Effect of Chelating Agents and Stress Factors on Zinc Content in Paneth Cells and Prostate Gland Cells

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The effects of chelating agents dithizone and sodium diethyldithiocarbamate on zinc content in Paneth cells and prostate gland cells were studied on rats preliminary exposed to stress factors (exercise and immobilization). Dithizone induced irreversible triphasic and sodium diethyldithiocarbamate reversible biphasic changes in zinc metabolism in cells. Preliminary stress potentiated the damaging effect of dithizone.

Key Words: *chelating agents; stress; Paneth cells; prostate gland; zinc*

Complexes of chelating agents with metals (chelates) are products and wastes of chemical, pharmaceutical, and other branches of industry [1,2,4]. Many drugs belonging to this family of compounds *in vivo* may interact with cellular zinc and induce cell changes [2,4].

Chelate-forming (cytochemically detectable) zinc is accumulated in some body cells, such as β-cells of islets of Langerhans, Paneth cells, prostate gland (PG) cells, *etc*. [3,4]. Zinc not detectable by cytochemical reactions is tightly bound with bioligands (more than 200 metalloenzymes etc.) and supports structural integrity and function of cell membranes [3,4]. Administration of dithizone (chelating agent) is followed by zinc binding in animal β-cells and their injury, while another chelating agent sodium diethyldithiocarbamate (DDC) does not induce damage despite zinc binding in these cells [2,4].

In furtherance of these studies, we studied the dynamics of zinc content in Paneth cells and PG cells taking into account their similar morphofunctional features. The function of these cells are excretion and production of antimicrobial peptides (defensins) [5-9,11]. It was also important to detect the effects of chelating agent and stress factor on these cells.

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MATERIALS AND METHODS

Experiments were carried out on 284 outbred male rats weighing 210-325 g at the age of 0.5-1 years. Stress was induced by physical exercise (swimming for 2 h in a water bath, water temperature 32°C) or immobilization (fixation in the supine position to a frame with soft ties for 6 hours). Dithizone (50 mg/kg) and DDC (500 mg/kg) were injected intraperitoneally.

For preparing dithizone solution, 0.6 ml 25% ammonium hydroxide solution and 400 mg dithizone were transferred in a glass-stoppered flask containing 30 ml of distilled water, the mixture was stirred in a water bath (70°C) for 10 min, then filtered through an ashless filter. The filtrate was a 1% aqueous ammonia solution of dithizone used for injections. DDC was injected as 10% aqueous solution.

The animals were decapitated 0.5 h-5 days after injection of the chelating agent. All experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609EEC).

In special experimental series, we examined the combined effect of stress factors and dithizone on intracellular zinc content. Dithizone was injected directly after immobilization and 2 h after the start of exercise. The animals were sacrificed 5 days after dithizone injection.

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Portions of ileum and PG were excised; frozen sections (20-60 μ) were prepared and fixed for 12 h in cold (4°C) acetone. Fixed pieces were processed through xylene (2×15 min), a 1:1 xylene-paraffin mixture (30 min at 40°C), liquid paraffin (2×1.5 h at 56°C), and embedded in paraffin. The sections (5-10 μ) were deparaffinized twice with xylene and alcohols (3 min each), treated for 1 min with 0.01% 8-quinolyl(p-toluenesulfonamide) (8-TSQ) in acetone, rinsed with distilled water for 5 min, and embedded in glycerin. Slides were examined under a fluorescent microscope with FS-1 and ZhS-18 filters. Intracellular zinc content was assessed microfluorometrically. We developed a method based on the comparison of fluorescence intensity of standard 8-TSQ-zinc solution and the luminescence intensity of the studied cells [4,5]. Calibrators were prepared filling the quartz cuvette with a standard solution of specified zinc concentration. The cuvette was placed on microscope stage, and microscope objective was immersed to a certain depth. The calibration curve was constructed based on fluorometry data (ordinate) and zinc concentration (μg/g) in standard solutions (abscissa). Intracellular zinc concentration (μg/g) was determined using a zinc standard curve based on data from cell fluorometry.

