Effect of Liver Macrophage Depression on the Development of Liver Metastases of HA-1 Tumor in Mice

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Gadolinium chloride (5 mg/kg) administered to mice 24 h before intravenous transplantation of HA-1 hepatoma cells decreased the volume density of tumor implants in the liver, reduced the intensity of degenerative and necrotic changes developing under the effect of growing tumor metastases, and prolonged the life span of tumor-bearing mice. Development of metastases was not associated with changes in cathepsin B activity in the liver, while activity of cathepsin L decreased only during the early period (4 days) after injection of gadolinium chloride. Injection of gadolinium chloride led to labilization of liver cell lysosomes because of overload with gadolinium chloride particles. The positive effect of gadolinium chloride was probably associated with depression of liver macrophages at the stage of tumor cell invasion and with subsequent migration of monocytes/macrophages preventing the growth of formed metastatic nodes in the liver.

Key Words: *metastases; macrophage depression; liver; cysteine proteases*

By the present time numerous experimental findings demonstrated the important role of macrophages in antitumor and antimetastatic defense [2,3]. It was shown that selective suppression of macrophage activity leads to stimulation of tumor growth and metastasizing [2]. Some stimulators of the mononuclear phagocyte system produce opposite effects on the growth of the primary tumor and formation of metastases [2,3]. Macrophage stimulation not always inhibits the formation of metastases: any activating exposure leads to a phase shift with cytotoxic and growth-stimulating activity of macrophages, and only substances leading to long stabilization of macrophages in the cytotoxic phase exhibit a pronounced antimetastatic effect [3,11].

We studied the role of liver macrophages in the development of metastases of HA-1 hepatoma; this tumor selectively forms metastases in the liver in all routes of transplantation. The role of liver macrophages in organ specificity of metastases of this tumor is not clear. They can produce a protective effect and exterminate metastatic cells transported with the blood or, vice versa, they can promote the development of metastases by providing adhesion of tumor cell without their elimination. In order to elucidate the role of macrophages in the development of experimental metastases of HA-1 hepatoma in mouse liver we used the model of selective depression of macrophages induced by gadolinium chloride ($GdCl_3$). $GdCl_3$ accumulates in lysosomes and suppresses receptor-mediated endocytosis and phagocytosis of macrophages without modifying endocytosis of endothelial and parenchymatous cells of the liver [6].

MATERIALS AND METHODS

Experiments were carried out on adult male A/Sn mice from Institute of Cytology and Genetics. Experimental

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metastases in the liver were induced by injection of HA-1 hepatoma cells into the lateral caudal vein (10^5 cells/mouse). $GdCl_3$ was injected intravenously in a dose of 5 mg/kg 24 h before tumor cell transplantation [6]. This dose of $GdCl_3$ selectively blocks Kupffer cells. Animals receiving tumor cells alone served as controls.

Some mice were decapitated 4 and 11 days after transplantation, the blood and liver were collected, and activities of cathepsins B and L were measured using Z-L-Phe-L-Arg-MCA and Z-L-Arg-L-Arg-MCA (Sigma) as the substrates, respectively [4,5,9]. Activity of cathepsin L was evaluated using CA-074, a selective cathepsin B inhibitor. Stability and vulnerability of lysosomes in hypotonic medium were evaluated by free activity of acid phosphatase in liver homogenate [10].

Liver samples for morphological studies were fixed in 10% formalin. Volume density of tumor implants and the presence of destructive changes in the liver tissue were studied under a light microscope on histological sections stained with hematoxylin and eosin by morphometrical methods [1]. Other animals were observed until their death and the life span from the moment of transplantation was determined.

In a special series of experiments $GdCl_3$ was injected to intact mice in doses of 5-10 mg/kg and Gd concentration in the liver was measured by inductively coupled plasma atomic emission spectroscopy on a JI-70 spectrometer (Joben Ivon). Liver samples were pretreated by the blow-pipe and then wet ashing in a mixture of hydrochloric and nitric acid. GdII 342, 247 nm strain served as the analytical strain of gadolinium. Basal level of GD was 0.005 μg/cm^3, the concentrations varied from 0.01 to 10 μg/cm^3.

The data were processed by the method of parallel series of variation statistics (the data on the life span were processed by paired analysis), the differences were evaluated using Student's *t* test (the differences were considered significant at $p<0.05$).

