Dietary Se and tumor glutathione peroxidase and superoxide dismutase activities

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The purpose of this study was to examine tumor activity of the radical scavenging enzymes selenium dependent glutathione peroxidase (SeGSHPx), cupro,zinc-superoxide dismutase (CuZnSOD), and manganese dependent SOD (MnSOD) from rats fed varying amounts of selenium and to compare the effects due to diet with those due to the development of the tumor. Enzyme activities were measured in mammary tumors from DMBA-treated rats fed 0.035, 0.1, 1.0, and 2.0 mg Se/kg diet for 25 weeks (n = 58, 33, 24, and 14 tumors from 8, 8, 7, and 8 tumor-bearing rats, respectively). Increasing dietary Se had no effect on tumor SeGSHPx, non-SeGSHPx, and MnSOD activities. In contrast, tumor CuZnSOD and total SOD activities decreased with increasing dietary Se. Tumor size had a significant effect on MnSOD, SeGSHPx, non-SeGSHPx, and total GSHPx activities with increasing activity observed with increasing tumor weight. Malignant tumors were unique in two ways with respect to the activity of antioxidative protective enzymes. Tumor SeGSHPx activity was unresponsive to dietary Se while CuZnSOD and total SOD activities were significantly reduced with increasing dietary Se compared to erythrocytes, liver, and spleen from the same animals. Thus, the overall effect of high dietary Se was to decrease the ratio of total SOD to total GSHPx activity ($P < 0.003$). Tumor size had no effect on the ratio. This ratio of total SOD/GSHPx activity was several-fold higher in tumors than in other tissues from the same animals.

Keywords: selenium; cancer; DMBA-induced tumors; glutathione peroxidase; superoxide dismutase

Introduction

Superoxide dismutase (SOD, superoxide oxidoreductase, E.C. 1.15.1.1) along with glutathione peroxidase (GSHPx, glutathione: hydrogen peroxide oxidoreductase, E.C. 1.11.1.9) and catalase form the primary intracellular enzymatic defenses against toxic oxygen metabolites.[1] Such metabolites have been implicated in the damage caused by ionizing radiation[2] and chemical carcinogens.[3] SOD functions to remove superoxide radicals by reducing them to hydrogen peroxide, which is then removed by GSHPx or catalase. There are two forms of SOD, a mitochondrial enzyme which requires manganese for activity (MnSOD) and another containing copper and zinc (CuZnSOD) which is located in the cytosol.[4] CuZnSOD activity is dependent on copper for activity, and the activity of this enzyme in blood and many tissues was shown to reflect dietary copper intake.[5] SeGSHPx requires selenium (Se) for activity, although there is also peroxidase activity within cells that is independent of Se and is referred to as non-Se dependent GSHPx (non-SeGSHPx). The activity of SeGSHPx varies with dietary intakes of Se.[6] GSHPx is considered more important than catalase at physiological peroxide concentrations as the K_m for peroxide is 1 μM for GSHPx compared to 1.1 M for catalase.[4]

The activity of these enzymes has been examined

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in tumor cell lines[2] and in transplanted tumors,[7,8] and comparisons have been made between malignant tumors and adjacent normal tissue.[9,10] In these studies, wide variations in activity have been reported. Very little information is available about the activity of these enzymes in tumors from animals that have been fed a defined diet. Supplemental Se has been shown to reduce cancer incidence in numerous studies,[11] and blood Se levels are reduced in cancer patients compared to matched healthy controls.[12,13] Animals with tumors have also been shown to have reduced Se status as assessed by SeGSHPx activity[14,15] and reduced retention of a ^{75}Se tracer.[16]

The purpose of the present study was to examine the activity of the protective enzymes: SeGSHPx, non-SeGSHPx, CuZnSOD, and MnSOD in tumors from rats fed varying amounts of Se to determine if the differences in tumor enzyme activities that have been reported may be a reflection of varying intakes of Se. The Se contents of the diets ranged from one-third to 1, 10, and 20 times the recommended intake of Se for rats (0.035, 0.1, 1, and 2 mg Se/kg diet, respectively). The response of tumor enzymes to dietary Se were compared with the activity seen in erythrocytes, liver, and spleen. The effects of tumor size on enzyme activity was also examined. Finally, as SOD and GSHPx function in concert with each other, the effects of both dietary Se and tumor weight on the ratio of total SOD to total GSHPx activity was examined.

