Failure of heparin, superoxide dismutase, and catalase to protect against decompression sickness

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Catron PW, Thomas LB, McDermott JJ, Holt MA, Harabin AL, Flynn ET. Failure of heparin, superoxide dismutase, and catalase to protect against decompression sickness. Undersea Biomed Res 1987; 14(4):319–330.—The effects of heparin (HEP), superoxide dismutase (SOD), and catalase (CAT) on the course of decompression sickness (DCS) were studied in anesthetized dogs (Canis familiaris). Animals were divided into 4 groups: a drug assay group (n = 4) received HEP + SOD or HEP + SOD + CAT but were not dived; a control group (n = 14) was dived without drug treatment; a HEPSOD group (n = 11) received HEP + SOD predive and postdive; and a HEPSODCAT group (n = 15) received HEP + SOD + CAT before diving. All dived animals were subjected to repetitive air dives to 10 ATA until pulmonary artery pressure at least doubled within 10 min postdive. Physiologic variables were measured for 3 h postdive or until death. Animals were not recompressed. More early deaths occurred in the HEPSOD (7/11) and HEPSODCAT (8/15) groups than in the control group (5/14). All dived animals developed pulmonary hypertension, systemic hypotension, hemoconcentration, acidosis, hypoxemia, and interstitial pulmonary edema postdive. Drug therapy did not alter these responses to decompression. We conclude that without recompression, treatment with either HEP + SOD OR HEP + SOD + CAT does not improve the outcome of severe DCS in this animal model.

The lung, by virtue of its central location within the circulation, is a major target for bubble emboli liberated in venous blood during decompression. It is not surprising then, that caisson workers, divers, and aviators occasionally develop substernal chest pain, cough, and dyspnea as symptoms of decompression sickness (DCS) (1, 2). Hypotension and cyanosis are frequently associated findings. Pulmonary edema has been observed experimentally (3), and at autopsy in human cases of DCS (4).

The lung injury of venous air embolism is similar to that which follows stressful decompression. In both cases, pulmonary hypertension, impairment of gas exchange, and pulmonary edema result (3, 5). A considerable body of experimental work has
implicated the granulocyte as a mediator of lung injury in air embolism (6, 7). Additional work has shown that superoxide dismutase (SOD) or catalase (CAT) protects against this lung injury, suggesting that superoxide ion or hydrogen peroxide (H₂O₂) released from activated granulocytes may be responsible for the injury (6). Because lung injuries of venous air embolism and DCS are similar, we undertook a study to determine if SOD and CAT would lessen the injury associated with stressful decompression.

MATERIALS AND METHODS

Forty-four experiments used male mongrel dogs divided into 4 groups: a drug assay group (n = 4); a control group (n = 14); a heparin (HEP) plus SOD (HEPSOD) group (n = 11); and a HEP + SOD + CAT (HEPSODCAT) group (n = 15). Mean weights (± sd) of each group were: drug assay 13 ± 1.4 kg; control 13.5 ± 1.5 kg; HEPSOD 12.8 ± 1.1 kg; and HEP SODCAT 13.6 ± 1.0 kg.

Experimental protocol

Fasted animals were anesthetized with a loading dose of sodium pentobarbital (30 mg/kg, i.v.), endotracheally intubated, and mechanically ventilated with air. Anesthesia was maintained by a continuous infusion of sodium pentobarbital (0.068 mg ⋅ kg⁻¹ ⋅ min⁻¹, i.v.), supplemented with i.v. boluses of 50 mg as needed to suppress corneal reflex and spontaneous ventilation. A pressure-cycled ventilator (Bird Products, Palm Springs, CA) was used during surgical preparation. At all other times, a constant volume ventilator (Penlon Ltd, Abingdon, England) was used. The ventilator was adjusted before each experiment to provide physiologic values of arterial pH, oxygen partial pressure (P0₂), and carbon dioxide partial pressure (PcO₂). No further changes in ventilator volume or rate were made during the experiment. A 7-French Swan-Ganz catheter was advanced through a femoral vein into the pulmonary artery to measure pulmonary artery pressure (PAP) and cardiac output (CO). Fluid-filled catheters were passed into the mid-thoracic aorta and retrograde across the aortic valve into the left ventricle to measure systemic arterial and left ventricular end-diastolic pressures (LVEDP). Catheter whip prevented the accurate determination of LVEDP. Vascular pressures were measured using Gould P 23 pressure transducers and Gould pressure processors (Gould, Cleveland, OH). All vascular pressures were referenced to midchest level. Cardiac output was measured by thermodilution (Edwards Laboratories, Santa Ana, CA). All animals were instrumented with electrocardiographic leads, a rectal temperature probe, and a urinary bladder catheter. Baseline predive measurements included core body temperature, CO, hematocrit (Hct), arterial P0₂, PCO₂, and pH, and vascular pressures. Following baseline measurements and drug administration, the vascular catheters were filled with a volume of heparinized saline (5 U/ml) equivalent to catheter volume, and animals were subjected to a simulated air dive to 10 ATA in an animal recompression chamber (Hahn and Clay, Houston, TX). For all dives, the descent rate was 3.0 ATA/min (3-min descent) and the ascent rate was 1.8 ATA/min (5-min ascent). For the 1st dive of each experiment the time at 10 ATA was always 14 min. After the animal had returned to the normal surface pressure of 1 ATA, the systolic PAP was monitored for 10 min. If systolic
PAP at least doubled during this time, no further dives were made. If lesser PAP increase occurred, repetitive dives, each with a 3-min descent, 5 min at 10 ATA, and a 5-min ascent, were made until systolic PAP at least doubled during the postdive observation period of 10 min. Animals were not treated with recompression.