RESULTS

Gadolinium was detected in the liver as soon as 5 min after its intravenous injection (34.40±3.56% of the dose injected); its content peaked after 1 h (79.90±4.18%) and then gradually decreased (56.70±4.03% after 24 h and 16.0±0.8% after 5-7 days). The preparation accumulated in lysosomes (mainly, in macrophage lysosomes) during the entire period of the study (12 days). Labilization of lysosomes was observed in these animals, which was seen from increased free acid phosphatase activity in mouse liver homogenate 24, 48, 72 h and 7 days after injection of $GdCl_3$ (Fig. 1). We previously observed a decrease in numerical density and size of liver macrophages in mice 24 h after

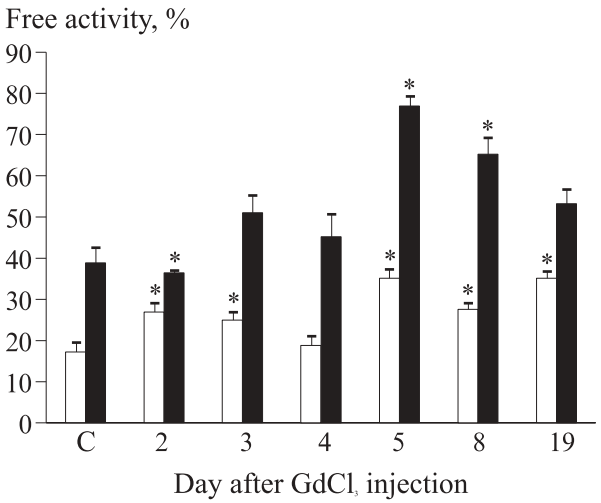

Fig. 1. Effect of $GdCl_3$ on stability and hypoosmotic resistance of mouse liver lysosomes. Light bars: free activity of acid phosphatase (lysosomal marker enzyme) in mouse liver homogenate before treatment in 0.125 M sucrose solution; dark bars: this activity after such treatment. Free enzyme activity is expressed in percent of total activity after incubation of the homogenate in 0.2% Triton X-100 solution. C: control. *$p<0.05$ compared to intact liver.

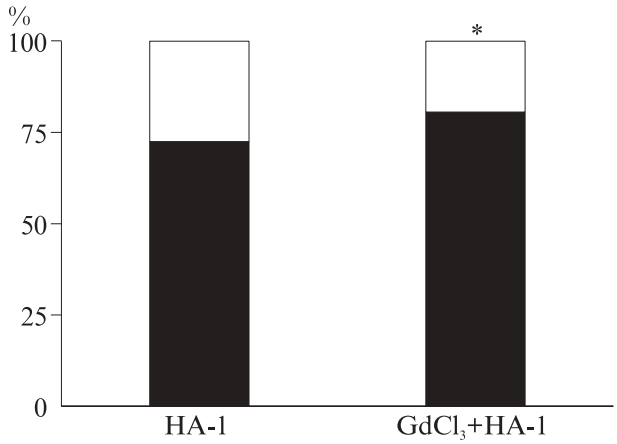

Fig. 2. Volume density (μ/μ^3) of HA-1 hepatoma implantats. Dark bar: liver tissue; light bar: tumor. *$p<0.01$ compared to HA-1.

injection of $GdCl_3$ and a 4-5-fold decrease in the volume density of secondary lysosomes of liver macrophages compared to intact animals [12]. The rate of colloid carbon phagocytosis during this period appreciably decreased [12]. These results attest to depression of liver macrophages in the early (24-48 h) period after injection of $GdCl_3$ and are in line with published data [6]. After 48 h numerical density of macrophages in the liver returned to normal due to migration of monocytes/macrophages.

Mouse survival experiments showed that the mice injected with $GdCl_3$ 24 h before tumor cell transplantation lived longer ($p<0.01$) than the corresponding controls (12 mice per group).

Morphometric studies of liver sections showed that $GdCl_3$ 3-fold increased the volume of degene-

