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# Forum Review

# Cross Talk of Nitric Oxide, Oxygen Radicals, and Superoxide Dismutase Regulates the Energy Metabolism and Cell Death and Determines the Fates of Aerobic Life

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## ABSTRACT

**Although oxygen is required for the energy metabolism in aerobic organisms, it generates reactive oxygen and nitrogen species that impair a wide variety of biological molecules, including lipids, proteins, and DNA, thereby causing various diseases. Because mitochondria are the major site of free radical generation, they are highly enriched with enzymes, such as Mn-type superoxide dismutase in matrix, and antioxidants including GSH on both sides of inner membranes, thus minimizing oxidative stress in and around this organelle. We recently showed that a cross talk of nitric oxide and oxygen radicals regulates the circulation, energy metabolism, reproduction, and remodeling of cells during embryonic development, and functions as a major defense system against pathogens. The present work shows that Cu/Zn-type superoxide dismutase, which has been postulated for a long time to be a cytosolic enzyme, also localizes bound to inner membranes of mitochondria, thereby minimizing oxidative stress in and around this organelle, while mitochondrial association decreases markedly with the variant types of the enzyme found in patients with familial amyotrophic lateral sclerosis. We also report that a cross talk of nitric oxide, superoxide, and molecular oxygen cooperatively regulates the fates of pathogens and their hosts and that oxidative stress in and around mitochondria also determines cell death in the development of animals and tissue injury caused by anticancer agents by some carnitine-inhibitable mechanism.** *Antioxid. Redox Signal.* 5, 475–484.

## INTRODUCTION

THE LARGE AMOUNTS OF ATP required by mammalian tissues are generated predominantly by oxidative phosphorylation in mitochondria, a process that results in the conversion of a small fraction of the inspired oxygen to the superoxide radical, hydrogen peroxide, and related metabolites even under physiological conditions (40). The enzymes in peroxisomes also generate superoxide radical and hydrogen peroxide (41). Most, but not all, reactive oxygen species react rapidly with a variety of molecules and thereby modulate cellular functions or damage cellular constituents, such as lipids, proteins, and DNA (1, 24, 42). Recent studies (23) revealed that a cross talk of reactive oxygen and nitrogen species regulates the circulation, energy metabolism, reproduction, embryonic development, and remodeling of tissues, and functions as a major defense system against pathogens. Thus, these reactive species should safely be metabolized selectively and effectively at or near the sites of generation. Because mitochondria and peroxisomes are the major sites for the generation of superoxide and related metabolites, it is important to understand the mechanism by which cells minimize oxidative stress in and around these organelles. The present work describes the presence of a super system driven by a cross talk of nitric oxide (NO), superoxide, and molecular oxygen that cooperatively regulates the circulatory status

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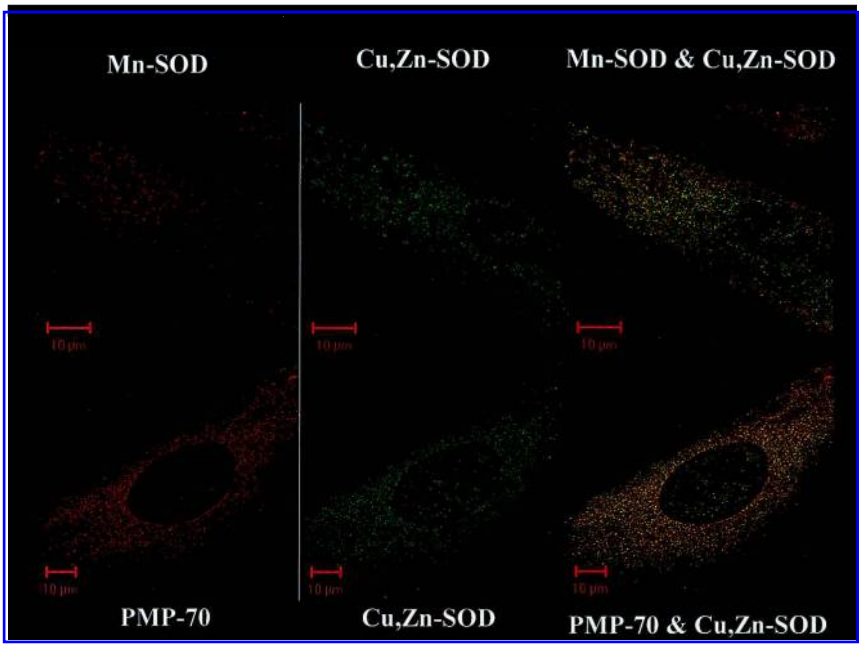


**FIG. 1. Subcellular localization of two SOD isozymes.** Subcellular localization of Mn-SOD (**left upper panel**), Cu,Zn-SOD (**middle upper panel**), and PMP-70 (**left lower panel**) within the same fibroblast was analyzed by a confocal microscopy using specific antibodies against these proteins. Cu,Zn-SOD localized associated with Mn-SOD-positive mitochondria. Some part of Cu,Zn-SOD (**middle lower panel**) also localized associated with peroxisomes stained with anti-PMP-70 antibody. The **right upper and lower panels** show the superimposed images of Cu,Zn-SOD-positive cells with Mn-SOD- and PMP-70-positive cells, respectively. The results indicate that Cu,Zn-SOD is localized bound predominantly to mitochondria and partly to peroxisomes.

and mitochondrial energy transduction, and functions as a potent defense system against pathogens. We also report that oxidative mechanism in and around mitochondria underlies the pathogenesis of familial amyotrophic lateral sclerosis (FALS) and cancer associated with chronic inflammation, such as viral hepatitis and ulcerative colitis.

