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# Effect of Selenium on the Antioxidative Enzymes in Rats with Mammary Tumor Induced by 7,12-Dimethylbenz(*a*)anthracene

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*Summary* The biological impact of selenium on the levels of antioxidant enzymes in Wistar rats bearing mammary tumor induced by dimethylbenz(*a*)anthracene was investigated. Control rats and tumor-bearing rats were fed a normal diet or one containing 5 mg sodium selenite/kg diet from the day of tumor induction. The reduced levels of ceruloplasmin, ascorbic acid, and $\alpha$-tocopherol seen in the serum of tumor-bearing rats on the normal diet were found to be increased by the selenium treatment. The activities of superoxide dismutase and catalase in tumor-bearing rats were decreased significantly when compared with those of control rats, whereas selenium administration caused a considerable recovery of the activities of these enzymes in the rats with tumors. The increase in the levels of these enzymes was found to be predominantly significant in the liver. These observations clearly suggest an antioxidant role for selenium in experimental mammary tumor.

*Key Words:* mammary tumor, DMBA, selenium, superoxide dismutase, catalase

Two of the current hypotheses for increased free radical generation in cancer are the coupling of the carcinogen with DNA and defective antioxidative systems resulting in the overgeneration of reactive oxygen species. The cellular defenses, enzymic and non-enzymic antioxidants, are protective against the reactive oxygen species in tumorigenesis [1]. Several investigators have attempted to understand the effect and mechanism of selenium on carcinogen metabolism [2] and carcinogen-DNA binding [3]. The protection offered by supplemental selenium appears to result from inhibition of both initiation and promotion phases of car-

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cinogenesis [4], and selenium deficiency has been shown to impair the detoxifying response of antioxidants against the acute oxidative stress generated by the carcinogen [5].

The protective effect of superoxide dismutase and catalase in macrophage- and in reactive oxygen-mediated DNA strand breaking in mammary tumor cells has been shown [6, 7]. L'Abbe *et al.* reported the reciprocal responses of selenium levels and the status of antioxidative enzymes during 7,12-dimethylbenz($\alpha$)anthracene (DMBA)-induced mammary carcinogenesis in rats [8]. Also, Chidambaram and Baradarajan examined the effect of selenium on glutathione peroxidase activity and the status of lipid peroxidation in rats with induced tumors [9].

This study was undertaken to determine the impact of selenium on nonenzymic antioxidants as well as on the activities of superoxide dismutase and catalase against the acute oxidative stress during DMBA-induced mammary tumorigenesis in rats.

## MATERIALS AND METHODS

*Animals.* Female Wistar albino rats weighing around 40–50 g were obtained from Fredrick Institute of Plant Protection and Toxicology, Padappai, Tamil Nadu, India. Rats were maintained under hygienic conditions and were fed standard rat pellets (Hindustan Lever Ltd., Bombay, India) and water *ad libitum.*

*Chemicals.* Epinephrine, 2,4-dinitrophenyl hydrazine, $\alpha,\alpha'$-dipyridyl, sodium azide, *p*-phenylene diamine hydroxide, sodium selenite, 2,3-diaminonaphthalene, and DMBA were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of analytical grade.

*Animal treatment.* For the preparation of DMBA emulsion, 25 mg of DMBA was added to 0.5 ml of sunflower oil, which was followed by 0.5 ml of 0.89% sodium chloride solution; and the mixture was vortexed to form an emulsion. For induction of mammary tumors, 1 ml of DMBA emulsion was injected subcutaneously into each rat by air pouch technique [10]. The rats were categorized as control rats (Group 1), selenite-administered control rats (Group 2), rats with tumor (Group 3) and selenite-administered rats with tumor (Group 4). Each group contained six rats. Rats in Groups 2 and 4 were fed sodium selenite at the rate of 5 mg/kg diet. The tumor-bearing rats were palpated once a week to determine the progress of tumor development. The rats were killed by cervical dislocation after 24 weeks.

*Analytical methods.* Selenium levels in serum was determined by the method of Watkinson [11]. Ascorbic acid and $\alpha$-tocopherol in serum were quantified following the methods of Omaye *et al.* [12] and Quaife and Dju [13], respectively. Serum ceruloplasmin was quantified as stated by the method of Ravin [14]. Plasma was separated from blood samples containing 3.7% trisodium citrate as anticoagulant (0.1 ml/ml blood). Erythrocytes were washed three times with isotonic saline and hemolyzed by the addition of 0.015 M Tris-HCl buffer, pH 7.2.

