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Depot-Zinc Therapy of Systemic Lupus Erythematosus in B/W Mice[1] (41276)

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Abstract. An improved method of zinc administration, employing parenteral depot injection, was used as an experimental therapy in a mouse model of systemic lupus erythematosus. The depot method was used to produce a sustained increase in circulating plasma zinc levels for 7-day periods between injections. Female New Zealand Black/New Zealand White (NZB/NZW) mice were treated either with once weekly depot zinc, or with vehicle alone (controls) for 24 weeks. The zinc treatment produced a significant reduction in glomerular lesions. These data provide further evidence of the beneficial effect of zinc in inflammatory diseases.

Information is accumulating that treatment with pharmacological doses of zinc may be beneficial in certain inflammatory diseases. Perhaps the most direct clinical evidence of this type is the original report by Simkin (1) of positive effects of oral zinc therapy in human rheumatoid arthritis. Both positive (2) and negative (3) results have subsequently been reported on studies of zinc therapy in rheumatoid arthritis. Some confusion may exist because the absorption of oral zinc is greatly influenced by whether the zinc is administered with food (4). Chvapil (5) reported that zinc will inhibit certain inflammatory cells, including both macrophage and neutrophil functions. So far there has not been evidence of an inhibitory effect of zinc on lymphocyte function, at least in concentration ranges likely to be attainable *in vivo*. Indeed, zinc seems to be a nonspecific mitogen for lymphocytes. Many human disorders are characterized by a significant inflammatory response. On the basis of previous positive effects of zinc therapy on the inflammatory response, we have elected to evaluate zinc in an animal model of an autoimmune disorder.

One good animal model for a human inflammatory disease is the hybrid NZB/NBW (B/W) mouse model of systemic lupus erythematosus (SLE). These animals develop antinuclear antibodies (ANA) and antibodies directed against DNA. Female B/W mice die prematurely with immune complex nephritis (6). In the present work, we studied female B/W mice to determine if zinc therapy would influence autoantibodies, glomerulonephritis, and longevity.

The administration of zinc to animals when one wishes to achieve continuous elevations of plasma zinc over a period of weeks to months is not an easy task. Zinc in water and food tends to be avoided by animals who often will dehydrate or starve themselves to avoid zinc. Obviously, one could administer zinc by intraperitoneal injection or intragastric instillation and obtain reliable administration, but the blood levels after such administration tend to decrease back to normal after a few hours. Thus, these approaches require frequent administration, up to four times a day 7 days a week, if one wishes to obtain continuous elevation of the circulating plasma zinc level. To circumvent these problems, we developed a depot form of parenteral zinc administration (7). Injections given at intervals ranging from once a week to once a month were used to maintain a continuously elevated plasma zinc level. The present study represents the first therapeutic application of the depot zinc method.

Materials and Methods. *Preliminary study of depot-zinc administration in mice.* A technique for parenteral depot zinc ad-

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ministration[3] developed in our laboratory has been previously shown to be effective in rats and monkeys (7). This procedure eliminates the need for the frequent administration required to maintain continuous elevation of plasma zinc levels. Furthermore, plasma zinc concentrations achieved with parenteral depot zinc can be maintained at much higher levels than can normally be obtained by oral administration.

Because depot-zinc therapy has not been used previously in the mouse, a preliminary study used healthy mice to measure plasma zinc levels in response to a given depot-zinc dose. In this preliminary study, zinc pamoate (Parke Davis lot DS11930X115A), as a 200 mg/ml suspension in benzyl benzoate–castor oil, 60:40, v/v, was injected subcutaneously into the back once weekly. A dose of 55 mg of zinc/kg body wt was selected on the basis of previous studies in rats. The volume of drug injected ranged from 0.03 to 0.10 ml during the study because of weight variation from growth. Injections of 0.02 ml or less were used, with multiple injections given as needed to give the full dose. A simultaneous control group received a comparable volume of the benzyl benzoate–castor oil vehicle following the same regimen. Blood was collected via cardiac puncture from two mice of the control and test groups every 2 weeks for 24 weeks using sterile plastic syringes and zinc-free heparin to avoid contamination. Blood was generally drawn 7 days after a depot injection, just prior to the next weekly treatment. Thus, the plasma zinc values represent the points of minimal effect of the previous injections. In earlier work in rats we have shown that the timing of blood drawing is not critical, since the plasma zinc levels are quite stable with the depot-zinc method over the entire period between injections. The samples were centrifuged in acid-washed plastic test tubes to isolate the plasma and plasma zinc concentrations were determined by atomic absorption.