## MITOCHONDRIAL LOCALIZATION OF SUPEROXIDE DISMUTASE (SOD) ISOZYMES

Three types of SOD isozymes, Cu,Zn-SOD, Mn-SOD, and extracellular SOD (EC-SOD) rapidly dismutate the superoxide radical (40). Whereas Mn-SOD and EC-SOD are localized in the mitochondrial matrix and on the outer surface of cell membranes, respectively, Cu,Zn-SOD has been believed to reside in the cytosol (16). In the absence of SOD, the superoxide radical is relatively stable, undergoing spontaneous dismutation with a half-life of 5 s; this reaction is extremely slow as compared with the SOD-catalyzed dismutation ($K = 10^9\ M^{-1}\ s^{-1}$). Given the hazardous nature of superoxide, this radical should be metabolized effectively at or near the sites of generation. It should be noted that the viscosity of a cytosol enriched with macromolecules and low-molecular-weight solutes is fairly high and, hence, motional freedom of cytosolic constituents is highly limited. Thus, collision of macromolecular enzymes and their substrates in cytosol would occur rather slowly to suppress the rate of enzymatic reactions. Because substantial amounts of superoxide radical are generated on both sides of mitochondrial inner membranes, we hypothesized that Cu,Zn-SOD might localize bound to mitochondrial membranes, thereby facilitating the effective dismutation of this reactive radical. With this concept in mind, we studied the subcellular localization of Cu,Zn-SOD in rat tissues and cultured human fibroblasts (26) with the use of antibodies specific for Cu,Zn-SOD, Mn-SOD, or PMP-70, a specific marker protein for peroxisomes (20). Subcellular fractionation, Nycodenz gradient centrifugation, and immunoblot analysis using specific antibodies showed that Cu,Zn-SOD is localized predominantly in mitochondria and peroxisomes of rat liver and brain (26).

Confocal immunofluorescence microscopy revealed that anti-Cu,Zn-SOD antibody in cultured human fibroblasts colocalizes with antibodies to Mn-SOD and PMP-70 (Fig. 1). These results indicate that Cu,Zn-SOD associates with mitochondria and peroxisomes in various cells in the brain, liver, and skin, and thereby effectively dismutates the superoxide radical at the site(s) of generation. Given the toxic nature of superoxide and high viscosity of the cytosol that restricts a diffusion-limited interaction between the enzyme and its substrate, it is not surprising that the two SOD isozymes are associated with the inside (Mn-SOD) and outside (Cu,Zn-SOD) of mitochondrial inner membranes (Fig. 2).

Immunoblot analysis revealed that digitonin released Cu,Zn-SOD from mitochondria without affecting the localization of cytochrome *c*. In contrast, depolarization of mito-

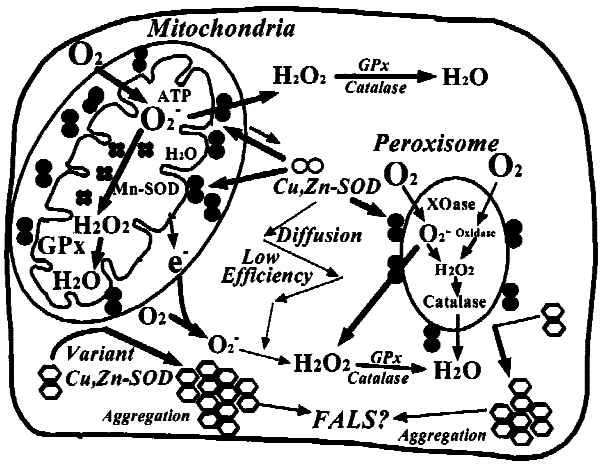


**FIG. 2. Mitochondrial localization of the two SOD isozymes.** Superoxide radicals ($O_2^-$) generated within the matrix and on the outside of mitochondrial inner membranes are dismutated efficiently at the sites of their generation by Mn-SOD and Cu,Zn-SOD, respectively. The superoxide radical generated by peroxisomes is also dismutated effectively on their cytoplasmic surface. Mutation of Cu,Zn-SOD found in patients with FALS markedly decreases the affinity of the enzyme to mitochondria and peroxisomes (25, 26). Presumably due to physicochemical changes in the molecular surface of Cu,Zn-SOD, the variant form of the enzyme readily undergoes aggregation (15, 19, 25, 29, 37). GPx, glutathione peroxidase; $H_2O_2$, hydrogen peroxide; XOase, xanthine oxidase.

chondria by phosphate and $Ca^{2+}$ induced the release of both Cu,Zn-SOD and cytochrome *c*. Thus, Cu,Zn-SOD seems to localize in the intermembranous space of mitochondria, but the nature of its association with this organelle differs from that of cytochrome *c*. Consistent with this notion, Cu,Zn-SOD was found to localize within the intermembranous space of mitochondria (33).

