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EXPERT REVIEW

Superoxide Dismutase Administration, A Potential Therapy Against Oxidative Stress Related Diseases: Several Routes of Supplementation and Proposal of an Original Mechanism of Action

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ABSTRACT Oxidative stress, involved in many diseases, is defined as an impaired balance between reactive oxygen species (ROS) production and antioxidant defences. Antioxidant enzymes such as superoxide dismutase (SOD) play a key role in diminishing oxidative stress. Thus, the removal of ROS by exogenous SODs could be an effective preventive strategy against various diseases. The poor bioavailability of exogenous SODs has been criticized. However, improvements in SOD formulation may overcome this limitation and boost interest in its therapeutic properties. Here, we provide a review of animal and human studies about SODs supplementation in order to evaluate their therapeutic value. Protective effects have been observed against irradiation, carcinogenesis, apoptosis and neurodegeneration. SODs administration has also been reported to alleviate inflammatory, infectious, respiratory, metabolic and cardiovascular diseases and genitourinary and fertility disorders, raising the question of its mechanism of action in these diverse situations. Some authors have shown an increase in endogenous antioxidant enzymes after exogenous SODs administration. The induction of endogenous antioxidant defence and, consequently, a decrease in oxidative stress, could explain all the effects observed. Further investigations need to be carried out to test the hypothesis that SODs supplementation acts by inducing an endogenous antioxidant defence.

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ABBREVIATIONS

AAPH	Hydrochloride 2,2′-azobis-2-amidinopropane
AIDS	Acquired immunodeficiency syndrome
ARE	Antioxidant response element
CAT	Catalase
Cu/Zn-SOD	Copper/zinc-superoxide dismutase
DIVEMA	Divinyl ether and maleic anhydride
DNA	Deoxyribonucleic acid
EC-SOD	Extracellular-superoxide dismutase
Fe-SOD	Iron-superoxide dismutase
FIV	Feline immunodeficiency virus
GPx	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HIV	Human immunodeficiency virus
HO°	Hydroxyl radical
Mn-SOD	Manganese-superoxide dismutase
Nrf2	Transcription factor nuclear-factor-E2-related factor
$O_2^{\circ-}$	Superoxide anion radical
PEG	Pegylated
PMA	Phorbol 12-myristate 13-acetate
ROS	Reactive oxygen species
SOD	Superoxide dismutase

INTRODUCTION

A paradox in metabolism is that, while aerobic organisms require oxygen for their existence, oxygen is also a highly reactive molecule that can cause a lot of damage. Reactive

oxygen species (ROS), physiological metabolites resulting from respiration such as the superoxide anion radical ($O_2^{\circ-}$), the hydrogen peroxide (H_2O_2), or the hydroxyl radical (HO°), are very unstable and rapidly damage other substances including DNA, membrane lipids and proteins (1). However, a complex network of antioxidant metabolites and enzymes work together to prevent this oxidative damage (Fig. 1). ROS also have useful cellular functions, such as redox signalling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (2).

Oxidative stress is defined as an impaired balance between ROS production and antioxidant defences, resulting in the accumulation of oxidative products (Fig. 1). It is involved in many diseases, such as inflammatory or cardiovascular diseases (3). The removal of free radicals by exogenous antioxidant compounds could thus be an effective precautionary measure against various diseases. Several kinds of supplements containing antioxidant compounds have been described, including those with metabolites such as selenium or vitamins as well as antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPx), which have longer lasting effects because of a lower rate of exhaustion.

One of the most critical oxidative radicals, the $O_2^{\circ-}$ is biologically significant because of its capacity to generate other more reactive species such as HO° (4) (Fig. 1). The best physiological defence against $O_2^{\circ-}$ is the enzyme superoxide dismutase, which converts two superoxide anions into one molecule of hydrogen peroxide and one of oxygen (5,6).

