ONCOLOGY

Effect of Gadolinium Chloride on the Growth and Metastasizing of Lewis Pulmonary Adenocarcinoma in Mice

S. Ya. Zhanaeva, T. V. Alekseenko, and T. A. Korolenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 146, No. 11, pp. 548-551, November, 2008
Original article submitted May 4, 2008

A single intraperitoneal injection of $GdCl_3$ in doses of 14 or 28 mg/kg to mice with intravenously transplanted Lewis pulmonary adenocarcinoma on days 3 or 8 after tumor transplantation reduced the mean number of tumor metastases in the lungs. The effect of $GdCl_3$ was more pronounced if it was injected at the stage of tumor dissemination (day 8). The positive antimetastatic effect of $GdCl_3$ was presumably due to its capacity to macrophage depression. The direct (not mediated through mononuclear phagocyte system cells) effect of $GdCl_3$ on tumor cells cannot also be excluded.

Key Words: *Lewis pulmonary adenocarcinoma; metastases; macrophages; gadolinium chloride*

Gadolinium (Gd), a rare-earth metal, is widely used in modern industrial technologies, biology, and medicine. In medicine, complexons or chelates (most often EDTA derivatives) with Gd salts are most often used as contrast agents in examinations of patients by the nuclear magnetic resonance method, for detection of metastases of tumors of different types. In biology, Gd salts are used for modeling the suppression of the macrophage-mediated immunity in studies of the functional role of macrophages in the development of some pathological processes, such as granulomatous inflammation, fibrosis, biliary obstruction of the liver, tumor processes, *etc.* [5,6]. Gadolinium chloride forms a poorly soluble Gd hydroxide colloid in the bloodstream at neutral pH values. This colloid is eliminated from the bloodflow mainly by resident macrophages. After intravenous injection, Gd is detected in Kupffer's cells, hepatocytes, hepatic polymorphonuclear cells, pulmonary, splenic, and bone marrow macrophages, and mesangial cells of renal glomeruli. Intravenous Gd induces partial depression and apoptosis of liver phagocytes, splenic red pulp, and lung vessel macrophages within 24-48 h postinjection [5,6,10]. In low doses (5-10 mg/kg), this lanthanide suppresses mainly hepatic macrophages. Being injected repeatedly or in high doses, it affects macrophages in other organs (abdominal, pulmonary, splenic).

We previously showed that preinjection of $GdCl_3$ 24 h before intravenous transplantation of HA-1 tumor or pulmonary adenocarcinoma suppressed the development of metastases and prolonged animal lifespan [2,3].

Here we studied the effect of macrophage depression by $GdCl_3$ on natural metastatic process of Lewis pulmonary adenocarcinoma (APA) forming a solid node at the site of intramuscular transplan-

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** s.ya.zhanaeva@physiol.ru. S. Ya. Zhanaeva

0007-4888/08/1465-0612

tation of tumor cells (TC) and multiple hematogenic metastases in 100% mice.

MATERIALS AND METHODS

Experiments were carried out on adult male C57Bl/6 mice from vivarium of Institute of Cytology and Genetics. The animals were kept in groups (n=6-8) at natural light on PK 120-1 granulated fodder (Laboratorsnab) and water *ad libitum*. Tumor (APA) cells were transplanted into the right hip muscle (0.5×10^6 TC per mouse). After 3 or 8 days, the animals were intraperitoneally injected with water solution of $GdCl_3$ (14 or 28 mg/kg). Controls received no injections. Three mutually perpendicular sizes of tumor nodes were repeatedly measured with a slide gage over the course of tumor growth and its volume was evaluated. The mice were decapitated 20 days after TC transplantation, body weight was measured and tumor weight was evaluated by the difference between the weight of the limb with tumor and contralateral limb. The lungs were fixed in 10% formalin and metastases were counted under a binocular magnifying glass. The incidence of tumor metastases was evaluated by the percentage of animals in the group with APA metastases in the lungs.

The effect of $GdCl_3$ injected in a dose of 14 mg/kg on day 10 after APA transplantation was evaluated in a special series of experiments. The mice were decapitated after 1, 3, 7, and 10 days. The liver, lungs, and tumor were collected and weighed. The concentration of Gd in organs was evaluated by inductively-coupled plasma atomic emission spectrometry on a PV 8490 spectrometer (Phillips). Liver specimens were subjected to blowpipe and then wet ashing in a mixture of hydrochloric and nitric acid. GdII bands at 342.2 and 335.0 nm served as Gd analytical bands. Gadolinium concentrations in tissues were evaluated for the 0.002-0.100 vol.%, the threshold detection level being 0.001 vol.% Analytical studies were carried out at Laboratory of Spectral Analysis (Khimpolitech Firm) by E. G. Obrazovskii, T. B. Sryvtseva, and G. I. Akulova.