Blood corticosterone levels were determined fluorometrically [3].

The data were processed statistically using Statistica 6.0 software. For all quantitative signs in the compared groups, the arithmetic means and standard errors ($X\pm m$) were calculated. The normality of experimental data distribution was verified using Kolmogorov–Smirnov test. The parametric Student's t test was used for group comparisons; the differences were significant at $p<0.05$.

RESULTS

Administration of dithizone to rats induced intravital cytochemical reaction in Paneth cells and epithelial cells in terminal compartments of PG. In the frozen sections, red granules were found in these cells. The intensity of the reaction peaked 30 min after injection, while after 2 hours the product of cytochemical reaction was not detected.

Administration of DDC also induced intravital intracellular cytochemical reaction with zinc. In contrast to dithizone, product of this reaction was not stained and therefore was not revealed under a light microscope.

In control (intact) rats, yellow-green luminescent granules in the cytoplasm of Paneth cells and epithelium of terminal compartments of PG were detected on paraffin sections treated with fluorochrome 8-TSQ.

In this group of rats, zinc content in Paneth cells and PG cells was 95.0±7.3 and 68.0±5.2 μg/g, respectively (Table 1). In 30 min after dithizone injection, zinc content in Paneth cells decreased by 49% and in PG cells by 44% ($p<0.001$); after 2 h, by 38 ($p<0.001$) and 18% ($p<0.05$), respectively; after 8 h, by 55 and 46% ($p<0.001$), after 1 day, by 38 and 25% ($p<0.001$); after 2 days, by 47 and 43% ($p<0.001$); after 5 days, by 23 and 22% ($p<0.05$).

Thirty minutes and 2 h after DDC injection, zinc was hardly detected in cells, because of its binding with the chelating agent (Table 2). After 8 h, zinc content in Paneth cells and PG cells decreased by 76 and 78% ($p<0.001$), respectively; after 1 day, by 28 ($p<0.01$) and 26% ($p<0.01$), respectively; after 2 days, by 25% ($p<0.05$) and 20%; after 5 days, by 9 and 6%. Thus, intracellular zinc content returned to the initial level 8 h-5 days after DDC injection. The third phase was not observed due to the lack of destructive changes in cells.

These findings (Tables 1 and 2) suggest that dithizone induces irreversible and DDC reversible zinc deficiency in cells. This can be explained by the fact that the first agent damages the cells, and the second

TABLE 1. Zinc Content in Rat Paneth Cells and PG Cells at Different Terms after Dithizone Injection (Microfluorometry Data; $\bar{X}\pm m$)

Time after injection	Zinc content, μg/g	Paneth cells	PG cells
Control (n=14)	95.0±7.3	68.0±5.2	
30 min (n=11)	48.0±5.3***		38.0±4.1***
2 h (n=11)	60.0±4.2***	56.0±5.5*	
8 h (n=13)	43.0±2.9***	37.0±4.3***	
1 day (n=11)	36.0±2.1***		40.0±2.9***
2 days (n=10)	50.0±5.1***		39.0±3.2***
5 days (n=13)	73.0±6.7*	53.0±5.0*	

Note. Here and in Tables 2-4: *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the control.

one does not induce destructive changes. The greater was cell destruction, the more pronounced was zinc deficiency. Therefore, irreversible intracellular zinc deficiency can be indicative of irreversible (destructive) changes in cells.

Thus, dithizone generates triphasic changes and DDC biphasic changes in intracellular zinc content. The first phase is characterized by primarily decrease in the concentration of cytochemically detectable zinc due to its blockade with chelating agents.

