

## S-ALLYLCYSTEINE INHIBITS CIRCULATORY LIPID PEROXIDATION AND PROMOTES ANTIOXIDANTS IN N-NITROSODIETHYLAMINE-INDUCED CARCINOGENESIS

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Effects of S-allylcysteine (SAC), an organosulfur compound of garlic, on circulatory lipid peroxidation and antioxidant levels were evaluated in N-nitrosodiethylamine (NEDA)-induced hepatocarcinogenesis in Wistar rats. Significantly elevated thiobarbituric acid reactive substances in the circulation of rats bearing carcinoma indicated the higher levels of lipid peroxidation which was accompanied by significantly decreased levels of antioxidants ( $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol, reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase) when compared with controls. Lipid peroxidation has been implicated as a major cause in cancer development. SAC-administered rats showed the inhibition of tumor incidence and lipid peroxidation with simultaneous elevation in antioxidants. We suggest that SAC exerts its chemopreventive effects by decreasing lipid peroxidation and enhancing the levels of antioxidants in NDEA carcinogenesis by reducing the formation of free radicals.

**Key words:** *antioxidants, hepatocarcinogenesis, lipid peroxidation, N-nitrosodiethylamine, S-allylcysteine*

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## INTRODUCTION

Hepatocellular carcinoma is one of the most common cancers and one of the most lethal [30]. Chemoprevention involving the use of synthetic or natural products to inhibit or reverse the carcinogenic process is an effective approach to control cancer [24]. Epidemiologic and clinical studies have shown that consumption of garlic (*Allium sativum*) reduced the risks of cancer incidence [21, 32]. Garlic and its organosulfur compounds [including S-allylcysteine (SAC)] have been reported to possess antitumorogenic, antibiotic, detoxifying and other properties [3, 8, 31]. Free radical scavenging properties and the antioxidant health effects of SAC has been reported [7, 19]. SAC had been demonstrated to protect vascular endothelial cells from hydrogen peroxide-induced injury [41] and hepatocytes from carbon tetrachloride toxicity [26]. SAC was found to decrease the doxorubicin-induced toxicity in the heart and liver of mice [25]. The antiproliferative effects of SAC have been documented against canine mammary human HCT-15 colon, A549 lung, SKMEL-2 skin, umbilical vein endothelial cells, smooth muscle, melanoma, neuroblastoma, prostate (LNCaP) cancer cell lines [35, 39]. Results of Yeh and Lijuan [42] reported the cholesterol lowering effects and inhibition of hepatic cholesterol synthesis by SAC. The expression of cell surface gangliosides, which are tumor-associated markers of cell differentiation and transformation, was significantly inhibited by SAC [39]. SAC is recognized to be effective in blocking benzopyrene-, 7,12-dimethylbenz(a)anthracene (DMBA)- and dimethylhydrazine (DMH)-induced preneoplastic lesions [1, 18]. Furthermore, SAC was shown to inhibit the DMH-induced colon cancer and N-methylnitrosourea-induced mammary tumorigenesis [34].

It is well known that N-nitroso compounds act as strong carcinogens in various mammals including primates [23]. Foodstuffs such as milk and meat products, salted fish, alcoholic beverages and a few varieties of vegetables are the principal sources of nitroso compounds [37]. N-nitrosodiethylamine (NDEA) has been suggested to cause oxidative stress and cellular injury due to the enhanced formation of free radicals. NDEA has been shown to be metabolized to its active ethyl radical metabolite, and the reactive product interacts with DNA causing mutation which would lead to carcinogenesis [2]. Experimental, clinical and epidemiological

studies have provided evidences supporting the role of reactive oxygen species in the etiology of cancer [28].

Several biochemical markers have been suggested for biomonitoring the actions of anticancer agents. Estimation of circulatory lipid peroxidation end products [thiobarbituric acid reactive substances (TBARS), non-enzymatic antioxidants – reduced glutathione (GSH),  $\beta$ -carotene, ascorbic acid and  $\alpha$ -tocopherol, activities of enzymatic antioxidants – superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] as biochemical markers is a reliable method for screening the action of chemopreventive agents. In the present study, a systematic investigation of the protective influences of SAC against NDEA-induced hepatic carcinoma has been carried out by analyzing the circulatory TBARS, non-enzymatic and enzymatic antioxidants as biochemical end points of chemoprevention

## MATERIALS and METHODS

### Chemicals

NDEA was purchased from Sigma Chemical Co., USA. SAC was kindly provided as gift by Wakunaga Pharmaceuticals Co. Ltd., Japan. All other chemicals and biochemicals used in our experiment were of analytical grade.

### Animals

Male Wistar rats (6–8 weeks old) were obtained from Central Animal House, Faculty of Medicine, Annamalai University. The animals were housed in polypropylene cages and provided with food pellets (Mysore Snack Feed Ltd., Mysore, India) and water *ad libitum*. Animals were handled, ethically treated and sacrificed according to the rules and instructions of Ethical Committee for Animal Care of the Annamalai University in accordance with the Indian National Law on animal care and use.