The results were statistically processed using Wilcoxon—Mann—Whitney nonparametrical and Student t test.

RESULTS

Single intraperitoneal injection of $GdCl_3$ did not modify the growth of primary APA node: the mean weight of the tumor on day 20 after transplantation and changes in its volume in the course of its growth were virtually the same in experimental and control animals (Table 1, Fig. 1). However, $GdCl_3$, injected according to all protocols, did not the incidence of tumor metastases, but 2-4-fold reduced their mean number in the lungs (Table 1). Antimetastatic activity of $GdCl_3$ depended mainly on the time of injection and was more pronounced after $GdCl_3$ injection on day 8 after TC transplantation. The mean number of metastases in this group was 2-fold lower than in animals injected with $GdCl_3$ on day 3 after tumor transplantation and 4.3 times lower than in controls (Table 1). No stimulation of antimetastatic activity of $GdCl_3$ with increasing its dose was detected, though the general toxic effect increased, judging by animal mortality.

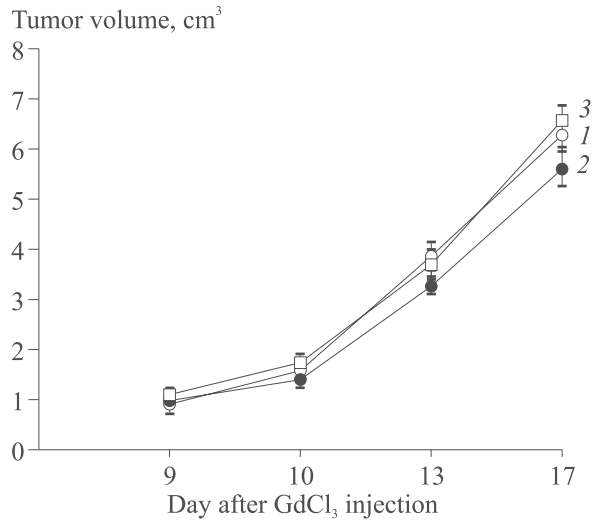

Fig. 1. Effect of a single intraperitoneal injection of $GdCl_3$ (14 mg/kg) on the dynamics of APA growth in C57Bl/6 mice. *1*) control; *2*) injection on day 3; *3*) on day 8.

We previously showed on models of experimental metastases of HA-1 tumor in the liver and of APA in the lungs that injection of $GdCl_3$ 24 h before intravenous transplantation of TC significantly inhibited metastasizing of these tumors. In these experiments the effects of macrophage stimulation and depression on the metastatic process was realized at the level of colonization of the metastasis target organs by TC [2,3]. In the present experiment, a more pronounced antimetastatic effect of $GdCl_3$ was obtained when the drug was used at the stage of tumor dissemination (on day 8 after tumor transplantation). According to published data, the first micrometastases of APA in mouse lungs emerged on days 6-8 after intramuscular transplantation of the tumor; no signs of tumor metastases were detected on day 3 after tumor transplantation [11].

More pronounced antimetastatic effect of $GdCl_3$ injected on day 8 after tumor transplantation was presumably explained by its effect on TC which already migrated into lung tissue, but not on the

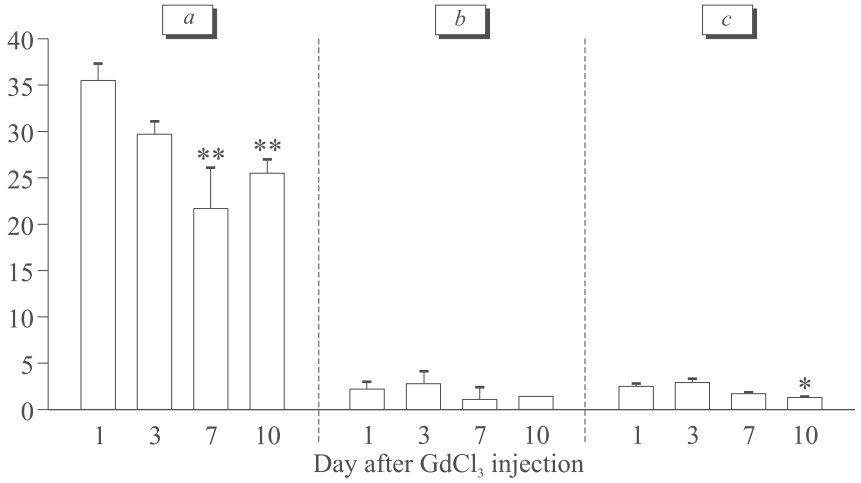

Fig. 2. Content of Gd in the liver (*a*), lung (*b*) or tumor (*c*) during different periods after single intravenous injection of $GdCl_3$ (14 mg/kg). The content of Gd in organs is expressed in % of injected dose in conversion to 1 g wet tissue. *$p<0.05$; **$p<0.01$ compared to day 1 after $GdCl_3$ injection.

