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Sex and Age Mortality Responses in Zinc Acetate-Treated Mice

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Trace elements have received a great deal of experimental attention in regard to the pivotal roles that they play in normal cellular processes (Chvapil 1973; Chvapil et al. 1972). Many recent investigations have been directed to the bioprotective functions of zinc against the pathophysiological effects induced by other trace metals. These include the prevention of renal proximal tubular necrosis and hyperglycemia associated with nickel treatment (Waalkes et al. 1985), blockage of gold-, lead-, and cadmium-related erythropoietic depression (Lutton et al. 1984), and inhibition of cadmium-generated testicular lesions (Mason and Young 1964). In relation to the above, the intrinsic functions of zinc have been reported to be associated with cellular integrity at the enzymatic level (Finelli et al. 1975). Conversely, it has been published that zinc in excess of its essential trace levels exhibits pronounced toxicity effects. Perturbations associated with zinc include depression of overall animal growth rate (Smith and Larson 1946), decreased activities of cardiac and hepatic cytochrome oxidases (Van Reen 1953), and retarded peritoneal macrophage mobilization (Zukosko et al. 1974).

In regard to trace metal treatment or exposure, a number of variables are known to affect the expression of toxicity concerning its time course and degree. For example, known variables are route of administration (English et al. 1981), anionic component of the test substance (Hopfer et al. 1976), and sex and age of the recipient animal (Kostial et al. 1974, Hogan 1982). Concerning the latter, little, if any, data have been reported dealing with sex- and age-related responses to excess zinc in mammalian systems. The primary purpose of the short communication presented here focuses on the determination of median lethal dose in sexually immature, i.e., juvenile, and adult female and male mice following a single zinc acetate insult. In addition, variation of lethality responses was examined within the age and sex groups to a divided treatment of a lethal dosage of zinc acetate, the injections of which were separated by various intervals.

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MATERIALS AND METHODS

Mice of the ICR strain were used throughout these studies. Food and water were freely available at all times. Age for the adult animals ranged between 10 to 12 weeks and for the juveniles, 3 to 4 weeks. Average weights for adult females and males were 32 and 35 g with body weight ranges from 29 to 37 g and 32 to 41 g, respectively. Seventeen and 18 g were the average weights for juvenile females and males with ranges, respectively, of 15 to 19 g and 15 to 20 g. Zinc acetate, $ZnAc_2$ (Matheson Coleman and Bell, Norwood OH, 45212), was administered by a single intraperitoneal injection. Stock solution of $ZnAc_2$ was prepared the day of injection (day 0) with serial dilutions made and used for the injection series. Treatment was based on mg $ZnAc_2$/kg body weight. The injection series differed for the two age groups; the juveniles require substantially larger dosages to induce lethality as determined by pilot studies designed to ascertain maximum tolerance levels to $ZnAc_2$. Ten animals comprised each injection subgroup and from the four dosages that were administered per group, mortality ratios (the numbers dead compared to the initial numbers injected) were recorded for each subgroup of each age and sex group. Using these ratios, the median lethal dosage (LD50) and its 95% confidence intervals were calculated for days 1,3,5,7, and 14 using the method of moving averages according to Thompson and Weil (1952). The LD50 is, of course, used as an index of sensitivity or vulnerability to an exogenous factor.

RESULTS AND DISCUSSION

The mortality responses of juvenile and adult mice receiving a single injection of $ZnAc_2$ are shown in Table 1. Most lethality occurs by day 7 for all groups even though there are some further deaths noted to day 14. Although not shown in the table, the period of observation was extended through day 28, during which there were no statistically significant differences noted among groups concerning LD50 values from the two-week values of a comparable group. As soon as possible following death, autopsies were conducted. Neither congestion nor necrosis was observed in the peritoneal cavity of $ZnAc_2$-treated mice, however, vasodilation of abdominal vessels was quite extreme, and the heart appeared to be arrested in diastole.

It is noted that the mortality on a given day following $ZnAc_2$ is strikingly different for juvenile and adult mice. For the female groups, considering day 7 LD50 values, the juveniles require approximately two and one-half times more $ZnAc_2$ to induce the fifty percent killing level. The same trend is apparent for juvenile males at day 7. It will be further noted that there are no statistically significant differences in mortality responses according to sex. There are no statistically significant differences between adult female and male LD50 values. This is the case for the juvenile females and males as well. Viewing the mortality ratios, there was earlier killing observed on days 1 and 3 of the

Table 1. Time course of lethality for juvenile and adult mice injected with zinc acetate showing differences in LD50 between juveniles and adults.