Postdive measurements of vascular pressures, arterial blood gases, and Hct were made 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 min after surfacing. Measurements of CO were made in triplicate 15, 30, 60, 120, and 180 min postdive. Total white blood cell counts were measured predive and at 120 min postdive.

Following data collection at 180 min postdive, the anesthetized animals were killed with an i.v. bolus of KCl. The lungs were removed, inflated with 10% buffered formalin solution, and prepared for routine histologic examination. In 2 animals each of the control and HEPSODCAT groups, a complete autopsy was performed. Sections of all major organs, including brain and spinal cord, were taken for histologic examination.

Drug dosage and administration

Heparin (porcine) was administered 30 min predive as a single i.v. bolus of 300 U/kg. Animals of the HEPSOD group received either bovine erythrocyte SOD (BESOD (2500–3000 U/mg), Sigma Chemical Corp., St. Louis, MO) or polyethylene glycol conjugated SOD (PEG SOD (2000 U/ml), Enzon Corporation, Piscataway, NJ) in addition to HEP.

Bovine erythrocyte SOD was used in 9 experiments. In the first 2 experiments, BESOD was infused i.v. at a rate of 1 mg · kg⁻¹ · h⁻¹. The infusion was begun 30 min predive, interrupted during the dive, restarted postdive, and continued for the duration of the experiment. In the next 7 experiments, a loading dose of 1.40 mg/kg was given as an i.v. bolus immediately predive; this was followed by an i.v. infusion of 1 mg/kg, which began immediately postdive and continued for the duration of the experiment. If a 2nd dive was required, a 2nd i.v. loading dose of 0.4 mg/kg was given before the dive. The aim of this regimen was to maintain a plasma level of BESOD equivalent to that produced by an indefinitely long infusion of 1 mg · kg⁻¹ · h⁻¹. Loading dose calculations assumed a volume of distribution equal to that of plasma and a half-life of 30 min. Two animals of the HEPSOD group received PEG SOD as a single i.v. dose of 2 mg/kg 30 min predive.

In the second half of the study, PEG SOD and polyethylene glycol conjugated CAT (PEG CAT) were used. All animals of the HEPSODCAT group received i.v. boluses of HEP, PEG SOD, and PEG CAT. PEG SOD was given in a dosage of 2 mg/kg and PEG CAT in a dosage of 200,000 U/kg. These drugs were administered as single predive boluses. Control animals were given i.v. saline predive, in volumes equivalent to that required for drug administration.

Drug assays

Total SOD activity was measured using the technique of Crape et al. (9). SOD is quantified by the ability of the plasma to inhibit the reduction of cytochrome C by superoxide ion produced by the reaction of xanthine with xanthine oxidase. One unit of activity is defined as the amount of SOD-containing plasma required to inhibit the
rate of reduction of cytochrome C by 50%. CAT was measured with the technique of Beers and Sizer (10). Activity is defined as the amount of enzyme reacting with 1 mM H₂O₂ and calculated using an extinction coefficient of 0.0436 μmol⁻¹ · cm⁻². Measurements of SOD and CAT were made at least in triplicate for all samples.

Drug assays were performed in 4 experiments. The protocol and drug administration were the same as those of the experimental groups, except animals were not dived. Blood samples were drawn before, at various times until 210 min, and after drug administration. Enzyme activity levels were measured in 2 animals receiving HEP + BESOD (with loading doses and continuous infusions as described above) and in 2 animals receiving HEP + PEGSOD + PEGCAT.