Hemoglobin and protein levels were estimated by the methods of Drabkin and Austin [15] and Lowry *et al.* [16], respectively. Hemolysate and the homogenates of liver and kidney were used for the assay of superoxide dismutase following the method of Misra and Fridovich, which is based on the inhibition of the oxidation of epinephrine to adrenochrome transition by superoxide dismutase [17]. The catalase activity in hemolysate and the homogenates of liver and kidney were assayed by the method of Sinha [18]. The statistical significance between different groups was compared by Student's *t*-test.

## RESULTS

The levels of selenium, ceruloplasmin, ascorbic acid, and $\alpha$-tocopherol in the serum of rats of the various groups are presented in Table 1. A significant decrease in the levels of antioxidants was noted in the serum of tumor-bearing rats compared with the levels for control animals. Whereas the increase in antioxidant levels was nonsignificant for selenium-administered control rats compared with untreated control rats. Selenium administration to rats with tumors caused a 32, 19, 16 and 16% increase in the serum levels of selenium, ascorbic acid, ceruloplasmin, and $\alpha$-tocopherol, respectively, when their levels were compared with those of tumor-bearing rats fed the normal diet.

The activity of superoxide dismutase was found to be significantly ($p<0.001$) decreased in liver, kidney, and erythrocytes of tumor-bearing rats when compared with that of the control rats (Table 2). No significant differences were discerned in the superoxide dismutase activity between selenium-administered and untreated

Table 1. Values of non-enzymatic antioxidants in serum of rats.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Selenium ($\mu$g/liter) | 0.075±0.009 | 0.082±0.011$^{N.S}$ | 0.059±0.007** | 0.087±0.01*** |
| Ceruloplasmin (mg/dl) | 3.95±0.46 | 4.07±0.42$^{N.S}$ | 3.14±0.35* | 3.72±0.26** |
| Ascorbic acid (mg/dl) | 2.67±0.39 | 2.71±0.35$^{N.S}$ | 2.06±0.24** | 2.53±0.12*** |
| $\alpha$-Tocopherol (mg/dl) | 1.32±0.16 | 1.45±0.18$^{N.S}$ | 1.03±0.12** | 1.23±0.09** |

Values are expressed as mean±SD for 6 rats. *$p<0.05$, **$p<0.01$, ***$p<0.001$, $^{N.S}$Non-significant. Compared between Groups 1 and 2, Groups 1 and 3, and Groups 3 and 4.

Table 2. Activities of superoxide dismutase in erythrocytes, liver, and kidney of rats.

| Source | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Erythrocytes | 3.18±0.43 | 3.23±0.35$^{N.S}$ | 1.98±0.24*** | 2.82±0.36*** |
| (50% inhibition of autoxidation reaction/min/mg Hb) | | | | |
| Liver | 6.15±0.74 | 6.27±0.67$^{N.S}$ | 3.69±0.48*** | 5.76±0.65*** |
| (50% inhibition of autoxidation reaction/min/mg protein) | | | | |
| Kidney | 3.09±0.32 | 3.14±0.36$^{N.S}$ | 1.63±0.21*** | 2.84±0.28*** |
| (50% inhibition of autoxidation reaction/min/mg protein) | | | | |

Values are expressed as mean±SD for 6 rats. ***$p<0.001$, $^{N.S}$Non-significant. Compared between Groups 1 and 2, Groups 1 and 3, and Groups 3 and 4.

Table 3. Activities of catalase in erythrocytes, liver, and kidney of rats.

| Source | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Erythrocytes | $0.17 \pm 0.019$ | $0.18 \pm 0.021^{N.S}$ | $0.11 \pm 0.017^{***}$ | $0.15 \pm 0.023^{*}$ |
| ($\mu$M of $H_2O_2$ consumed/min/mg Hb) | | | | |
| Liver | $66.73 \pm 7.98$ | $69.18 \pm 7.54^{N.S}$ | $37.14 \pm 5.12^{***}$ | $59.28 \pm 6.02^{**}$ |
| ($\mu$M of $H_2O_2$ consumed/min/mg protein) | | | | |
| Kidney | $7.03 \pm 0.679$ | $7.21 \pm 0.941^{N.S}$ | $4.65 \pm 0.468^{***}$ | $6.12 \pm 0.709^{**}$ |
| ($\mu$M of $H_2O_2$ consumed/min/mg protein) | | | | |

Values are expressed as mean $\pm$ SD for 6 rats. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{N.S}$Non-significant. Compared between Groups 1 and 2, Groups 1 and 3, and Groups 3 and 4.

control rats. However, selenium administration to the rats with induced tumors increased or recovered the activities of superoxide dismutase in the liver, kidney, and erythrocytes significantly ($p<0.001$).

