Intralysosomal Accumulation of Gadolinium and Lysosomal Damage during Selective Depression of Liver Macrophages *in Vivo*

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Kinetics of gadolinium accumulation was studied by inductively coupled plasma-emission spectroscopy after intravenous injection of this agent (7.5 mg/kg) to CBA mice. Gadolinium exhibits lysosomotropic properties (long-term selective accumulation in lysosomes *in vivo*). Gadolinium uptake by hepatic cells attained maximum 1 h after its intravenous injection and remained at this level during the next day. Accumulation of gadolinium in hepatocytic lysosomes disturbed their osmotic properties (as was seen from the increase in free acid phosphatase activity, which persisted for 19 days). Serum activities of β-D-galactosidase and β-D-glucuronidase also increased (24-72 h and day 19). Selective depression of liver macrophages (24-48 h) was accompanied by a decrease in serum chitotriosidase activity. We conclude that accumulation of gadolinium in lysosomes of liver macrophages leads to their damage and elimination of a certain population of macrophages (primarily large cells). Changes in activity of serum lysosomal enzymes also reflect repopulation of liver macrophages.

Key Words: *gadolinium chloride; depression of liver macrophage; lysosomal enzymes*

Gadolinium chloride (GC) is widely used for modeling selective depression of liver macrophages [2-6]. Selective elimination of a subpopulation of large Kupffer cells [3,4,6], inhibition of receptor-mediated endocytosis and phagocytosis of carbon particles in liver macrophages [6,15], increase in the number of mRNA transcripts, and stimulation of TNF-α, IL-1, and IL-6 production were observed 24-48 h after intravenous injection of GC [5,7]. The phagocytic function of macrophages recovered and population of liver macrophages is restored after 3 and 4 days, respectively [4-6]. It was hypothesized that the mechanism of gadolinium accumulation in lysosomes is similar to that for other lysosomotropic agents, and this accumulation is accompanied by labilization of lysosomes and leads to cell damage [13]. The consequences of GC accumulation and its effect on lysosomal functions remain little studied.

Here we studied the kinetics of GC accumulation in the liver and its effects on hepatocyte lysosomes and activity of lysosomal enzymes in blood serum. Among lysosomal enzymes we chose β-N-acetylhexosaminidase, a marker enzyme of liver macrophages, and β-D-glucuronidase and β-D-galactosidase, easily solubilized matrix lysosomal enzymes, whose activity varies more pronouncedly in comparison with membrane-bound enzymes. Suppression of functional activity of Kupffer cells was also assessed by the changes in activity of blood serum chitotriosidase, a novel macrophagal enzyme [1].

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MATERIALS AND METHODS

The experiments were carried out on mature male CBA mice weighing 20-25 g. (Vivarium of Research Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk). GC (kindly provided by Prof. M. Hardonk) was injected intravenously (into the caudal vein) in a dose of 7.5 mg/kg [4,6]. The animals were sacrificed on minutes 0, 5, 15, 30, 60, and on day 1, 5, 7, 14, 30, 37 postinjection. Liver homogenate was prepared and lysosomal vulnerability was evaluated (by the increment in free acid phosphatase activity in hypotonic medium) as described previously [13]. Activities of β-D-galactosidase and acid phosphatase were also determined [13,14]. Serum activity of chitotriosidase was assessed fluorimetrically using 4-methylumbelliferyl β-D-N,N',N"-triacetylchitotrioside (Sigma) as the substrate [1,9]. Fluorescence was measured on a Perkin-Elmer 650-10S spectrofluorimeter. We previously showed that stimulation of macrophages with β-1,3-D-glycans elevated serum chitotriosidase activity in mice and rats [1,9].

Concentration of GC in the liver was assessed in dehydrated tissue by adsorption spectroscopy. The measurements were performed on a JI-70 spectrometer (Jober Ivon) in Katalizator Ltd (Novosibirsk). The spectrum analytical lines were GdII 342 and 247 nm. The detection limit for GC was set at 2σ. The background concentration was 5 ng/cm^3 and the concentration range from 10 ng/cm^3 to 10 μg/cm^3; standard deviation <0.005.

For morphometric electron microscopy liver specimens were fixed in a mixture of glutar- and paraformaldehyde in 0.1 M phosphate buffer, dehydrated, and embedded in Epon-812. Ultrathin sections were examined under a JEM-100S electron microscope. The ultrastructural stereometric indices were assessed at ×5000 and ×15,000.

The data were analyzed statistically using Student *t* test at $p<0.05$.

