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ORGAN SPECIFIC ZINC DEFICIENCY IN TESTICULAR FEMINIZATION RATS: HORMONE-METAL INTERACTION

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Summary: In the present study, we observed that zinc concentration in the testes of testicular feminization rats was significantly lower than control animals. Reduced zinc concentration was not observed in any other organ systems including serum, liver, kidney and adrenal gland. The contents of two other trace elements, copper and manganese in all tissues examined were comparable. The organ-specific zinc deficiency in Tfm rats was associated with a parallel decrease in the activity of the testicular metalloenzyme alkaline phosphatase. These findings substantiate the suggested relationship between zinc and androgen action.

Zinc is present in high concentrations in male accessory sex organs (1). In both humans and animals nutritional zinc deficiency has been reported to cause primary hypogonadism (2,3). Testicular function has been reported to be diminished as a consequence of dietary zinc deficiency (4) or conditioned zinc deficiency as occurs in sickle cell anemia (5). Testicular zinc metabolism is regulated by the action of androgens. Castration decreases, while testosterone administration increases prostatic zinc concentration in male rats (6). The interaction of zinc and testicular steroidogenesis may be best studied in rats with testicular feminization syndrome, genetic mutants with an abnormal steroid response. Affected Tfm males are characterized by a female phenotype, inguinal testes with well-developed Leydig cells but arrested spermatogenesis and end organ insensitivity to testosterone and other androgens (7). Zinc has been reported to affect androgen metabolism at several different levels (8-10). Prasad and associates (3,11) suggested that zinc may specifically affect testicular steroidogenesis. Identification of the zinc quantitative profile of Tfm rats in different androgen target tissues might provide insight into the interaction between zinc and steroid action.

FOOTNOTES: Tfm, Testicular Feminization

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TABLE 1

		Testes	Liver	Kidney	Adrenals
Copper ng/mg dry weight	Normal	11.74 ± 3.79	22.30 ± 8.68	40.97 ± 22.67	8.72 ± 5.14
	Tfm	10.72 ± 3.12	15.08 ± 0.90	27.53 ± 14.45	5.12 ± 2.87
		N.S.	N.S.	N.S.	N.S.
Zinc ng/mg dry weight	Normal	207.0 ± 29.0	89.10 ± 11.0	89.20 ± 26.30	42.14 ± 25.76
	Tfm	126.0 ± 34.0	82.10 ± 16.00	89.00 ± 18.10	34.42 ± 17.35
		S.D.	N.S.	N.S.	N.S.
Manganese ng/mg dry weight	Normal	4.626 ± 2.534	8.114 ± 1.488	4.331 ± 0.881	7.855 ± 4.455
	Tfm	4.638 ± 1.209	9.743 ± 4.141	3.731 ± 1.042	9.412 ± 5.150
		N.S.	N.S.	N.S.	N.S.

Tissue copper and zinc contents of normal and Tfm rats compared. Tissues from 10 normal control and 10 Tfm rats were analyzed except in serum and testes where tissues were taken from 10 animals in both control and Tfm. For trace metal analysis, an aliquot of tissue was weighed and the tissue dried at 110°C for three to five days until a constant weight was obtained. Dry ashing was performed in a Muffle Furnace (Sybron, Dubuque, Iowa) at 500°C for 24 hours, followed by wet ashing at the same temperature after addition of 1 ml of concentrated nitric acid. The ashed material was reconstituted with 1 ml of 0.1 N nitric acid. Metal analyses were carried out using a Perkin-Elmer 703 atomic absorption spectrophotometer equipped with an HGA 500 graphite furnace and an AS-1 autosampler. For tissue digests zinc was measured using the spectral line 307.6 nm whereas serum samples were analyzed at 213.8 nm. For all samples copper was analyzed using 324.7 nm and manganese at 279.5 nm. Program settings for zinc, copper and manganese analysis were: Drying 110°C/30 seconds, charring 500°C/40 seconds and atomization 2040°C/8 seconds; Drying 110°C/35 seconds, charring 900°C/30 seconds and atomization 2300°C/10 seconds; Drying 120°C/100 seconds, charring 1100°C/60 seconds and atomization 2300°C/8 seconds. Values shown are means ± standard deviation. N.S. indicates difference between control and Tfm rats non-significant, and S.D. indicates significant difference both at 99 percent confidence limits.

