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Increased Levels of Hepatic and Renal Metallothionein in the Rat and Guinea Pig after Percutaneous Application of Zinc Chloride

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Metallothionein (MT) is a cytoplasmic, low molecular weight, cysteine rich, heat stable protein (Cherian and Goyer 1978, Dunn et al. 1987). It was detected in various organs including liver, spleen, pancreas, testes, lung, intestine, brain, heart, adrenal, lacrimal and parotid glands (Chen and Ganther 1975, Hart et al. 1985, Heilmeir and Summer 1985, Onosaka and Cherian 1981, Waalkes and Klaassen 1985, Wormser and Calp, 1988a, Zelazowski and Piotrowski 1977). MT can be induced by several metals (Eaton et al. 1980, Maitani and Suzuki 1982, Mogilnicka et al. 1975, Waalkes and Klaassen 1985) chemicals and drugs (Brzeznicka et al. 1987, Goering et al. 1985, Klaassen 1981, Wormser and Calp 1988b).
The most powerful inducers of metallothionein are cadmium and zinc. These metals cause significant enhancement of MT rate of synthesis in a variety of organs such as liver, pancreas, intestine and several secretory glands (Dunn et al. 1987, Wormser et al. 1988a).

Water soluble zinc salts are common contaminants of the environment. They are used in metal galvanization and in some plastic products. Zinc exposure may occur from the use of various cosmetics such as deodorants, body lotions and shampoos. Zinc chloride bioavailibility can also be increased by its clinical use as a topically applied astringent and antiseptic agent. Since zinc-containing preparations are widely used in skin treatments, it was of interest to examine the effect of percutaneous application of this metal on induction of metallothionein in the rat. In the present study dose-response relationship and the cumulative effect of topically applied zinc chloride have been demonstrated. For comparison, metal-binding protein induction by the same route of exposure has been also tested in the guinea pig.

MATERIALS AND METHODS

Guinea pigs (450-550g) and rats (200-250g, Wistar-Sabra strain)

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were housed in stainless cages and exposed to 12-hr light/12-hr dark cycle in a temperature controlled room (22-24°C). Food and water were provided ad libitum. The animals were housed singly to prevent biting.

Zinc ointment was prepared by emulsifying $ZnCl_2$ (gold label, Aldrich, Milwaukee, WI, USA) in 200μl liquid paraffin. An appropriate quantity of soft paraffin was gradually added and mixed well to form a homogenized preparation. The vehicle (control) contained the same components without $ZnCl_2$.

The backs of guinea pigs (5X5 cm) and rats (3X3 cm) were shaved (Krups shaver) and on the following day a thin layer of $ZnCl_2$ ointment at selected concentrations was applied to the skin. Five rats or guinea pigs were in every dose group. The animals were treated once daily for 3 days. In most of the experiments the animals were not bandaged to avoid skin irritation. However, similar results were obtained when a bandage was used. Twenty four hr after the last treatment the animals were sacrificed by cervical dislocation after ether anesthesia. The liver, kidney and lung were removed for MT determination. The effect of repetitive applications of zinc on metal-binding protein induction was studied in rats after topical application of 5% $ZnCl_2$ ointment (once/day) for 0 (without treatment), 1, 2 and 3 days. The animals were sacrificed as above 24 hr following the last application and liver, kidney and lung were removed for MT determination.

MT levels were determined by the hemoglobin radioassay method of Onosaka et al. (1978) as described by Eaton and Toal (1982) with the modifications developed by Wormser and Calp (1988b). Tissues were homogenized (1:3 w/v) in 10mM Tris-HCl pH 7.4 at 4°C by Kinematica homogenizer. The homogenates were centrifuged at 10,000g for 15 min at 4°C and the resulting supernatants were heated (100°C) for 2 min followed by 10,000g centrifugation at 4°C. The supernatants (appropriately diluted with Tris-HCl buffer) were incubated with 200μl $CdCl_2$ 4μM containing ^{109}Cd 1μCi/ml (Amersham, Buckinghamshire, England), and 100μl Tris-HCl buffer 10mM pH 7.4 in an Eppendorf polyethylene microcentrifuge tube. After 10 min of incubation at room temperature, 10μl of NaOH 0.1N were added following 100μl of 2% bovine hemoglobin (type II Sigma, St. Louis, MO, USA) in water. The samples were incubated at 100°C for 2 min, cooled on ice, and centrifuged at 10,000g for 2 min. An additional aliquot of 100μl hemoglobin was added, heat denaturation and centrifugation were repeated. An aliquot of the supernatant fraction (300μl) was recentrifuged and the resultant supernatant (200μl) was counted for ^{109}Cd radioactivity. Appropriate blank (no sample) and total (no sample and hemoglobin) were included in each assay. MT concentrations were calculated by using purified rabbit MT type II (Sigma, St. Louis, MO, USA) as a standard in each experiment. Results are expressed as mean ±SD using the Mann-Whitney (2 tailed) for statistical evaluation.