Materials and methods

Animals and diets

Weanling female Sprague-Dawley rats (weight 40 ± 1 g, Charles River Canada, St. Constant, Que.) were randomized by body weight into groups of 20 rats each. Rats were housed in individual stainless steel wire-mesh bottom cages (22° C, 12 hr light-dark cycle) and water and food were consumed ad libitum for the 25-week duration of the experiment. Body weight and food consumption were determined weekly. Rats were fed a casein-based diet containing 20% fat (3:1, lard:corn oil). The diet has been described in detail previously.[6] The basal diet contained 0.035 ± 0.007 mg Se/kg diet by fluorometric determination,[17] and additional Se was added in the form of sodium selenite to the other diets to provide 0.1, 1.0, and 2.0 mg Se/kg diet.

Tumor induction and pathology

After 5 weeks (56 days of age), mammary tumors were induced by intragastric administration of 4.3 mg 7,12-dimethylbenz(a)anthracene (Sigma Chemical Co., St. Louis, MO) dissolved in corn oil. Rats were palpated weekly for tumors and the number, location, and size of tumors recorded. During the 25th week, rats were killed by exsanguination from the abdominal aorta while under sodium pentobarbital anesthesia (6 mg/100 g body wt). The skin was separated from underlying tissue and all rats were examined for nonpalpable tumors. Tumors, liver, and spleen were removed and weighed. Tumors were split in half: one half was used for the enzyme determinations and the other half used for histopathology. Mammary tumor pathology was confirmed according to IARC guidelines for mammary neoplasms.[18] Only animals with tumors confirmed as adenocarcinomas by histology were used for this study.

Biochemical methods

Tissues were rinsed in ice-cold saline, blotted, and stored at −70° C until used for enzyme determinations. On the day of analysis, tissues were thawed at 4° C and homogenized in 10 volumes of cold Triton X-100 (0.2%, vol/vol). Reagents were from Sigma Chemical Co. (St. Louis, MO), and all reagents and samples were maintained at 4° C throughout the preparative steps. A portion of the homogenate was used for the determination of total SOD, GSHPx, and total protein. The remainder of the homogenate was extracted with chloroform/ethanol and used for the determination of CuZnSOD.

Tissue SOD activity was measured using an automated modification of the xanthine-xanthine oxidase cytochrome c method described previously,[6] using an ABA-200 discrete analyzer (Abbott Laboratories, Mississauga, Ont., Canada). The assay cuvette contained 240 μL of a mixture of: 20 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.2 μM ferricytochrome c, 50 μM xanthine, and 10 μM fresh potassium cyanide. The reaction was initiated with sufficient xanthine oxidase to give a reaction rate, in the absence of sample, of 0.025 A/min at 30° C using a 550/650 filter. SOD activity was determined using 1.25 to 10 μL of sample. Total SOD was determined on the tissue homogenate, CuZnSOD on the chloroform/ethanol extract, and MnSOD determined by difference. MnSOD has been measured in crude tissue homogenates by adding 1 mM CN to inhibit the CuZnSOD activity.[19] We have found that this procedure, however, does not fully inactivate the CuZnSOD activity even up to 5–10 mM CN and more satisfactory results are obtained by extraction and measurement of the CuZnSOD activity directly.[5] Erythrocyte CuZnSOD activity was determined using a similar method at pH 10.0.[20]

GSHPx activity was determined by an automated modification of the coupled assay of Paglia and Valentine.[21] The assay mixture was prepared fresh daily and contained: 150 mM potassium phosphate buffer (pH 7.0), 5 mM EDTA (disodium salt), 0.5 mM sodium azide, 2 mM glutathione (reduced, crystalline free acid), 0.24 mM NADPH, and 1 U/mL glutathione reductase. SeGSHPx activity was determined using 0.25 mM hydrogen peroxide or 0.3 mM t-butylperoxide as substrate. The blank activity was significantly lower with 0.3 mM t-butylperoxide than with hydrogen peroxide. Total GSHPx activity was determined using 1.2 mM t-butylperoxide as substrate and non-SeGSHPx determined by difference. Reactions were carried out

