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Role of Superoxide Dismutase in Cancer: A Review[1]

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Abstract

Diminished amounts of manganese-containing superoxide dismutase have been found in all the tumors examined to date. Lowered amounts of the copper-zinc-containing superoxide dismutase have been found in many, but not all, tumors. At the same time, tumors have been shown to produce superoxide radicals. It is shown how diminished enzyme activities along with radical production may lead to many of the observed properties of cancer cells. The apparent exploitation of the differences between normal and cancer cell superoxide dismutase activity in the treatment of cancer is discussed.

The enzyme SOD[4] (superoxide oxidoreductase, EC 1.15.1.1) is believed to be present in all oxygen-metabolizing cells but lacking in most obligate anaerobes, presumably because its physiological function is to provide a defense against the potentially damaging reactivities of the superoxide radical ($O_2^{\cdot-}$) generated by aerobic metabolic reactions (60). This enzyme catalyzes the reaction

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

(59). Fridovich and coworkers have provided substantial evidence that this enzyme is necessary for survival in all oxygen-metabolizing cells. This work has been reviewed elsewhere (34) and will not be discussed in this review.

Four different forms of SOD have been found to date (33). One of these, which is found in the cytosol and intermembrane space of mitochondria of eukaryotic cells, contains copper and zinc and is entirely unrelated, except in its activity, to the other three. An example of this SOD is the erythrocuprein found in bovine and human RBC. There are 2 kinds of SOD that contain manganese. One of these is found in the matrix of mitochondria (98), and the other is found in the matrix of bacteria such as *Escherichia coli* (47) and *Streptococcus mutans* (95). The fourth type of SOD contains iron and has been found in the periplasmic space of *E. coli* (39, 104).

In Vitro Studies of SOD in Cancer

Malignant cells are known to differ biochemically in many ways from normal cells. Yamanaka and Deamer (102) were the first to show that the SOD activity in transformed cells was abnormal. In this study, it was found that SOD activity was present in lung fibroblast WI-38 cells at levels somewhat higher than in human embryonic lung tissue. Gel electrophoresis patterns revealed bands corresponding to both the cytosol (Cu-Zn SOD) and mitochondrial (Mn SOD) enzymes. In SV40-transformed cells, the band from Mn SOD was diminished or absent, but total SOD activity was somewhat higher than in normal WI-38 cells. This implies that the Cu-Zn SOD activity increased severalfold after transformation.

In Vivo Studies of SOD in Cancer

The first *in vivo* observations of altered SOD activities in malignant neoplastic tissues were reported at nearly the same time by 2 different groups (24, 84). Dionisi *et al.* were the first to report SOD activity in *in vivo* cancer cells. They studied isolated mitochondria from bovine heart, Morris hepatoma 3924A, and Ehrlich ascites tumor (Lettré) mutant with respect to both SOD activity and superoxide radical formation. It was found that superoxide radicals were generated in the mitochondrial membrane of both normal and malignant cells and that in normal cells these radical anions were the precursors of hydrogen peroxide formation. Hepatoma mitochondria did not possess SOD activity and did not generate hydrogen peroxide. Ehrlich ascites tumor mitochondria contained a small amount of SOD activity but also did not generate hydrogen peroxide. Hence, it was concluded that superoxide ions were the precursors of hydrogen peroxide formation, the reaction being catalyzed by SOD. It was also found that in the Morris hepatoma the rate of superoxide formation was 5 times that of bovine heart. In the Ehrlich ascites tumor cells, the rate of $O_2^{\cdot-}$ production was nearly equal to that of bovine heart. Hence, this group found that both SOD activity and superoxide generation may be different in *in vivo* tumor cells.

Before this report was published, our group at The University of Iowa had also studied SOD activity in Ehrlich ascites tumor cells (84). Using disc polyacrylamide gel electrophoresis and removal of enzymatic activity by cyanide and ethanol-chloroform, it was shown that crude extracts from normal mouse liver had both Cu-Zn SOD and Mn SOD, while extracts from Ehrlich ascites tumor cells contained only Cu-Zn SOD. Since some tumor cells have a diminished number of mitochondria, it was possible that the loss of Mn SOD activity could be due to a loss of mitochondria. To check this possibility, disc gel electrophoresis was also performed on extracts of isolated mitochondria from normal liver and tumor cells. Isolated normal mouse liver mitochondria were found to contain both Cu-Zn SOD and Mn SOD. In contrast, when an equal amount (per mg protein) of extract of isolated mitochondria from Ehrlich ascites tumor cells was applied to the gels, only bands caused by Cu-Zn SOD appeared. Even when 10 times

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[4] The abbreviations used are: SOD, superoxide dismutase; Cu-Zn SOD, the copper- and zinc-containing SOD; Mn SOD, the manganese-containing SOD; cAMP, cyclic adenosine 3':5'-monophosphate; NRK, normal rat kidney; $^1O_2$, singlet oxygen; DDC, diethyldithiocarbamate.