Treatment Group	Day	Mortality Ratios[a] by Treatment				LD50 (mg/kg)	95% Confidence[c] Limit Range (mg/kg)
Juvenile							
Female	1	0/10	0/10	0/10	6/10	——[b]	—— – ——
	3	0/10	2/10	5/10	8/10	115.2	102.7-129.2
	5	0/10	2/10	6/10	10/10	109.1	101.0-117.8
	7	0/10	6/10	6/10	10/10	101.4	93.2-110.3
	14	0/10	6/10	7/10	10/10	99.6	91.8-108.0
Male	1	0/10	0/10	1/10	4/10	——[b]	—— – ——
	3	0/10	2/10	7/10	6/10	115.2	98.4-134.9
	5	0/10	2/10	7/10	8/10	110.1	99.3-122.0
	7	0/10	2/10	8/10	10/10	105.2	98.2-112.7
	14	0/10	6/10	8/10	10/10	97.8	90.5-105.6
Adult							
Female	1	0/10	5/10	6/10	10/10	45.2	41.5- 49.2
	3	0/10	6/10	6/10	10/10	44.4	40.8- 48.3
	5	0/10	6/10	7/10	10/10	44.2	40.7- 47.9
	7	0/10	7/10	8/10	10/10	42.0	38.2- 46.2
	14	0/10	7/10	8/10	10/10	42.0	38.2- 46.2
Male	1	0/10	3/10	2/10	2/10	——[b]	—— – ——
	3	0/10	4/10	7/10	4/10	50.4	39.2- 64.9
	5	0/10	4/10	7/10	8/10	46.0	41.4- 51.1
	7	1/10	4/10	8/10	8/10	44.2	39.3- 49.8
	14	1/10	5/10	8/10	9/10	42.0	37.5- 47.1

[a] Injection series for juvenile and adult mice were 80,96,115, and 138 mg/kg and 35,42,50, and 60 mg/kg, respectively. Fractions of a ratio are the number dead on a designated day over the number initially treated (day 0) with the four dosages of the injection series.

[b] Ratio value was not given in the tables used for the LD50 and confidence limit range determinations.

[c] Comparisons for which confidence limits do not overlap are significantly different.

adult females than adult males in response to the $ZnAc_2$ injections but by day 5 male lethality is comparable to that of the female.

Table 2 illustrates survival responses of adult and juvenile mice to divided treatments of a lethal dosage of $ZnAc_2$. The total dosage for each group was approximately one and one-half times the LD50 level that induced 100 percentage killing by day 7 following injection. As shown in the table, no major effects on survival percentages are seen in either age group if the separation time

Table 2. Survival of ICR mice receiving a split dosage treatment of zinc acetate.

Group[a]	Separation Interval[b] (Days) between Doses	Percentage Survival[c] on Days[d] 1	3	5	7	14
Adult						
Female	1/2	35	35	30	15	10
	1	55	55	55	50	45
	2	100	100	100	95	95
Male	1/2	45	40	35	20	15
	1	60	60	55	45	40
	2	100	100	95	95	95
Juvenile						
Female	1/2	100	90	90	90	90
	1	100	95	95	90	85
	2	100	100	100	100	100
Male	1/2	100	95	90	85	85
	1	100	100	90	80	80
	2	100	100	100	100	100

[a] Twenty animals comprise each subgroup.

[b] Adult total dosages were 32 mg/kg on day 0 and 32 mg/kg at the intervals indicated above following the initial injection. Juveniles dosages separated as the adults were two treatments of 75 mg/kg each.

[c] Number of animals alive on a given day/number of animals originally injected X 100.

[d] Days represent times following the second $ZnAc_2$ injection.

is 2 days. However, when the interval between injections was reduced to 24 h, there was an abrupt reduction in the percentage survival to about one-half of the 48-h percentages within the female and male adult groups. Such striking declines are not observed in the juvenile subgroups, although juvenile survival percentages are reduced by an approximate total of 20% on day 14 compared to the day 1 percentages. As observed in the table, an even more dramatic reduction in survival in the adult subgroups is observed when the second dosage is administered 12 hours after the first. Survival percentages for the juveniles do not follow this pattern; the percentages of survival for female and male juveniles for the 12-hour split dose closely mimic those of the 1-day separation interval values, as determined between the days 1-14 period of observation. It is interesting to note that from the lethality studies shown in Table 1, there were few deaths noted between days 7 and 14; this trend appears to be the case for the split dosage treatments as well.

Some have reported differential sex and age responses to toxicity of trace elements (Kostial et al. 1974), while others reported only age-related response differences (Hogan 1982). A variety of postulates are plausible concerning these data, suggesting that younger mice are capable of tolerating substantially greater levels of $ZnAc_2$ than their adult counterpart. It is possible that adult mice are not as efficient as young ones in eliminating excess $ZnAc_2$. If this were the case, accumulation of damaging levels of zinc in vulnerable tissues of adults might occur more readily; this would account for the lower LD50 values associated with the adult groups. In addition, it is possible that juvenile and adult mice may have the same capacity for $ZnAc_2$ elimination, but vary in the rate and/or extent to which they are capable of repairing zinc-induced lesions following in the wake of zinc exposure. If the zinc-associated lesions caused only sublethal damage and if the repair efficiency were greater in juveniles, juvenile mice would require more $ZnAc_2$ to reach the necessary injury level causing death. Data from split dosage studies support this notion, i.e., juvenile male and female mice show greater survival percentages day 7 following a split dosage treatment separated by 12 h, compared to those of male and female adult mice. Kostial and co-workers (1974) have reported the same effect for rats treated with lead acetate. If the juvenile repair processes were more efficient, the 12-h interval would not be sufficient to allow the accumulation of damage leading to death. The same difference in survival percentages between juveniles and adults is apparent for the 24-h split dosage separation treatment. These differences disappear for the 48-h separation time for dosages, where survival responses of age groups show no significant differences. Additional studies are needed to further test the postulate on variable repair efficiencies. However, whatever the mode(s) of action of higher levels of $ZnAc_2$ in ICR mice which cause mortality, it is noteworthy that younger female and male animals are considerably more tolerant of zinc acetate insult than the older ones.

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