## IMPORTANCE OF MITOCHONDRIAL LOCALIZATION OF CU,ZN-SOD

About 20% of patients with FALS are associated with point mutation of the gene encoding Cu,Zn-SOD; >70 different types of mutation of the gene have been reported with FALS. Transgenic mice expressing the human type of variant Cu,Zn-SOD also showed motor neuron dysfunctions, and the resulting progeny developed clinical signs found in patients with FALS (19, 37). However, the mechanism by which variant Cu,Zn-SODs cause motor neuron degeneration is largely unknown.

We previously showed that Cu,Zn-SOD localizes bound to mitochondria and peroxisomes in rat brain, liver, and cultured human fibroblasts (26). To elucidate the role of subcellular localization of Cu,Zn-SOD in the pathogenesis of FALS, we analyzed the association of wild-type and variant enzymes (G37R, G41S, H46R, N86S, and I113T) to mitochondria. We found that the association of variant Cu,Zn-SODs to mitochondria was decreased significantly compared with that of the wild-type enzyme. Thus, effective dismutation of the superoxide radical in and around the intermembranous space in mitochondria-enriched cells and tissues would be impaired in patients with FALS (Fig. 2). The replaced amino acids in the FALS-related variant SODs are localized preferentially in the β-barrels of the enzyme that play important roles in the assembly of the two subunits and/or in determining the surface properties of the enzyme (15). Thus, mutation of the Cu,Zn-SOD gene at different sites might affect the surface properties of the enzyme required for the interaction with mitochondria, thereby increasing the oxidative stress in mitochondria-enriched cells and tissues.

## SUPER SYSTEM REGULATES CIRCULATION AND ENERGY METABOLISM

NO functions as an endothelium-derived relaxing factor (28, 29) and regulates intracellular calcium status (36). Because NO rapidly reacts with molecular oxygen ($k = 6 \times 10^6$ $M^{-2}$ $s^{-1}$), the lifetime of NO increases significantly under physiologically low oxygen concentrations (30, 44). Therefore, the change in local oxygen tensions in and around cells and tissues might affect biological functions of NO. In fact, the activities of NO to induce arterial relaxation (44) and to inhibit the respiration and ATP synthesis of cells (30), mitochondria (22), and some bacteria, including *E. coli* (47), strictly increased under physiologically low oxygen tensions (Fig. 3). When NO inhibits mitochondrial respiration, cellular ATP levels decrease transiently and return to control levels soon after the disappearance of its inhibitory effect. Mitochondria regulate cellular calcium homeostasis by an ATP-dependent mechanism. As NO inhibits the ATP synthesis in tumor cells, it strongly increases cytosolic levels of calcium $[Ca^{2+}]_i$ particularly under low oxygen tensions (30).

Because oxygen concentrations in mitochondria are significantly lower than those in cytosol, NO might affect the metabolism more strongly in the former than in the latter. Kinetic analysis using different respiratory substrates revealed that the reversible inhibition of mitochondrial respiration by NO reflects the inhibition at the site of cytochrome *c* oxidase. Cytochrome *c* oxidase is the terminal complex of the mitochondrial respiratory chain responsible for ~90% of oxygen consumption in mammals (2). The oxidase contains two hemes (cytochrome a and cytochrome $a_3$) and two copper centers ($Cu_A$ and $Cu_B$). Oxygen binds to the reduced form of a binuclear center consisting of cytochrome $a_3$ ($Fe^{2+}$) and $Cu_B$ ($Cu^{1+}$) in the complex. NO binds to the oxygen binding site of the $Fe^{2+}$ of cytochrome $a_3$ ($k = 0.4$–$1.0 \times 10^8$ $M^{-1}$ $s^{-1}$) and slowly dissociates from the enzyme ($k = 0.1$–$0.01$ $M^{-1}$ $s^{-1}$) (4–6, 10, 11, 13, 39). NO binds preferentially to the reduced form of $Cu_B^{1+}$, but also binds to $Cu_B^{2+}$ to form $Cu^{1+}$-$NO^+$, which is then converted to nitrite (7). Oxygen binds only to the reduced form of binuclear center ($Fe^{2+}$-$Cu^{1+}$). Thus, binding of NO to the oxidase increases the apparent $K_m$ of respiration for oxygen (10, 18, 46). Because two molecules of NO