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the amount of mitochondria was examined, no Mn SOD activity bands were present. These observations show that there was no detectable manganese-containing mitochondrial matrix enzyme activity in the tumor cells; the small amount of SOD activity reported by Dionisi *et al.* was probably due to some residual Cu-Zn SOD present in the intermembrane space of the mitochondrial membranes. Since they have shown that Ehrlich ascites mitochondria do not produce H_2O_2 as do normal mitochondria, it follows that the manganese-containing enzyme is required for H_2O_2 production at this site.

Our group at The University of Iowa has also measured SOD activity in various types of mouse liver (68). We have shown by both direct assay and gel electrophoresis that normal mouse liver contains both Cu-Zn SOD (122 units/mg) and Mn SOD (35 units/mg) activity. Regenerating liver, a rapidly dividing normal cell system, also contained both forms of the enzyme. Moreover, the Cu-Zn SOD and Mn SOD activities per mg protein changed with time after partial hepatectomy. Cu-Zn SOD activity per mg protein was found to be at a minimum at 4 days postsurgery (46 units/mg protein), while Mn SOD activity per mg protein peaked at 2.5 days (65 units/mg protein) and then fell back to normal levels by 4 days. In regenerating mouse liver, DNA synthesis starts at about 2 days after surgery, and the first wave of nearly synchronous cell division occurs at 4 days postsurgery (43, 86). On the other hand, we have found that H6 hepatoma tumor cells, which are also a rapidly dividing cell system, contain Cu-Zn SOD (40 units/mg protein) but no Mn SOD. From these studies, it can be concluded that normal mouse liver, whether quiescent or dividing, possesses both forms of SOD activity. On the other hand, hepatoma tumor cells contain Cu-Zn SOD, but no detectable Mn SOD activity. Thus, it appears that in the liver system the loss of Mn SOD activity is characteristic of tumor cells and not of normal cells.

We have also recently measured SOD activity in whole crude homogenates from several other mouse tumor systems. Normal thymus lymphocytes were found to have 15.5 units of total SOD activity per mg protein, with 5.5 units per mg protein being Mn SOD.[5] In contrast, L1210 leukemia cells contained 26.3 units of total SOD activity per mg protein with none of the activity due to Mn SOD. Yamanaka *et al.* (101) have also reported that L1210 cells have no Mn SOD, using polyacrylamide gel electrophoresis to indicate the presence of the enzyme. Recently, we have shown that mouse mammary carcinoma C3H has 15 units total SOD activity per mg protein.[6] None of this activity is due to Mn SOD.

We have also measured SOD activity in 2 *in vivo* tumors derived from tissue of neural crest origin. Mouse S91 melanoma crude homogenate had 67 units of total SOD activity per mg protein, again with no activity due to Mn SOD.[7] In contrast, neuroblastoma tumor cells contained a small amount of Mn SOD (70). However, the level of enzyme activity was much smaller than normal. We found that normal mouse brain extract had total SOD activity of 9.0 units/mg protein, with 4.5 units/mg protein being Mn SOD. Prenatal brain extract had total SOD activity of 5.4 units/mg protein, with 2.5 units/mg protein due to Mn SOD. Extracts from neuroblastoma tissue had both lowered Cu-Zn and lowered Mn SOD, with 3.8 units total SOD activity per mg protein and 1.3 units per mg protein due to Mn SOD. To date, this is the only tumor that appears to have Mn SOD activity. However, since this tumor is very invasive, it is quite possible that the Mn SOD activity is due to normal cells which are mixed with the tumor cells. An example of this mixture occurs when Ehrlich ascites tumor cells are implanted in the leg muscle of the mouse. Pure Ehrlich ascites cells have no Mn SOD, but when allowed to invade the muscle a small amount of Mn SOD is measured due to the contribution from normal muscle cells.[8] In order to resolve whether Mn SOD is indeed present in neuroblastoma cells, we are currently studying *in vitro* neuroblastoma cells. It would not surprise the authors if Mn SOD were present in this tumor cell, as it is in many ways an atypical tumor cell that has many characteristics of normal cells. *In vitro* neuroblastoma cells constitute one of the few tumor systems that can be caused to differentiate and become nontumorigenic (80). Thus, parallel to its other strange properties, this tumor may have Mn SOD. Even if this proves true, it is possible that the tumor has Mn SOD, but none in its mitochondria, so that it may be actually much like the other tumors. In any case, both of the tumors of neural crest origin show lowered levels of Mn SOD although neuroblastoma tumors may have non-zero amounts. In the latter case, what may be important is not the actual amount of SOD, but rather the amount of SOD relative to O_2^- that is present. If there is not enough SOD to handle the flux of O_2^-, then damage may result.

