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Inhibition of Lethality in Endotoxin-Challenged Mice Treated with Zinc Chloride

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Zinc chloride protected against lethality in mice undergoing endotoxin shock.

The precise mechanism by which endotoxin induces shock is not completely elucidated, but considerable evidence has accumulated suggesting that lysosomal labilization is a crucial event in the pathogenesis of the shock syndrome. Numerous investigators have shown that there is an increase in the activities of circulating lysosomal hydrolases in endotoxin shock (1, 9, 14) as well as an increased fragility of hepatic lysosomes (9, 16). The lysosome is known to contain many potent mediators of inflammation and shock (10, 13), particularly proteases (2, 13). Indeed, it was shown that intravenous administration of exogenous proteases is sufficient to induce shock in experimental animals (11). Presumably the proteases can act on tissue and serum proteins to produce toxic peptide fragments, such as bradykinin (12), which contribute to circulatory collapse. The observation that steroids known to stabilize lysosomal membranes reduce mortality in endotoxin shock (6) supports the hypothesis that the release of lysosomal mediators is an important step in the pathogenesis of the syndrome. Furthermore, antiproteases have been used to obviate the lethal effects of proteases released from lysosomes during endotoxemia (2).

The success of previous investigators in reducing mortality to endotoxin-induced shock by using lysosomal stabilizing agents and antiproteases prompted us to test the hypothesis that zinc could protect against lethality induced by endotoxin. There is evidence that the zinc ion (Zn^{2+}) can participate in the stabilization of membranes (14). Zinc is known to facilitate the isolation of intact plasma membranes (15); it can inhibit the spontaneous hemolysis of erythrocytes when given orally to rats (3); and, finally, zinc inhibits in vitro the release of enzymes from lysosomes obtained from rat liver (4, 8). In addition, the zinc ion can strongly inhibit cathepsin B (10), an acid protease which can catalyze the formation of bradykinin from kininogen (1).

Male B6CBF1 mice obtained from Cumberland View Farms, Clinton, Tenn., were injected intraperitoneally with zinc chloride dissolved in normal saline 1 h before challenge by the same route with 0.75 mg of *Salmonella typhosa* lipopolysaccharide (Difco Laboratories, Detroit, Mich.). Specific enzyme inhibitors were administered intraperitoneally, but cortisone acetate (Upjohn Co., Kalamazoo, Mich.) was injected subcutaneously. The mice used weighed 20 to 25 g and were 8 to 9 weeks old.

We found that zinc chloride at a dose of 0.40 mg per mouse effectively protects against lethality induced by endotoxin as measured by mortality at 24 h (Table 1). This time was chosen because final survival data were essentially the same. At this time 0.75 mg of endotoxin was sufficient to cause 100% mortality in the untreated group, whereas almost complete survival (97%) was achieved in the zinc chloride-treated group. The protection afforded by zinc in our experiments is comparable with that achieved with cortisone. Furthermore, zinc was more effective in protecting mice against endotoxin challenge than the several specific protease inhibitors tested (Table 1).

It is significant that the degree of protection was dependent on the amount of zinc administered (Table 1). Increasing or decreasing the optimum dose of 0.40 mg per mouse by only 0.20 mg resulted in a substantial reduction in the degree of protection. The time of administration was also important (Table 1). Zinc is apparently much less effective when injected after endotoxin challenge.

The potency of zinc as a protective agent in endotoxin shock is further illustrated in Fig. 1, where the mortality as a function of the amount of endotoxin administered at constant zinc concentration is plotted. Zinc afforded 80% protection, even when the normally lethal endotoxin dose was doubled (Fig. 1). By defining the dose reduction factor as the ratio of the amount of endotoxin (milligrams per mouse) required to cause death in 50% of the treated population

TABLE 1. *Twenty-four-hour mortality of B6CBF1 mice challenged with Salmonella typhosa endotoxin and treated with $ZnCl_2$, cortisone, or antiproteolytic agents*[a]

Agent	Dose (mg)	Time of injection	Mortality (ratio)	Mortality (%)
Saline	0.20 ml	1 h before endotoxin	25/25	100
$ZnCl_2$	0.080	1 h before endotoxin	10/10	100
$ZnCl_2$	0.20	1 h before endotoxin	7/10	70
$ZnCl_2$	0.40	1 h before endotoxin	1/30	3
$ZnCl_2$	0.60	1 h before endotoxin	3/10	30
$ZnCl_2$	0.80	1 h before endotoxin	4/10	40
$ZnCl_2$	0.40	3 h before endotoxin	1/10	10
$ZnCl_2$	0.40	simultaneously with endotoxin	3/10	30
$ZnCl_2$	0.40	30 min after endotoxin	7/10	70
$ZnCl_2$	0.40	3 h after endotoxin	7/10	70
Trasylol	5.0	1 h before endotoxin	7/20	35
OTI	5.0	1 h before endotoxin	10/10	100
STI	5.0	1 h before endotoxin	10/10	100
EACA	20	1 h before endotoxin	10/10	100
LBTI	5.0	1 h before endotoxin	4/10	40
Cortisone	5.0	1 h before endotoxin	0/10	0