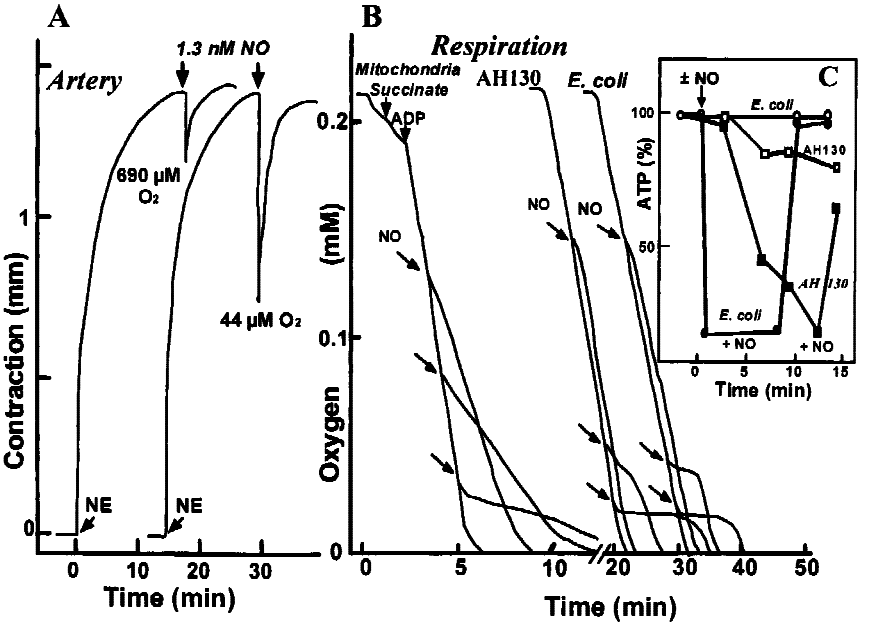


**FIG. 3. Oxygen tension regulates biological activities of NO.** Relaxation of the norepinephrine (NE)-treated aortic rings (**A**) and inhibition of the respiration (**B**) and ATP synthesis (**C**) in *E. coli*, mitochondria, and ascites hepatoma AH-130 cells by NO were determined at different oxygen tensions. NO induced arterial relaxation more strongly at low oxygen than at high oxygen tensions. NO reversibly inhibited the respiration of mitochondria (in the presence of 0.1 m*M* ADP and 5 m*M* succinate), AH-130 cells, and *E. coli;* the lower the oxygen tension, the stronger the inhibitory action of NO. During the inhibition of the respiration, cellular ATP levels decreased in a reversible manner, suggesting that NO functions as a physiological modulator of energy metabolism the potency of which is regulated by local oxygen tensions (22, 30, 44, 47).

compete with one molecule of oxygen, the $IC_{50}$ of NO increases in proportion to the square of the oxygen concentration (27, 43). The binding of NO to $Cu^{1+}$ might be the major pathway for the rapid inhibition of the oxidase (see Fig. 3).

## DETERMINATION OF APOPTOSIS BY A CROSS TALK OF NO AND SUPEROXIDE RADICALS

NO interacts with the terminal oxidase and enhances the generation of superoxide and related metabolites, which increases the intracellular free calcium ion $[Ca^{2+}]_i$ and activates $Ca^{2+}$-dependent phospholipase $A_2$, thereby opening membrane permeability transition (MPT) pores. It should be noted that small amounts of either hemoglobin or erythrocytes completely reversed the NO-inhibited respiration of cells (30). Thus, the biological activities of NO might be strong in extravascular cells with low oxygen tensions, but low in the circulation and hyperoxic tissues. Because oxygen tensions in the peritoneal cavity and the ascites fluid are fairly low, energy metabolism in ascites tumor cells would be inhibited strongly by NO derived from peritoneal macrophages that express high levels of inducible NO synthase (iNOS). In fact, NO released from peritoneal macrophage strongly inhibits the respiration and ATP-dependent processes in tumor cells (30). Because NO depolarizes the membrane potential, elevates the cytosolic $Ca^{2+}$, and releases cytochrome *c*, it might trigger the sequence of events leading to apoptosis of cells. In fact, macrophage-derived NO might play critical roles in host defense against tumor cells by inhibiting their energy metabolism and inducing apoptosis particularly under low oxygen tensions in the peritoneal cavity and in intestinal lumen.