The activity of catalase in liver, kidney, and erythrocytes of rats are depicted in Table 3. The catalase activity in liver, kidney, and erythrocytes of tumor-bearing rats was found to be significantly ($p<0.001$) lower than that of the control rats, whereas it increased significantly in liver ($p<0.01$), kidney ($p<0.01$), and erythrocytes ($p<0.05$) in selenium-administered, tumor-bearing animals. The increase in activity was more pronounced in tissues than in erythrocytes. However, the catalase activity in liver, kidney, and erythrocytes of selenium-administered control rats differed little when compared with that of untreated control rats.

## DISCUSSION

DMBA has been recognized as an initiator of mammary cancer in rats [19]. DMBA metabolites bind DNA covalently to form an adduct by either one- and/or two-electron oxidation or monooxygenation primarily with both syn- and anti-dihydrodiol epoxides in the bay region [20, 21]. This tumor promoter (DMBA metabolites-DNA adduct) induces the formation of the superoxide anion radical, which is responsible for the formation of reactive molecular oxygen and hydrogen peroxide during tumor promotion [22]. Several investigators reported that superoxide dismutase and catalase activities were significantly depressed by this tumor promoter [23, 24]. Yamaguchi *et al.* suggested that the reduction in liver catalase activity in the tumor bearer may be due to a down-regulation of the catalase gene induced by a certain humoral factor(s) [25]. Observations from our earlier studies revealed that a significant reduction in glutathione peroxidase activity was associated with a concomitant increase in the status of lipid peroxidation in tumor-bearing rats [9]. The significant reduction in serum selenium and glutathione peroxidase activity in tumorous rats and the increase in them in selenite-treated tumorous rats largely depend on the chemical form, status, retention behavior, and incorporation of selenium into enzymic selenoprotein, i.e., selenium-dependent glutathione peroxidase. In the present investigation, the levels of non-enzymic antioxidants as well as the activities of superoxide dismutase and catalase were

found to be significantly reduced in tumor-bearing rats, while the tumorous rats treated with selenite showed a significant increase in the activities of superoxide dismutase and catalase. Hence, the significance of the role of selenium in antioxidant stress is underlined.

Selenium is associated predominantly with glutathione peroxidase (75 to 85%) in erythrocytes of rats [26]. The antioxidative role of selenium is largely dependent on glutathione peroxidase, while the other antioxidative enzymes play a complementary role to glutathione peroxidase during oxidative stress in any diseased condition. Thus the reduced activity of antioxidative and protective mechanisms during tumorigenesis results in the accumulation of higher concentrations of oxygen species. The oxidative stress during tumorigenesis can be manipulated by adjusting the expression of superoxide dismutase and catalase in eukaryotic cells [27, 28]. Greenberg *et al.* identified a genetic locus, the superoxide response regulon, which is responsible for the regulation of the respective proteins with demonstrable antioxidant roles against superoxide stress [28]. It can be, therefore, concluded that the decreased activity in the antioxidative enzymes present in the tumor bearer may be due to reduction in the synthesis of the protein portions of these enzymes. Thus, the resulting excess of non-detoxified free radicals may mediate some molecular events linked to tumor promotion that cause cell injury and death.

Dietary selenium is capable of altering the incidence of DMBA metabolites-DNA adducts by inhibiting the initiation phase of mammary carcinogenesis [4]. This is due to the reduced formation of adducts, viz., anti- and syn-diol epoxides of deoxyguanosine and deoxyadenosine by selenium administration [21, 29]. Selenium supplementation to the rats with DMBA-induced tumor resulted in 58 and 32% reduction in the rate of formation of anti-dihydrodiol epoxide adducts bound to guanosine and adenosine, respectively [4]. Selenium administration, therefore, can reduce the generation of superoxide anion by inhibiting the formation of DMBA metabolites-DNA adducts during mammary tumorigenesis in rats. Our earlier studies provide evidence suggesting a pivotal physiological role for selenium in the protective mechanism against uncontrolled lipid peroxidation in tumor-bearing rats [9].

The formation of DMBA metabolites-DNA adduct during mammary tumorigenesis in rats causes an increased generation of superoxide anion. This could be accompanied by a subsequent accumulation of free radicals corresponding to an increase in the lipid peroxidation coupled with reduction in the activities of antioxidative enzymes. The free radical-scavenging enzymes, superoxide dismutase and catalase, are increased in tumor-bearing rats by selenium supplementation, thereby decreasing reactive oxygen species toxicity in cellular organelles. We thus conclude that administration of selenium to rats with mammary tumor confers a decrease in the levels of reactive oxygen species in cellular organelles, thereby preventing cell damage and subsequent cell death.

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