Reduced zinc content in cells promotes secretion of stress hormones by the adrenal glands and of blood level of corticosterone increases. The latter stimulates synthesis of metallothioneins promoting zinc accumulation in cells (second phase). This phase is observed 2 h after dithizone injection and from 8 h to 5 days after DDC administration.

In control (intact) rats, blood corticosterone content was 1020.0±72.4 nmol/liter (Table 3). Two hours after injection of dithizone and DDC, hormone concentration increased by 23 and 27% ($p<0.05$), respectively. Exercise and immobilization produced similar results (34 and 33%, respectively, $p<0.05$). Similar changes with the introduction of helants and under stress indicate that the second chelating agent — induced phase is a result of adrenal activation. This phase did not occur in adrenalectomized animals after injection of chelating agents.

In contrast to DDC, dithizone induces damage to intestinal and PG cells and the development of the third phase (secondary decrease in intracellular zinc content). The greater was this damage, the more potent was the third phase. In this case, reduced level of zinc in cells reflects the degree of damage. Under the action stress factors, intracellular accumulation of zinc in cells is a result of inhibition their secretory activity.

Taking into account multidirectional effects of dithizone and stress, it was of interest to study the influence of stress factors on the damaging effect of dithizone on cells.

Five days after dithizone injection, zinc content in Paneth cells was decreased by 20% ($p<0.05$), while in case of preliminary forced swimming and immobilization this parameter was reduced by 35% ($p<0.001$) and 36% ($p<0.001$); in PG cells the corresponding values were 25% ($p<0.05$), 32% ($p<0.01$), and 41% ($p<0.001$), respectively (Table 4).

Thus, preliminary stress enhanced dithizone-induced zinc deficiency in rats. This is because the intracellular content of chelating agent interacting with zinc *in vivo* was much higher than in case of dithizone injections without preliminary stress. These data suggest that zinc blockade plays a role in the mechanisms underlying damaging effect of dithizone. DDC also binds zinc, but in contrast to dithizone it stabilizes cell membranes and therefore does not damage the cells.

Dithizone forms a stable complex with zinc and only substances with higher zinc complex stability constants, *e.g.* metallothioneins can displace zinc from its complex with dithizone [1,10]. Dithizone released from the complex by metallothioneins labilizes cell membranes inducing their alteration [2].

TABLE 2. Intensity of Cytochemical Reaction with 8-TSQ in Paneth Cells and PG Cells at Different Terms after DDC Injection ($X±m$)

Time after injection	Zinc levels, μg/g	
	Paneth cells	PG cells
Control (n=14)	95.0±7.3	68.0±5.2
30 min (n=11)	1.0±0.2***	1.0±0.4***
2 h (n=10)	1.0±0.3***	1.0±0.2***
8 h (n=12)	23.0±1.8***	15.0±0.7***
1 day (n=10)	68.0±5.3**	50.0±4.1**
2 day (n=11)	72.0±6.7*	55.0±5.0
5 day (n=12)	86.00±6.70	64.0±4.5

TABLE 3. Effect of Chelating Agents and Stress Factors on Blood Corticosterone Concentration in Rats ($X±m$)

Group of animals	Corticosterone, nmol/liter
Control (n=16)	1020.0±72.4
2 h after dithizone injection (n=12)	1254.0±93.6*
2 h after DDC injection (n=12)	1301.0±100.4*
2 h after exercise (n=12)	1365.0±102.3**
Directly after immobilization (n=13)	1354.0±105.2**

TABLE 4. Effect of Dithizone Administration after Stress Exposures on Zinc Content in Rat Paneth Cells and PG Cells ($\bar{X}±m$)

Group of animals	Zinc levels, μg/g	
	Paneth cells	PG cells
Control (n=16)	95.0±7.3	68.0±5.2
Dithizone (n=12)	76.0±6.2*	51.0±4.9*
Exercise+dithizone (n=13)	62.0±4.4***	46.0±4.5***
Immobilisation+dithizone (n=15)	61.0±5.7***	40.0±6.8***

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