When depolarized, mitochondria release apoptosis-related proteins, including cytochrome *c*, and play important roles in the determination of death and survival of cells (34). Release of cytochrome *c* from mitochondria is mediated by adenine nucleotide translocase (ANT) via a $Ca^{2+}$-mediated mechanism. Thus, the NO-induced inhibition of cytochrome *c* oxidase appears to initiate a cascade of events that might determine the death and survival of cells. It should be noted, however, that such an increase in $[Ca^{2+}]_i$ is not always the consequence of mitochondrial dysfunction because ATP levels and calcium homeostasis can be maintained by increasing the rate of glycolysis. In fact, peritoneal macrophages become strongly resistant to NO after the increase of their glycolytic activity. In this context, Rakhit *et al.* (35) reported that preconditioning of cardiomyocytes by an NO donor induced mitochondrial depolarization, but protected cells from anoxic injury. Brown and Borutaite (9) also reported that NO induces three types of reaction of mitochondria: reversible inhibition of their respiration, stimulated generation of superoxide, hydrogen peroxide, and peroxynitrite, and irreversible inhibition followed by induction of mitochondrial MPT. In

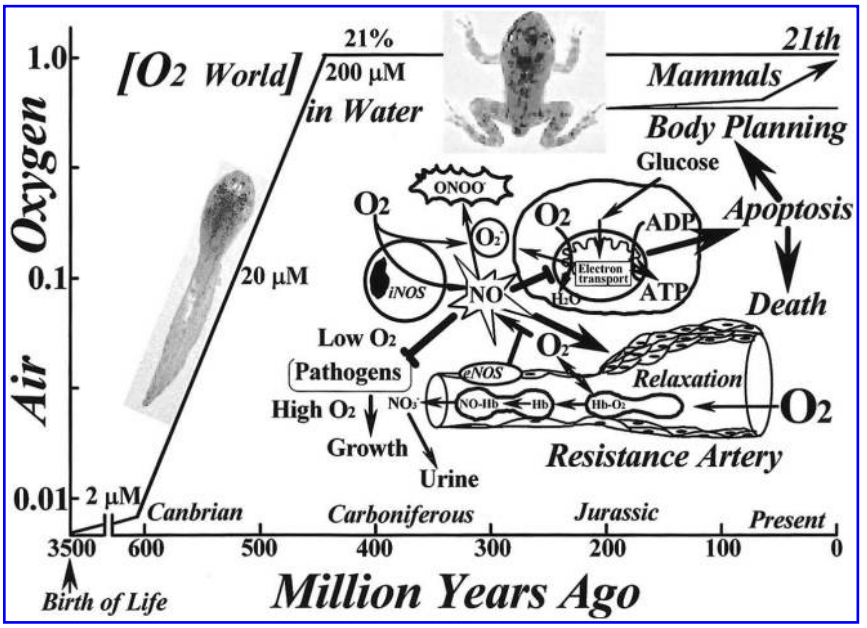


**FIG. 4. The cross talk of $O_2$, NO, and superoxide radical ($O_2^-$) pivotally regulates the energy metabolism at resistance arteries and mitochondria.** NO increases oxygen delivery to tissues by inducing vasorelaxation, but inhibits electron transport and ATP synthesis in mitochondria and aerobic bacteria predominantly by interacting with their terminal oxidases. Lowering oxygen tensions increases the biological activities of NO. Inhibition of electron transport releases free electrons and increases the generation of the superoxide that rapidly reacts with NO. Such a pivotal action of NO and the cross talk of the three radicals constitute a super system that cooperatively regulates the circulatory status and energy metabolism, and functions as a defense mechanism against pathogens. The super system acquired by higher organisms during evolution seems to play critical roles in the evolution, embryonic development, remodeling of tissues, and metamorphosis of various animals (22, 23) and in the pathogenesis of age-related diseases (22, 25). eNOS and iNOS, endothelial and inducible NO synthase, respectively; Hb, hemoglobin; $ONOO^-$, peroxynitrite.

some cases, inhibition of mitochondrial respiration by NO can induce either necrosis when glycolysis is insufficiently compensated or apoptosis by activating the mitochondrial pathway to induce MPT. Thus, the relative ratio of the dependency of cells to generate ATP by mitochondria to that by activated glycolysis seems to be an important factor that determines the fate of cells exposed to NO. Because mitochondrial release of apoptosis-related proteins can be triggered by functional and/or structural modifications of ANT and voltage-dependent anion channel in their membranes by a variety of ligands, including free radicals, enhanced release of free electrons followed by generation of the superoxide radical by NO might facilitate the sequence of events leading to apoptosis. Figure 4 shows the cross talk of NO, superoxide radical, and molecular oxygen that cooperatively regulates the circulation, energy metabolism, and apoptosis, and functions as a potent defense mechanism against pathogens and cancer.