Statistical analyses

Statistical analyses were done using either t tests [with Bonferroni correction (11)] or an analysis of variance. Analysis of variance tested the hypothesis that a given physiologic measurement in control and treated animals was different at one or more times. This hypothesis was evaluated by fitting physiologic data from all animals to a 12-parameter model that estimated one parameter (a group mean) for each measurement time. The residual error from this model was compared with that from a 24-parameter model in which parameters were estimated for both control and treated groups. Parameters were estimated by a least squares method using a FORTRAN version of the iterative parameter estimation program of Bailey and Homer (12). Residual error was compared using an F test. An alpha level of 0.05 was considered statistically significant.

RESULTS

Drug assay experiments

Continuous infusion of BESOD, with one or two loading doses as described, increased plasma SOD activity approximately threefold, from a mean activity of 9 U/ml to a mean of 26 U/ml. Single i.v. doses of PEG SOD produced higher levels of plasma SOD activity (158 and 208 U/ml in 2 animals) which remained high over the 240 min of the experiment. Single i.v. doses of PEG CAT increased plasma CAT activity from immeasurably low, pre-injection levels (< 25 U/ml) to 3282 and 2339 U/ml, and these increases were maintained for the duration of the experiment.

Infusion of BESOD produced no significant changes in vascular pressures, CO, temperature, Hct, or arterial blood gases. Likewise, the combination of PEG SOD and PEG CAT did not appreciably change arterial blood gases, Hct, or temperature. Mean arterial pressure (MAP) and PAP fell in both animals during the 1st h after injection of PEG SOD and PEG CAT and then slowly approached baseline values during the next 2 h. MAP fell by 25 mmHg in 1 animal and by 60 mmHg in the other. Clear changes in CO were apparent only in the animal with the largest drop in MAP. In this animal, CO decreased by more than 50% (from 2.6 to 1.1 liter/min) and then partially recovered. The time course of the drop and recovery of CO paralleled that of MAP.
Leukocyte and platelet counts were measured in 2 animals before and 2 h after administration of HEP + SOD + CAT. No significant change in platelet counts occurred. Total leukocyte counts remained unaltered in 1 animal but fell in the other from a pretreatment value of 6200 white blood count (WBC)/ml to 3700 WBC/ml at 2 h posttreatment.

Decompression experiments

Five of 14 controls, 7 of 11 HEPSOD animals, and 8 of 15 HEPSODCAT animals died early. The 7 early deaths in the HEPSOD group included the 2 animals treated with PEG SOD. The early deaths occurred within 30 min of surfacing. Of the animals that died early, 0 of 5 controls, 2 of 7 HEPSOD, and 3 of 8 HEPSODCAT animals were dived twice. Thus, although there were more early deaths in the treated animals, only these had multiple dives.

Of animals surviving for 3 h postdive, 0 of 9 controls, 0 of 4 HEPSOD, and 2 of 7 HEPSODCAT animals had 2 dives. The severity of DCS in the 3-h survivors was not significantly altered by treatment with either HEP + SOD or HEP + SOD + CAT. In all 3 groups of dived animals, pulmonary artery systolic (Fig. 1) and diastolic pressures rose to 2–3 times predive values within 15 min of surfacing, and returned to near baseline levels by 1 h postdive. MAP (Fig. 2) and CO (Fig. 3) fell postdive in all animals and did not recover.

Hematocrit rose in all dived animals during the first 15 min after surfacing from a predive mean of 38% to a mean postdive value of 55%, and remained at this level for

![Graph](image)

Fig. 1. Systolic pulmonary artery pressure, mmHg; Dive time is indicated by the vertical line; n indicates the number of animals in which the specified measurement was accomplished.
the duration of the experiment. Arterial pH fell in most animals by 0.15–0.20 pH units during the first 45 min postdive and then stabilized. Arterial PCO₂ increased by a small amount (5–7 mmHg) in all 3 groups during the first 60 min postdive, and then returned to near baseline values during the next 2 h. Arterial PO₂ (Fig. 4) dropped precipitously during the first 30 min postdive and then slowly recovered as the experiment continued. HEPSOD- and HEPSODCAT-treated groups were not statistically different from controls or from each other with regard to PAP, MAP, CO, Hct, temperature, or arterial pH, PO₂, or PCO₂.

Diving did not significantly change platelet or leukocyte counts in control or HEPSOD animals, but was associated with significant decreases in platelets \( P < 0.001 \) and leukocytes \( P < 0.001 \) in animals of the HEPSODCAT group (Table 1).