In 1968, McCord and Fridovich characterised the SOD enzyme from bovine erythrocytes, by asking what could dissociate $O_2^{\circ-}$ produced by xanthine oxidase into oxygen and H_2O_2 (7). Today, several common forms of SOD are known: they are oligomeric proteins cofactored with copper and zinc, or manganese, or iron. SOD subunits exhibit a two-domain structure: one domain contains α-helices and the second is composed of both α-helices and β-sheets. The metal binding site occurs between these two domains and the ligands of the metal ions are composed of histidine and aspartate side-chains (8). In mammals, there are 3 isoforms of SOD, produced by distinct genes: copper/zinc SOD (Cu/Zn-SOD), a homodimer of 32 kDa, localized in the cytosol or mitochondrial inter-membrane space; manganese SOD (Mn-SOD), a homotetramer of 88 kDa, localized in the mitochondria (matrix and inner membrane), and Cu/Zn form (EC-SOD), an extracellular tetrameric glycoprotein of 135 kDa (5,8,9). Some bacteria contain an iron SOD (Fe-SOD), others Mn-SOD, and some contain both (8). In plants, 3 isoforms are present: mitochondrial Mn-SOD, a Cu/Zn-SOD in the cytosol and the chloroplasts and a Fe-SOD in the chloroplasts (6,10).

The main role of SODs in all aerobic organisms is to neutralize the $O_2^{\circ-}$ produced in the cytosol, mitochondria and endoplasmic reticulum of cells. However, the SOD can also have a pro-oxidant effect because the dissociation of the $O_2^{\circ-}$ produces H_2O_2, which is toxic to cells. It is to remove this dangerous H_2O_2 that the presence of others antioxidant systems, such as CAT and GPx enzymes, becomes necessary (7) (Fig. 1). An imbalance in the ratio of SOD to CAT and

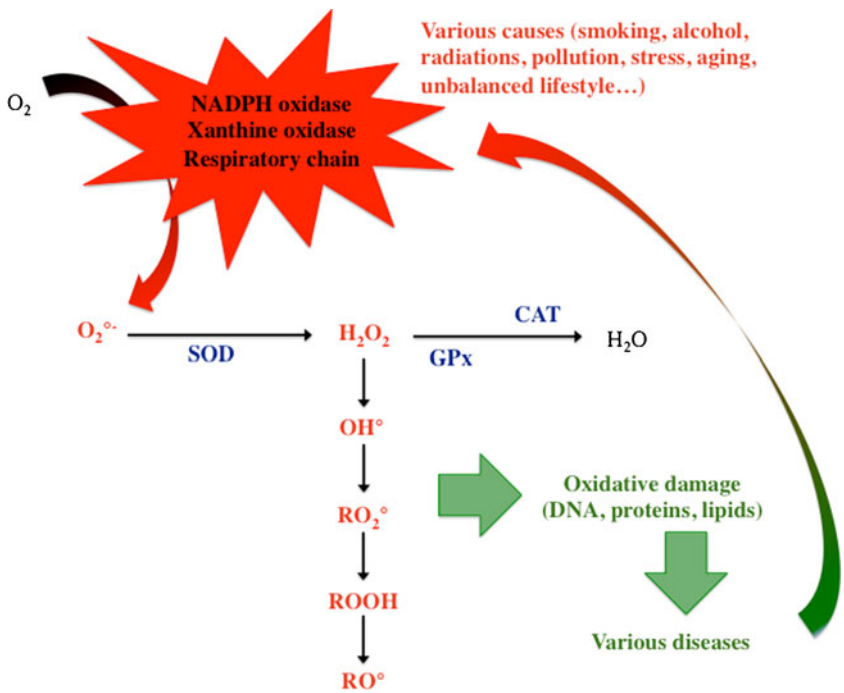

Fig. 1 Mechanism of oxidative stress induction. An impaired balance between ROS (reactive oxygen species) production and antioxidant defences results in the accumulation of oxidative products.

GPx could be involved in some diseases. For example, Down syndrome patients display an increased Cu/Zn-SOD/GPx and CAT activity ratio (11,12).