TABLE 1. Effect of $GdCl_3$ on the Growth and Metastasizing of APA in Mice ($M\pm m$)

$GdCl_3$ dose, mg/kg	Day of injection	Tumor weight on day 20 after tumor transplantation, g	Mean number of metastases per mouse	Incidence of tumor metastases, %	Number of mice dead by day 20 after tumor transplantation, %
Control	—	5.40±0.22	16.90±1.01	100	10
14	3	5.50±0.29	7.70±1.31*	100	20
	8	5.40±0.19	3.90±0.67*	100	30
28	3	5.10±0.19	7.80±1.19*	100	40
	8	5.60±0.39	5.20±1.81*	100	40

Note. *$p<0.05$ compared to the control.

primary node cells. On the other hand, it is highly probable that the antimetastatic effect of this agent is mediated by its macrophage-suppressing activity. According to published reports, $GdCl_3$ in the studied doses suppressed macrophages not only in the liver, but also in the lungs [5-7]. Significant inhibition of experimental HA-1 and APA metastases was attained in the above-mentioned studies by using $GdCl_3$ and other compounds, reducing functional activity of macrophages, while preinjection of macrophage stimulators stimulated metastasizing of these tumors [2,3]. A similar effect of tissue macrophages stimulation and suppression on metastatic activity of experimental tumors was previously described [1,7,9]. As for experimental metastases, we can assert that the antimetastatic effect was due to $GdCl_3$ capacity to macrophage suppression (because the organs were colonized by TC over the first 24 h after its injection, when signs of macrophage suppression were maximally manifest). However, in spontaneous metastatic process, when lung colonization by TC is slower and the duration of macrophage suppression induced by Gd is limited by 2 days, after which not only the count and functional activities of macrophages in the liver and other organs are restored, but even signs of stimulation of the macrophage component of immunity appear, this seems to be less likely.

In order to clear out the probability of direct effect of Gd on TC located in the primary tumor nodule and on lung cells (main target for APA), we studied the time course of Gd accumulation in liver, lung, and tumor tissues after intravenous injection of $GdCl_3$. It was found that Gd was captured from the blood stream mainly by liver cells: its percentage in the liver 1, 3, 7, and 10 days after injection into the tail vein changed from 35.5 to 21.7% of the injected dose, while in the lung and tumor tissue, it was just 2.9-1.1% during different periods (Fig. 2). Hence, despite the fact that Gd content in the tumor and lung tissue was by one order of magnitude lower than in the liver, the metal was present in these tissues and could have some effect on TC and on lung and tumor macrophages.

REFERENCES

1. S. Ya. Zhanaeva, T. V. Alekseenko, and T. A. Korolenko, *Byull. Sibirsk. Otdelen. Rossiisk. Akad. Med. Nauk*, No. 3, 121-126 (2007).

2. S. Ya. Zhanaeva, T. A. Korolenko, B. G. Nekrasov, *et al.*, *Byull. Eksp. Biol. Med.*, **140**, No. 10, 450-452 (2005).
3. S. Ya. Zhanaeva, T. A. Korolenko, E. V. Nikitenko, *et al.*, *Ibid.*, **137**, No. 6, 660-663 (2004).
4. T. A. Korolenko, M. A. Dergunova, T. V. Alekseenko, and S. Ya. Zhanaeva, *Ibid.*, **142**, No. 10, 369-372 (2006).
5. L. C. Adding, G. L. Bannenberg, and L. E. Gustafsson, *Cardiovasc. Drug Rev.*, **19**, No. 1, 41-59 (2001).
6. M. J. Hardonk, F. W. Dijkhuis, C. E. Hulstaert, and J. Koudstaal, *J. Leukoc. Biol.*, **52**, No. 3, 296-302 (1992).
7. C. Lewis and C. Murdoch, *Am. J. Pathol.*, **167**, No. 3, 627-635 (2005).
8. J. P. Mizgerd, R. M. Molina, R. C. Steams, *et al.*, *J. Leukoc. Biol.*, **59**, No. 2, 189-195 (1996).
9. R. T. Prehn, *Theor. Biol. Med. Models*, **3**, 23 (2006).
10. B. Singh and A. de la Concha-Bermejillo, *Int. J. Exp. Pathol.*, **79**, No. 3, 151-169 (1998).
11. M. Wald, T. Olejar, P. Pouckova, and M. Zadinova, *Life Sci.*, **63**, No. 17, L237-L243 (1998).