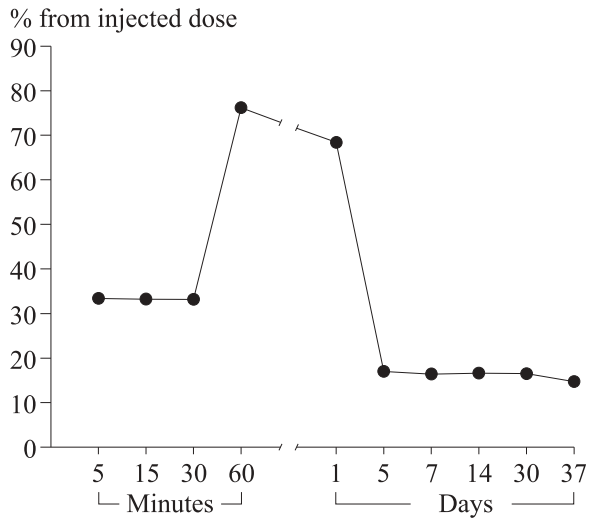

Fig. 1. Uptake of gadolinium by mouse hepatic cells after single injection of GC (7.5 mg/kg). Abscissa: time postinjection. Each group comprised 10 mice. *$p<0.001$ in comparison with the data obtained 5 min after GC injection.

RESULTS

GC uptake by the liver (up to 70% of the injected dose) attained maximum on minute 60 postinjection and remained at high level during the next day (Fig 1). During days 5-37, GC uptake decreased to 16% (Fig. 1). These data attest to a rapid capture of GC molecules in the liver during the first postinjection day followed by a long-term accumulation of GC by liver cells (specifically, by hepatocytes). After repopulation GC can be recaptured by newly recruited macrophages.

Electron microscopy of liver specimens showed that 24 h after GC injection, the density and size of liver macrophages markedly decreased (Table 1). Similar changes were observed 48 h after GC injection. Most macrophages had oval shape, smooth surface, and more electron dense cytoplasm. The relative volumes of primary and secondary lysosomes decreased compared to the control (Table 1). The total number of leukocytes after admini-

TABLE 1. Effect of Gadolinium Chloride on Morphometric Parameters of Liver Macrophages in Mice

Index		Control	Experimental
Macrophage density (per 1 mm^2)		1053.0±60.5	788.00±69.10*
Cytoplasm cross-section area, μ^2		31.70±2.93	19.50±2.50*
Relative volume, %	mitochondria	4.30±0.44	3.60±0.39
	primary lysosomes	4.00±0.37	2.70±0.22*
	secondary lysosomes	7.60±1.08	1.70±0.64**

Note. *$p<0.01$, **$p<0.001$ compared to the control.

stration of CG did not change, but the content of peripheral blood monocytes decreased on days 1-3 (to 3.50±0.35 and 2.6±0.3, respectively, *vs.* 5.40± 0.50 in the control) and increased on days 7-37. This probably reflected the deficiency of monocytopoiesis inhibitor factors against the background of selective elimination of liver macrophages [6].

Activities of serum β-D-glucuronidase and β-D-galactosidase increased on day 3 after GC injection; β-D-galactosidase activity remained at this high level throughout the observation period (19 days; Fig. 2). The peculiarities of solubilization of these enzymes can be explained by differences in their half-lives (10 and 60 min for β-D-galactosidase and β-D-glucuronidase, respectively) [13,14].

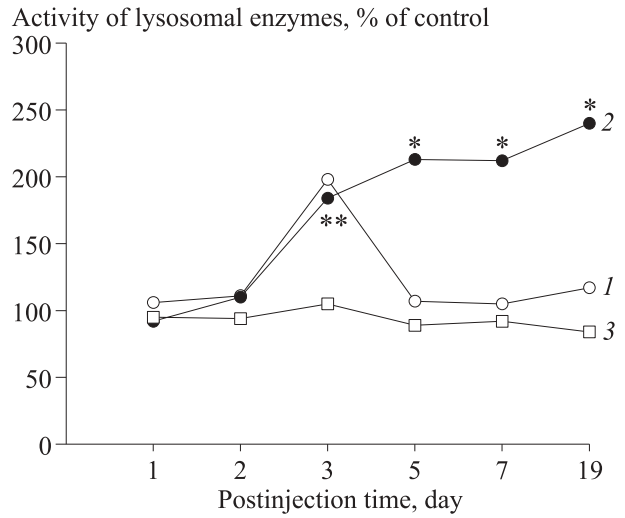

Fig. 2. Effect of GC on serum activities of β-D-glucuronidase (*1*), β-D-galactosidase (*2*), N-acetyl-β-D-hexosaminidase (*3*) in mice. **p*<0.05, ***p*<0.001 compared to the control.

