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Superoxide Dismutase Activity, Caeruloplasmin Activity and Lipoperoxide Levels in Tumour and Host Tissues of Mice Bearing the Lewis Lung Carcinoma

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Abstract—*Superoxide dismutase (SOD) activity, plasma caeruloplasmin activity and the level of whole tissue and subcellular lipoperoxides have been determined in normal and neoplastic tissues from control and tumour-bearing mice, measurements being made nine, twelve and fifteen days after the inoculation of Lewis lung carcinoma cells. SOD activity of host liver and lung tissues did not vary significantly from those of the control animals. Blood SOD activity of the tumoured animals was markedly elevated on the ninth and twelfth days after inoculation, decreasing to control levels on the fifteenth day. Tumor SOD diminished from an activity on the ninth day which was greater than that for control lung to a level significantly lower than that for control lung on the twelfth and fifteenth days after inoculation. The presence of a tumour did not appear to affect plasma caeruloplasmin oxidase levels. The lipoperoxide level of hepatic tissue rose significantly as the tumour progressed. In the lung tissue the lipoperoxides decreased from a level four times higher on the ninth day to one not significantly different from that of the controls. Tumour lipoperoxides were about twice the level of hepatic tissue and of the order of ten-fold greater than those of lung. The level of lipoperoxide in the plasma of tumoured mice did not differ markedly from that of control mice. Assays of lipoperoxide in subcellular fractions of liver, lung and tumour tissue revealed that the elevated lipoperoxide was principally synthesized in the endoplasmic reticulum.*

INTRODUCTION

LIPID peroxidation has been implicated as a feature of a large variety of pathological conditions, including atherosclerosis, aging, encephomalacia, ceroid lipofuscinosis, chronic pancreatitis, haemolytic anaemia, iron toxicity, liver necrosis, lung damage, oleonalyde poisoning, reproductive dysfunction and testicular atrophy[1, 2]. Lipid peroxidation could also be implicated in the carcinogenic process since free radicals are formed by the activation of many carcinogens and in the process of photocarcinogenesis[3]. Antioxidants, agents which quench oxygen-derived radicals, have been shown to suppress carcinogenesis in laboratory animals[4, 5]. In addition, it has been widely claimed that there is an inverse correlation between the intake of dietary antioxidants such as selenium and the incidence of various forms of cancer in human populations, suggesting that there could be a link between cancer and peroxide formation[6]. The damaging actions of peroxidized lipids upon cell membranes, that is, protein polymerization and cross-linking, phospholipid degradation, polypeptide scission and chemical alterations of amino acids could provide the basis of an hypothesis which would encompass the initiation of carcinogenesis[7] and provide an explanation for many of the characteristic biochemical and physical phenomena of cancer cells.

Superoxide dismutase (SOD, EC1:15:1:1), which catalyses the dismutation of superoxide anions to hydrogen peroxide, glutathione peroxidase (GSH-Px, EC1:11:1:9) and catalase (EC1:11:1:6), which reduces hydrogen peroxide and lipoperoxides to water and fatty acids respectively, have low activities in neoplastic tissues[8–12]. In measurements made from eight human colon carcinoma excisions, the mean malonaldehyde level (as an index of lipid peroxidation) was elevated by about $3\frac{1}{2}$-fold in

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comparison to non-malignant tissue from the same colons, but SOD and GSH-Px activities were raised while catalase activity was reduced[12]. Conversely, other investigations of human tumours indicate that SOD activity is low[13, 14]. Tissue from the Lewis lung carcinoma has been reported to have a low activity of SOD and GSH-Px, and elevated levels of thiobarbituric acid-reactive material (TARM)[15, 16]. The increase in TARM is associated with elevated lipid peroxidation because the only substrate pool available to support peroxidation of this magnitude is the polyunsaturated fatty acid (PUFA) of the intracellular and plasma membranes of tumour cells.

