Inhibition of liver glutaminase activity by Diallyl disulfide in experimentally induced hepatoma in mice

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Abstract

Background: Cancer cells addiction to glutamine, an essential non-essential amino acid, has stirred up the interest in researchers across the globe. Increased glutamine metabolism (glutaminolysis) is a hallmark of cancer. Targeting glutaminolysis via glutaminase inhibition emerges as a promising strategy to disrupt cancer metabolism and tumor progression. Diallyl disulfide (DADS), a major organosulfur compound derived from garlic, is known for its anticancer properties. The mechanisms of action of DADS include activation of metabolic enzymes that detoxify carcinogens, suppression of the formation of DNA adducts, antioxidant effects, regulation of cell-cycle progression, induction of apoptosis, and inhibition of angiogenesis and metastasis.

Aim: to assess the effect of diallyl disulfide on liver glutaminase activity in experimentally induced hepatoma in mice.

Materials & Methods: Swiss albino male mice were divided into four groups - normal, control, preventive and curative groups. Hepatoma was induced by intraperitoneal injection of Ehrlich ascites carcinoma (EAC) cells. DADS (100 mg/kg body weight/mouse/day) was orally fed to protective and curative group mice for a stipulated time period. Mice of all the groups were sacrificed, and liver tissue glutaminase activity were measured.

Results: The present study shows a significant decrease in glutaminase activity in protective ($p > 0.001$) and curative groups ($p > 0.001$) as compared to control group.

Conclusion: DADS at the dosage employed shows inhibitory effects on liver glutaminase activity which may be attributed to anti-inflammatory properties of DADS, specifically in suppression of NF-kB signalling pathway.

Key words: diallyl disulfide, glutaminase, glutaminolysis, hepatoma, cancer, glutamine

Introduction

The process of tumorogenesis is often associated with altered metabolism of two major nutrients, glucose and glutamine[1]. Glucose is considered to be the most important source of energy in proliferating and transformed cells as these cells perform elevated glycolytic activity i.e., "Warburg effect"[2]. Equally important is glutamine, an essential non-essential amino acid that provides a stable nitrogen and carbon pool for protein, nucleotide, and lipid biosynthesis[3]. Glutaminase is the key enzyme of glutamine metabolism. Increased glutamine metabolism (glutaminolysis) is a hallmark of cancer. Glutaminolysis is the major mechanism for TCA anapleurosis to replenish TCA intermediates diverted for nucleotide synthesis for generating cellular energy[4]. Targeting glutaminolysis via glutaminase inhibition emerges as a promising strategy to disrupt cancer metabolism and tumor progression[4,5]. Diallyl disulfide (DADS), a major organosulfur compound derived from garlic, is known for its anticancer properties[6]. The mechanisms of action of DADS include activation of metabolic enzymes that detoxify carcinogens, suppression of the formation of DNA adducts, antioxidant effects, regulation of cell-cycle progression, induction of apoptosis, and inhibition of angiogenesis and metastasis[7]. The present study is undertaken to assess the effect of DADS on liver glutaminase activity in Ehrlich ascites carcinoma (EAC)-induced hepatoma in mice.

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Materials & Methods:

Experimental animals:

Twenty-four Swiss albino male mice weighing 25-30 g were randomly selected from the Institutional animal house and employed in the present study. Mice were kept in plastic well aerated cages and were served standard animal feed and water *ad libitum.* DADS was procured from Sigma-Aldrich Pvt. Ltd. USA. All other chemicals that we have used in the present study were of A.R. grade. Ethical clearance was obtained from Institutional Animal Ethical Committee. The experiments were conducted as per the norms of CPCSEA, New Delhi.

Invivo- cell line maintenance and induction of hepatoma:

The EAC tumor-bearing Swiss albino mice were kindly provided by the Amala Cancer Research Institute, Thrissur, Kerala. EAC cells were thereafter propagated by weekly intraperitoneal injection of freshly drawn ascitic fluid (0.5 ml) from a donor mouse bearing ascites tumor of 8-10 days old into healthy Swiss albino male mice at our Institutional animal house. Transplantation was carried out using sterile disposable syringes under aseptic conditions. Liver histology showed areas of fibrotic and necrotic changes with hyperchromatism in EAC-bearing mice.

Animal study groups and experimental design:

The mice were divided into 4 groups - normal group (Group-I), control group [EAC induced hepatoma bearing mice] (Group-II), protective group [DADS treated-EAC induced hepatoma bearing mice] (Group-III) and curative group [EAC induced hepatoma bearing- DADS treated mice] (Group-IV).

The details of animal groups and experimental design; animal maintenance, DADS (100 mg/kg body weight) dosage, animal sacrifice and liver extractions and processing, are as explained in our earlier study[8]. The liver tissue processing for the estimation of glutaminase activity[9] is as follows. To 0.3 g liver tissue, 4.7 ml of cold phosphate buffer (pH 7.4) was added and thoroughly homogenized for 5 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was employed for the enzyme assay. Data analysis was done using SPSS software version 24 (IBM, Armonk, NY, USA) and Student 't' test was employed for statistical analysis. The 'p' value < 0.05 was considered significant.

