SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITIES IN TUMORS

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1. Introduction

Superoxide dismutase (SOD) is believed to play a key role in the enzymatic defence of the cell against oxygen toxicity, the belief being substantiated by the ubiquitous presence of this enzyme in aerobic organisms and by an impressive body of evidence for a protective role of SOD both in vitro and in vivo (cf. reviews [1,2]).

It was of obvious interest to study SOD activity in tumors. Dionisi et al. [3] reported SOD to be absent from tumor mitochondria. On the other hand, our preliminary data [4] and recent results of Rotilio and collaborators [5] pointed to a low SOD activity in the cytosol of tumor cells. In this communication we confirm and extend our preliminary observations showing tumors to be poor in cytosolic cyanide-sensitive SOD, compared to normal tissues.

In addition, a decline in the SOD activity of the Hepatoma 27 mitochondria (as compared to liver mitochondria) is found to be due solely to a lower content of the cytoplasmic-type Cu–Zn-enzyme localized in the intermembrane space of mitochondria; no change in the eumitochondrial cyanide-insensitive enzymatic SOD activity could be observed. The activity of glutathione peroxidase (GP), another enzyme thought to be 'the first line of defence against oxidative damage' [6–8], was relatively very low in both cytosol and mitochondrial matrix of Hepatoma 27, suggesting the presence of a single enzyme in these two cell compartments rather than of two differently controlled isoenzymes.

2. Methods

2.1. *Experimental tumors*

These were grown as outlined in the table 1.

2.2. *The SOD assay*

The standard method of Beauchamp and Fridovich [9] was used (xanthine oxidase/Nitro Blue Tetrazolium (NBT)) with the only modification being that the temperature was 30°C. Linear rates of Blue Formazan accumulation were monitored with a 'Unicam SP 8000' recording spectrophotometer at 560 nm.

2.3. *The SOD activity in cytosol*

Tumors or normal tissues were minced thoroughly with scissors and homogenized in a Potter-Elvehjem homogenizer in a medium containing 0.25 M sucrose, 0.01 M Tris–HCl, pH 7.5. The homogenate was centrifuged for 1 h at 105 000 × *g*. The resulting clear supernatant was referred as the 'cytosolic' fraction.

2.4. *The SOD activity in mitochondria*

Mitochondria were isolated from rat liver and Hepatoma 27 by a conventional procedure [10] with slight modification and the purity of the final prepa-

Table 1
Conditions of tumor growth

Tumor	Animals	Time of growth	Route of transplantation
Hepatoma 27	Random-bred albino rats	3–4 weeks	Subcutaneous
Zajdela hepatoma	Random-bred albino rats	1 week	Intraperitoneal
Walker 256 carcinosarcoma	Random-bred albino rats	2 weeks	Subcutaneous
Adenocarcinoma 755	C57B1 mice	2 weeks	Subcutaneous
Sarcoma 180	Random-bred albino mice	11 days	Subcutaneous
Ehrlich ascites carcinoma	C57B1 × CBA mice	1 week	Intraperitoneal
Lewis lung carcinoma	C57B1 mice	10 days	Intramuscular

ration was monitored by electron microscopy. Mitochondria were sonicated for 2 × 15 s in an MSE ultrasonic disintegrator, type L667 (20 kcycles/s, maximal output) and then centrifuged at 105 000 × g for 1 h. The supernatant was assayed for SOD. The cyanide-insensitive SOD was determined in the presence of 1 mM cyanide in the reaction mixture.

2.5. *The SOD activity in mitochondrial matrix*

Prior to sonication, mitochondria were osmotically shocked (20 min in 10 mM Tris–HCl, pH 7.5) to disrupt the outer membrane and washed to remove the soluble intermembrane enzymes.

The NBT-reductase activity was checked carefully in all samples and found to be negligible or absent in the cytosolic fractions. 'Mitochondrial' supernatants reduced NBT, but, too slowly to interfere significantly with the SOD assays and could be easily compensated by adding aliquots of the sample being assayed for SOD to both the sample and the reference cells.

GP activity was measured according to [11] in the same fractions as SOD.

3. Results

A survey of the SOD activity in the cytosolic fractions of various rat and mouse normal and tumor tissues is presented in table 2. All the activities listed

Table 2
Cytosolic SOD activity in tumors and normal tissues

Tissue	Animals	Specific activity[a] (units/mg protein of the 'cytosolic' fraction)	Activity[a] (units/g tissue)
Liver	Rats	180 ± 17 (6)	10 800 ± 1100 (6)
Liver	Mice	240 ± 50 (7)	15 100 ± 3200 (7)
Lung	Mice	120 ± 26 (4)	4400 ± 200 (4)
Heart	Mice	67 ± 16 (3)	2400 ± 400 (3)
Spleen	Mice	62 ± 20 (2)	3100 ± 100 (2)
Brain	Mice	91 ± 20 (2)	1900 ± 400 (2)
Walker 256 carcinosarcoma	Rats	25 ± 6 (4)	640 ± 90 (4)
Zajdela hepatoma	Rats	17 ± 1 (2)	420 ± 10 (2)
Hepatoma 27	Rats	36 ± 4 (6)	2000 ± 200 (6)
Lewis lung carcinoma	Mice	24 ± 4 (3)	610 ± 40 (3)
Sarcoma 180	Mice	26 ± 1 (2)	990 ± 40 (2)
Adenocarcinoma 755	Mice	11 (1)	500 (1)
Ehrlich ascites carcinoma	Mice	15 (1)	240 (1)

[a] Averages ± SD are given for the indicated number of experiments

in table 2 were more than 95% sensitive to KCN and heat treatment. In agreement with the data of others' [12,13], liver is found to be richest in SOD. Heart, lungs, brain and spleen specific activities (left column) were not very much different from each other (0.5–0.25 that of liver). The ratios of total activities (right column) are roughly similar but the difference between liver and other tissues is even more pronounced. A somewhat lower total SOD activity in brain tissue is probably due to a relatively smaller partial volume of cytoplasm in brain cells.