### Treatment schedule

Animals were randomized and divided into four groups (n = 6 in each group). Animals in group I (untreated control) received neither NDEA nor SAC. Group II (NDEA) animals received single intraperitoneal injection of NDEA (200 mg/kg) followed by weekly subcutaneous injections of carbon tetrachloride (3 ml/kg) for 6 weeks. Group III (NDEA +

SAC) animals received single intraperitoneal injection of NDEA (200 mg/kg) followed by weekly subcutaneous injections of carbon tetrachloride (3 ml/kg) for 6 weeks as in group II (NDEA). In addition, the animals were administered with SAC (200 mg/kg) [5, 31, 34] on alternate days by oral gavage. Group IV (SAC) animals received SAC alone as in group III (NDEA + SAC).

### Biochemical determinations

Thiobarbituric acid reactive substances (TBARS) released from endogenous lipid peroxides reflecting the lipid peroxidation process were assayed in plasma by the method of Yagi [40] and in erythrocytes by Donnan procedure [12]. GSH was determined by the method of Ellman [14]. GP<sub>x</sub> activity was evaluated by following utilization of hydrogen peroxide by the method of Rotruck et al. [29]. Activities of SOD and CAT were assayed by Kakkar et al. [20] and Sinha [33], respectively.  $\beta$ -Carotene [6] ascorbic acid [27],  $\alpha$ -tocopherol [4] and hemoglobin [13] were also determined in all groups.

### Statistical analysis

The data were presented as means  $\pm$  SD and were subjected to analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (DMRT). The results were considered significant if the p value was 0.05 or less.

## RESULTS

Table 1 shows the effects of SAC on NDEA-induced tumor incidence. The NDEA-treated group showed 100% tumor incidence whereas in groups III (NDEA + SAC) and IV (SAC) no malignancies were seen.

Table 2 depicts the levels of TBARS in plasma and erythrocytes of control and experimental groups.

Table 1. Effect of SAC on NDEA-induced hepatocarcinogenesis

Groups	Treatment	No. of animals	No. of tumor bearing animals	% of tumor incidence
I	Normal	6	0	0
II	Control NDEA	6	6	100
III	NDEA + SAC	6	0	0
IV	SAC	6	0	0

Table 2. Effects of SAC on plasma and erythrocyte TBARS in control and experimental groups

Groups	Plasma TBARS (nmoles/ml)	Erythrocyte TBARS (pmol/mg Hb)
Normal	2.16 $\pm$ 0.19 <sup>a</sup>	1.80 $\pm$ 0.12 <sup>a</sup>
NDEA treated	4.40 $\pm$ 0.34 <sup>b</sup>	3.64 $\pm$ 0.21 <sup>b</sup>
NDEA + SAC	2.96 $\pm$ 0.29 <sup>c</sup>	2.20 $\pm$ 0.16 <sup>c</sup>
SAC	1.96 $\pm$ 0.18 <sup>a</sup>	1.52 $\pm$ 0.22 <sup>a</sup>

Values are means  $\pm$  SD, n = 6. Values not sharing a common superscript differ significantly (p < 0.05, Duncan's multiple range test)

Table 3. Effects of SAC on circulatory enzymatic and non-enzymatic antioxidants in control and experimental groups

Parameters	Group I Normal	Group II NDEA	Group III NDEA + SAC	Group IV SAC
Plasma				
GSH (mg/dl)	38.18 $\pm$ .59 <sup>a</sup>	18.72 $\pm$ 1.71 <sup>b</sup>	33.33 $\pm$ 1.69 <sup>c</sup>	44.44 $\pm$ 2.30 <sup>d</sup>
Vit. C (mg/dl)	1.83 $\pm$ 0.14 <sup>a</sup>	1.12 $\pm$ 0.06 <sup>b</sup>	1.65 $\pm$ 0.06 <sup>a</sup>	2.03 $\pm$ 0.10 <sup>a</sup>
Vit. E (mg/dl)	1.64 $\pm$ 0.05 <sup>a</sup>	0.88 $\pm$ 0.04 <sup>b</sup>	1.42 $\pm$ 0.13 <sup>a</sup>	1.85 $\pm$ 0.08 <sup>a</sup>
Vit. A (mg/dl)	1.26 $\pm$ 0.07 <sup>a</sup>	0.72 $\pm$ 0.03 <sup>b</sup>	1.04 $\pm$ 0.06 <sup>a</sup>	1.48 $\pm$ 0.09 <sup>a</sup>
Erythrocytes				
GSH (mg/dl)	41.28 $\pm$ .08 <sup>a</sup>	24.8 $\pm$ 2.89 <sup>b</sup>	34.02 $\pm$ 2.59 <sup>a</sup>	52.4 $\pm$ 0.08 <sup>d</sup>
GPx (U <sup>A</sup> /mg Hb)	11.49 $\pm$ .87 <sup>a</sup>	6.32 $\pm$ 0.29 <sup>b</sup>	10.73 $\pm$ 0.52 <sup>a</sup>	16.82 $\pm$ 1.24 <sup>d</sup>
SOD (U <sup>B</sup> /mgHb)	2.69 $\pm$ 0.17 <sup>a</sup>	1.38 $\pm$ 0.11 <sup>b</sup>	2.12 $\pm$ 0.10 <sup>c</sup>	3.15 $\pm$ 0.13 <sup>d</sup>
CAT (U <sup>C</sup> /mg Hb)	2.49 $\pm$ 0.09 <sup>a</sup>	1.32 $\pm$ 0.07 <sup>b</sup>	2.16 $\pm$ 0.06 <sup>c</sup>	3.19 $\pm$ 0.09 <sup>d</sup>

