphoglucoisomerase is helpful for the conversion of glucose-6-phosphate to fructose-1,6-biphosphate and it regulates the growth of several types of cells. This is an indicator of metastatic growth and increases specifically after metastasis. This may be one of the reasons for its elevated levels in the liver (22). The phosphoglucoisomerse activity was found to be more in group II hepatocarcinoma induced animals when compared to group I. The function of aldolase is to convert fructose-1,6-biphosphate to glyceraldehyde-3-phosphate and is an important step in glycolysis. Since glycolysis is most favourable pathway that promotes the invasion and metastasis of tumour cells. Aldolase levels were elevated in cancer induced animals (group II) but the extent of increase is less when compared to other enzymes (23).

Gluconeogenesis takes place in liver, this reveals that any disease that affect liver also alters the levels of gluconeogenic enzymes (24). Glucose-6-phosphatase hydrolyses glucose-6-phosphate before being liberated as glucose into the circulation. There will be a decreased rate of glucose-6-phosphate mediated dephosphorylation in malignant cells (25). The result also depicts that there is a marked fall in glucose-6-phosphatase levels in diseased rats (group II) when compared to normal.

Fructose-1,6-biphosphatase is a gluconeogenic enzyme and is helpful for the synthesis of smaller sub-

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</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphatase</td>
<td>31.83±1.60*</td>
<td>19.89±1.93</td>
<td>26.44±1.53*</td>
<td>29.13±0.83NS</td>
</tr>
<tr>
<td>Fructose 1,6-biphosphatase</td>
<td>23.05±1.02*</td>
<td>18.76±2.01</td>
<td>21.08±1.21*</td>
<td>22.13±0.97NS</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D; n=6. Statistical significance: (*p<0.01, and NS-Not Significant). One way ANOVA followed by Tukey test. Units are expressed as follows: Glucose-6-phosphatase and fructose-1,6-biphosphatase - Nanomoles of inorganic phosphorus liberated per min per mg protein.
strates from which glucose is synthesized. It synthesizes glucose-6-phosphate from pyruvic acid. Fructose-1,6-biphosphatase levels were found to be decreased in hepatoma induced rats (group II) when compared to normal. Fructose-1,6-biphosphatase levels were found to be decreased during the tumour growth which may block the synthesis of glucose (26).

The DNA content directly relates to the size of the tumour and is an indicator of tumour prognosis (27). There is an increase in the RNA content in tumour bearing animals (group II), but not as significant as DNA. Increase in RNA content may be due to increased transcription. The decreased protein content in cancer induced animals (group II) may be due to decreased RNA polymerase synthase and also due to increase in protein degradation rate and implies the underlying metabolic imbalance. The total protein content was recovered in the group III drug treated animals. Moreover, the histological studies reveals that, the liver sections of group II aflatoxin B1 induced rats (Figure 2) shows marked congestion of central vein and intense cytoplasmic granules in hepatocytes this was found to be due to nuclear segregation (28). Alteration in the synthesis of ribosomal precursors by various agents which bind to DNA and RNA synthesis and protein synthesis may also be partly responsible for ultrastructural changes observed.

The liver section of group III drug treated rats shows almost normal architecture as depicted in Figure 3 which is due to the anticarcinogenic activity of the AMC, there is hyperplasia of parenchymal cells in group II animals and contributes to the development of hepatocellular carcinoma in two ways. Firstly by cell proliferation and secondly by carcinogen altered hepatocytes appear better to survive in presence of hepatotoxin (29). The liver section of Figure 3 can be compared from Figure 2 which have normal architecture without any signs of carcinoma and Figure 4 which was from drug treated rats shows almost similar structure to Figure 2. In group IV animals there is no significant change in the glycolytic enzymes, gluconeogenic enzymes, DNA and RNA content and protein content. This suggests that the AMC exerts anticarcinogenic activity without causing any alterations in biochemical pathways in normal condition.

From the above experimental results it may be concluded that the allylmercaptocaptopril has an ability to modulate the function of glycolytic and gluconeogenic enzymes, DNA and RNA synthesis in hepatocellular carcinoma with its anticarcinogenic activity. It has a definite beneficial role against aflatoxin B1 induced hepatocellular carcinoma.

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