Yamanaka *et al.* (101) have recently measured SOD activities in human leukemia cells. Total SOD activities in myelocytic, monocytic, and lymphocytic leukemia cells were increased as compared to mature normal blood cells of similar types. With polyacrylamide gel electrophoresis, it was shown that Mn SOD was diminished or absent, "especially in immature type malignant cells."

Thus, a large number of studies by several different investigators have shown a lack of Mn SOD activity in most tumor cells. A diminished amount of Mn SOD activity has been reported in all tumors measured to date, with no exceptions. Yamanaka's group in Japan, Dionisi's in Italy, and our group in the United States have concluded that this lack of Mn SOD is found generally in tumor cells (24, 68, 101). Peskin's group in Russia has developed a different theory about SOD activity in cancer cells (73, 74). They believe that both cytosol and mitochondrial SOD activities are lowered in the tumor cells of mice and rats. Thus, for hepatoma 27, Zajdela hepatoma, and Lewis lung carcinoma, they found that the ratio of cytosol SOD activities to those in homologous normal tissues was 0.1 to 0.2. They also found the total specific SOD activity in mitochondria

[5] L. W. Oberley, D. G. Luttenegger, G. R. Buettner, S. K. Sahu, and S. W. H. C. Leuthauser. Superoxide dismutase activity in L1210 leukemia cells, manuscript in preparation.

[6] S. W. H. C. Leuthauser, L. W. Oberley and S. K. Sahu. Superoxide dismutase activity of mouse C3H mammary carcinoma, manuscript in preparation.

[7] S. W. H. C. Leuthauser, L. W. Oberley and S. K. Sahu. Superoxide dismutase activity of mouse S91 melanoma, manuscript in preparation.

[8] S. W. H. C. Leuthauser and L. W. Oberley. Superoxide dismutase activity of Ehrlich ascites tumor cells grown intramuscularly, manuscript in preparation.

from hepatoma 27 to be about 3.5-fold lower than in liver mitochondria. In the presence of 1 mM cyanide, both tumor and liver mitochondria displayed similar SOD activities of approximately 2 to 3 units/mg protein. They concluded that there is no loss of mitochondrial SOD and that the net decrease in the enzyme merely reflects the decline of the Cu-Zn SOD localized in the intermembrane space of the mitochondria. However, the authors believe that this conclusion is totally unjustified for several reasons. First, it is very doubtful that they are working with isolated liver mitochondria because the SOD activity in the mitochondria that they have isolated is almost entirely Cu-Zn SOD (34 units/mg protein, of which only 3 units/mg protein is Mn SOD). By way of contrast, we have reported almost 90% Mn SOD in isolated liver mitochondria (68). Thus, it appears that their mitochondria were contaminated with cytosol protein. Moreover, we have shown that at least 5 to 8 mM cyanide is necessary to inhibit the Cu-Zn SOD totally (68). Tyler has also shown that 1 mM cyanide inhibits the Cu-Zn SOD by only 70%, while 2 mM inhibits by 86%, and 3 mM inhibits by 91% (94). Hence, in the work reported by Peskin, if there is any cytosol enzyme in these mitochondrial preparations, its activity will not be totally inhibited by 1 mM cyanide. The small amount of residual activity that they measured in isolated mitochondria is thus most probably due to the Cu-Zn and not to Mn SOD. Moreover, it is also possible that the activity measured is due not to Cu-Zn or Mn SOD but to some other compound with SOD activity. Gel electrophoresis is the best way to ascertain the identities of the various compounds with SOD activity.

There is, however, evidence to favor at least part of Peskin's hypothesis that cytosol SOD is lowered in tumor cells. We found that H6 hepatoma had lower Cu-Zn SOD than does normal liver (68). Lankin and Gurevich (51) measured total SOD activity in mice given injections of Ehrlich's ascites carcinoma cells and found the activity at most times to be lower than normal. The total activity was maximal during the period of intensive growth (6 to 9 days after injection); at this time, the SOD activity actually exceeded that found in the livers of normal mice. However, this observation does not agree with our studies. We found that, 8 days after injection, Ehrlich ascites tumor cells had about one-half the total SOD activity of normal liver cells (84). At the terminal stage of development of Ehrlich's carcinoma, Lankin and Gurevich found that the SOD activity in tumor cells was greatly lowered. In these studies, total SOD can be equated with cytosol SOD, inasmuch as the mitochondrial SOD is missing in these cells.

Bozzi *et al.* (10) have measured cytosol SOD activity in normal liver and tumor tissue from mice and rats. Liver cells from normal mice and Ehrlich ascites tumor-bearing mice had 1 μg of cytosol SOD per mg of protein, while all tumor cells had lowered levels of cytosol SOD. Thus, in mice, Ehrlich ascites tumor cells had 0.25 μg SOD per mg protein, while transplantable methylcholanthrene-induced rhabdomyosarcoma MCI-A cells had 0.50 μg per mg protein. In rats, Yoshida ascites tumor cells had 0.53 μg SOD per mg protein, and Novikoff hepatoma ascites tumor cells had 0.50 μg SOD per mg protein.