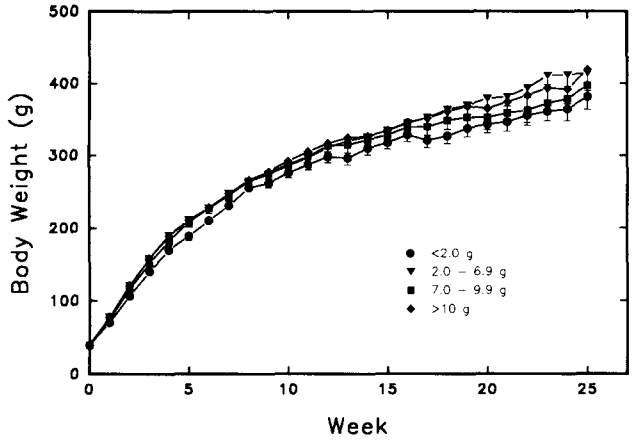

Figure 1 Effect of total tumor weight/animal on body weight. Rats were fed diets containing varying amounts of Se for 25 weeks. All animals had at least 1 malignant tumor present at necropsy and were classified according to the total tumor weight per animal at necropsy (n = 7–8 per group).

at 37° C using an ABA-200 discrete analyzer with a 340/380 nm filter. The sample cuvette contained 500 μL of assay mixture and 1.25–10 μL of homogenate. The reaction was initiated by the addition of substrate. One unit of activity catalyzes the oxidation of 1.0 μmol of reduced NADPH per minute.

Statistics

Results were examined using analysis of variance and significant differences between means were determined by the least significant difference method. Regression equations and Pearson correlation coefficients were calculated. All statistical procedures were done using the CSS statistical software package (Stat Soft, Tulsa, OK).

Results

The body weights of rats with different sized tumors present at necropsy are shown in *Figure 1*. The total tumor burden per animal had no significant effect on body weight, although rats with the smallest tumors (<2 g) tended to have lower body weights. In addition, there were no significant differences in food consumption among animals with different sized tumors (data not shown).

The GSHPx and SOD activities in erythrocytes, liver, and spleen of tumor-bearing rats fed the different amounts of Se are shown in *Table 1*. SeGSHPx activity increased with increasing dietary Se in erythrocytes, liver, and spleen. Dietary Se had no effect on CuZnSOD or MnSOD activities in these tissues.

The effects of dietary Se on the activity of enzymes which catalyze the intracellular removal of radicals from tumor tissue are shown in *Table 2*. SeGSHPx activity was low in tumors compared to other tissues and the Se form of the enzyme accounted for more than 85% of the total activity. In contrast with erythrocytes, liver, and spleen, tumor SeGSHPx activity did not vary with dietary Se. Tumor MnSOD activity was highly variable and could not be detected in 32 of 131 tumors examined. The lack of MnSOD activity was not confined to particular rats as many animals had some tumors with MnSOD activity and other tumors with no detectable activity. The absence of MnSOD activity was not related to growth rate, tumor type, or to the amount of protein/g tumor tissue (data not shown). In those tumors with detectable activity, MnSOD activity did not vary with dietary Se. CuZnSOD and total SOD activities decreased as dietary Se increased ($P < 0.05$). As a consequence, the ratio of total SOD to total GSHPx activity declined in tumors from rats fed increasing amounts of Se ($P < 0.05$).

Since tumor weights varied greatly in this study (range 0.059–21.91 g, mean 2.13 g, median 0.81 g) tumors were classified into 5 size categories based on weight at necropsy. There were no significant differences in the mean tumor weights of the different dietary Se groups. Tumor SeGSHPx and total GSHPx activities were significantly higher in the larger tumors compared to smaller tumors ($P = 0.004$ and $P = 0.0002$, respectively) (*Table 3*). A similar effect was seen in MnSOD ($P < 0.02$) and total SOD activities ($P = 0.003$).

In contrast with tumors, the size of tumors present had no effect on erythrocyte, liver, or spleen SOD or GSHPx activities, except for a slight increase in spleen SOD in animals with large tumors ($P = 0.03$) (*Table 4*).

In tumors, the overall effect of increasing dietary Se was to decrease the ratio of total SOD to total GSHPx activity ($P < 0.003$) (*Table 5*). This ratio did not vary with tumor size. In addition, the SOD/GSHPx ratio in tumors was much higher than in other tissues from the same animals due to the much lower GSHPx activity (corresponding liver ratios 21–45; spleen ratios 33–43; erythrocyte ratios 12–20).