## OXIDATIVE INJURY OF MITOCHONDRIA AND THEIR DNA IN PATIENTS WITH CHRONIC INFLAMMATION AND CANCER

Because large amounts of reactive oxygen and nitrogen species are generated by activated leukocytes, inflammation is one of the major causes that impair cellular constituents, such as lipids, proteins, and DNA. Oxidative modification of DNA frequently causes mutation that underlies the mechanism of carcinogenesis. In fact, the incidence of cancer significantly increases in patients with chronic hepatitis and ulcerative colitis (32). Persistent infection with hepatitis B or C virus ultimately results in the development of hepatocellular carcinoma, the process of which is fairly rapid in individuals with viral hepatitis and high levels of alanine aminotransferase in plasma. Treatment of hepatitis patients with interferon reduces the incidence of hepatocellular carcinoma even if hepatitis viruses are not eliminated. Chronic inflammation induced by hepatitis viruses thus plays important roles in hepatocarcinogenesis. Generation of reactive oxygen species is markedly enhanced by inflammatory reactions in the liver of individuals infected with hepatitis viruses (38), and oxidative stress is a potent inducer of DNA mutations (3). Recent studies revealed that cellular constituents in the liver of patients with chronic hepatitis are modified significantly by reactive oxygen and nitrogen species. Immunohistochemical and biochemical examinations revealed a marked expression of iNOS in hepatocytes with a diffuse lobular pattern in all liver samples from patients with viral hepatitis (17). The amount of nitrotyrosyl proteins was significantly higher in liver biopsies from viral liver disease than from nonviral liver disease. Furthermore, NO-mediated nitration

of proteins is markedly increased in the inflammatory liver tissue from patients with chronic hepatitis; the extent of nitrosylated proteins increased with the increase in the histological severity of chronic viral hepatitis. The presence of a substantial amount of nitrotyrosyl proteins suggests the occurrence of both NO and superoxide radicals that cause oxidative injury of proteins and DNA during the course of long-lasting inflammation.

Mutations accumulate to a greater extent in mtDNA than in nuclear DNA (14). Expression of the entire mitochondrial genome is necessary for the maintenance of mitochondrial functions, suggesting that small changes in the sequence of mtDNA might result in profound impairment of their functions. Therefore, we hypothesized that the increase in oxidative stress in individuals with chronic inflammation might enhance the mutation of mtDNA and the impaired mitochondria generate substantial amounts of free radicals, thereby increasing the risk of cancer through the enhancement of DNA mutation in mitochondria and nucleus. To test this hypothesis, we sequenced the mtDNA in cancerous and noncancerous regions of the liver of individuals with hepatocellular carcinoma, and compared the sites and frequencies of mutations with those detected in normal liver (32). The mtDNA sequence obtained from the liver specimens of the control subjects contained three single-base mutations, all of which were located in the D-loop (Fig. 5). The mtDNA sequences obtained from the cancerous tissue specimens contained numerous mutations, at least half of which were also apparent in the mtDNA obtained from the paired noncancerous liver specimens. mtDNA harboring certain mutations might generate abnormal RNAs and/or proteins, the latter of which may promote leakage of electrons from the mitochondrial electron transport chain. The amounts of endogenously produced reactive oxygen species might thus be increased in cells with mutant mtDNA. The resulting oxidative modification of DNA therefore may contribute to the initiation and/or promotion of hepatocarcinogenesis.

The high frequency of mtDNA mutations in noncancerous liver tissues of individuals with hepatocellular carcinoma suggests that hepatocytes in such tissues may already have undergone the initial stage of malignant transformation during chronic inflammation. The finding that most of the mutations detected were homoplasmic in nature indicates that the mutated mtDNA had become dominant in both cancer tissues and hepatocytes in noncancerous regions of the inflammatory liver. Given the clonal nature and large number of copies of mtDNA, mutation of the mitochondrial genome in noncancerous regions of the liver of individuals with chronic hepatitis is indicative of genomic instability and likely contributes to the hepatocarcinogenesis.