Several studies regarding the effects of SOD on animals were carried out by Emerit at the end of 70's. In 1983, he initiated the first human study in a case of fibrosis. Then, Edeas studied the effects of the bovine SOD on AIDS in 1993 (13). Finally, the antioxidant enzyme was used in several therapeutic studies with promising results, as reviewed by Nordmann and Ribiere (7). However, clinical research into this molecule was slowed to a great extent because of the removal from the market of bovine SOD following the Creutzfeldt-Jacob epidemic in 1991. Nowadays, the availability of non-bovine SODs again boosts their therapeutic interest. Indeed, several SODs, extracted from plants or produced in bacteria, are used in animal and human studies. Use of SODs mimetics and the study of SOD-overexpressed models have also been developed in order to better understand SODs effects and their mechanisms of action.

There are numerous studies regarding the administration of SODs in very different pathological situations, raising the question of how a single molecule could have so many possible applications. We decided to carry out a review of these very heterogeneous results, in order to attempt to answer the following questions: (i) What pathological situations can be improved by the administration of SODs? (ii) What mode of administration is efficient? (iii) What hypothesis could explain its mechanism of action?

BENEFICIAL EFFECTS OBSERVED AFTER SOD ADMINISTRATION

Positive effects have been observed in diverse situations after SODs administration. A review of the studies published in the last 30 years is presented below, and summarized in Table I.

First, several groups have shown an increase in resistance to irradiation and a decrease in its side effects following SODs supplementation. Concerning the resistance to irradiation, Epperly *et al.* (14) and Greenberger *et al.* (15) have shown that the survival of mice given an intravenous injection of human Mn-SOD is greater than that of control mice (90% *vs.* 58%) after total-body irradiation. The physiological functions of several rodent organs (lung (16,17), oesophagus (18,19), head and neck area (20,21), oral cavity (22), pelvic region (23), and bladder (24–27)) are better preserved after different SODs administrations near the irradiated area, before or after radiation.

The modification of tissues by irradiation is manifested by the development of fibrosis in the irradiated zone. The protective effects of SODs administration on the appearance of post-radiation fibrosis have been investigated by several groups. A study on cultured fibroblasts from human skin with chronic radiotherapy damage has demonstrated the decrease of pro-fibrotic markers in the extracellular matrix after bovine Cu/Zn-SOD incubation (28). Rabbani *et al.* (17) have shown that subcutaneous injection of bovine Cu/Zn-SOD prevents collagen deposition, which lies at the origin of the fibrosis, in irradiated rodents. Lefaix *et al.* (29) have observed that curative SODs treatment (intramuscular injections) for 3 weeks reduces fibrotic areas in irradiated pigs. Finally, clinical studies in humans have shown a decrease in radiotherapy-induced fibrotic areas with topical application of tomato Cu/Zn-SOD (30) or intramuscular injection of bovine Cu/Zn-SOD (31), near the damaged areas for several days.

Several authors have also demonstrated a beneficial effect of SODs supplementation on cell resistance. The inhibition of damage on DNA plasmids incubated with SOD shown by Takehara *et al.* (32) suggests a decrease in apoptosis following incubation with SODs. A study in mice given oral melon SODs supplementation for 28 days has demonstrated a decrease in hepatocyte apoptosis (20% *vs.* 72% in controls) and improved resistance to the induced hemolysis of red blood cells (33). Our work (unpublished data) has confirmed these results in hamsters, by demonstrating an increase in the latency to AAPH (2,2′-azobis-2-amidinopropane hydrochoride)-induced lysis following oral supplementation with melon SODs for 1 month both in total blood cells and in red blood cells (Fig. 2). The experiment consisted in generation of alkoxyl and peroxyl radicals measured by electron paramagnetic resonance as previously described by Prost *et al.* (34,35). Notin *et al.* (36) have also shown that oral supplementation with melon SODs increases blood resistance to haemolysis of horses in training. Leskova (37) have also shown that bovine Cu/Zn-SOD injection in a cat model of hemorrhagic shock leads to improved cell resistance by protecting the phospholipid bilayer of the plasma membranes of hepatocytes and adipocytes in cats.

Numerous positive effects of SODs administration have been demonstrated in inflammatory diseases. First, several studies have been carried out in rat models of oedema induced in the paw. Regnault *et al.* (38) and Jadot *et al.* (39) have demonstrated a preventive effect of different SODs administrations on both oedema volume and the associated inflammation.