Discussion

Tumors were found to be a unique tissue with respect to SOD and SeGSHPx activities. Although a few rats in this study had large tumors (up to 21.9 g), the mean size was 2.1 g and the mean total tumor weight per animal was 4.1 g. The presence of these tumors did not significantly affect body weight or food consumption during the 25-week duration of the study, indicating that enzymatic differences were not a result of anorexia.

SeGSHPx activity in tumor tissue, unlike most other tissues examined, did not vary with dietary Se. Normal mammary gland SeGSHPx activity was shown to plateau at relatively low intakes of Se (approximately 0.1 mg Se/kg diet).[22,23] The Se requirement for maximal tumor SeGSHPx activity appears to be much lower than for other tissues which, in this study, plateaued at dietary intakes of 1–2 mg Se/kg diet. The Se form of the enzyme, however, accounted for almost all of the GSHPx activity in tumors. This lack of response by tumor SeGSHPx to dietary Se

Table 1 GSHPx and SOD activities of tumor-bearing rats fed different amounts of Se for 25 weeks

		Se (mg/kg diet)				
		0.035	0.1	1.0	2.0	*P* value
RBC	SeGSHPx[1] (U/mL)	52.7 ± 2.3[a]	60.5 ± 2.3[a]	80.3 ± 1.8[b]	81.4 ± 3.2[b]	<0.0001
	CuZnSOD[2] (U/μL)	31.3 ± 0.8	29.4 ± 1.1	29.5 ± 0.7	30.5 ± 1.1	ns
	SOD/GSHPx Ratio[3]	20.0 ± 0.8[c]	16.4 ± 1.0[b]	12.3 ± 0.3[a]	12.6 ± 0.5[a]	<0.0001
Liver	SeGSHPx[1] (mU/mg)	431 ± 39[a]	644 ± 27[b]	768 ± 46[bc]	787 ± 40[c]	0.0001
	Non-Se GSHPx[4] (mU/mg)	82 ± 10[a]	119 ± 9[b]	133 ± 6[bc]	159 ± 11[c]	0.0005
	Total GSHPx[5] (mU/mg)	513 ± 48[a]	763 ± 34[b]	901 ± 51[bc]	946 ± 50[c]	0.0001
	CuZnSOD[2] (U/mg)	13.3 ± 0.5	15.4 ± 1.1	16.0 ± 1.1	16.2 ± 0.8	ns
	MnSOD[6] (U/mg)	7.4 ± 0.6	6.2 ± 1.1	6.1 ± 0.9	6.4 ± 1.4	ns
	Total SOD[7] (U/mg)	20.7 ± 0.8	21.6 ± 0.9	22.1 ± 0.9	22.6 ± 1.1	ns
	SOD/GSHPx Ratio[8]	44.8 ± 1.1[b]	28.3 ± 1.5[a]	23.9 ± 1.2[a]	24.8 ± 1.6[a]	<0.001
Spleen	SeGSHPx[1] (mU/mg)	123 ± 7[a]	141 ± 3[ab]	154 ± 4[b]	157 ± 2[b]	0.0002
	Non-Se GSHPx[4] (mU/mg)	49 ± 3	52 ± 4	49 ± 4	51 ± 3	ns
	Total GSHPx[5] (mU/mg)	172 ± 5[a]	193 ± 5[b]	203 ± 6[b]	207 ± 5[b]	0.0007
	CuZnSOD[2] (U/mg)	4.4 ± 0.4	4.2 ± 0.1	3.6 ± 0.2	4.0 ± 0.4	ns
	MnSOD[6] (U/mg)	2.9 ± 0.7	4.2 ± 0.8	4.1 ± 0.5	3.2 ± 0.9	ns
	Total SOD[7] (U/mg)	7.3 ± 0.5	8.5 ± 0.9	7.7 ± 0.6	7.1 ± 1.1	ns
	SOD/GSHPx Ratio[8]	42.6 ± 2.9	44.0 ± 4.7	38.1 ± 3.2	33.9 ± 4.7	ns

Note: Values are mean ± SEM for 8, 8, 7, and 8 rats. All rats had at least 1 malignant tumor present at necropsy. Values on the same line with different superscripts are significantly different ($P < 0.05$).
[1] Se-dependent glutathione peroxidase activity.
[2] Cupro,zinc-superoxide dismutase activity.
[3] Ratio of total superoxide dismutase to total glutathione peroxidase activity; both activities expressed in mU/mL; RBC CuZnSOD assay is 30 times more sensitive than tissue assay. Therefore, ratio was divided by 30 for comparison with tissue ratios.
[4] Non-Se dependent glutathione peroxidase activity.
[5] Total glutathione peroxidase activity.
[6] Manganese dependent superoxide dismutase activity.
[7] Total superoxide dismutase activity.
[8] Ratio of total superoxide dismutase to total glutathione peroxidase activity; both activities expressed in mU/mg protein.