Lowered cytosol SOD is not a universal characteristic of tumor cells because at least 3 exceptions have been found. First, Yamanaka and Deamer found that after SV40 transformation total SOD activity was higher than in normal lung fibroblast WI-38 cells (102). Since no mitochondrial SOD was present, this meant that the cytosol activity increased after transformation. This tumor may be different from the others measured because it is composed of fibroblasts, whereas most tumors are made up of epithelial cells. However, in another epithelial cell tumor, Petkau *et al.* (78) measured little change in total SOD activity. At the center and margin of 7,12-dimethylbenz[a]anthracene-initiated rat mammary carcinoma, the concentrations of SOD were 54 ± 10 (S.E.) and 117 ± 38 μg/g, respectively, while in the tumor as a whole it was 104 ± 32 μg/g. The latter value is not significantly different from 113 ± 35 μg/g, the enzyme concentration in mammary tissue from lactating rats. Lastly, our work in mouse leukemia cells[5] and Yamanaka's in human leukemia cells (101), as described earlier, show that these malignant cells actually have higher Cu-Zn SOD activity than do their normal counterparts.

It thus appears that lowered Cu-Zn SOD activity is a common, but not universal, characteristic of tumors. This low Cu-Zn SOD activity may be related to cell division, because our studies show that in the rapidly dividing cell systems, regenerating liver and H6 hepatoma, Cu-Zn SOD activity is low (68).

To summarize, in a number of cases, differences in the activity of SOD have been found between normal and cancerous cells. This difference is manifested usually but not always as lowered Cu-Zn SOD activity and always as a lower Mn SOD activity. This loss of Mn SOD has been found in spontaneous, transplanted, virally induced, *in vitro*, and *in vivo* tumor cells. The generality and importance of this observed loss of Mn SOD enzymatic activity remains to be determined.

Superoxide Radical in Cancer Cells

If the rate of production of superoxide ion in tumor mitochondria is comparable to that found in the mitochondria from normal tissue, then the loss of Mn SOD would result in a net increase in the level of superoxide ion in the tumor cell. This could have vast metabolic consequences due to production of chemical species derived from superoxide. Indeed, Fridovich (34) has provided impressive evidence that SOD is needed to maintain life in all oxygen-metabolizing cells. On the other hand, if the production of superoxide in tumor cell mitochondria is greatly reduced compared to normal mitochondria, then the loss of Mn SOD should not lead to any such harmful effects. In this case, research should focus on the loss of superoxide-producing ability and not on the loss of Mn SOD. From these considerations, it can be seen that, in order to establish that the loss of Mn SOD is important in cancer, it is also necessary to show the production of superoxide in tumor cell mitochondria.

As has been mentioned, one study has been performed which shows that tumor cell mitochondria do produce $O_2^{\cdot-}$. Using adrenochrome formation as an indicator of the presence of superoxide, Dionisi *et al.* (24) found that mitochondrial membrane fragments from bovine heart and Ehrlich ascites tumor cells had nearly the same rate of superoxide

formation, while mitochondrial fragments from Morris hepatoma had nearly a 5 times higher rate than the other cell types. Since the adrenochrome formation in all 3 cases was reduced to essentially zero in the presence of SOD, it was concluded that superoxide was responsible for the adrenochrome formation. We have been able to show $O_2^{\cdot-}$ production by mouse H6 hepatoma tumor mitochondria using this same method.[9] Thus, it appears that cancer cells do indeed produce $O_2^{\cdot-}$.

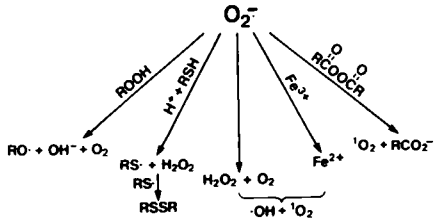

Chart 1. Reaction pathways of superoxide.

Possible Consequences of $O_2^{\cdot-}$ in Cancer Cells

Critics of the role of superoxide in cancer have pointed out that it is not known whether the loss of Mn SOD is a cause or effect of cancer. After all, there are numerous enzyme changes that occur in cancer (both additions and deletions). What evidence is there that the loss of Mn SOD is any more important than any of the other enzyme changes characteristic of the malignant phenotype? The answer to this question may lie in the function of the enzyme SOD. In contrast to most other enzymes, the principal role of SOD seems to be to act as a protective enzyme. Thus, its absence can lead to widespread metabolic consequences. In order to understand these consequences, it is necessary to consider what is known about the chemistry of the superoxide radical. Proposed pathways are shown in Chart 1. It should be emphasized that there is considerable evidence for each of these pathways, but none have been proven beyond doubt. It is not the purpose of this review to analyze the evidence for and against each pathway, but rather to relate the evidence for each pathway in the cancer cell. Therefore, one pathway, the formation of alkyloxy radicals (75), will not be discussed further, because no evidence has yet been found for it in the cancer cell.

