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Differential Effect of Parenteral Zinc on the Course of Various Bacterial Infections[1] (39931)

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There is considerable interest in the effects of endogenous and exogenous trace metals on the course of infections. The interest arises, in part, from the essential role trace elements have in diverse biological functions (1) and from the documented redistribution of endogenous trace metals that occurs during infections (2). Exogenous trace metals appear to present a potential health hazard as environmental pollutants by compromising certain aspects of the host's defense against disease (3). In this regard, lead and cadmium enhance susceptibility to bacterial endotoxins, presumably by altering the reticuloendothelial system (4, 5), and enhance the susceptibility of rats to *Escherichia coli* infection with toxicity attributed to the endotoxin content of bacteria (6).

In contrast, we have demonstrated the prophylactic efficacy of parenteral zinc administration in endotoxemic rats (7). The protection afforded by zinc against endotoxin-induced mortality appears to be limited to intraperitoneally administered toxin and involves, in some as yet undefined manner, reduction in absorption of the toxin from the peritoneal cavity (7).

Diet-induced zinc deficiency, on the other hand, appears to lower resistance of rats to certain gram-negative microorganisms (8). Therefore, a series of studies was performed to determine the effect of acute and chronic zinc treatment of rats on susceptibility to experimentally induced bacterial infections with various gram-negative as well as gram-positive organisms.

Materials and methods. Male, Sprague-Dawley rats, weighing 257 g ± 14 (SE), were housed 10 per cage in a room maintained at 22 to 24° and lighted from 6 AM to 6 PM. Animals were fed Wayne Lab-Blox and water *ad libitum*. Zinc, as zinc chloride, in physiological saline was administered ip, 0.2 to 1.6 mg/100 g body weight, 1 hr prior to bacterial challenge in acute studies and, in addition, in chronic studies every 12 hr (0.8 mg/100 g) during the early postinfection period. Control rats received an equivalent volume of physiological saline in place of zinc chloride.

Bacterial challenge was performed by ip or sc administration of cultures of either the live vaccine strain (LVS) *Francisella tularensis, Salmonella typhimurium* (MIT), or *Streptococcus pneumoniae* (Ia5), at the doses, per 100 g body weight, shown in the figures. The concentration of organisms in each inoculum was determined by turbidometric methods just prior to injection. All concentrations were confirmed by subsequent colony counts. Mortality incidence was determined for a 7-day observation period following infection with the various organisms. Plasma zinc concentration was determined by atomic absorption spectrometry (9). Fisher's exact method (10) was used to determine statistical significance of the mortality data obtained at specified time intervals; P values equal to or less than 0.05 were considered significant.

Results. An acute dose of ip administered zinc chloride in normal rats induces a transient increase in plasma zinc concentration for approximately 24 hr. An apparent maximum concentration occurs within 1 to 3 hr (the time of bacterial challenge) following administration of the salt (Table I). Zinc pretreatment (1.6 mg/100 g body weight) significantly reduced survival incidence of rats during the 7-day period following chal-

[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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lenge with 7.1×10^6 or 1.2×10^8 *S. typhimurium* when compared to untreated controls (Fig. 1A). However, no significant zinc-enhanced susceptibility was evident when an overwhelming infection with 1.0×10^9 organisms was employed. Infection with 1.9×10^5 organisms was innocuous in zinc-treated and untreated rats. Mortality incidences at 7 days for zinc-treated rats given either 7.1×10^6 or 1.2×10^8 *S. typhimurium* were 70 and 80%, compared to 10% at either dose of microorganism for untreated rats, respectively. The zinc-enhanced susceptibility to *S. typhimurium* infection was also evident when lower doses of zinc chloride were used (Table II). In an additional study, the administration of 2.5×10^8 heat-killed *S. typhimurium* organisms did not result in mortality in either zinc-treated (1.6 mg, $N = 10$) or untreated ($N = 10$) rats.

In contrast with the results obtained with *S. typhimurium,* zinc treatment did not enhance mortality incidence in animals challenged with either 1.7×10^7 or 1.4×10^8 *F. tularensis* (Fig. 1B), but rather a significant enhanced survival incidence was evident during the initial 48-hr postinfection period in rats infected with 1.4×10^8 organisms. Although not statistically significant, zinc pretreatment prevented mortality in rats challenged with 1.7×10^7 organisms. Twenty-five percent of untreated rats succumbed to this dose of microorganisms (Fig. 1B).