Depot-zinc treatment for the SLE mouse model. Eight female New Zealand Black and eight male New Zealand White mice were maintained and allowed to breed continuously (8). Female F_1 offspring received depot-zinc injections beginning at 4 weeks of age. They were treated once weekly for 24 weeks. Based on the results of the preliminary study, a zinc pamoate dose of 400 mg/kg (55 mg/kg total zinc) was used in the SLE treatment protocol. Control F_1 female offspring were given comparable volume injections of the benzyl benzoate–castor oil vehicle. A small number of mice from various groups were bled at 8, 16, and 24 weeks to monitor plasma zinc levels and for collection of serum samples for anti-DNA antibody determinations. All test mice were sacrificed after 24 weeks of treatment to end the study.

Histology and analysis. Complete autopsies were performed on all treated and control mice. Samples of kidney, liver, spleen, and thymus were fixed in Carnoy's fluid and embedded in paraffin. Four-micron sections were prepared for examination. All sections were stained with hematoxylin and eosin and examined by light microscopy. Slides of kidney tissue were labeled with numerical codes and were examined by one of us (S.E.W.) in a "blind" manner, i.e., without knowledge of whether the tissue was from a zinc-treated or vehicle-treated animal. The extent of renal damage was quantified using an adaptation of the method of Piraini and others (9). Abnormalities were counted in 20 glomeruli of each section of kidney yielding an overall glomerular lesion score for each mouse. Lesions counted were: thickening or hypercellularity of the mesangial stalk, focal glomerular hypercellularity, basement membrane thickening, diffuse glomerular hypercellularity, fibrinoid changes, and crescent formation. Anti-DNA antibody determinations utilized the Farr technique described by Steinberg *et al.* (10) and adapted by Walker and Bole (11). Results were expressed as the percentage of ^{14}C-labeled double-stranded DNA bound by 0.015 ml of heat-inactivated mouse serum.

Results. *Plasma zinc levels.* Figure 1 illustrates the results of the preliminary study of depot-zinc administration in healthy mice using a dose of 55 mg of

[3] Patent pending.

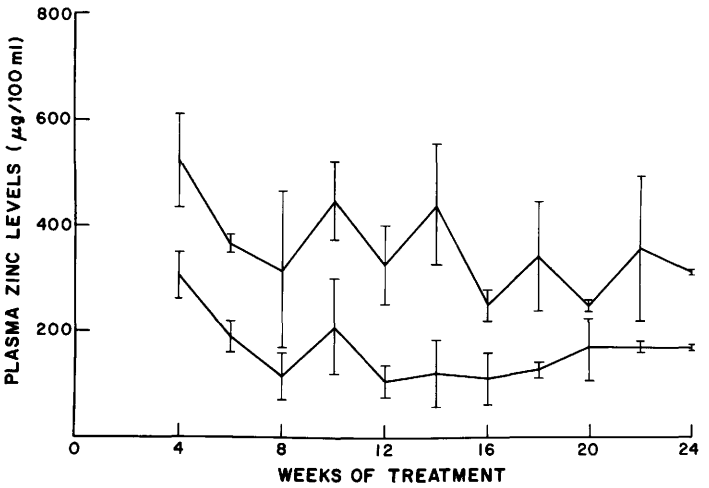

FIG. 1. Mean and ranges of plasma zinc levels of zinc pamoate- and control vehicle-treated mice over time. (The levels at 4 and 10 weeks may be somewhat spuriously elevated, perhaps due to sample contamination, as the control values appear to be abnormally high.)

elemental zinc/kg body wt. Circulating plasma zinc levels were effectively increased 1.5 to 3.0-fold by depot-zinc administration as compared to vehicle-treated controls. This approximate difference was maintained over the duration of the experiment.

In depot-zinc-treated and vehicle-treated B/W mice, we did not bleed the experimental animals excessively because we did not want to jeopardize their survival. Therefore, samples were taken from four animals on three occasions. The mean plasma zinc concentrations of the zinc-treated mice and the vehicle-treated mice were significantly different ($P < 0.01$) and generally comparable to the study shown in Fig. 1. The mean zinc level in zinc-treated mice was 1.7 times greater compared to the zinc level in control mice during the study.

Histology. Examination of renal tissue showed that the mean glomerular lesion score was decreased significantly in treated mice. Figure 2 displays individual scores of the zinc-treated versus the vehicle-treated SLE model mice. The vehicle-treated group mean score of 32.3 (±10.3) and zinc-treated group mean score of 21.8 (±10.4) are significantly different (*t* test, $p < 0.02$). Ten of sixteen zinc-treated scores were more than one standard deviation below the control group mean score.