Then, the anti-inflammatory properties of SODs administrations have been shown in rat models of induced polyarthritis (40,41). Many of the clinical signs observed in the rat closely resemble those of human rheumatic diseases and the Fiessinger-Leroy-Reiter syndrome. Several studies of arthritic diseases in humans have also shown beneficial effects of bovine Cu/Zn-SOD on the knee (42–47), finger joint (48), elbow (49) and temporomandibular joint (50), after intra-articular injections.

Table I Summary of Positive Effects Observed After SODs Administration in Several Studies

Pathological situations	References	Routes of administration and SOD nature
Irradiation resistance and side effects	(14,15)	Intravenous injection of human Mn-SOD
	(16)	Intratracheal injection of human Mn-SOD
	(17)	Subcutaneous injection of bovine Cu/Zn-SOD
	(18)	Oesophageal injection of human Mn-SOD
	(19,22)	Oral administration of human Mn-SOD
	(20)	Aerosolized bovine Cu/Zn-SOD
	(21,23,27,29,31)	Intramuscular injection of bovine Cu/Zn-SOD
	(24,26)	Intramural (bladder) injection of bovine Cu/Zn-SOD
	(25)	Submucosal infiltration of bovine Cu/Zn-SOD
	(28)	Incubation with bovine Cu/Zn-SOD
	(29)	Intramuscular injection of bovine Mn-SOD
	(30)	Topical application of green tomato Cu/Zn-SOD
Cell resistance	(33,36)	Oral administration of melon SODs (Cu/Zn, Mn and Fe)
	(37)	Injection of bovine Cu/Zn-SOD
Inflammatory diseases	(38)	Oral administration of bovine Cu/Zn-SOD
	(39–41,51)	Intraperitoneal injection of different SODs of several sources
	(42–50)	Intraarticular injection of bovine Cu/Zn-SOD
	(52)	Intravenous injection of of bovine Cu/Zn-SOD
	(53)	Subcutaneal injection of bovine Cu/Zn-SOD
	(54)	Oral administration of Mn-SOD
Carcinogenesis	(55)	Oral administration of melon SODs
Infectious diseases	(13)	Incubation with bovine Cu/Zn-SOD
	(56)	Oral administration of melon SODs
Nervous tissue and superior functions	(57,59)	Intravenous injection of bovine Cu/Zn-SOD
	(58,60)	Oral administration of melon SODs
Carbohydrate-lipid metabolism	(37)	Injection of bovine Cu/Zn-SOD
	(61,62)	Oral administration of melon SODs
Cardiovascular diseases	(62)	Oral administration of melon SODs
	(63)	Injection of bovine Cu/Zn-SOD
	(64)	Intravenous injection of human Cu/Zn-SOD
Respiratory diseases	(65,67)	Aerosolized bovine Cu/Zn-SOD
	(66,69,71,72)	Intravenous injection of bovine Cu/Zn-SOD
	(68,70)	Aerosolized human Mn-SOD
	(73)	Inhalation or intravenous injection of human Cu/Zn-SOD
	(74)	Intratracheal injection of human Cu/Zn-SOD
Genitourinary disorders and male fertility	(75)	Incubation with non-specified SOD
	(76–79)	Local (penis) injection of bovine Cu/Zn-SOD

A beneficial effect has also been demonstrated in ischemia-reperfusion models. The deleterious consequences of paw ischemia (51) and intestine ischemia (52) have been shown to be attenuated after different SODs administrations in rodents.

Finally, the prevention of colonic inflammation (leukocyte recruitment, vascular cell adhesion molecule-1 expression) by subcutaneous injection of bovine Cu/Zn-SOD (53) or oral administration of recombinant Mn-SOD (54) has been observed in animal models of induced colitis.

Some groups have also described the effects of SODs administration on carcinogenesis. Takehara *et al.* (32) have observed, *in vitro*, the inhibition of damage induced by certain radicals ($O2^{\circ-}$, HO° and hypochlorite) on DNA plasmids incubated with SOD. This observation suggests that the administration of SODs could inhibit the induction of tumours. Moreover, the prevention of tumour progress (induced by inflammation) has been shown in mice following oral administration of melon SODs (55).