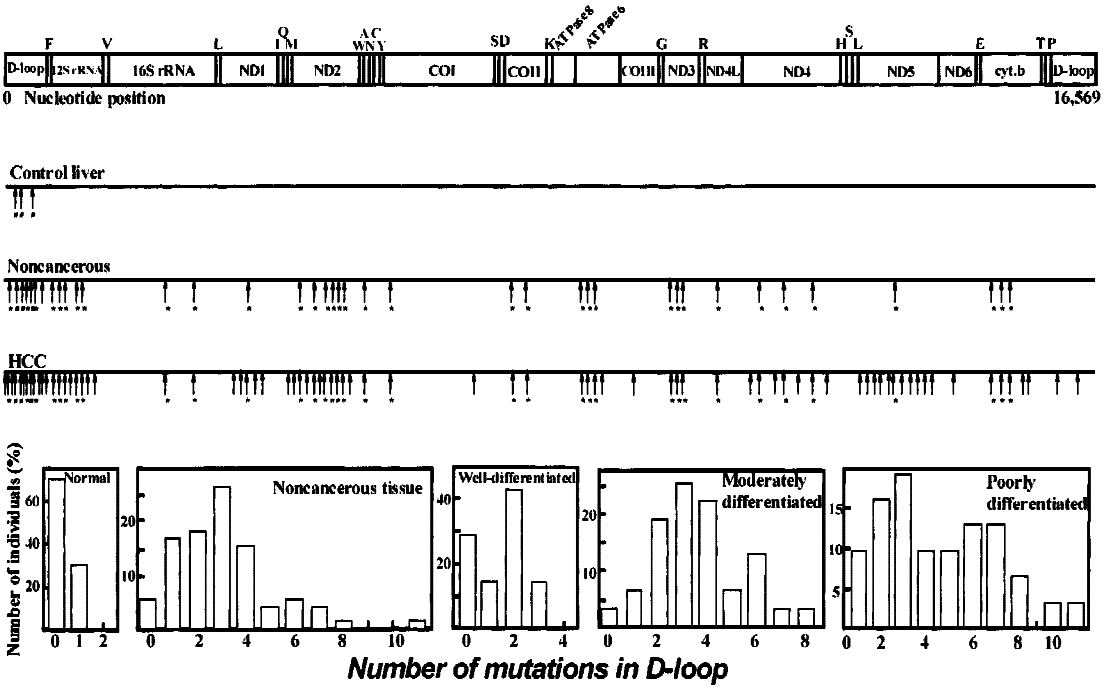


**FIG. 5. mtDNA mutation in patients with chronic hepatitis and liver cancer.** mtDNA samples obtained from a normal human subject and from a cancer patient with chronic hepatitis were sequenced and the sites of their mutations compared (arrows). mtDNA specimens obtained from the cancerous (HCC) and noncancerous portion of the liver of a patient contained numerous numbers of mutations. The extent of mutation in the D-loop regions of mtDNA in cancer specimens from patients with HCC showing different stages of histological malignancy is also compared. The numbers of the ordinates show the % of individuals having different numbers of mtDNA mutations in their D-loops (32).

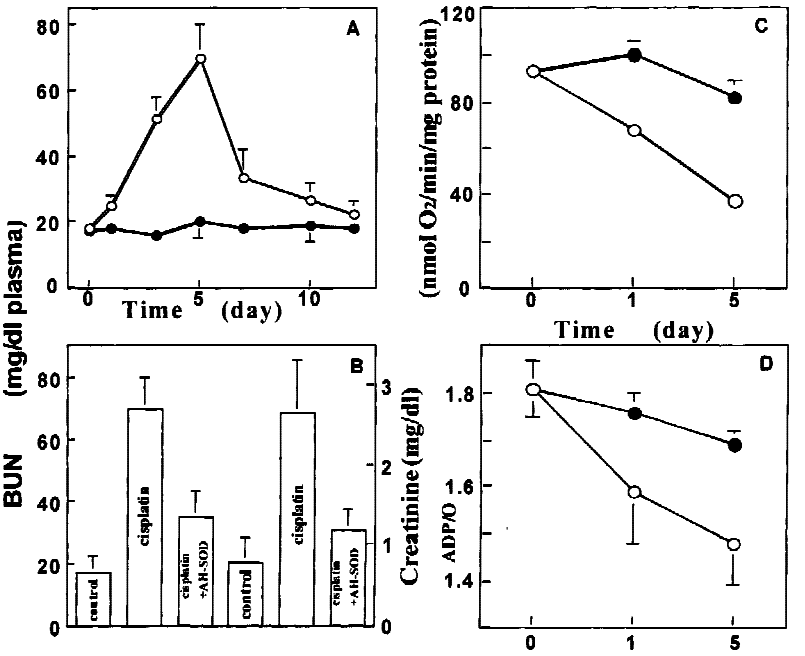


**FIG. 6. Inhibition of cisplatin nephropathy by targeting SOD.** After administration of 5 mg/kg cisplatin to rats with (●) or without AH-SOD (○), plasma levels of blood urea nitrogen (BUN; **A** and **B**) and creatinine (B) and renal mitochondrial respiration (**C**) and respiratory control index (**D**) were compared using two animal groups. AH-SOD rapidly undergoes glomerular filtration, binds to renal brush border membranes, and accumulates within proximal tubule cells, thereby effectively dismutating the superoxide radicals inside cells. Cisplatin strongly impaired renal mitochondria and induced nephropathy by some AH-SOD-inhibitable mechanism (12, 21, 31).

## MITOCHONDRIA AS A POTENTIAL TARGET FOR CHEMOTHERAPY

Although hazardous free radicals underlie the mechanism of carcinogenesis, they also play important roles in the treatment of patients with cancer, such as radiation and chemotherapy. However, these methods are highly limited because of their strong side effects in normal cells and tissues. Thus, prevention of the side effects of anticancer agents without suppressing their tumoricidal activity is of critical importance in treating patients with cancer. Because *cis*-dichlorodiammine platinum (II) (cisplatin) preferentially accumulates in the kidney and gastrointestinal tract and generates reactive oxygen species in and around their mitochondria and nucleus, this anticancer agent exhibits strong side effects to impair these tissues. To decrease oxidative stress effectively and site-specifically in the kidney, we synthesized a hexamethylenediamine-conjugated SOD (AH-SOD) that rapidly undergoes glomerular filtration, accumulates in renal proximal tubule cells, and selectively dismutates the superoxide radical without being excreted in the urine (21).