Mortality incidence due to *S. pneumoniae* infection, by either ip (7.1×10^3) or sc (4.0×10^3) inoculation, in zinc-treated rats was significantly less, during the early postinfection period, than that observed for untreated rats (Fig. 1C). At no time during a 7-day observation period did the mortality incidence of the zinc-treated rats exceed that of the untreated controls. Intraperitoneally administered *S. pneumoniae* at doses of either 2.0×10^1 or 7.1×10^3 was highly lethal and approximately 95% of the untreated animals died within 2 to 3 days. Zinc pretreatment significantly enhanced survival incidence only during the very early postinfection period. Subcutaneous inoculation with a dose of microorganisms of the

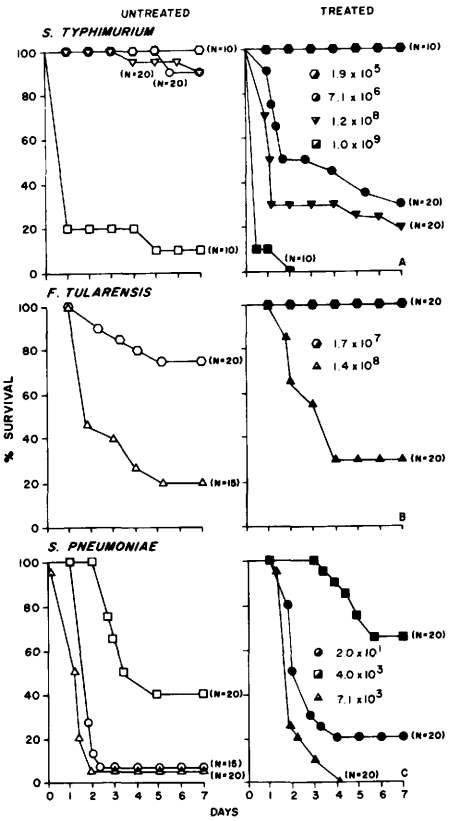

FIG. 1. Effect of a single ip zinc pretreatment on the course of (A) *S. typhimurium*, (B) *F. tularensis*, and (C) *S. pneumoniae* infections. Zinc, as $ZnCl_2$ in physiological saline (1.6 mg/ml/100 g body weight) was administered 1 hr prior to bacterial challenge. All microorganisms were administered ip at the doses per 100 g body weight shown in the figures; in addition, *S. pneumoniae* was also administered sc (□——□). Untreated control rats received an equivalent volume of saline in place of $ZnCl_2$.

TABLE I. PLASMA ZINC CONCENTRATIONS AT VARIOUS TIMES AFTER $ZnCl_2$ OR PHYSIOLOGICAL SALINE IN NORMAL RATS.

Time (hr)	Plasma zinc (μg/100 ml)[a]	
	Zinc chloride[b]	Saline[b]
1	966.0 ± 62.4 (5)	187.3 ± 3.5 (3)
3	1098.0 ± 140.4 (5)	182.0 ± 2.3 (3)
6	795.6 ± 11.6 (5)	171.0 ± 6.6 (3)
24	203.2 ± 6.5 (5)	169.7 ± 3.7 (3)
27	182.0 ± 3.4 (5)	174.0 ± 4.2 (3)

[a] Values are means ± SE for the number of animals shown in parentheses.

[b] Either $ZnCl_2$, 1.6 mg/100 g body wt, or an equivalent volume of physiological saline was administered at zero time.

same order of magnitude as used in ip studies (10^3) resulted in prolongation of time to death and reduction in mortality incidence in zinc-treated and untreated rats. Again, zinc pretreatment significantly enhanced survival incidence in sc challenged rats only during the early postinfection period. At 7 days, 65% of the zinc-treated and 40% of the untreated rats survived sc *S. pneumoniae* challenge.

Pretreatment combined with multiple zinc administration after ip challenge with either 2.0×10^8 *F. tularensis* or 2.0×10^1 *S. pneumoniae* appeared to enhance resistance to the infections (Fig. 2) when data obtained for similarly infected, zinc-treated (Fig. 2) and untreated (Figs. 1B and C) rats were compared. When data for single and multiple zinc treatments were compared statistically, multiple zinc appeared to benefit only the *S. pneumoniae*-challenged rats with regard to survival incidence at 7 days. However, there was some suggestion that multiple zinc treatments in 2.0×10^8 *F. tularensis*-challenged rats provided additional protection, albeit not significant, when data for single (Fig. 1B) and multiple (Fig. 2) zinc treatments were compared. Subsequent rechallenge of survivors in the *F. tularensis* group with multiple zinc treatment produced no additional mortality. All untreated rechallenge control rats succumbed to the rechallenge dose. Similarly, rechallenge of survivors in the *S. pneumoniae* group multiply treated with zinc produced, but to a lesser extent, enhanced survival incidence (40%) when compared to rechallenge controls (12.5%).