Three pathways are of particular interest in the field of cancer. Superoxide can: oxidize SH groups to S—S via RS· (5) (Pathway 1); dismute to form H_2O_2 + ground-state oxygen (34, 79) (Pathway 2); react with ferric ion to form ferrous ion (11, 30) (Pathway 3). Each of these pathways can probably lead to large changes in cell metabolism. Considering Pathway 1 initially, sulfhydryl groups are constituents of many proteins. Oxidation of these groups to disulfides can result in protein conformational changes with possible activation or inactivation of key enzymes. For instance, 2 sulfhydryl-dependent enzymes, linked with the plasma membrane, play a determinant role in regulating the intracellular levels of cAMP. Adenylate cyclase is involved in the synthesis of cAMP and phosphodiesterase is involved in its breakdown (82). cAMP has been postulated by several investigators to be a regulator of cell division (35). Moreover, both of these enzymes, as well as cAMP levels, have been found to be abnormal in several tumor systems (71, 72). Many other examples of sulfhydryl-dependent enzymes and proteins can be documented. As further evidence for the role of this pathway in cancer, Apffel and Walker (4) have also reported an increase in protein disulfide reductase activity in tumors. This activity may be increased in response to the increased amount of disulfides produced in tumors by $O_2^{\cdot-}$. The role of sulfhydryl groups in carcinogenesis has been documented and discussed by Harington (42). Moreover, Apffel has found (2, 3) that the proliferation of murine tumor cells depends upon a free exchange between thiols and disulfides; when suspended in Hanks' medium and incubated for 1 hr with 1 mM concentrations of the sulfhydryl-blocking agents iodoacetate or *N*-ethylmaleimide, such cells are still viable but are no longer transplantable. All these observations are consistent with increased levels of $O_2^{\cdot-}$ in the tumor cell.

The second important pathway, dismutation to form H_2O_2 and ground-state oxygen, occurs either with or without SOD. Dismutation occurs much faster in the presence of SOD than in its absence. The importance of this reaction in the formation of ·OH and $^1O_2$ will be discussed later.

The last pathway may lead to perhaps the most widespread changes. Superoxide can denote electrons to metals to change their oxidation state. For instance, it has been shown that $O_2^{\cdot-}$ can react with Fe^{3+} to produce Fe^{2+} (11, 30). Reactions of this sort can lead to vast metabolic consequences due to large changes in the oxidation-reduction potential of the cell. Many enzymatic reactions require metals as cofactors, and a change in their oxidation-reduction state will surely affect these reactions. Fridovich has recently shown that $O_2^{\cdot-}$ is apparently a very diffusible substance able to migrate large distances and even through membranes if they do not contain SOD (57). Thus, in the cancer cell, which is low in SOD, changes may occur far from where the $O_2^{\cdot-}$ was originally produced. There is evidence that the changes in iron status may be particularly important in cancer. Picolinic acid, a specific iron chelator, has been shown to reversibly inhibit the growth of cultured mammalian cells (29). Untransformed NRK cells were reversibly arrested in the G_1 stage of the growth cycle. This G_1 arrest induced in NRK cells by picolinic acid could be prevented by the addition of Fe^{3+} to the tissue culture media (28). Transformed cells showed responses that were dependent upon the transforming virus and were blocked in different stages of the cell cycle. Picolinic acid was toxic to transformed cells but not to normal cells.

Besides changing the oxidation-reduction state of various metal ions, this reaction of $O_2^{\cdot-}$ with metals is important because it can lead to hydroxyl radical (·OH) formation. The hydroxyl radical is the most powerful oxidizing radical known to arise in a biological system. We and others have shown that Fe^{2+} generated from $O_2^{\cdot-}$ can catalyze a Fenton-type production of ·OH from H_2O_2 (11, 30). This reaction sequence has been verified by Halliwell (41) and by McCord and Day (58). There is evidence that ·OH is formed in tumor cells. Dimethyl sulfoxide, an effective ·OH scavenger (12), caused both biochemical and morphological differentiation of erythroleukemia cells (36) and promyelocytic leukemia

[9] I. B. Bize and L. W. Oberley, unpublished observations.

cells (17) and morphological differentiation of mouse neuroblastoma cells (50). Moreover, it has been suggested recently that ·OH activates guanylate cyclase (64, 65). Cyclic 3′:5′-GMP has also been proposed as a regulator of cell division, and higher levels of both cyclic 3′:5′-GMP and guanylate cyclase have been found in malignant tissue (23, 87, 96).