King-Holtzman hybrid rats with testicular feminization from the Stanley-Gumbreck colony and their normal litter mates were obtained from the International Foundation for the Study of Rat Genetics and Rodent Pest Control, Oklahoma City. The animals were kept in an air conditioned and light-controlled room and fed ad libitum with water and Rodent Laboratory Chow (Ralston Purina Company). The rats were numbered individually for random selection at 120 days of age. Rats were anesthetized with ether. Blood was obtained from the abdominal aorta and allowed to clot at 4°C for two hours in trace-metal free plastic tubes. Serum was separated by centrifugation at 4°C and stored at - 20°C until analyzed. Androgen target organs including testes, liver, kidneys and adrenal

TABLE 2

	N	Normal	Tfm	Difference
Testes	20	319.4 ± 61.8	225.0 ± 76.4	S.D.
Liver	10	5.06 ± 1.71	7.12 ± 3.70	N.S.
Kidney	10	1199.0 ± 354.5	1245.3 ± 421.0	N.S.
Adrenal	10	62.14 ± 26.86	63.93 ± 24.08	N.S.

Alkaline phosphatase activity in tissue homogenates of normal and Tfm rats compared. Protein concentration was determined by the method of Lowry et al (21) or the Biuret method (22). DNA content was determined using the diphenylamine reaction (23). Alkaline phosphatase activities was assayed using p-nitrophenyl phosphate as the substrate (24). N is the number of animals analyzed. Values shown are means ± standard deviation (I.U./gm protein). N.S. indicates difference between control and Tfm rats non-significant, and S.D. indicates significant difference both at 95 percent confidence limits.

glands (22-24) were removed, rinsed with double-deionized water, blotted dry with filter paper and weighed. The tissues were stored frozen at -20°C until analyzed.

Tissue trace metal contents were determined after dry ashing at 500°C. Serum samples were analyzed after appropriate dilution with 0.1N nitric acid. Analyses were performed by flameless atomic absorption spectrophotometry using a graphite furnace. Detail procedures were outlined in Table 1. Activities of alkaline phosphatase were assayed in the tissue homogenates as described in Table 2.

The testes of Tfm rats (N=20) (0.341 ± 0.090 gm wet weight) were significantly smaller than those of normal controls (N=20) (0.532 ± 0.124 gm wet weight). However, no significant difference was observed in protein/unit wet weight or DNA/unit wet weight. In Tfm rats the other organs examined (liver, kidney and adrenal) were comparable in size, protein and DNA content. Serum copper, zinc and manganese concentration of Tfm rats (1.593 ± 0.140 μg copper/ml, 1.270 ± 0.199 μg zinc/ml and 2.587 ± 1.185 ng manganese/ml) were not different from those of controls (1.473 ± 0.333 μg copper/ml, 1.361 ± 0.361 μg zinc/ml and 2.518 ± 1.104 ng manganese/ml) indicating that Tfm rats were not deficient in any of these elements. Tissue trace metal analysis indicated that Tfm rat testes had significantly reduced (99 percent confidence level) zinc concentration when compared to the controls. Testicular copper and manganese concentration and

copper, manganese and zinc levels of the other organs were not significantly different. Table 1 presents the tissue trace metal concentrations expressed in terms of unit dry weight. The same difference between Tfm and normal control rats was observed when the metal content was expressed in terms of unit wet weight, unit soluble protein or unit DNA. In all cases, testicular zinc concentration of Tfm rats was significantly lower (at 99 percent confidence level) than control rats (29.89 $\pm$ 4.65 µg/gm wet weight in control compared with 17.16 $\pm$ 4.56 µg/gm wet weight in Tfm; 481 $\pm$ 79 ng/mg protein in control compared with 291 $\pm$ 117 ng/mg protein in Tfm; 19.43 $\pm$ 2.66 µg/mg DNA in control compared with 7.85 $\pm$ 2.97 µg/mg DNA in Tfm). The reduced testicular zinc content of the Tfm rats is organ specific and might reflect a specific abnormality of the testicular feminization syndrome.