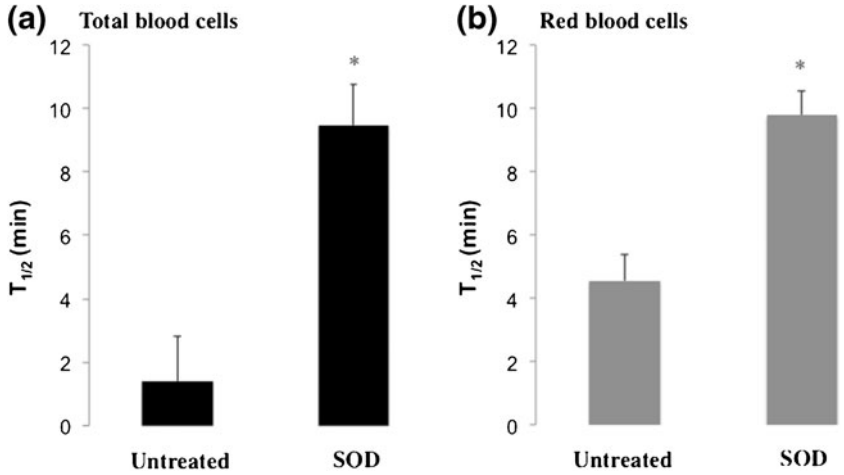

Fig. 2 Increased resistance of total blood cells and red blood cells after oral SOD supplementation. Oral supplementation with melon SODs (80U/day) for 28 days in healthy hamsters led to increased resistance to AAPH-induced lysis in total blood cells (**a**) and red blood cells (**b**). Resistance is expressed in minutes, and corresponds to the time necessary for the lysis of 50% of the cells ($T_{1/2}$) after the radical attack. *: $p < 0.05$; SOD-treated *vs.* untreated animals.

Some authors have, in addition, established the beneficial effects of SODs supplementation in infectious diseases. Webb *et al.* (56) have demonstrated the beneficial effects of oral melon SODs supplementation in FIV-infected cats. Indeed, FIV infection involves modifications in the lymphocyte profile (CD4+/CD8+ ratio), which are restored by SODs supplementation. This beneficial effect improves the organism's defence against AIDS. Edeas and colleagues have carried out studies of human AIDS (13). They have shown, *in vitro*, that bovine Cu/Zn-SOD inhibits HIV replication, and that no viral transmission is possible in the presence of the enzyme. They have also demonstrated the protection of DNA by bovine Cu/Zn-SOD, similar to the findings of Takehara *et al.* (32).

Several groups have also shown positive effects of SODs administration on nervous tissue damage and higher brain functions. Concerning nervous tissue protection, Wengenack *et al.* (57) have shown the improved survival of rat CA1 neurons, following global cerebral ischemia, after an intravenous injection of bovine Cu/Zn-SOD. An increase in neurogenesis has also been demonstrated in a mouse model of restraint stress after oral melon SODs administration (58). Hamm *et al.* (59) have observed the prevention of motor deficits in a rat model of brain ischemia-reperfusion by the intravenous injection of bovine Cu/Zn-SOD. Then, the oral administration of melon SODs has been shown to improve cognitive functions, such as memory, learning and concentration both in mice (58) and in human patients (60).

Studies of carbohydrate and lipid metabolism have also demonstrated beneficial effects of SODs administration. The prevention of non-alcoholic steatohepatitis has been shown after 2–3 months of oral supplementation with melon SODs in animal models of diet-induced obesity (61,62). These studies have also shown that SODs prevent body weight increase and obesity markers (cholesterol, triglycerides, leptin, insulin…). Finally, Leskova (37) has shown a decrease in the concentration of chylomicrons and very-low-density lipoproteins in the venous blood of central vessels after an injection of bovine Cu/Zn-SOD in cats.

Positive effects of SODs on cardiovascular diseases have also been shown. Décordé *et al.* (62) have demonstrated that oral administration of melon SODs prevents aortic lipid deposition in an animal model of diet-induced atherosclerosis. Other authors have shown that injection of bovine (63) or human (64) Cu/Zn-SOD reduces blood pressure in rat models of hypertension.