The reaction of H_2O_2 with Fe^{2+} in the presence of $O_2^{\cdot-}$ can also apparently produce $^1O_2$ (48, 49). Both ·OH and $^1O_2$ have been shown to cause lipid peroxidation (48, 49). Indirect evidence suggests that $^1O_2$ may be formed in the cancer cell. For instance, retinoids, which are thought to scavenge $^1O_2$ or free radicals (31), slow down the growth of *in vitro* and *in vivo* tumor cells (56). Moreover, vitamin A and several of its naturally occurring and synthetic analogs have been shown to prevent the development of benign and malignant chemically induced epithelial tumors *in vivo* (8, 9, 14, 21, 40, 66, 67, 83, 90, 91) and to prevent or reverse carcinogen-induced changes in prostate gland and tracheal epithelial cells in organ culture (13, 19, 52, 53). Inhibition of the development and growth of transplantable tumors has been demonstrated with rat chondrosarcoma (44, 88, 93), mouse mammary adenocarcinoma (81), and murine S91 melanoma (27). *In vitro,* retinoids were found to inhibit the growth of 2 malignant murine melanomas, showing the effect of the drug to be nonimmunological and due to a direct effect on tumor cell growth (55). Thus, if these drugs do indeed work by scavenging $^1O_2$, a role for this species is indicated in cancer. $^1O_2$ may perhaps be generated by other pathways besides the reaction between Fe^{2+} and H_2O_2. Thus, as shown in the last pathway of Chart 1, superoxide has been reported to react with diacylperoxides to generate $^1O_2$ (20).

In summary, many of the reaction pathways of $O_2^{\cdot-}$ can lead to toxic intermediates. Which of these are truly important is still in question, but the lack of Mn SOD is certain to have drastic consequences to the cell because of damage to key subcellular structures by oxygen-derived radicals. This damage may be responsible for many of the properties of the cancer cell.

Treatment of Cancer

Thus far we have presented evidence that the loss of Mn SOD is intimately related to the cancerous phenotype. This loss of Mn SOD may have enormous practical value because of the potential use of $O_2^{\cdot-}$ in cancer therapy. The rationale behind the use of $O_2^{\cdot-}$ in therapy is the following. If equal amounts of $O_2^{\cdot-}$ can be delivered to both cancer cells and normal cells, then the cancer cell should be preferentially killed because it has lower Mn SOD activity. Indeed, there is evidence that many of the existing cancer treatments actually are using this rationale because many of the anticancer drugs have been shown to produce $O_2^{\cdot-}$. For example, *in vitro* DNA chain breakage by the glycopeptidic antitumor antibiotic bleomycin was enhanced by the addition of the xanthine-xanthine oxidase system (45). The effect of the xanthine oxidase system disappeared completely when SOD was added. From these results, it was concluded that superoxide radical is one of the mediators for the enhancement of the DNA chain breakage action of bleomycin. Sausville *et al.* (85) has recently shown that DNA degradation by bleomycin requires oxygen and Fe(II). Reducing agents such as ascorbate and H_2O_2, as well as $O_2^{\cdot-}$, greatly increase the DNA degradation. These observations have led Sausville *et al.* (85) to propose the following model for the action of bleomycin. Bleomycin can bind to DNA in the absence of metal ion or reducing agent. Fe(II) can then attach to the bleomycin and thus form a ternary complex. The ternary complex can produce a species which degrades DNA. Reducing agents, including $O_2^{\cdot-}$, enhance the breakage by regenerating Fe(II) from Fe(III) and thence continuing the reaction. This model does not identify the nature of the toxic species. Since this proposed mechanism was similar to that observed by us (11) and others (30, 41, 58) for the production of hydroxyl radical from xanthine-xanthine oxidase, the reviewers thought that this radical might also be responsible for the degradation of DNA by bleomycin. Using the technique of spin trapping, we have observed that bleomycin and Fe(II) produce ·OH (69). Because of the high reactivity of ·OH, it is likely that this radical is responsible for the toxicity caused by bleomycin. Since bleomycin binds preferentially to DNA, the net result is that we have a site-specific free radical. As mentioned earlier, reducing agents such as H_2O_2 or $O_2^{\cdot-}$ are necessary for bleomycin to degrade DNA effectively. What is the source of reducing agent in the tumor cell? Since tumor cells apparently have lower levels of Mn SOD and many have diminished amounts of Cu-Zn SOD, tumor cells should have greatly increased levels of $O_2^{\cdot-}$. Moreover, $O_2^{\cdot-}$ has been shown recently to be produced in tumor cell nuclei (7). The increased levels of $O_2^{\cdot-}$ in tumor cells as compared to normal cells may explain the differential toxicity exhibited between normal and malignant cells upon treatment with bleomycin.