The administered cisplatin rapidly accumulated in the kidney, oxidatively impaired mtDNA, decreased mitochondrial functions, induced apoptosis of renal tubule cells, and finally caused severe nephropathy (Fig. 6). Intravenous administration of nephrophilic AH-SOD inhibited the cisplatin-induced mtDNA injury, mitochondrial dysfunctions, and tubule cell apoptosis, thereby preventing renal dysfunction. Thus, the superoxide radicals generated in and around mitochondria and nucleus in renal proximal tubule cells play critical roles in the pathogenesis of cisplatin-induced nephropathy. Recent studies in this laboratory (31) revealed that AH-SOD inhibited the renal injury caused by cisplatin without suppressing the tumoricidal activity of this drug and strongly improved the survival of tumor-bearing animals. Thus, targeting AH-SOD to renal proximal tubules might permit administration of sufficiently high doses of anticancer agents having nephrotoxic nature, thereby effectively killing extrarenal cancer cells without causing renal injury. AH-SOD has thus therapeutic potential in inhibiting the toxicity of nephrophilic agents that oxidatively damage renal tubule cells.

## EFFECT OF CARNITINE ON THE SIDE EFFECTS OF ANTICANCER AGENTS

We recently found that free forms of long-chain fatty acids released by calcium-activated phospholipase $A_2$ perturbed membrane/lipid bilayers of mitochondria, thereby enhancing the oxidative injury of mitochondrial functions to induce MPT pores, a prerequisite reaction to cytochrome *c* release to trigger the chain reactions leading to apoptosis. Because L-carnitine forms conjugates with free forms of long-chain fatty acids and enhances β-oxidation to generate ATP, this amino acid may suppress the fatty acid-induced mitochondrial injury and apoptosis. Because the kidney and small intestine are highly enriched with carnitine transporters OCTN2 (45) and are the critical sites for eliciting the side effects of cisplatin, we hypothesized that carnitine might protect these tissues from toxic side effects of the agent in both tissues. In fact, oral administration of carnitine strongly inhibited the cisplatin-induced mitochondrial injury and apoptosis of ep-

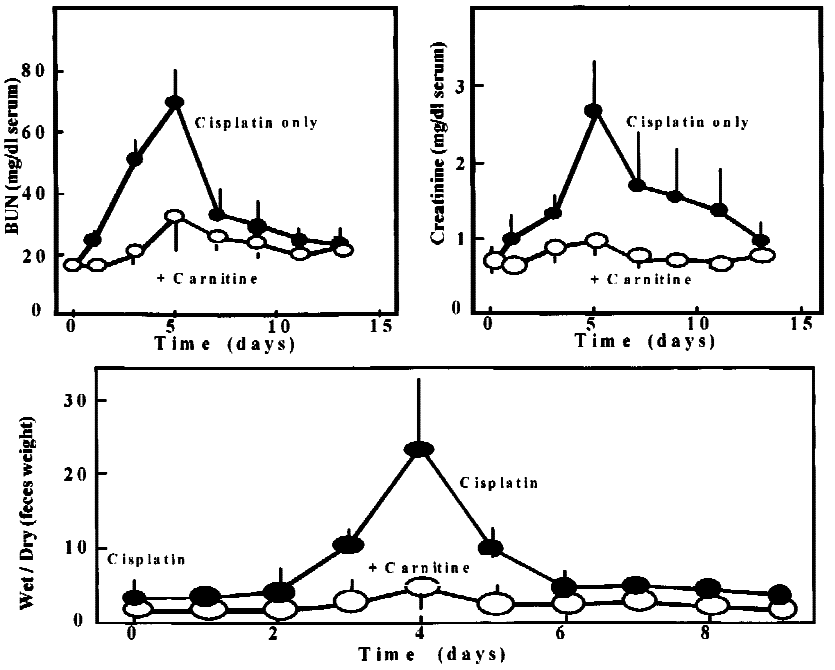


**FIG. 7. Inhibition of cisplatin-induced injury of the kidney and intestine by L-carnitine.** After intraperitoneal administration of 5 mg/kg cisplatin with (40 mg/animal, open circles) or without L-carnitine (closed circles), plasma levels of blood urea nitrogen (BUN) and creatinine were compared using two animal groups (**upper panels**). Under identical conditions (**lower panel**), the occurrence of diarrhea was compared with carnitine-treated (open circles) and untreated (closed circles) groups (12).

ithelial cells in the kidney and small intestine (Fig. 7); carnitine protected the functions of both organs and suppressed the increase in plasma blood urea nitrogen and creatinine and the occurrence of diarrhea and mucosal atrophy (12). Thus, site-specific protection of mitochondria and mtDNA from hazardous anticancer agents by targeting SOD and/or carnitine may have therapeutic potential in patients with cancer to minimize the side effects of anticancer agents without decreasing their tumoricidal activity.

## ABBREVIATIONS

AH-SOD, hexamethylenediamine-conjugated superoxide dismutase; ANT, adenine nucleotide translocase; EC-SOD, extracellular superoxide dismutase; FALS, familial amyotrophic lateral sclerosis; iNOS, inducible nitric oxide synthase; MPT, membrane permeability transition; NO, nitric oxide; SOD, superoxide dismutase.

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