TABLE II. EFFECT OF PRETREATMENT WITH VARIOUS AMOUNTS OF ZINC CHLORIDE ON SURVIVAL INCIDENCE OF RATS 24 hr AFTER CHALLENGE WITH *S. typhimurium*.

Group[a]	1	2	3	4	5
$ZnCl_2$[b] (mg/100 g body weight)	0	0.2	0.4	0.8	1.6
Survival incidence (%)	90	70	50	40[c]	30[c]

[a] Ten rats per group.
[b] Zinc chloride dissolved in physiological saline was administered ip 1 hr prior to ip inoculation with 1.9×10^8 *S. typhimurium* organisms per 100 g body weight.
[c] $P < 0.05$ vs untreated controls.

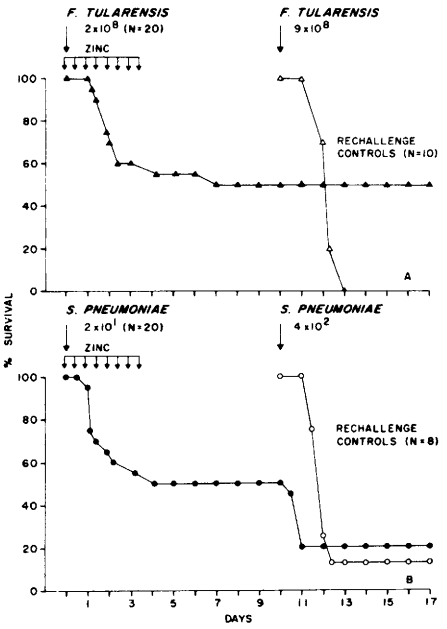

FIG. 2. Effect of multiple ip zinc treatments on the course of *F. tularensis* and *S. pneumoniae* infections. Zinc, as $ZnCl_2$, in physiological saline (1.6 mg/ml/100 g body weight) was administered 1 hr prior to bacterial inoculation and subsequently at 12-hr intervals (0.8 mg/0.5 ml/100 g body weight). Number of organisms shown represents the dose/100 g body weight administered ip at 0 and 10 days. Rechallenge controls were untreated.

In order to determine whether zinc itself or some induced humoral factor affected microbial growth, pooled plasma samples from zinc-treated and untreated rats were inoculated with each of the organisms studied. Plasma obtained from zinc-treated, uninfected rats (1116 μg/dl of zinc) was found to inhibit growth of *S. pneumoniae* organisms significantly (Table III) when compared to the extent of growth observed in plasma from untreated rats (117 μg/dl). Plasma, whether obtained from zinc-treated or untreated rats, did not support growth of *F. tularensis* or *S. typhimurium* during a 6-hr incubation period.

Discussion. Results presented in this study provide evidence that parenteral zinc administration, in spite of its ability to stimulate peripheral granulocytosis (11) and its documented prophylactic efficacy in ip induced endotoxemia (7), significantly com-

TABLE III. ANTIBACTERIAL ACTIVITY OF PLASMA SAMPLES FROM ZINC-TREATED AND UNTREATED RATS.

	Plasma organisms (No./ml)[a]			
	Treated (1116 μg/dl)[b]		Untreated (117 μg/dl)[b]	
Microorganism	0 hr	6 hr	0 hr	6 hr
F. tularensis	299 ± 9	142 ± 9	310 ± 16	120 ± 16
S. pneumoniae	124 ± 24	277 ± 15[c]	114 ± 15	807 ± 10
S. typhimurium	145 ± 11	97 ± 8	144 ± 6	87 ± 8

[a] Plasma from three rats in each group was obtained 1 hr after either ip $ZnCl_2$ (1.6 mg/100 g body weight) or saline injection and pooled. Two-tenths milliliter of a bacterial suspension was added to 1 ml of plasma at zero time and incubated at 37° for 6 hr. Bacterial counts were performed in triplicate. Values are means ± SE.

[b] Mean plasma zinc concentration; $N = 3$.

[c] $P < 0.001$ vs untreated controls.

promises the host's resistance to an endotoxin-producing gram-negative microorganism. The lack of demonstrable lethality in zinc-treated or untreated rats challenged with 2.5×10^8 heat-killed *S. typhimurium* together with the observation that the quantity of zinc employed in these experimemts affords significant protection against massive amounts of *S. typhimurium* endotoxin (7) argues the possibility that the observed zinc-enhanced lethality in gram-negative sepsis was attributable to endotoxin. This contention is in marked contrast to the findings of Cook *et al.* (6), employing lead and cadmium, who suggested that the metal-enhanced toxicity in infection (*E. coli*) is probably due to bacterial endotoxin. Providing that their conclusion is valid, it appears that zinc, in comparison to lead and cadmium, enhances lethality through a different mechanism when endotoxin-producing gram-negative infections are involved. Although the data have not been presented, we have observed in additional experiments, that zinc pretreatment also enhances mortality incidence in rats infected with *E. coli*.