It has been reported, however, that hydrogen peroxide, a likely precursor of lipoperoxide, is not formed in the mitochondria of neoplastic tissues[17]. Furthermore, it has been claimed that there is no potential *in vitro* for peroxidation reactions to occur in animal tumours[18], and that most tumour cells are much less susceptible to lipid peroxidation[19].

The presence of a tumour evokes deleterious biochemical changes within non-cancerous tissues of the host[20–24]. Mice bearing the Lewis lung carcinoma have been shown to have significantly raised levels of hepatic lipoperoxide[15].

The present study investigates the site of the prodigious synthesis of lipoperoxides in tumoured animals, and the role of SOD in tumour growth and the protection of host tissues from the tumour-associated lipid peroxidation.

The TARM level in whole tissue, mitochondria, endoplasmic reticulum and cytosol of liver, lungs and tumour of tumour cell-inoculated mice has been measured and compared with that of saline-inoculated controls. Whole tissues and red blood cells have been assayed for SOD activity, and the plasma for the superoxide anion radical scavenger, caeruloplasmin.

Since tumour growth is a dynamic process it is probable that there are continuous metabolic changes during the progression of neoplastic disease. In the present study, therefore, the tumour and host tissues were monitored on the ninth, twelfth and fifteenth days after tumour inoculation.

MATERIALS AND METHODS

Chemicals

All chemicals were of the highest grade commercially available and, unless stated otherwise, were obtained from Fisons Scientific Apparatus, Loughborough, Leics, U.K. Thiobarbituric acid, Coomassie blue G, *O*-dianisidine dihydrochloride and the haemoglobin determination kit were supplied by Sigma (London) Chemical Co., Poole, Dorset, U.K.; riboflavin by Koch Light, Colnbrook, Bucks, U.K.; and potassium cyanide and EDTA (diaminoethanetetra-acetic acid) by British Drug Houses Ltd., Poole, Dorset, U.K.

Animals and treatment

Male C-57 BL/10 ScSn mice were purchased from Bantin and Kingman, Hull, Yorks, U.K., and maintained on experimental No. 1 SQC (B. P. Nutritional) diet in high density polypropylene cages. Lewis lung tumour cells were maintained by regular serial intramuscular transplantation into one flank of a syngeneic host every 14 days. When the animals had reached 35 ± 5 g in weight, twenty-four were inoculated with freshly-prepared tumour cells in the manner previously described[25], while a further nine mice were inoculated with phosphate-buffered saline (PBS) and retained as controls. Groups consisting of eight tumour-bearing and three control mice were killed by decapitation nine, twelve and fifteen days after tumour inoculation.

Blood was pooled into two groups of four experimental animals and one group of three control animals on the day of each killing. The liver and lungs of the mice were rapidly excised, thoroughly rinsed in isotonic saline and weighed before being homogenized at 10–20% w/v in ice cold buffer containing 0.25 M sucrose, 0.1 mM EDTA and 1 mM Tris–HCl (pH 7.4). The tumours were treated in a similar manner except that each tumour was divided into approximately two equal parts, one half being homogenized as above and the other in buffer containing a 1% w/v bovine serum albumin (BSA) additive.

A portion of each homogenate was retained for the whole-tissue assays, while the remainder was separated into mitochondrial, microsomal and cytosolic subcellular fractions by means of differential centrifugation[26]. The mitochondrial and microsomal fractions were washed in buffer and respun, and stored at −20°C with the cytosols prior to analysis.

Assays

The protein concentration of the subcellular fractions was determined by the method of Spector[27]. Tumour microsomes and mitochondria were fractionated in buffer containing BSA, present in order to confer extra stability

to the fragile intracellular organelles of the carcinoma[28].

TARM was determined in whole tissue, in microsomal, mitochondrial and cytosolic fractions, and in plasma by means of a fluorimetric assay essentially as described by Satoh[29]. SOD activity was measured in whole tissue and erythrocytes using the photochemical augmentation assay devised by Misra and Fridovich[30]. Caeruloplasmin activity of pooled plasma from control and experimental animals was assayed by the method of Schosinsky *et al.*[31].