The testis specific zinc deficiency in Tfm rats was further corroborated by the finding of reduced alkaline phosphatase activity in the testes. The alkaline phosphatase activity in International Units/gm protein is shown in Table 2. Testicular alkaline phosphatase was significantly lower (at 95 percent confidence level) in Tfm rats. No significant difference was observed in any other tissue homogenate. Serum alkaline phosphatase activities in Tfm rats (284.0 $\pm$ 126.0 I.U./L) were comparable to that of controls (322.3 $\pm$ 57.7 I.U./L). Alkaline phosphatase is a zinc metalloenzyme (21). The decreased enzyme activities in testes of Tfm rats is compatible with an organ specific zinc deficiency.

Discussion:

Plasma zinc concentration has been suggested to be an index of zinc deficiency especially coupled with measurement of alkaline phosphatase activity in the blood (15). Tfm rats have normal levels of plasma zinc and alkaline phosphatase activity indicating that Tfm rats are not zinc deficient. This fact is also evidenced by the absence of external zinc deficiency symptoms and normal growth rate of Tfm rats. The reduction of zinc content and alkaline phosphatase in the testes of Tfm rats is therefore primarily due to the functional defect of the testes.

Copper and zinc metabolism in both animals and human subjects are closely related (16). The present study indicates that copper metabolism in Tfm rats is normal.

Manganese metabolism apparently is also normal. The abnormal zinc metabolism in the testes suggests that a certain event (or events) in testicular feminization specifically interferes with normal zinc metabolism in the testes. Under normal circumstances, leutinizing hormone is the major hormone affecting testicular zinc level (17). In Tfm rats serum leutinizing hormone concentration is higher than in control animals. This should result in increased accumulation of zinc in the testes. The present result contradicts this prediction. Thus, leutinizing hormone may not be the direct effector of testicular zinc content, or that there is an increased mobilization of zinc from the testes in Tfm rats.

The most striking abnormality of androgen metabolism in Tfm rats is the inability to concentrate dihydrotestosterone in the cells (17). This was suggested to be the consequence of a reduced receptor number (7) caused by either a defect in synthesis or assembly of the receptor (7). The formation of metal complexes of steroid sex hormones is well established (18). Steroid hormones especially testosterone have been shown to exhibit ionophoric activity in biological membranes. Hechtor (18) also suggested that steroid-receptor complex may serve as a metal carrier and shuttles metal ions through cell membranes and to specifc metal binding sites in the cell. Whether the testicular zinc deficiency in Tfm rats is a consequency of the failure to concentrate dihydrotestosterone or of the reduced steroid receptor number is a question to be resolved by further research.

On the other hand, zinc has been reported to affect steroid receptor binding in several systems including the cytosolic dihydrotestosterone receptor (9,10,19). The binding affinity of the receptor could be enhanced or depressed depending on the zinc concentration in the system (19). This change in binding affinity might be due to an alteration of the configuration of the receptor induced by the metal (10). Thus, the reduced dihydrotestosterone-receptor complex formation in Tfm rats may be due to the decreased testicular zinc content leading to decreased receptor number or assembly of defective receptors with lower binding affinity for dihydrotestosterone. Under either circumstance the testes will fail to concentrate enough dihydrotestosterone in the cells, resulting in insensitivity to stimulation by androgens as is characteristic of testicular feminization. However, the observation that the other androgen sensitive organs such as

kidneys of Tfm rats which also have deficient androgen receptors (20) do not show decreased zinc levels cannot be accounted for by this explanation.

Nevertheless, the present study raises an interesting question. What is the relationship between zinc, androgens and testicular development? It illustrates the complexity of hormone-metal interaction and suggests the necessity of further research to understand the interaction of hormone and metal during normal testicular development.

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