Table 2 Effect of dietary Se on tumor superoxide dismutase and glutathione peroxidase activities in rats fed different amounts of Se for 25 weeks

	Se (mg/kg diet)				
	0.035 (n = 58)	0.1 (n = 33)	1.0 (n = 24)	2.0 (n = 14)	Diet effect *P* value
CuZnSOD (U/mg protein)	7.85 ± 1.0[c]	4.56 ± 0.4[c]	4.19 ± 0.6[ab]	2.38 ± 0.7[a]	<0.05
MnSOD	4.56 ± 0.7(22ND)[1]	4.37 ± 0.5(4ND)	3.26 ± 0.5(3ND)	3.08 ± 0.9(3ND)	ns
Total SOD	9.79 ± 1.2[b]	8.47 ± 0.6[c]	7.36 ± 0.8[ab]	6.15 ± 0.8[a]	<0.05
SeGSHPx (mU/mg protein)	35.8 ± 3.1	32.2 ± 2.1	30.0 ± 3.7	43.8 ± 3.1	ns
Non-Se GSHPx	4.0 ± 0.6(23ND)	7.5 ± 1.7(10ND)	7.0 ± 1.6(9ND)	7.0 ± 1.6(5ND)	ns
Total GSHPx	37.5 ± 3.1	36.7 ± 2.9	32.6 ± 4.5	47.4 ± 3.3	ns
Ratio $\frac{\text{Total SOD}}{\text{Total GSHPx}}$	282 ± 24[b]	265 ± 27[b]	266 ± 31[b]	130 ± 11[a]	<0.05

Note: Values are the mean ± SEM for the number of tumors indicated in brackets. Only histologically confirmed malignant tumors were included. Values on the same line with different superscripts are significantly different ($P < 0.05$). No significant interactions between diet and tumor weight effects were seen.
[1] Mean is value for tumors with detectable activity. ND denotes the number of samples with no detectable activity.

Table 3 Effect of tumor weight on tumor superoxide dismutase and glutathione peroxidase activities

	Tumor weight range (g)					
	<0.2 (n = 12)	0.21–0.5 (n = 32)	0.51–1.0 (n = 32)	1.01–2.0 (n = 23)	2.01+ (n = 32)	Tumor wt effect P value
CuZnSOD (U/mg protein)	3.7 ± 1.2	5.5 ± 0.8	6.1 ± 1.1	5.9 ± 1.0	6.7 ± 1.3	ns
MnSOD	1.9 ± 0.3[a](6ND)[1]	3.5 ± 0.5[ab](10ND)	4.5 ± 0.7[b](5ND)	5.5 ± 0.9[b](7ND)	5.5 ± 0.8[b](7ND)	<0.02
Total SOD	3.2 ± 0.4[a]	7.1 ± 0.9[ab]	9.6 ± 1.2[b]	9.1 ± 1.2[1]	10.5 ± 1.6[b]	0.003
SeGSHPx (mU/mg protein)	23.4 ± 6.3[a]	30.1 ± 3.0[ab]	31.7 ± 2.9[ab]	37.9 ± 3.9[bc]	44.1 ± 3.6[c]	0.004
Non-Se GSPHx	3.4 ± 0.9(5ND)	4.9 ± 0.8(14ND)	4.7 ± 1.0(16ND)	6.6 ± 1.8(6ND)	7.9 ± 1.5(7ND)	ns
Total GSHPx	23.4 ± 5.4[a]	31.1 ± 3.1[ab]	33.3 ± 3.1[ab]	42.1 ± 3.8[bc]	49.8 ± 3.9[c]	0.0002
Ratio $\frac{\text{Total SOD}}{\text{Total GSHPx}}$	189 ± 52	268 ± 28	318 ± 35	232 ± 29	221 ± 22	ns

Note: Values are the mean ± SEM for the number of tumors indicated in brackets. Only histologically confirmed malignant tumors were included. Values on the same line with different superscripts are significantly different ($P < 0.05$). No significant interactions between diet and tumor weight effects were seen.
[1] Mean is the value for tumors with detectable activity. ND denotes the number of samples with no detectable activity.