Various studies have also shown beneficial effects of SODs administration in several respiratory disorders. Different SODs administrations have been shown to provide protection against pulmonary oxygen toxicity in some animal studies (65–71). Assa'ad *et al.* (72) have demonstrated an attenuation of chronic allergic asthma in rabbits after intravenous injection of bovine Cu/Zn-SOD. Tanaka *et al.* (73) have shown that inhalation or intravenous injection of human Cu/Zn-SOD protects mice against pulmonary emphysema. Finally, human Cu/Zn-SOD intratracheal administration has been shown to decrease manifestations of respiratory distress syndrome in premature human neonates (74).

Finally, some positive effects of SODs supplementation have been shown in genitourinary disorders and male fertility. Cocchia *et al.* (75) have shown that the addition of SOD to semen extenders improves the quality of chilled equine semen (mobility and integrity of sperm cells). This beneficial effect on fertility could be attributed to a decrease in the oxidative stress experienced by the semen, as measured by levels of extracellular signal-regulated kinase activity.

In several human studies, there have been marked improvements in Peyronie's disease after an injection of bovine Cu/Zn-SOD into the indurated areas of the penis (76–79).

In conclusion, SODs supplementation exhibits beneficial effects in various pathological situations. Oxidative stress and inflammation are involved in all these diseases.

SEVERAL ROUTES OF SOD ADMINISTRATION

Several routes of SODs administration have been described, as shown above and in Table I.

Some authors have demonstrated the efficacy of oral SODs supplementation, as part of the diet or by force-feeding (19,33,38,54,80).

Many authors administer SODs by subcutaneous (17,53), intravenous (15,52,57,64,69,81), intraperitoneal (40,51), intramuscular (21,27,29), or local (16,18,24,45,76) injections.

Others have chosen to deliver SODs directly to the damaged body part. For example, Campana *et al.* (30) have administered vegetal Cu/Zn-SOD as an ointment to women with palpable skin fibrosis following breast irradiation, in order to evaluate the local anti-fibrotic effects of the enzyme. Finally, aerosolized SODs have been used for several studies concerning respiratory disorders (20,65,68).

However, all these routes of administration have limitations. We cannot imagine that a high molecular weight protein like the SOD can enter into the cells. Even if it could reach the blood circulation, the half-life of SODs in human plasma is only a few minutes (82) because of their elimination from the circulation by the kidneys (81,83,84). Indeed, *in vivo* studies have shown that injected SOD accumulates preferentially in the kidney than in other organs. Consequently, therapeutic levels of SOD are not maintained in plasma or non-renal tissues (81). This is probably why, in numerous studies, SODs were administered close to the damaged organ.

Moreover, the oral administration of SODs is also not very effective because of the degradation of the protein before it reaches the intestinal barrier (33,80,85,86) or its elimination in the faeces (87).

Improvements in the pharmacological formulation of SODs could overcome these limitations.

Improvement of Delivery to Targeted Cells After Blood Administration

Several modifications to increase the half-life of SODs in the plasma or their delivery to target cells have been undertaken.

Corvo *et al.* (88,89) have explored the utility of pegylated liposomes (PEG-liposomes) in targeting bovine Cu/Zn-SOD to arthritic sites after intravenous administration. They have demonstrated that the half-life of this SOD in the circulation is prolonged and that the enzyme can be targeted to inflamed sites more efficiently *via* PEG-liposomes.

Moreover, inclusion in cationic liposomes has been found to significantly enhance the antioxidant effect of Cu/Zn-SOD in induced oxidative damage of the jejunal mucosa and experimental colitis (90,91). This effect was caused by the increased cell adhesion of the liposomes as compared to free SOD.

Swart *et al.* (81) have demonstrated a prolongation of the half-life of bovine Cu/Zn-SOD when it is coupled to a copolymer of divinyl ether and maleic anhydride (DIVEMA). With this approach, they have shown the improved delivery of this SOD into target cells and a concomitant increase in its effectiveness.