At autopsy, most animals had widespread evidence of intravascular bubbling, with bubbles in the pulmonary and mesenteric vessels, and in the vena cavae. On histologic examination, all dived animals that survived for 3 h postdive and most that died early had evidence of interstitial pulmonary edema in the form of perivascular cuffs of edema fluid. Alveolar filling was not seen. HEPSOD and HEPSODCAT groups were not different from controls in terms of the frequency or extent of interstitial pulmonary edema. Vascular congestion with occasional perivascular hemorrhage was seen in the liver, intestine, lymph nodes, urinary bladder, cerebrum, and cerebellum. One control and 1 HEPSODCAT animal had diffuse mucosal hemorrhage throughout the mucosa of the small intestine.
SOD AND CATALASE IN DECOMPRESSION SICKNESS

DISCUSSION

Decompression sickness and venous gas embolism produce similar responses from the lungs including pulmonary hypertension, impairment of gas exchange, and interstitial pulmonary edema. Pulmonary edema develops without elevation of LVEDP and is presumably the result of increased permeability of the pulmonary endothelium (3, 13).

Studies of venous air embolism suggest that granulocytes may be important mediators of the lung injury. Granulocytes are sequestered in the lungs during venous air embolism (14). In histologic studies of air embolism, granulocytes have been found adherent to air bubbles within pulmonary arterioles and in intimate contact with areas of injured endothelium (15). Ultrastructural studies of air embolism have shown leukocytes adherent to an electron-dense film at the blood-bubble interface, which may consist of denatured plasma proteins (15). Similar ultrastructural changes at the blood-bubble interface have been reported by Philp (16) during decompression sickness in the rat.

Physiologic as well as structural evidence supports the concept that granulocytes may be important mediators of the lung injury of air embolism. Flick et al. (7) showed that in sheep, granulocyte depletion markedly attenuated the increase of pulmonary vascular permeability that normally follows air embolism. Studies in sheep have also shown that granulocytopenia protects against increased pulmonary vascular perme-
ability resulting from other types of microemboli, including thrombin (17), bone marrow (18), and glass beads (19).

The mechanism(s) by which granulocytes mediate lung injury after air embolism has not been completely defined. There is evidence that superoxide ion, perhaps released from activated granulocytes, may be an important mediator of the injury. Flick et al. (6) reported that SOD combined with HEP attenuated the lung injury of air embolism. Both drugs were required for the protective effect, the magnitude of which was similar to that afforded by granulocyte depletion. In a separate study using
the same model, Flick et al. (20) reported that CAT alone had a similar protective effect. These studies suggest that superoxide ion or its conversion product \( \text{H}_2\text{O}_2 \) or both are important mediators of bubble-induced lung injury in air embolism.

The fundamental event that leads to injury in both DCS and venous air embolism is the formation of intravascular bubbles. In DCS however, intravascular bubbles are formed throughout the systemic as well as the pulmonary circulation. Systemic endothelial injury has been documented in DCS (21), and the widespread occurrence of bubble-induced endothelial injury is probably responsible for the plasma volume loss and hemoconcentration characteristic of severe cases of this illness.

We expected that SOD would offer some protection against DCS in our experimental model but found that it did not. We thought initially that this might be due to a relative overproduction by SOD of \( \text{H}_2\text{O}_2 \), a known cytotoxin (22). The addition of CAT to the prophylactic regimen should have resulted in the conversion of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \), but the combination did not improve the outcome in animals that survived for 3 h positive. It is possible that SOD or SOD + CAT may have offered some protection, because among the animals that died early, more of the HEPSOD and HEPSODCAT groups had multiple dives than did controls. Unfortunately, given the binomial nature of the data set (death/no-death), the sample size is too small to demonstrate a protective effect (or lack thereof) with a reasonable degree of confidence.

Drug dosages used in these experiments were more than adequate to achieve the desired pharmacologic effect. Dosages of SOD and CAT were equal to or greater than those used in other reported studies of air embolism and oxygen toxicity in which the drugs were effective prophylactically (6, 23). In the drug assay experiments, plasma levels of SOD and CAT activity were greatly augmented by drug administration, and remained at a high level throughout the experiments.

Heparin alone was not tested in this study. Prophylactic HEP has been previously studied in a number of animal models of DCS. The results of these studies have been conflicting, with HEP being protective in some cases but not in others (15, 24–26). To our knowledge, no studies of HEP in DCS demonstrate it to have a deleterious effect.