Kidney damage was primarily of three types: thickening of the basement membrane, focal glomerular hypercellularity, and general hypercellularity. The damage was qualitatively similar in both groups, but quantitatively greater in the control mice. Out of 20 glomeruli examined from each

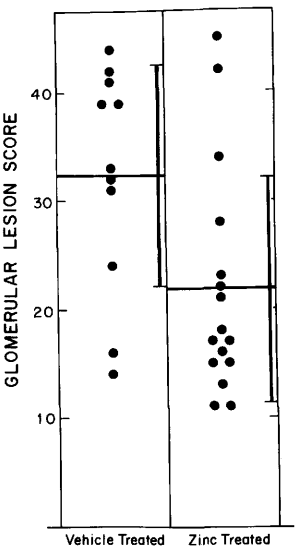

FIG. 2. Glomerular lesion scores of vehicle-treated and zinc pamoate-treated NZB/NZW F_1 test mice. The horizontal lines indicate the mean values (32.3 for control and 21.8 for zinc treated), and the vertical brackets indicate ±1 SD.

kidney section, the zinc-treated mice averaged 5.2 normal glomeruli with only 3 of 16 (18.8%) having no normal glomeruli. The control mice averaged only 1.6 normal glomeruli per examination with 7 of 11 (63.6%) having no normal glomeruli.

There were no significant differences between the control and zinc-treated groups in the sections of liver, spleen, and thymus. There was also no significant difference in body weights of the control and zinc-treated animals prior to sacrifice.

Anti-DNA antibody levels. Anti-DNA antibody levels were determined from serum samples taken just before autopsy. The levels in the control group ranged from 20 to 55% DNA bound while the zinc-treated group values ranged from 20 to 65%. The mean value for the control group was 36.3 (±13.6) and the mean value for the zinc-treated group was 40.3 (±18.7) indicating no significant effect of the drug on this variable.

Discussion. Since we did not wish to bleed the experimental animals excessively, we did the preliminary study illustrated in Fig. 1 using nonlupus animals to determine the response to depot-zinc administration. In general, the 55 mg/kg dose of depot zinc worked quite well, producing about a twofold continuous elevation in the plasma zinc levels. This depot method has previously been used successfully in rats and monkeys in our laboratory (7), and these data demonstrated its usefulness in mice. By means of injections once a week, substantial, consistent, and reasonably controlled elevations in plasma zinc levels were obtained.

The main point of this paper, shown in Fig. 2, indicates that there is a statistically significant effect on reduction of the kidney damage in SLE model zinc-treated mice. There is, however, considerable variation in the zinc-treated animals, and in fact, a complete overlap with the range of the control animals. We can only speculate that those zinc-treated animals in the upper part of the kidney damage score range either did not obtain significant elevations of plasma zinc (we have no data on these specific animals) or that other factors over which we have no control intervened to produce high levels of damage. The essence of the data then is that zinc protects against kidney damage on the average, but other factors may be present that can mitigate against its protective effect.

The mechanism of the protective effect is of considerable interest, but cannot be resolved by our study. One factor which appears to be ruled out is differences in ANA levels as a result of zinc therapy, as the antibody levels were equal in the two treatment groups. The effect of zinc would seem to be more likely in the area of a decreased inflammatory response to a given level of antibody. As indicated in the introduction, zinc is known to inhibit both neutrophil and phagocyte activity (5), and if activity of these cell types is involved in the lupus lesion, inhibition of such cells may explain the mechanism of zinc action.

It remains a theoretical possibility that the pamoate content of the zinc pamoate depot injection could be involved in protecting against the renal lesions. Control animals received injections of vehicle, but these did not contain a pamoate salt. However, we think an involvement of pamoate rather than zinc is unlikely based on a preliminary test using zinc carbonate rather than zinc pamoate in the treatment regimen. The two zinc carbonate-treated mice in this test had kidney damage scores in the low twenties indicating zinc as the effective agent. This aspect needs to be investigated further, and an additional full-scale study is currently underway using zinc carbonate as the zinc salt.

In general then, it appears that depot injections once a week of zinc pamoate, which elevate the plasma zinc levels about twofold normal, result in a significant lessening of renal damage in the SLE mouse model. These results are encouraging in that therapy in human lupus and other autoimmune disorders involving significant inflammatory response may ultimately be benefitted by zinc therapy.

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