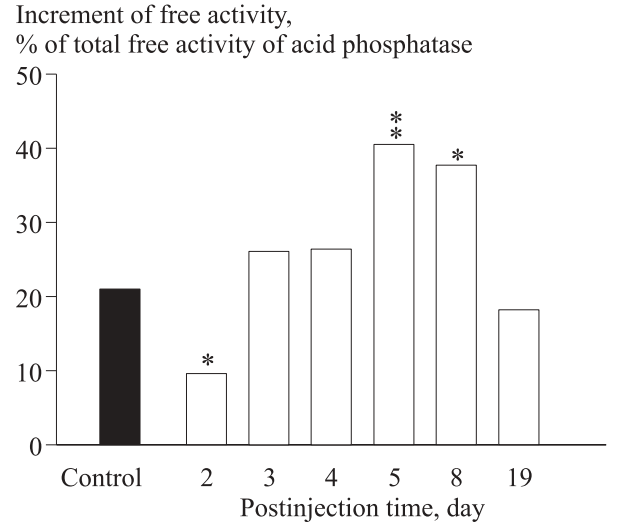

Fig. 3. Effect of GC (7.5 mg/kg) on vulnerability of mouse hepatic lysosomes in hypotonic medium. **p*<0.05, ***p*<0.01 compared to the control.

Increased enzyme activity observed from day 3 can be explained by the appearance of a new pool of macrophages after depression and elimination of GC-overloaded cells. Increased β-D-galactosidase activity in the serum is considered as more sensitive index of ischemic damage to hepatic cells than transaminase activity [13,14]. We observed no significant changes in N-acetyl-β-D-hexosaminidase activity (macrophage marker) throughout the observation period (Fig. 2).

In GC-treated mice, the sensitivity of lysosomes to the damaging action of hypotonic medium (assessed by the increment in free acid phosphatase activity) increased, especially on postinjection days 5-8 (Fig. 3). The increase of this index attests to lysosome enlargement and labilization, as well as to the prevalence of secondary lysosomes under conditions of GC load.

We previously demonstrated elevation of serum chitotriosidase activity during stimulation of liver macrophages in mice and rats [1]. In this study, we observed a decrease in chitotriosidase activity under conditions of depression of liver macrophages on postinjection day 2 (121.50±12.20 *vs.* 282.90± 23.72 nmol methylumbelliferone/ml/h in the control, *n*=10). These changes agree with the results of electron microscopy. They should be further studied in view of new data on the difference of the cell origin of chitotriosidase in mice and humans [1,9].

Intravenous injection of GC (5-10 mg/kg) to mice and rats is widely used as a model of selective depression of liver macrophages [7-10]. In these cases, no significant inhibition of functional activity of other macrophage pools (pulmonary and splenic) was observed [6]. Some papers demonstrated the protective effect of GC (10-20 mg/kg) against experimental interstitial pneumonia, which suggests suppression of alveolar and interstitial macrophages [11,12].

We showed that accumulation of GC during the period of its maximum uptake (the day 1 postinjection) and during the following decrease of its concentration (days 5-37) led to structural and functional disturbances in liver lysosomes. Accumulation of GC in lysosomes of liver macrophages and depression of the affected macrophages was accompanied by a decrease in osmotic vulnerability on the day 2 and increase in this parameter during repopulation on days 5 and 8 (Fig. 3). The hypotonic increment of the free activity of acid phosphatase was determined as a surpass of the enzyme activity measured in liver homogenate in hypotonic medium (0.125 M sucrose at 0°C during 30 min) over its activity in isotonic medium (0.25 M sucrose). The fall of hypotonic increment of acid phosphatase can be explained by a decrease in the num-

ber of lysosomes and the change of the ratio between the primary and secondary lysosomes (Table 1). Solubilization into the blood serum (predominantly, increase in β-D-galactosidase activity) did not occur during depression (24-48 h), but took place during repopulation of macrophages, which can also indicate ischemia of liver cells. Selective depression of liver macrophages was accompanied by a decrease in chitotriosidase activity. Probably, accumulation of gadolinium in liver macrophage lysosomes damages them and eliminates a certain population of the macrophages (predominantly, large cells) by apoptosis.

Gadolinium (similarly to lanthanum) belongs to trivalent chemically active lanthanides (rare-earth elements), playing an important role in modern industrial technologies. In experimental biology, these elements are used for selective inhibition of liver macrophages. Gadolinium was shown to affect hepatocytes by decreasing cytochrome P-450 activity, which in addition to depression of liver macrophages demonstrated its protective action against some xenobiotics. Gadolinium promotes proliferation activity of liver cells probably due to up-regulation of TNF-α secretion. Therefore, new data appeared on a wide spectrum of biological action of this agent. The selectivity of gadolinium action on liver macrophages is discussed. Increasing the dose of GC provokes hepatic necroses related to enhanced secretion of TNF-α, which triggers the cascade of cell events accompanied by elimination (probably by apoptosis) and repopulation of liver macrophages. Our data attest to lysosomotropic properties of gadolinium and its long-term accumulation in the hepatic cells. The changes in lysosomal enzymes indicate involvement of these subcellular organelles in the process of gadolinium accumulation in macrophages and probably in their repopulation.

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