Likewise, the antitumor antibiotic streptonigrin causes DNA strand breaks *in vivo* (16). The antibiotic has been shown to generate the superoxide anion upon reduction and autoxidation *in vitro* (38, 100), and the superoxide anion has been shown to cause strand breaks in closed circular double-stranded DNA (18, 103). These observations have led to a proposed mechanism in which the antibiotic generates superoxide during a reduction-oxidation cycle, and this radical brings about single-strand breaks (18, 100). It has also been shown that superoxide radicals are formed by the oxidation-reduction cycling of the antitumor anthracycline antibiotics daunomycin and Adriamycin (37). It was found that NADPH and purified cytochrome P-450 reductase caused oxygen consumption from these drugs in excess of the amount of drug present. A reduction-autoxidation cycle of quinone groups was postulated. During this cycle, the cooxidation of sulfite may be initiated. This latter reaction was inhibited by SOD, suggesting that $O_2^{\cdot-}$ was formed. H_2O_2 was also generated, presumably by nonenzymatic dismutation of superoxide. Rat liver microsomes also catalyzed this oxidation-reduction cycling, which was accompanied by the peroxidation of lipids. These experiments suggested that the formation of oxygen radicals followed by lipid peroxidation may be the basis for the cardiotoxic effects of these drugs.

Recently, Thayer (92) has shown that Adriamycin stimulates superoxide formation in submitochondrial particles. Adriamycin at a concentration of 400 μM stimulated the rate of $O_2^{\cdot-}$ formation 6-fold to 25 nmol/min/mg. Measurements

of the relative catalase activity of blood-free tissues of rabbits and rats indicated that heart contained 2 to 4% of the catalase activity of liver or kidney. The author concluded that an enhanced production of $O_2^{\cdot-}$ and H_2O_2 and the relatively low catalase content of heart tissue may be factors in the cardiotoxicity induced by Adriamycin chemotherapy.

Bachur *et al.* (6) have extended these measurements and proposed a unifying theory behind their mechanisms of action. They have found that highly active, quinone-containing anticancer drugs, Adriamycin, daunorubicin, carminomycin, rubidazone, nogalamycin, aclacinomycin A, and steffimycin (benzanthraquinones); mitomycin C and streptonigrin (*N*-heterocyclic quinones); and lapachol (naphthaquinone) interact with mammalian microsomes and function as free radical carriers. These quinone drugs augmented the flow of electrons from NADPH to molecular oxygen. This reaction was catalyzed by microsomal protein and produced a free radical intermediate form of the drugs as determined by electron spin resonance spectroscopy. Several nonquinone anticancer agents were tested and were found to be inactive in this system. Since quinone anticancer drugs are associated with chromosomal damage that appears to be dependent on metabolic activation of these drugs, Bachur *et al.* proposed that intracellular activation of these drugs to a free radical state is primary to their cytotoxic activity. As free radicals, these drugs, because of their high affinity and selective binding to nucleic acids, have the potential to be "site-specific free radicals" that bind to DNA or RNA and either react directly or generate oxygen-dependent free radicals such as $O_2^{\cdot-}$ or $\cdot OH$ to cause the damage associated with their cytotoxic actions.

Thus, a wide number of anticancer drugs seem to involve $O_2^{\cdot-}$ in their mode of action. The differential toxicity of $O_2^{\cdot-}$ to tumor cells as compared to normal cells may be brought about by the lack of Mn SOD in tumor cells. This, perhaps coupled with increased $O_2^{\cdot-}$ production in tumor cells, can easily explain the differential toxicity.

One novel way of using this difference in SOD activity between normal and malignant cells has been proposed by Lin *et al.* (54). They propose to inhibit Cu-Zn SOD with DDC. Since normal cells still have Mn SOD, they should survive this treatment, whereas tumor cells, having only Cu-Zn SOD, would not. Lin *et al.* have done preliminary experiments with normal Chinese hamster cells (DON). The cytocidal effect of DDC on DON cells was dependent on the DDC concentration and exposure time. After 8 to 10 days of incubation with 10^{-9} M DDC, no change in DON cell survival was noted; however, incubation with 10^{-4} M DDC showed marked toxicity. When DDC-treated cells were irradiated, they did not survive as well as did cells treated with radiation or DDC alone. The combined effects of hyperthermia and DDC were dramatic. Cells treated for 8 min at 43° with 10^{-4} M DDC or for 10 min at 47° with 10^{-5} M DDC showed significant decrease in survival. These results suggested that DDC, which acts on Cu-Zn SOD, may be a powerful sensitization agent in tumor therapy.

Models for Cancer

If the loss of Mn SOD is important in cancer, this fact must: (*a*) explain and be consistent with previous observations about cancer; and (*b*) serve as the basis for new predictions. This model of cancer has the particular beauty of being able to reconcile new concepts of the origin of cancer with some of the older theories. In particular, it is possible to unify Warburg's hypothesis about the cause of cancer with modern theory that says that changes in DNA or its expression are the causative agents. Warburg (97) believed that cancer originated from the irreversible injury of respiration. This injury resulted in replacement of respiration energy by "fermentation energy" (glycolysis). This theory has largely been discredited in modern times, mainly because experiments have implied that cancer is caused by changes in DNA (62). The DNA theory has been given added force in recent years with the finding that most activated carcinogens are indeed mutagens (63). The 2 theories can now easily be reconciled if one realizes that the gene for Mn SOD is thought to be located in the nuclear genome (99). Thus, a defect in DNA or its expression could easily lead to a loss of Mn SOD which could in turn lead to mitochondrial damage from oxygen-derived radicals. The mitochondrial damage then could lead to increased use of glycolysis for energy. This theory does not necessarily explain the origin of cancer, but it does explain many of the observed properties of the cancer cell.