Then, the administration of polyamine-modified Cu/Zn-SOD could increase blood–brain barrier permeability and preserve enzymatic activity (57).

Finally, Clarke *et al.* (92) have demonstrated an increased half-life of a chimeric recombinant SOD, containing the enzymatic domain of Mn-SOD and the heparan-binding domain of EC-SOD, compared to native Mn-SOD.

Even if it is possible to increase the half-life of SODs into the blood circulation, this cannot explain the long-term effects observed by several authors. There is probably another mechanism of action involved in long term effects of SODs administration.

Improvement of Delivery to Intestinal Barrier After Oral Administration

Some authors have improved the delivery to the intestine of oral SODs by different kinds of encapsulation.

Among various delivery systems, wheat-gliadin biopolymers present a dual advantage: (i) their capacity to trap and delay the release of the active ingredient during the digestive process (93), and (ii) their bioadhesive properties, which improve and/or promote the delivery of the active ingredient to the intestinal mucosa (94).

Webb *et al.* (56) have confirmed the efficiency of a gliadin-complexed oral melon SODs in FIV-infected cats.

Others have demonstrated the antioxidant and anti-inflammatory properties of melon SODs encapsulated with gliadin (33,80). They have also shown that when SODs activity is preserved during the digestive process by its combination with gliadin, it is possible to elicit the pharmacological effects of this antioxidant enzyme *in vivo*.

Then, other coatings of different natures have been developed for different applications (95). Indeed, oral supplementation with vegetable oil-, shellac- or gum Arabic-encapsulated melon SODs could have antioxidant effects.

Bovine Cu/Zn-SOD liposome encapsulation, with or without addition of ceramides, seems to be beneficial for oral administration (96). Indeed, the blood antioxidant activity was increased after liposome encapsulated SOD administration compared to free SOD administration.

Encapsulating SOD would thus be a useful improvement to enable the enzyme to reach the intestinal barrier. However, even if the amount of SOD reaching the intestinal barrier is increased, a major limitation remains: SOD, which has a high molecular weight, may not be able to cross the intestinal barrier.

The question that now arises is how this protein can have so many beneficial effects without intestinal absorption.

HYPOTHESIS OF MECHANISM OF ACTION

Oxidative stress is involved in many diseases, including cardiovascular disease, neurodegenerative disorders, diabetes, cancer, arthritis and other inflammatory conditions described above (97).

The antioxidant properties of SODs are well known (5,6,8). Therefore, SODs administration could be hypothesized to act by reducing oxidative stress. Indeed, in some cases studied in this review, the authors have shown a decrease in oxidative stress after SODs supplementation.

A significant reduction in oxidative stress (ROS production) has been observed after oral supplementation of Mn-SOD in eukaryotic cells (pig myelomonocytes and mice splenocytes) following PMA (phorbol 12-myristate 13-acetate)-induced oxidative stress (54). Vouldoukis *et al.* (80) have demonstrated an inhibition of superoxide anion and peroxynitrite production by melon SODs incubation in murine peritoneal macrophages pre-activated with interferon-γ. A decrease in oxidative stress (ROS, 8-hydroxydeoxyguanosine, lipid peroxidation), induced by irradiation, has been shown in rodents after several SODs supplementation (17–19). A decrease in oxidative stress (lipid peroxidation), induced by immobilization, has also been observed in the nervous tissue of mice supplemented with oral melon SODs (58). Finally, melon SODs oral supplementation has been shown to lead to a decrease in stress proteins (several heat-shock proteins and nitric oxide synthases) along the gastrointestinal tract of pigs after weaning (98).

Some articles also describe a decrease in inflammation after supplementation with SODs.

For example, Vozenin-Brotons *et al.* (99) have shown an anti-inflammatory effect of bovine Cu/Zn-SOD in a co-culture model of skin fibrosis (decreased transforming growth factor-beta1 expression and levels of extracellular matrix components such as collagen). Vouldoukis *et al.* (80) have also demonstrated a decrease in inflammation (reduced tumour necrosis factor alpha level but increased level of interleukin-10, an anti-inflammatory cytokine) with melon SODs incubation in murine peritoneal macrophages pre-activated with interferon-γ. They have obtained the same result in mice given an intraperitoneal injection of interferon-γ, using oral melon SODs supplementation for 1 month. A decrease in inflammation induced by irradiation (as revealed by ED-1 labelling) has also been shown in rats given subcutaneous injection of bovine Cu/Zn-SOD (17).