We propose that our experimental findings with zinc in *S. typhimurium* infection, and perhaps in *E. coli* infection as well, may be the first demonstration of the consequences of zinc's ability to inhibit phagocytosis (12). In the present study, bacterial challenge was performed when plasma zinc levels were in excess of that used by Chvapil *et al.* (12) in their *in vitro* studies (60 μM $ZnCl_2$) to demonstrate zinc's inhibition on granulocyte phagocytosis and bactericidal activity. Apparently, zinc differs from cadmium with respect to its influence on various functions of phagocytes (12, 13) and this observation together with cadmium-induced alterations in reticuloendothelial function (4, 5) may explain why endotoxin in cadmium-treated rats is a relatively more important aspect of enhanced lethality when compared to our zinc results.

The same explanation for our observation with zinc on the susceptibility to *F. tularensis* infection is not tenable. Since it has been shown that this microorganism is phagocytized only in the presence of immune serum (14), it appears unlikely that zinc's ability to inhibit phagocytosis of this gram-negative microorganism, which lacks demonstrable endotoxin, in the nonimmune host is of prime importance in altering host defense. Although it is not clear at the present time why zinc enhances survival incidence in rats challenged with *F. tularensis*, one possible explanation is that zinc promotes either humoral or cell-mediated immunity or both. It has recently been shown that zinc is a T-cell mitogen (15) and promotes T-cell helper function (16). In addition, zinc deficiency in the nonimmune host promotes lethality by affecting the host defense system in rats challenged with *F. tularensis* (8). In this regard, it is interesting to note that rechallenge of zinc-treated, *F. tularensis*-infected rats indicated that these animals developed an immune response, in a relatively short time, which was sufficient to prevent mortality. Other factors, however, may be involved and cannot be further defined from the results of our studies.

A similar phenomenon concerning zinc's potentiation of the immune system may be operative in the ability of zinc to enhance survival in rats challenged sc with *S. pneumoniae*, another microorganism which is poorly phagocytized (17). However, this possibility does not appear to be applicable to ip challenged rats. The enhanced early resistance in ip challenged rats appears to be attributable to the ability of zinc to inhibit bacterial growth as demonstrated *in vitro*. Since plasma zinc levels are normalized within 24 hr, this effect can be expected to be of short duration. Therefore, it does not appear that inhibition of bacterial growth in blood contributes to the enhanced survival incidence in zinc-treated, sc infected rats. However, the possibility still exists that other tissues which retain elevated zinc levels for extended periods perturbs in some manner the growth of *S. pneumoniae*. In addition to our data demonstrating the inhibitory effect of zinc on bacterial growth, zinc has been reported to inhibit selectively certain active transport processes of bacteria (18) and to block reversibly the initial step in bacterial mating (20).

Regardless of the actual mechanism(s) involved, the results presented clearly demonstrated that zinc administration produces diverse effects on the course of bacterial infection dependent on the type of microorganism involved. Finally, our findings also suggest that, although zinc, in the amounts used in these studies, may prevent lethal aspects of endotoxemia arising from the escape of enteric endotoxins or of those produced as a consequence of gram-negative peritoneal infection, its ability to potentiate lethality associated with gram-negative sepsis seemingly cautions against its indiscriminate therapeutic application.

Summary. Intraperitoneal pretreatment of rats with zinc, as zinc chloride, 1 hr prior to bacterial challenge with *S. typhimurium*, *F. tularensis*, or *S. pneumoniae* enhanced mortality incidence in *S. typhimurium* infection only. Unlike previous reports concerning lead and cadmium, zinc's potentiation of lethality in an endotoxin-producing gram-negative infection does not appear to be attributable to heavy metal-induced increases in sensitivity to the toxic aspects of endotoxin. In contrast to the results obtained with *S. typhimurium*, single and multiple zinc treatments enhanced survival incidence during the early postinfection period in rats infected with *F. tularensis* or *S. pneumoniae*. These diverse observations can possibly be explained, in part, by zinc's ability to modify certain aspects of the host's defense mechanism such as leukocytosis, phagocytosis, and cell-mediated immunity as well as by zinc's inhibition of bacterial proliferation.

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