As mentioned earlier, this model for cancer is useful only if it leads to new predictions about cancer. In particular, this model would predict 3 important things: Prediction 1, loss of Mn SOD (or increased levels of $O_2^{\cdot-}$) should occur in all cancer cells; Prediction 2, loss of Mn SOD in normal cells should cause these cells to appear transformed in at least some ways or make the cells more susceptible to transformation; Prediction 3, addition of SOD to tumor cells or a reduction in $O_2^{\cdot-}$ flux should cause them to reacquire at least part of the normal cell phenotype.

With regard to Prediction 1, only the assay of a large number of tumors will show the generality of the loss of Mn SOD. The present evidence for generality has already been summarized. As has already been pointed out, it is probably the increase in net levels of $O_2^{\cdot-}$ which is important, not the loss of Mn SOD. Thus, cancer may result even without complete loss of Mn SOD if $O_2^{\cdot-}$ levels are high. This may be what occurs in neuroblastoma.

Prediction 2 involves studying the consequences of a loss of Mn SOD in normal cells. If the loss of Mn SOD is an important feature of cancer, then loss of the enzyme from normal cells should either produce characteristics of tumor cells or make the cell more susceptible to malignant transformation. There are already several lines of indirect evidence to indicate that this is true. Mitochondrial and cytoplasmic SOD activities have been determined in blood platelets, RBC, polymorphonuclear leukocytes, and lymphocytes from trisomy 21 patients (26, 89). The cytoplasmic enzyme showed an increase of 50% in these cells as compared to normal controls. This is not surprising, inasmuch as the gene which codes for this protein is located on Chromosome 21 (61). Hence, a direct gene-dosage effect is indicated. What is surprising is that the mitochondrial SOD is decreased by one-third in trisomy 21 patients. The gene for this enzyme is located on Chromosome 6 (61). This suggests the possibility of regulation of the activity of

mitochondrial SOD by cytosol SOD and/or superoxide ions. In any case, these observations may be significant because trisomy 21 patients have a 10 to 30% increased probability of developing acute leukemia. Hence, in this case, decrease in Mn SOD activity may be associated with the acquisition of the cancerous phenotype.

The Dubin-Johnson-Sprinz syndrome is another inherited disease which shows a deficiency of Mn SOD (76). This syndrome is an inherited form of conjugated hyperbilirubinanemia in which there is a progressive accumulation of both lipofuscin and melanin in the hepatocytes. Edwards (25) has noted an unusually high incidence of cancer in the Dubin-Johnson-Sprinz syndrome.

The SOD model for cancer would lastly predict that addition of SOD activity to tumor cells would enable them to reacquire at least some of the characteristics of normal cells (such as growth control). One cannot expect that the addition of SOD to tumor cells would enable the tumor cell to become completely normal, because some of the damage sustained by these cells is likely to be irreversible. This prediction is at present difficult to test for lack of a compound with SOD activity that can penetrate into the cell. Native SOD, either of the Cu-Zn or Mn form, does not enter the cell due to its large molecular weight (77). For this reason, it was not expected that SOD itself would affect tumor growth. However, preliminary experiments in our laboratory have shown that a single i.v. or i.m. injection of Cu-Zn SOD markedly increased the life span of animals carrying Ehrlich ascites tumor cells or Sarcoma 180 tumor cells in solid form.[10] This effect must be due to scavenging of superoxide outside the cell. This O_2^- may be produced from reactions inside the tumor cell since superoxide is highly diffusible, but it may also come from neutrophils and other blood elements outside the tumor cell (46). In any case, in order to test the above hypothesis further, it will be necessary to use low-molecular-weight compounds with SOD activity. Such compounds have been synthesized. Many copper coordination compounds have SOD activity (22). Some of these copper coordination compounds have already been shown to have antitumor activity (1, 15, 32). One of the main problems with these compounds to date has been their lack of solubility and difficulty in entering the tumor. These compounds, as well as adding SOD to liposomes, hold promise for cancer therapy in the future.

Prospects

Evidence has been presented for a relationship between SOD and cancer. Knowledge of this relationship may enable the clinician to find better treatments for cancer. Much work remains to be done on this subject. In particular, this review has not touched on the whole area of carcinogenesis. One of the major questions to be answered is what causes the loss of Mn SOD.

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