It may be seen that tumors are characterized by considerably lower cytosolic SOD activity. Thus for Hepatoma 27, Zajdela hepatoma and Lewis lung carcinoma the ratios of the specific SOD activities to those in the homologous tissues (liver [14,15] and lung [16]) are 0.1–0.2. Decreased SOD activity was not observed in our preliminary experiments on regenerating or post-natal developing rat liver and therefore it may be a characteristic of neoplastic growth rather than of proliferation or differentiation.

Dionisi et al. [3] claimed recently that it was 'a general feature of tumor mitochondria not to possess superoxide dismutase'. We found the specific SOD activity in mitochondria from Hepatoma 27 to be about 3.5-fold lower than in liver mitochondria (table 3), apparently in agreement with the data of Dionisi et al. [3] on Morris hepatoma. However, in the presence of cyanide, both tumor and liver mitochondria displayed rather similar specific SOD activities (sensitive to heat treatment) of approximately 2–3 units/mg protein.

The values of 9 and 11 units/mg protein were obtained for Hepatoma 27 and liver mitochondrial cyanide-insensitive SOD activities respectively when higher salt concentrations were present in the sonication medium, in agreement with the data on mitochondrial SOD being 'loosely bound' to the membrane [12,17]. It was further found that in osmotically shocked and washed mitochondria from Hepatoma 27 and liver the remaining SOD activity was cyanide-insensitive and again similar for both tissues.

These results clearly show that a net decrease in Hepatoma 27 mitochondrial SOD activity merely follows the decline of the cytosolic cyanide-sensitive Cu–Zn-type enzyme localized in the intermembrane space of mitochondria [12,17–19].

Evidently, there is no pronounced change in the level of the true mitochondrial procaryotic-type cyanide-insensitive enzyme. The same explanation is probably applicable to the data of Dionisi et al. [3] since those authors did not report sensitivity of mitochondrial SOD to cyanide in their experiments.

GP is also believed to be of primary importance for cell protection against oxidative damage [5–7]. Like SOD, GP is present in both cytosol and mitochondrial matrix [20]. However, there are not two entirely different isoenzymes as with SOD but rather a single enzyme in both cell compartments [20]. It was therefore of interest to compare the behaviour of GP and SOD upon neoplastic transformation. It may be seen (table 4) that the GP activity is drastically decreased not only in the cytosolic fraction of Hepatoma 27, but in the mitochondrial matrix as well. Thus GP intramitochondrial compartmentalization per se does not prevent its response to malignancy.

4. Discussion

It would be premature to speculate on the

Table 3
SOD activity in mitochondria from rat liver and Hepatoma 27

Tissue	Specific activity (units/mg protein)[a]			
	Without shock		Osmotically shocked	
	$-CN^-$	$+CN^-$	$-CN^-$	$+CN^-$
Liver	34 ± 8 (5)	3.0 ± 1.3 (4)	2.9 ± 0.5 (4)	1.3 ± 0.1 (4)
Hepatoma 27	10 ± 2.4 (7)	2.0 ± 0.2 (7)	2.4 ± 0.9 (3)	2.1 ± 0.4 (4)

[a] See footnote to table 2

Table 4
GP activity in liver and Hepatoma 27

Tissue	Specific activity[a] (units/mg protein)[a]		
	Cytosol	Mitochondria	
		Without shock	Osmotically shocked
Liver	159 ± 24 (6)	188 ± 42 (4)	108 ± 5 (3)
Hepatoma 27	15 ± 3 (12)	8.5 ± 2.3 (4)	8.5 ± 2.3 (5)

[a] See footnote to table 2

'sufficiency' or 'insufficiency' of the low SOD activity in tumors, since there are not sufficient data on the actual rates of $O_2^{\cdot}$ production in tumor cells. A greatly reduced enzymatic defence system against oxygen toxicity and a concomitant enrichment in antioxidants [21] may, however, indicate that cancerous tissues are 'switched' to a more primitive chemical mechanism of oxygen detoxication than the enzymatic one.

On the other hand, diminished activity of SOD, GP, catalase [22] and, possibly, other oxygen-protective enzymes in neoplastic tissues can arise from the general trend of tumors to decreased aerobic metabolism. Interestingly, the level of xanthine oxidase, which is known to be a potent generator of $O_2^{\cdot}$ radicals, is low in tumors [23].

Quite apart from the above considerations, data on SOD activity in cancer tissues should be of practical significance for cancer radiotherapy. $O_2^{\cdot}$ Radical was shown to play an important role in oxygen enhancement of radiation damage [24–26]. Radioprotective functions of SOD have been demonstrated both in vitro [27–29] and in vivo [30]. The low level of SOD in tumors compared to that in normal tissues could account at least partially for the positive effect of hyperbaric oxygen on the effectiveness of γ-therapy [31,32]. Data on SOD activity in human tumors (experiments in progress) would be of particular importance on this point.

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