Oxidative stress and inflammation are closely linked (97). Inflammation is one of the manifestations of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress. Thus, this decrease in inflammation could be explained by the antioxidant effects of SODs administration.

The chelating potential of exogenous SODs may partly explain the decrease of oxidative stress and inflammation observed after SODs supplementation. However, SODs half-life is only a few minutes in blood circulation, but more long effects were observed after SODs administration. Moreover, beneficial effects appeared after SODs oral supplementation although SODs cannot be absorbed. Altogether, these findings indicate that the decrease of oxidative stress and inflammation cannot be entirely explained by the chelating activity of administered SODs, and suggest that exogenous SODs have an additional influence independently of their antioxidant activity.

Interestingly, some authors have shown an increase in endogenous SODs after exogenous SOD administration, in correlation with the beneficial effects observed in several models.

Okada *et al.* (55) have demonstrated an increase in endogenous Mn-SOD activity in isolated murine cells after oral supplementation with encapsulated melon SODs for 1 month. The exposure of human fibroblasts to bovine CuZn-SOD enhances not only endogenous Mn-SOD activity but also its protein levels (28).

Other authors have confirmed this induction of endogenous SODs in several animal tissues, such as the hippocampus of mice (58), cat erythrocytes (56) and pig plasma (98).

Some authors have also demonstrated the induction of a global antioxidant defence involving not only endogenous SODs, but also endogenous CAT and GPx (33). Finally, some human studies have confirmed the induction of these enzymes in the serum and tracheal fluid after SODs supplementation (74,100,101).

The induction of all antioxidant enzymes avoids imbalance in the ratio of SODs to CAT and GPx, which could be involved in some diseases such as Down syndrome (11,12). Indeed, CAT and GPx remove the H_2O_2 produced after the dismutation of $O_2^{\cdot-}$ by SODs.

Use of antioxidant enzymes mimetics or study of their overexpression show beneficial effects similar to those obtained after SODs supplementation (102–104). These results confirm the hypothesis that exogenous SODs could act by inducing endogenous antioxidant enzymes.

Therefore, besides the chelating activity of exogenous SODs, induction of endogenous antioxidant defence appears to be an important mechanism of the beneficial effects observed after SODs supplementation. But how can this endogenous antioxidant defence be induced after exogenous SODs administration?

The induction of antioxidant enzymes could be regulated at the transcriptional level through a specific enhancer, the antioxidant response element (ARE), in the promoter of antioxidant enzyme genes. The transcription factor nuclear-factor-E2-related factor (Nrf2) has been implicated as the central protein

interacting with the ARE to modulate gene transcription, including that of antioxidant enzymes and inflammatory proteins (105–112).

But how exogenous SODs could regulate Nrf2/ARE pathway without intestinal absorption or cell penetration?

Some authors have shown that exogenous SODs is able to promote the activation of the immune system locally, and then induce a cascade leading to the activation of macrophages in the entire body (80). The exogenous SOD, as an antigen, could stimulate a protective immune response (113,114). The effects of SODs supplementation could be limited by homology. Indeed, in a rat model, heterologous forms of SODs (human, bovine or bacterial) have beneficial effects, but not a rat SOD (39,40,115–117). We could then imagine this immune response inducing endogenous antioxidant defences, possibly *via* the upregulation of Nrf2/ARE pathway.

The induction of endogenous antioxidant defence and, consequently, the decrease in oxidative stress and inflammation, is a strong hypothesis to explain all the effects observed in different pathological situations. This represents an original mechanism of action and suggests that exogenous SODs could have potential applications in several situations in which oxidative stress is increased.

Although several studies support this hypothesis, the mechanism of action by which exogenous SODs administration affects endogenous antioxidant defence remains to be elucidated.

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