Although we did not test SOD and CAT without HEP, it is unlikely that a deleterious drug interaction among HEP, SOD, and CAT was responsible for the failure of the combination to be protective. Flick et al. (6) found that HEP was a necessary addition to SOD for protection against air embolism in sheep, although the mechanism of action of HEP was unclear. In addition, in the drug assay experiments HEP did not interfere with plasma SOD and CAT activities.

It is unlikely that species variation accounted for the failure of SOD and CAT to protect against decompression-induced injury. Although most of the work implicating granulocytes and superoxide ion in air embolism has been done in sheep, Moosavi et al. (27) have shown that granulocytes also accumulate in the lungs of dogs during venous air embolism. In addition, granulocytopenia in dogs has been shown to prevent increases in extravascular lung water which would otherwise follow glass bead microembolism (28). Finally, superoxide ion is produced by canine granulocytes (29).

The experimental model used in this study was not as sensitive for detecting pulmonary edema as the lung lymph flow model developed in sheep by Staub et al. (30). We did observe, however, interstitial pulmonary edema in every animal pretreated with either SOD or SODCAT. Histologically, pulmonary edema in control
and treated animals appeared to be of equal severity. Therefore, even though we
could not measure lung lymph flow in these experiments, it is clear that SOD and
CAT did not prevent the development of pulmonary edema during DCS.

Our animal model corresponds most closely to the “blow-up” of a diver, a situation
in which the unplanned ascent of a diver results in a large burden of omitted
decompression and severe, often fatal, DCS. The results of our study indicate that
in the dog, HEP, SOD, and CAT do not modify the course of a blow-up and suggest
that liberation of superoxide ion and hydrogen peroxide by activated granulocytes is
not an important mechanism of its pathogenesis. It is possible that mechanical vas-
cular obstruction is more important in the pathogenesis of a blow-up than are the
various consequences of blood-bubble interactions. It is also possible that the seque-
lae of blood-bubble interactions are relatively more important mechanisms of injury
in less severe decompression insults, and that a beneficial drug effect was missed
because the experimental model was too severe. We cannot therefore conclude that
SOD or SOD/CAT dose would not be helpful in less severe degrees of DCS, but a
dramatic effect of the drugs would seem unlikely in view of their failure to modify
any aspect of DCS in these experiments.

We did not recompress animals in this study. Recompression therapy is the primary
treatment modality for DCS. Recompression restores organ blood flow by physical
compression of bubbles, and improves tissue oxygenation by augmenting the inspired
partial pressure of oxygen. It is possible that SOD and CAT might be useful as
adjuncts to recompression therapy to reduce reperfusion injury after tissue blood
flow is restored by recompression. Experimental studies of temporary coronary artery
occlusion have shown that SOD and CAT can reduce the size of the resultant infarct
(31). The drugs are believed to exert this effect by scavenging toxic oxygen radicals
that are produced during the period of reflow after the occlusion is relieved. It is
possible that SOD and CAT might act similarly in DCS. It would seem worthwhile
to study them in a different model as adjuncts to recompression therapy.

Some interesting effects of SOD and CAT on hemodynamics and formed elements
of blood were observed in these experiments. SOD + CAT has been reported to cause
hypotension during myocardial ischemia in the dog through a mechanism other than
decreased myocardial contractility (32). If myocardial contractility is unaffected by
the drugs, the occurrence of hypotension in the face of decreased CO suggests that
myocardial preload has been decreased, possibly through venous pooling. If vено-
dilation is the mechanism of SOD + CAT-induced hypotension, the failure of these
drugs to further decrease blood pressure and CO during DCS may indicate that
preload was already maximally depressed.

The fall in platelet and leukocyte counts after decompression were much larger in
animals of the HEPSODCAT group than in controls. Shortened platelet survival as
a consequence of decompression has been well documented (21). Although we did
not measure platelet survival, the data suggest that HEP + SOD + CAT increased
postdive platelet consumption. The effect of decompression on leukocyte kinetics is
less well established. Inwood (25) however, reported that in the rat, granulocytes
increase in mild-to-severe DCS but decrease in fatal cases. In the present study,
therapy with HEP + SOD + CAT resulted in a marked drop in leukocytes after
decompression but did not alter the severity of DCS. The mechanism of this effect
and its potential significance will require further investigation.
SOD AND CATALASE IN DECOMPRESSION SICKNESS

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No. (NIH) 85-23.

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poumon catalase
œdème pulmonaire plongée
granolocyte embolie gazeuse
maladie de décompression animal
superoxyde dismutase chien

REFERENCES