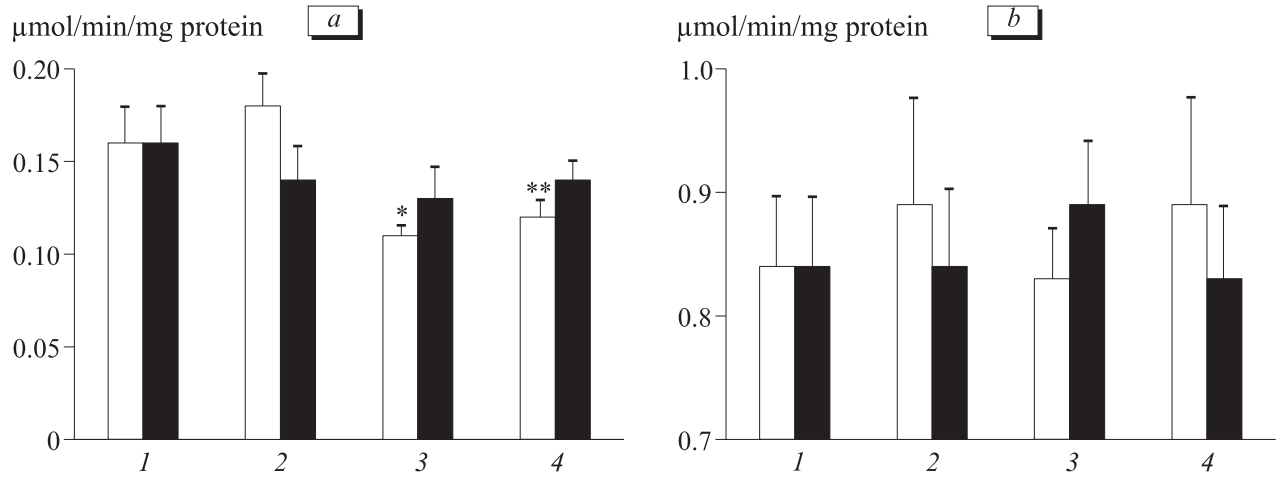

Fig. 3. Activities of cathepsins L (*a*) and B (*b*) in the liver of mice with experimental metastases of HA-1 hepatoma 4 (light bars) and 11 days (dark bars) after tumor cell transplantation. *1*) intact mice; *2*) untreated mice with metastases of HA-1 hepatoma; *3*) control mice without tumor injected with $GdCl_3$; *4*) mice with HA-1 hepatoma metastases injected with $GdCl_3$. $p<0.05$: *compared to *1*, **compared to *3*.

TABLE 1. Destructive Processes in the Liver of A/Sn Mouse on Day 11 after HA-1 Hepatoma Cell Transplantation (%, *M*±*m*)

Group	Changes, volume density of structures, μ/μ³		Binuclear hepatocytes, numerical density of profiles of structures in the section area, μ/μ²
	degenerative	necrotic	
Control	1.08±0.05	0.140±0.007	0.29±0.01
HA-1 hepatoma	8.030±0.021	4.72±0.10	0.94±0.02
$GdCl_3$	3.11±0.17⁺	0.47±0.02⁺	1.08±0.07⁺
$GdCl_3$+HA-1 hepatoma	6.94±0.18*	3.81±0.21*	0.97±0.04

Note. $p<0.05$ compared to: ⁺control, *HA-1 hepatoma.

rative and necrotic changes in control mice without tumor, but decreased the volume density of destructive changes in tumor-bearing mice (Table 1). Preinjection of $GdCl_3$ reduced the volume density of tumor implants in comparison with control animals (Fig. 2), which was presumably responsible for different involvement of the liver in destructive processes, on the one hand, and for prolongation of the life span, on the other hand.

Development of liver metastases did not change activities of lysosomal enzymes (cathepsins B and L) in comparison with intact liver (Fig. 3). In contrast, activity of cathepsin L in the liver decreased in mice with and without tumors at early terms (4 days) after injection of $GdCl_3$, but on day 11 this parameter did not differ from the control.

Proteolysis plays the key role in the mechanism of tumor cell invasion [13,14]. Lysosomal cysteine proteases (cathepsins B and L) are believed to initiate the proteolytic cascade in tumor cells by activating urokinase-type plasminogen precursors, which leads to activation of plasminogen and metalloproteinases. Stimulation of biosynthesis of these proteases correlates with metastatic potential of many mammalian tumor cells [13,14]; moreover, metastasizing of tumors characterized by high secretion of cathepsin L is appreciably suppressed by its specific inhibitors [7,8]. Our findings suggest that the decrease in cathepsin L activity in the liver can be responsible for suppression of tumor metastases after injection of $GdCl_3$ (Fig. 3). One more possible cause of metastases suppression is impaired phagocytic activity of macrophages, which is observed early after injection of $GdCl_3$ [6,12]. It is possible that liver macrophages have a pro-, but not antimetastatic effect towards HA-1 hepatoma, and stimulation of their activity is associated with more intense involvement. Selective depression of liver macrophages by $GdCl_3$ *in vivo* prevents the development of metastases in the liver, which is presumably due to decreased invasion of tumor cells, and contributes to prolongation of mouse life span.

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