Values are mean  $\pm$  SD, n = 6. Values not sharing a common superscript differ significantly (p < 0.05, Duncan's multiple range test). A = micromoles of GSH utilized/min/mg Hb, B = amount of enzyme required to inhibit 50% NBT reduction, C = micromoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg Hb

Lipid peroxidation was significantly higher in group II (NDEA) than in group I (control). Further, the levels of TBARS in plasma and erythrocytes were significantly lower in groups III (NDEA + SAC) and IV (SAC) compared with group II (NDEA) and group I (control), respectively.

Table 3 summarizes the levels of antioxidants in plasma and erythrocytes in control and experimental groups. The levels of  $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol, GSH, GPx, SOD and CAT were significantly lower in group II (NDEA); whereas in groups III (NDEA + SAC) and IV (SAC) the levels of antioxidants ( $\beta$ -carotene, ascorbic acid,  $\alpha$ -tocopherol, GSH, GPx, SOD and CAT) were increased significantly when compared with group II (NDEA) and group I (control), respectively.

## DISCUSSION

The results showed that administration of SAC could prevent NDEA-induced hepatocarcinogenesis in rats. It has been reported that NDEA is metabolized to alkylating reactants, which could interact with DNA molecule and initiate carcinogenesis [2]. Although many risk factors have been reported, lipid peroxidation plays an important role in hepatic carcinogenesis. Further, enhanced lipid peroxidation associated with depletion of antioxidants is a characteristic finding in a variety of malignancies.

In our study, the increased levels of TBARS in the rats of group II could be ascribed to the excessive generation of free radicals. Szatrowski and Nathan [36] have suggested that tumor cells produce substantial amount of hydrogen peroxide and reactive oxygen metabolites that are released into the circulation. Therefore, the increased susceptibility of plasma and red blood cells of NDEA-administered rats could be due to the production of reactive oxygen metabolites during the metabolism of NDEA or during the process of carcinogenesis. Decreased levels of TBARS in SAC-treated rats suggest the SAC could scavenge free radicals produced by NDEA. SAC has been reported to inhibit the lipid peroxidation and scavenge reactive oxygen species [19].  $\beta$ -Carotene and its derivatives are involved in epithelial cell differentiation and are known to prevent carcinogenesis. GSH is the major cytosolic thiol compound which plays important cellular functions including destruction of hydrogen peroxide, lipid peroxides and free radicals [15]. GSH

also preserves the cellular levels of active forms of  $\beta$ -carotene, ascorbic acid and  $\alpha$ -tocopherol [16].

GPx is capable of reducing free hydrogen peroxide and lipid hydroperoxides. SOD and CAT provide the first defence against oxygen toxicity by catalyzing the dismutation of superoxide anion to hydrogen peroxide and decomposition of hydrogen peroxide to water and molecular oxygen. Earlier reports showed the decreased activities of GPx, SOD and CAT in hepatoma [10].

Decreased activities of GPx, SOD and CAT in NDEA-treated rats could be due to overutilization of these non-enzymatic and enzymatic antioxidants to scavenge the products of lipid peroxidation. Tumor cells have been reported to sequester essential antioxidants from the circulation, in order to meet the demands of the growing tumor [9]. The enhancement of detoxifying and antioxidant enzymes in the circulation by SAC may be a major mechanism by which it ameliorates the deleterious effects of NDEA. Enhanced levels and activities of non-enzymatic and enzymatic antioxidants in SAC-treated rats indicate the chemopreventive potentials of SAC.

SAC has been reported to reduce the free radical-induced formation of 8-oxodeoxyguanosine in DNA [17]. Further, SAC is known to inhibit the mutagenicity of N-nitrosomorpholine and depress the binding of N-nitrosodimethylamine to rat liver cell DNA [11]. Thioallyl compounds such as SAC have been shown to inhibit cell proliferation [22]. Effectiveness of garlic and its organosulphur compounds is due to blocking DNA alkylation, a primary step in nitrosoamine carcinogenesis [38]. Furthermore, SAC was found to enhance circulatory antioxidants and inhibit 7,12-dimethylbenz(a)anthracene-induced buccal pouch carcinogenesis in hamsters [5]. These findings support results of our study suggesting that SAC could mediate its anticarcinogenic activity by modulating lipid peroxidation and could increase GSH and GSH-dependent enzymes. Further studies are in progress in our laboratory to evaluate the potential of SAC in counteracting NDEA-induced genotoxic and cytotoxic effects which would suggest the plausible clinical applications of SAC.

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