Statistical analyses

The data obtained from individual animals were analysed statistically using the unpaired Students' *t*-test. Differences between control and tumoured animals were considered significant when $P < 0.05$.

Since the Lewis lung carcinoma originally arose spontaneously as a primary carcinoma of the lung in a C57 BL/6 mouse[32], data derived from tumour tissue was arbitrarily related to that of control lung in order to provide an index of changes in SOD activity and lipid peroxidation as the tumour progressed. Comparisons between changes in tumour SOD activity and tumour growth (as estimated by increasing percentage of body weight) were made by linear regression analysis.

RESULTS

The SOD activity of liver and lung tissue from tumoured mice did not significantly differ from that of control animals. However, the erythrocyte SOD activity was raised about five-fold in the tumour-bearing groups on the ninth and twelfth days after innoculation. Tumour SOD activity decreased from a ninth day mean value slightly greater than that of control lung to activities significantly lower than those of control lung on the twelfth and fifteenth days (see Table 1). Linear regression analysis showed there to be a negative correlation coefficient on 0.94 between SOD activity and tumour growth.

The serum caeruloplasmin levels of the tumoured mice did not differ significantly from those of the controls. The whole-tissue TARM levels are given in Table 2. The presence of a tumour significantly raised the concentration of

Table 1. Superoxide dismutase activity in tumour tissue, and in lung, liver and erythrocytes of control and Lewis lung carcinoma-bearing C-57 BL/10 mice

Duration after inoculation of tumour cells (days)	Liver		Lung			Erythrocyte*	
	Control	Tumoured	Control	Tumoured	Tumour	Control	Tumoured
9	7.8 ± 0.5	7.4 ± 1.3	1.8 ± 0.4	1.7 ± 0.6	2.3 ± 0.7	2.2	8.0, 13.3
12	7.7 ± 1.0	7.3 ± 1.1	1.4 ± 0.2	1.6 ± 0.8	0.67 ± 0.18†	2.0	10.3, 8.8
15	7.7 ± 0.6	8.3 ± 1.2	1.6 ± 0.3	1.8 ± 0.2	0.29 ± 0.18†	2.1	3.9, 3.0

Results represent the mean ± S.D. of individual observations of 3 control and 8 tumour-bearing mice, expressed as units $\times 10^5$/g wet wt. tissue.

*Erythrocytes pooled from 3 control and 2 × 4 tumour-bearing mice, the results being expressed as units/mg haemoglobin.

†Significantly different from controls (P.B.S. injected) for $P < 0.05$. (Tumour tissue compared with control lung.)

Table 2. Thiobarbituric acid-reactive material in tumour tissue, and in liver, lung and plasma of control and Lewis lung carcinoma-bearing C-57 BL/10 mice

Duration after inoculation of tumour cells (days)	Liver		Lung			Plasma*	
	Control	Tumoured	Control	Tumoured	Tumour	Control	Tumoured
9	87.3 ± 19.6	114.2 ± 29.5	12.0 ± 1.3	50.6 ± 19.7†	133.1 ± 55.8†	2.70	1.60, 1.74
12	62.0 ± 26.5	215.7 ± 66.9†	13.0 ± 1.7	26.9 ± 9.0†	124.9 ± 54.1	1.45	1.88, 1.90
15	64.8 ± 10.7	252.9 ± 96.1†	10.3 ± 1.4	11.3 ± 3.1	153.4 ± 43.8	2.17	2.17, 1.42

Results represent the mean ± S.D. of individual observations of 3 control and 8 tumour-bearing mice, expressed as nmol malonaldehyde/g wet wt. tissue.

*Plasma pooled from 3 control and 2 × 4 tumour-bearing mice, expressed as nmol/ml.