Results:

It is evident from table-1 and chart-1 that glutaminase activity in liver tissue are significantly raised ($p<0.001$) in group-II as compared to group-I, whereas the same parameters are significantly lowered ($p<0.001$) in group-III and group-IV as compared to group-II.

Table-1 showing the liver tissue glutaminase activity in group-I, group-II, group-III and group-IV mice.

Groups ↓ / Parameter →	Glutaminase activity (units)
Group-I: Normal group (6)	18.37± 1.17
Group-II : Control group (6)	31.12*** ± 1.52
Group-III : Protective group(6)	22.11*** ± 0.95
Group-IV : Curative group (6)	24.82*** ± 1.38

Note:

- The number in parentheses indicates the number of mice.
- The values are expressed as their mean ± SD.
- Probability level - * $p<0.05$, ** $p< 0.01$, *** $p<0.001$.
- Glutaminase activity: 1 unit = µg NH_3 liberated /g liver/min.

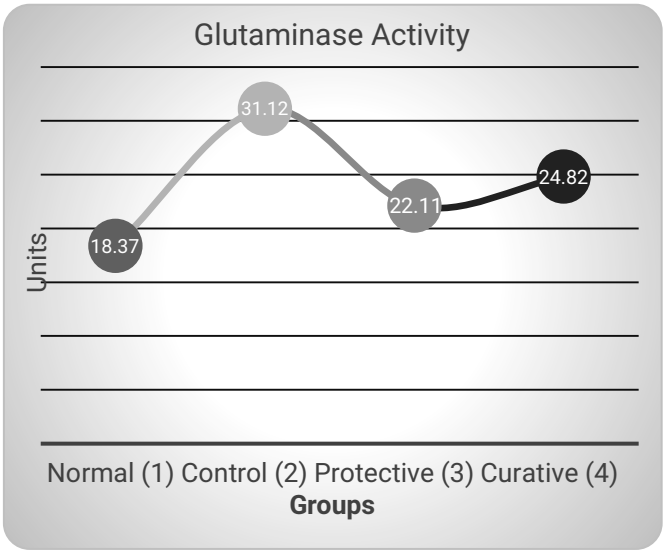

Chart-1 showing the liver tissue glutaminase activity in group-I, group-II, group-III and group-IV mice.

Discussion

The liver tissue glutaminase activity is significantly raised in group-II ($p<0.001$) as compared to group-I (refer table-1 and chart-1). It has been previously reported that amino acids, especially glutamine, play an important role in tumor cell proliferation[4,5] as they behave as "glutamine trappers"[10]. The conversion of glutamine to glutamate is catalyzed by mitochondrial glutaminase enzyme[11]. (refer figure-1)

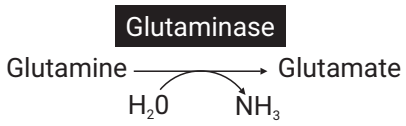

Figure 1

Glutamine, through glutaminolysis, serves as both anaplerotic re-feeder of the TCA cycle and NADPH demands fulfiller for growth[4]. Also, glutamine was found to be the most rapidly consumed amino acid by Ehrlich ascites carcinomas as well as by a number of hepatomas and carcinosarcomas proliferating in vivo[12,13].The rate of glutaminase activity correlates with the rate of tumor growth and is found to be over expressed in a variety of tumor models and human malignancies[14-16]. The observed significant raise in glutaminase activity in group-II (control) mice suggests that this carcinoma is highly glutamine dependent. Several studies have reported that glutaminase inhibitors have anti-proliferative activity in vitro and in vivo in mice in a wide range of cancers[5]. A significant decrease ($p > 0.001$) in glutaminase activity in preventive and curative groups (refer table-1 and chart-1) suggests that DADS at the dosage employed has inhibitory effect on glutaminase activity. Several other research studies have suggested that DADS exhibits its anti-inflammatory effects by suppressing nuclear factor kappa B (NF-kB)[17]. NF-kB, a proinflammatory transcription factor that is hypothesized to promote tumorigenesis by regulating the expression of genes that are involved in cell proliferation, apoptosis, and metastasis[17,18]. Suppression of NF-kB in tumor cells usually leads to tumor regression, which makes the NF-kB pathway a promising therapeutic target[17]. Research findings from Wang JB et.al[14] suggests that NF-kB is essential for glutaminase activation in cancer cells. We hypothesize that DADS at the dosage of 100 mg/kg body weight, by suppressing the NF-kB, might have in turn inhibited the expression of glutaminase enzyme and cancer progression.

Conclusion:

In conclusion, DADS at the dosage employed has inhibitory effects on liver glutaminase activity which may be attributed to anti-inflammatory properties of DADS, specifically in suppression of NF-kB signalling pathway. Targeting glutaminolysis via glutaminase inhibitors can inhibit oncogenic transformation. As prospects, more clinical trials with DADS and other organosulfur compounds in cancer research would perhaps give researchers and clinicians a deeper insight in to their therapeutic values.

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