[a] Each mouse received 0.75 mg of endotoxin intraperitoneally. LBTI, Lima bean trypsin inhibitor; OTI, ovomucoid trypsin inhibitor; STI, soy bean trypsin inhibitor; EACA, epsilon aminocaproic acid.

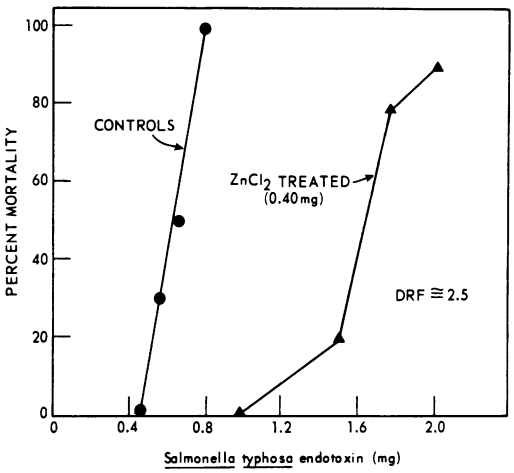

FIG. 1. *Mortality of control and zinc-treated mice versus endotoxin dose.*

relative to the same amount of endotoxin causing 50% mortality in the control group, we estimate a dose reduction factor for zinc of 2.5.

Evidence consistent with the hypothesis that zinc may protect against endotoxin by stabilizing lysosomal membranes is presented in Fig. 2. The relative circulating levels of serum β-glucuronidase in normal mice, and saline- or zinc chloride (0.40 mg per mouse)-treated mice after challenge with endotoxin, were determined at 30 min and 20 h. β-Glucuronidase was measured spectrophotometrically at 555 nm, using phenophthalein β-glucosiduronate as a substrate. We found marked elevation in the serum level of β-glucuronidase in the endotoxin-challenged mice, both at 30 min and at 20

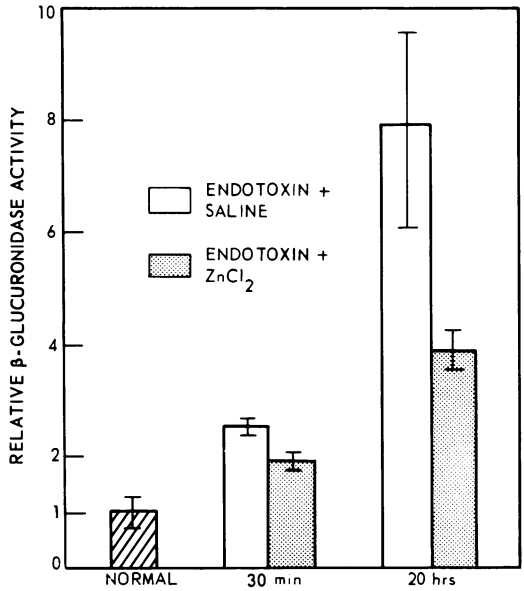

FIG. 2. *Serum β-glucuronidase activity for normal mice and saline- or $ZnCl_2$-treated mice challenged with endotoxin. Samples were collected at 30 min and 20 h after inoculation with endotoxin.*

h after challenge with endotoxin, relative to the controls. Presumably the increased level of serum β-glucuronidase partially reflects the leakage of enzymes into the systemic circulation from disrupted lysosomes. Furthermore, this elevation was significantly reduced when zinc was administered, suggesting that a mechanism by which zinc protects against endotoxin action could be stabilization of lysosomal membranes. Although we have no information at

present regarding the origin of the serum β-glucuronidase in endotoxin-challenged mice, it is likely that some of it is released from disrupted granulocytes or certain fixed macrophages (5).

It is becoming increasingly evident that lysosome disruption resulting in the release of harmful mediators such as proteases may be a major event in endotoxemia. Therefore, the application of agents such as zinc designed to stabilize lysosomes and/or inhibit lysosomal protease could prove useful in the treatment of endotoxemia and in the development of a better understanding of the means by which endotoxin induces shock.

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