†Significantly different from control (P.B.S. injected) for $P < 0.05$. (Tumour compared with control lung.)

peroxidized lipid in liver and lung but not in the plasma. Tumour TARM was of the order of ten-fold and two-fold respectively higher than that of lung and liver. The hepatic lipoperoxide level of the tumour-bearing mice tended to rise as the tumour progressed, whereas pulmonary lipoperoxide decreased from a significantly elevated level nine days after injection to the control level by the fifteenth day.

TARM from microsomal fractions of tumour, and host liver and lung was consistently and significantly increased (Fig. 1). On the ninth day after tumour cell inoculation mitochondrial TARM was significantly higher in hepatic tissue but not in pulmonary tissue, while tumour mitochondrial TARM was significantly higher than that of control lung (Fig. 1). Cytosolic TARM in the tumour was significantly higher than the TARM level of control lung cytosol at all three intervals of measurement (Fig. 1).

DISCUSSION

In the present study the Lewis lung carcinoma had no effect upon liver and lung SOD activity: the erythrocyte enzyme level was raised while tumour SOD decreased as the carcinoma grew in size.

The activity of erythrocyte SOD was presumably raised in response to elevated

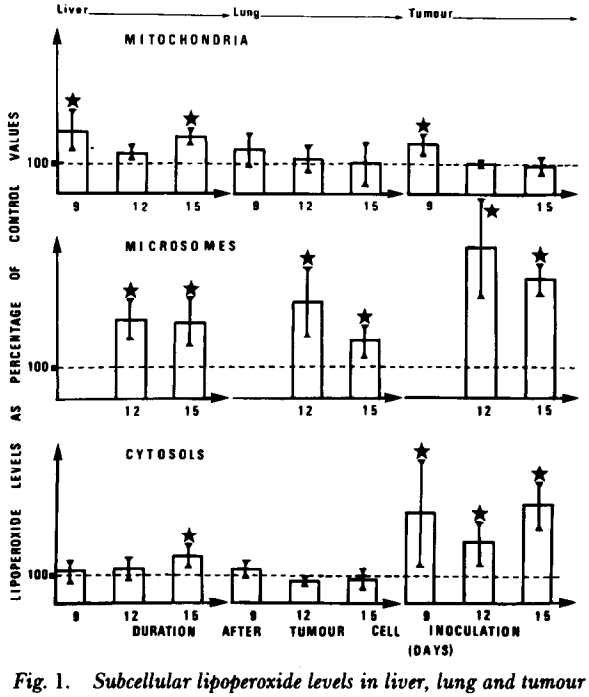

Fig. 1. Subcellular lipoperoxide levels in liver, lung and tumour tissue of C-57 BL/10 mice at various intervals after inoculation with Lewis lung carcinoma cells. Values expressed as percentages of control value (dotted line) on the same day. ★ Significantly different from control for $P < 0.05$ *(tumour compared with control lung).*

levels of superoxide anions ($^{\cdot}O_2^-$). It is possible that $^{\cdot}O_2^-$ and other active oxygen intermediates could attack and lyse red blood cells, resulting in the characteristic anaemia of cancer. The $^{\cdot}O_2^-$ ions could be released either by activated macrophages[33], by the breakdown of oxyhaemoglobin or, alternatively, by tumour cells themselves. Single i.v. or i.m. injections of SOD have been demonstrated to increase the life expectancy of animals with Ehrlich ascites tumour cells or Sarcoma 180 tumours[8]. Since SOD cannot enter the cell, this effect must be due to the scavenging of extracellular $^{\cdot}O_2^-$.

The decreasing tumour SOD which was associated with tumour growth may have resulted from end product inhibition by continually increasing levels of hydrogen peroxide[34, 35]. Elevated hydrogen peroxide could in turn have been the result of the low GSH-Px activity of the Lewis lung carcinoma[15].