Table 4 Effect of total tumor wt/rat on tissue glutathione peroxidase and superoxide dismutase activities

		Total tumor wt/rat (g)				
		<2	2–6.9	7–9.9	>10	P value
RBC	SeGSHPx (U/mL)	72.6 ± 4.7	62.2 ± 3.9	62.1 ± 6.1	65.1 ± 6.1	ns
	CuZnSOD (U/mL)	31.5 ± 0.8	30.3 ± 0.7	30.1 ± 1.4	29.0 ± 1.1	ns
	SOD/GSHPx Ratio	14.8 ± 1.2	16.6 ± 0.9	17.3 ± 2.3	15.6 ± 1.6	ns
Liver	SeGSHPx (mU/mg)	665 ± 36	597 ± 69	626 ± 108	592 ± 47	ns
	Non-Se GSHPx (mU/mg)	123 ± 10	114 ± 14	100 ± 18	115 ± 10	ns
	Total GSHPx (mU/mg)	787 ± 44	711 ± 82	725 ± 124	707 ± 56	ns
	CuZnSOD (U/mg)	16.0 ± 1.5	14.4 ± 1.1	15.3 ± 1.6	14.5 ± 1.1	ns
	MnSOD (U/mg)	6.8 ± 1.3	5.7 ± 1.0	6.0 ± 1.0	7.2 ± 0.9	ns
	Total SOD (U/mg)	22.8 ± 0.6	20.2 ± 0.8	21.3 ± 1.3	21.8 ± 0.9	ns
	SOD/GSHPx Ratio	28.8 ± 2.2	32.2 ± 3.6	31.6 ± 6.0	31.8 ± 4.6	ns
Spleen	SeGSHPx (mU/mg)	148 ± 8	146 ± 4	126 ± 9	143 ± 7	ns
	Non-Se GSHPx (mU/mg)	48 ± 3	54 ± 4	50 ± 5	51 ± 3	ns
	Total GSHPx (mU/mg)	196 ± 9	200 ± 6	176 ± 8	195 ± 9	ns
	CuZnSOD (U/mg)	3.6 ± 0.1	4.2 ± 0.2	4.4 ± 0.5	4.5 ± 0.3	ns
	MnSOD (U/mg)	3.1 ± 0.4[a]	3.8 ± 0.8[ab]	2.6 ± 0.9[a]	5.4 ± 0.9[b]	.05
	Total SOD (U/mg)	6.8 ± 0.4[a]	8.0 ± 0.8[ab]	7.0 ± 0.5[a]	9.9 ± 1.0[b]	.03
	SOD/GSHPx Ratio	35.0 ± 2.7[a]	40.2 ± 4.1[a]	39.8 ± 2.1[a]	51.1 ± 4.7[b]	.03

Note: Values are mean ± SEM for 6–8 animals per group. Each rat had at least 1 malignant tumor present at necropsy. Values on the same line with different superscripts are significantly different ($P < 0.05$).

Table 5 Effects of dietary Se and tumor weight on tumor superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities

		[Se] effect		Tumor wt effect		ANOVA		
Parameter	Regression equation[a]	CC(%)[b]	P	CC(%)	P	Diet	Wt	D*W[c]
CuZnSOD	6.55 − 1.43[Se] + 0.007 (Wt)	−19.6	<0.02	− 1.0	ns	0.05	ns	ns
MnSOD	4.12 − 0.88[Se] + 0.16 (Wt)	− 7.1	ns	16.6	0.05	ns	ns	ns
Total SOD	8.84 − 1.76[Se] + 0.27 (Wt)	−18.9	<0.02	12.8	ns	0.05	ns	ns
SeGSHPx	31.6 + 1.12[Se] + 1.16 (Wt)	6.23	ns	22.6	<0.01	ns	0.08	ns
Non-Se GSHPx	2.81 + 1.23[Se] + 0.34 (Wt)	18.5	ns	25.0	<0.01	ns	0.09	ns
Total GSHPx	33.3 + 1.20[Se] + 1.66 (Wt)	6.73	ns	29.9	<0.001	ns	0.01	ns
Ratio $\frac{\text{Total SOD}}{\text{Total GSHPx}}$	323 − 70.9[Se] − 4.92 (Wt)	−19.2	<0.03	− 7.5	ns	0.003	ns	ns