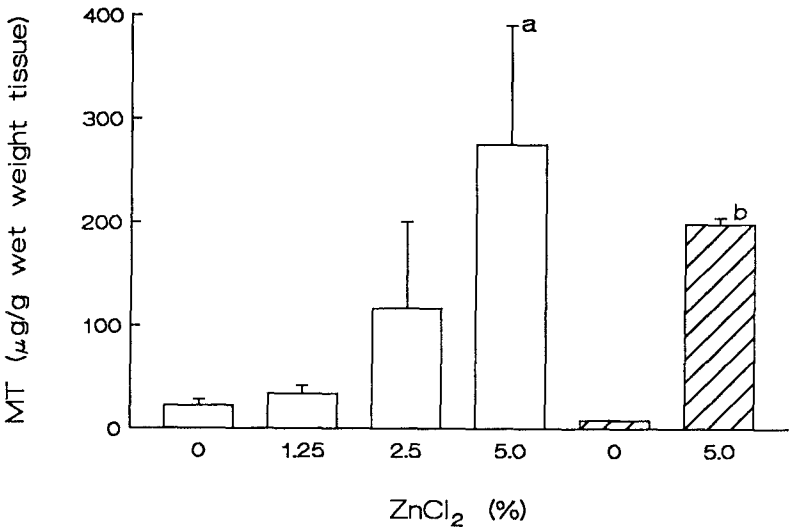

Figure 1. Dose-response relationship of hepatic MT induction in rats (open bars) and guinea pigs (hatched bars) treated topically with zinc ointment.
The animals were applied (once/day for 3 days) with $ZnCl_2$ ointment in the indicated concentrations (w/w). The controls (0%) were treated with the vehicle. Twenty four hr after the last treatment hepatic MT was determined as described in Materials and Methods.
[a]$p<0.05$; [b]$p<0.01$

RESULTS AND DISCUSSION

Application of zinc ointment on rat skin resulted in hepatic MT induction in a dose-dependent manner (Fig. 1). The metalloprotein was elevated by 5.3 and 12.3 fold in livers of rats treated (x3) with 2.5 and 5% $ZnCl_2$, respectively. Kidney and lung were weakly affected by this treatment (Table 1). The guinea pig was more influenced by the metal. Liver MT levels of the guinea pig were 24 times higher in the 5% $ZnCl_2$-treated than in the control animals (Figure 1), whereas renal and lung metalloprotein contents were increased by 3.1 and 1.4 fold, respectively (Table 1).
The correlation between hepatic MT levels and number of treatments is shown in Table 2. The metal-binding protein in the rat liver was induced by 4.4 and 10.6 fold after 2 and 3 zinc treatments, respectively. Renal and lung MT were only slightly affected even by 3 treatments of the metal.

The present study demonstrates the effect of topically applied zinc on induction of hepatic and renal MT synthesis both in the rat and guinea pig. The dose-dependent elevation of hepatic metalloprotein as well as the accumulated effect of zinc may indicate that the metal is absorbed through the skin after topical exposure of the metal (5%) for two and three days. In addition,

Table 1. MT induction in kidney and lung of the rat and guinea pig following topical treatment with zinc ointment.

species	$ZnCl_2$ (%)	MT (µg/g wet weight tissue) kidney	lung
rat	0	93±22	1.4±0.1
	1.25	105±31	1.3±0.3
	2.5	109±29	1.6±0.2
	5.0	116±33	1.5±0.3
guinea pig	0	44±7	2.1±0.2
	5.0	137±61*	3.0±0.6

The animals (5 of each dose group) were treated topically (once/day) for 3 days with $ZnCl_2$ ointment in the indicated concentrations (w/w). The controls (0%) were treated with the vehicle. Twenty four hr following the last treatment MT levels in kidney and lung were determined as described in Materials and Methods. *$p<0.05$

Table 2. Effect of repetitive applications of zinc ointment on MT levels in liver, kidney and lung of the rat.

number of applications	MT (µg/g wet weight tissue) liver	kidney	lung
0	25±6	88±23	1.3±0.1
1	27±8	95±19	1.4±0.2
2	115±85*	89±12	1.5±0.2
3	275±215*	116±33	1.5±o.3

The animals (5 of each group) were treated topically (once/day) with 5% $ZnCl_2$ ointment. Twenty four hr following the last application MT levels in liver, kidney and lung were determined as described in Materials and Methods. *$p<0.05$

skin irritation was observed after 3 applications, and to a lesser extent after 2 treatments, of 5% $ZnCl_2$ (data not shown). The damaged integrity of skin layers might enhance penetration of the metal into the circulation, from which it can be transferred to the liver and kidney for MT induction. However, a shorter duration of treatment (one application) neither influenced the skin (not shown) nor the hepatic and renal MT induction. This is in agreement with previous studies reporting on a weak percutaneous penetration of $ZnCl_2$ after short exposure to the metal (Wahlberg 1965).

Although it is generally accepted that heavy metals are not absorbed by the skin, it is highly important to consider the effect of topically applied metal salts on the biochemical-physiological functions of various organs when using metal-containing preparations in medical and cosmetic skin treatments.

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