The caeruloplasmin level in the plasma of tumoured mice did not appear to differ from that of the control animals. However, the observed level (0.024 ± 0.007 units/ml plasma) is very low in comparison to other species; only a tenth of the activity normally recorded for human and rat plasma. It is probable, therefore, that in mice caeruloplasmin has a relatively small role as an oxygen radical scavenger.

The present study indicates that there was an increase in the level of TARM in at least two different organs, namely liver and lung of tumour-bearing mice. In addition, the level of TARM in the tumour itself was remarkably high. The elevated TARM was presumably lipoperoxide and appeared to be predominantly synthesized in the endoplasmic reticulum of the tissues concerned. It would thus appear from these observations that the source of the increased formation of peroxidized lipid was the iron-dependent, NADPH-linked cytoplasmic system of the endoplasmic reticulum[36] rather than a more general non-enzymic process.

The elevated lipoperoxides in the liver and lungs of tumoured mice are not necessary produced by the same mechanisms as in the tumour, although there were similarities in the subcellular distribution of TARM. The antioxidant enzymes of host tissues, in common with those of tumour tissues, have been reported to have relatively low activities[35, 37, 38]. Hepatic catalase[37, 38] and SOD are particularly depressed in tumoured animals[35]. Thus, oxygen-derived radical species, such as superoxide ($^{\cdot}O_2^-$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), may be involved in the ele-

vation of lipoperoxide within both neoplastic and host tissues. Active oxygen intermediates could possibly catalyse the formation of an active iron species, for example perferryl iron, FeO_2^{2+} [36], which could itself initiate an increase in endoplasmic lipid peroxidation.

The results obtained with tumour tissue provide some evidence for a recent hypothetical model for cancer [35] which suggests that a change in DNA, or its expression, in the stem cell results in the loss of SOD and the ensuing increase in O_2^- and H_2O_2 produces a rise in the cyclic GMP/cyclic AMP ratio and intracellular glucose, which in turn inhibits or represses catalase and GSH-Px. This reduction in antioxidant defence enzymes of tumour cells and concomitant build-up of active oxygen intermediates would lead to elevated lipoperoxide levels, as was observed.

It was proposed as long ago as 1947 that there is a tendency for the tumour to influence enzyme systems of the tumour-bearing animal to become like those of the tumour itself [21]. Thus, tumours may reduce the capability of the host tissues to combat toxic oxygen-derived species and, as a result, there is an increase in the lipoperoxide levels in the non-neoplastic tissues.

The rise in hepatic and pulmonary lipoperoxide in mice bearing the Lewis lung carcinoma could be the result of other phenomena such as stress [39] or cell-mediated immunity [40], which arise as a consequence of tumour invasion. Furthermore, lipid peroxidation could be promoted by an increase in host tissue polyunsaturated fatty acid (PUFA). A rise in total and neutral lipids in hepatic microsomes of rats with benzo(a)pyrene induced sarcomas has been reported [41].

In the present experiment, pulmonary lipoperoxide levels decreased from an originally elevated level as the tumour grew, thereby indicating that the lung has an antioxidant factor(s) that is either absent or inferior in hepatic tissue. There is a recent report of cytosolic factors, present in the lung but not in the liver, which can inhibit both enzymic and non-enzymic lipid peroxidation [42]. Lung tissue is much more resistant to peroxidation than most other tissues, a resistance that correlates well with the relatively high vitamin E to PUFA ratio [43].

Many agents currently in use for cancer therapy act by raising the levels of active oxygen intermediates within the cell and are presumably selective against neoplastic cells due to their relatively inadequate antioxidant defence systems [8]. Many cytotoxic chemicals raise the content of lipoperoxide in liver and other normal tissues [44]. Should the elevated production of lipoperoxide in both neoplastic and normal tissues be a characteristic common to all or many types of cancer, it is possible that chemotherapeutic agents will exacerbate peroxidative damage in the non-malignant tissues of cancer patients. Elucidation of the relationship between tumour growth and lipid peroxidation could provide an alternative approach to the control of neoplastic disease.

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