[a] Least squares regression equation of enzyme activity (U/mg protein for SOD and mU/mg protein for GSHPx activity and Ratio) with concentration of Se in the diet (mg Se/kg diet) and tumor weight (g); n = 129.
[b] Pearson simple correlation coefficent.
[c] Diet-Tumor weight interaction.

agrees with the results we have reported during a ^{75}Se tracer study.[16] Other tissues from tumor-bearing rats retained less of the ^{75}Se label as dietary Se increased, but tumors did not show this relationship.

Although MnSOD activity did not vary with dietary Se, there were a large number of tumors with no detectable activity in the low Se group. Tumors were reported to be generally low but variable in CuZnSOD activity and low in MnSOD activity compared to normal cells.[24] In this study, tumor CuZnSOD and total SOD activities decreased linearly with increasing dietary Se, while MnSOD did not vary. Our results show that the relative proportion of the two forms of SOD in tumors changed with dietary Se. The majority of SOD was the CuZnSOD form in tumors from rats fed low Se, while MnSOD was the major form of SOD in tumors from rats fed high Se. These results demonstrate that elevated dietary Se can reduce tumor SOD activity, a feature that was not seen in other tissues in this study, or in other studies.[6,25] Further studies are required before any definite explanation for this different response by tumors to dietary Se can be determined. Whether the reduction was related to the loss of differentiation in tumors is worth investigating.

As tumors increase in size, the amount of necrotic tissue tends to increase, with a central hypoxic core and well-developed surface vascular system.[26] This was suggested to result in reduced activity of antioxidative protective enzymes.[26] Tumor necrosis factor was found to induce MnSOD synthesis in a human pulmonary adenoma cell line.[27] This induction may account for the increase in tumor MnSOD activity seen here with increasing tumor size, although whether tumor necrosis factor or the increase in necrotic tissue and the reduction in tissue protein contributed to the increase in enzyme activity cannot be established at this time. Even with the large increases (2–3.3 fold) in enzyme activities with increasing tumor size, tumors retained a relatively constant ratio of total SOD/total GSHPx activity.

SOD and GSHPx activities in erythrocytes, liver, and spleen, on the other hand, were not affected by the weight of tumors present, except for an increase in spleen SOD activity in rats with large tumors. We have shown previously that the activity of these enzymes in other tissues are reduced in rats with tumors, compared to rats fed the same amount of Se, but remaining tumor free.[14,15] Together these results suggest that the presence or absence of tumors can affect SeGSHPx activity in tissues other than the tumor, but in animals with malignant tumors, the size of the tumor has little further effect on enzyme activity.

The ratio of total SOD to total GSHPx activity indicates the relative production of peroxides via SOD versus its removal. When the effects of both dietary Se and tumor weight on tumor enzyme activities were examined, dietary Se was found to have a greater effect on the ratio than did the size of the tumor. Thus, tumors from rats fed high Se had a lower production of peroxides via SOD activity compared to tumors from rats fed lower amounts of Se.

Several groups have shown that Se inhibition of cancer is not mediated through increased activity of SeGSHPx, as normal mammary gland SeGSHPx activity does not increase with elevated intakes of Se.[28,29] The present study, however, examined mammary tumors from rats fed different amounts of Se. Similar to normal mammary glands, tumor SeGSHPx activity did not increase with dietary Se; however, CuZnSOD activity was reduced by more than 300% with high Se. Thus, tumors from rats fed elevated levels of Se produce less peroxides via SOD, while retaining the same SeGSHPx activity.

Tumor cells have been shown to be much less sensitive to lipid peroxidation than the corresponding normal tissue due to elevated α-tocopherol and decreased polyunsaturated fatty acid content.[30,31] The data from the present experiment suggest that the enzymatic changes due to dietary Se may also alter the antioxidant status of malignant tumors and thereby the sensitivity to lipid peroxidation. Examining the ratio of SOD to GSHPx activities may prove a useful measure of the superoxide production versus removal systems and hence the susceptibility to peroxidation.

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