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THE EFFECT OF GADODIAMIDE ON CANCER CELL LINES

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Aim: **Recent literature suggests that some human cancer cell lines possess a calcium cation receptor. Human myeloma cell lines have demonstrated stimulated cell proliferation by the gadolinium cation through this receptor, and osteosarcoma cell lines possess the same cation receptor. Although enhanced MRI is a very useful diagnostic tool for the treatment of sarcoma in the orthopedic area, incorporating the use of MRI contrast agents based on gadolinium raises the possibility of the stimulation of cancer cell growth.** ***Methods:*** **Human myeloma (RPMI 8226), osteosarcoma (Saos-2) and rat osteosarcoma (UMR-106) cell lines were exposed to various concentrations of common MRI contrast agent gadodiamide (Omniscan®) (5 μM, 50 μM, 500 μM, 5 mM, 50 mM) in a culture medium. The response of the cells was then assessed by measuring cell proliferation and DNA synthesis.** ***Results:*** **Treatment with 5 μM to 5 mM gadodiamide did not stimulate cell proliferation; only cells exposed to 50 mM gadodiamide showed suppressed proliferation rates.** ***Conclusions:*** **Since intravenously injected gadodiamide is diluted from 500 μM to 1 mM by patient blood flow at enhanced MRI examinations, the results of the present study suggest that gadodiamide has not effect on these types of cancer cells.**

Key Words: **Gadodiamide (Omniscan®), MRI contrast agent, myeloma, osteosarcoma, Ca receptor, cell proliferation.**

Nowadays, contrast-enhanced magnetic resonance imaging (MRI) is a very important medical tool for the diagnosis of tumorigenic disease. MRI contrast agents used as injectable mediums are based chiefly on gadolinium because of the significant magnetic signal generated by gadolinium chloride hexahydrate (Gd^{3+}) [1]. After injection of the contrast medium, increased blood flow to the tumor area results in the accumulation of the agent and a concomitantly-enhanced signal upon imaging [2, 3]. It is well known that the free gadolinium ion is harmful to living tissues [4, 5]. To temper such detrimental effects, MRI contrast agents have been modified to chelate structures in order to protect tissues from exposure to the free gadolinium ion [6, 7]. However, exposure of mesenchymal cells which have a calcium cation sensing receptor, to Gd^{3+} results in stimulation of cell proliferation and mediation of increased mitogenic responses [8–10]. Furthermore, it has recently been shown that some cancer cell lines — myeloma and osteosarcoma in particular — possess a similar calcium-sensing receptor [11–13] and myeloma cultured lines experience stimulated cell proliferation when exposed to Gd^{3+} [12]. Consequently, MRI contrast agents might stimulate the proliferation of these cancer cells. To date no published reports have analyzed this potential effect.

Our study was conducted with the use of the common MRI contrast agent, gadodiamide (Omniscan®), to examine this possibility by measuring DNA synthesis and the cell number of several, commercially available cancer cell lines exposed to this MRI contrast agent *in vitro*.

Culture of cancer cell lines. Human cancer cell lines myeloma (RPMI 8226) and osteosarcoma (Saos-2) (ATCC, Manassas, VA, USA) were grown in RPMI 1640 and McCoy's 5A (ATCC) in a 5% CO_2 humidified incubator at 37 °C. The culture mediums contained an antibiotic-antimicotic solution and 10% (15% for McCoy's 5A) fetal bovine serum (FBS) (Gibco BRL, Guithersburg, MD, USA).

Rat cancer cell line osteosarcoma (UMR-106) was purchased from ATCC and grown in Dulbecco's Modified Eagle's Medium (Gibco BRL) containing an antibiotic-antimycotic solution and 10% FBS.

After the initial culture, an adequate number of cells were harvested and re-seeded in 96-well culture plates at a density of 1000 cells/well and subsequently used to measure DNA synthesis. The remaining cells were seeded into 24-well plates at a density of 20 000/well for determining the cell number.

DNA synthesis. Each cell line was divided into six groups on Day 1. Groups 1 to 5 were exposed to gadodiamide (Omniscan®) (Amersham Health, Princeton, NJ, USA) (5 μM, 50 μM, 500 μM, 5 mM, 50 mM) in the culture medium; that is, the 5 mM group was treated with 10 μl of gadodiamide in 1 ml of culture medium, reflecting the rate commonly used in clinical therapeutics. Clinically, gadodiamide injected intravenously immediately before MRI imaging is diluted from 500 μM to 1 mM within the circulatory system. Group 6 was used as control. Twenty-four hours after changing the culture medium, 1.0 μCi of ^{3}H-thymidine (Dupont-NEN, Boston, MA, USA) was added to each well, the culture medium was aspirated 18 h thereafter and the cells were washed and collected by detachment with 0.25% trypsin (Gibco BRL). The myeloma cells, the cells were harvested onto a 1.2 μm glass filter (Whatman Inc., Clifton, NJ, USA), washed and dried. The rate of DNA synthesis was measured with the use of a scintillation counter (Packard Instrument Co., Downers Grove, IL, USA). Each experimental group consisted of 5 wells.

Cell number. Using the same experimental protocol outlined above, the cell number was counted with a hemocytometer on day 4. The assays were done in quintuple.

Statistical Analysis. Results are expressed as the mean ± SD (standard deviation of the mean). Assay results were analyzed for differences using

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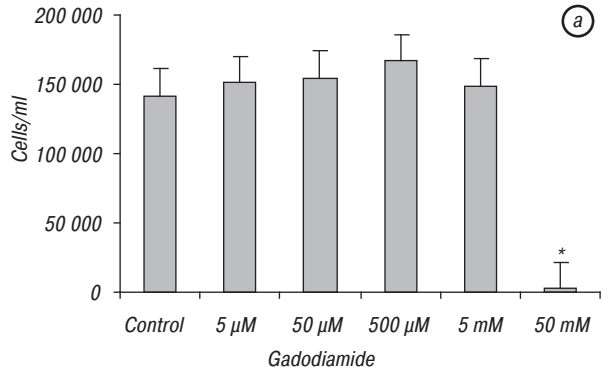
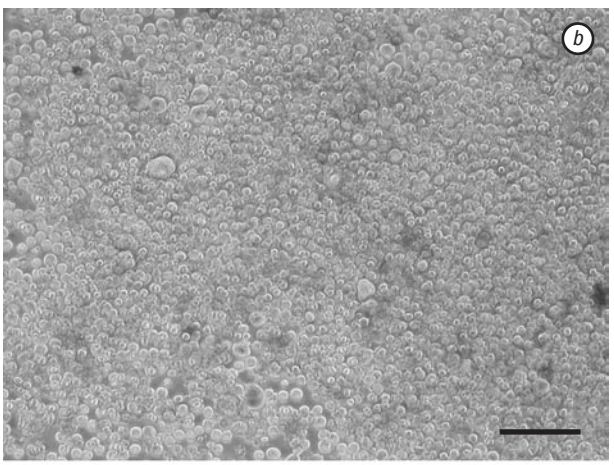
analysis of variance (ANOVA) followed by Fisher's protected least significant differences (PLSD) test, with *P*-value less than 0.05 considered significant.

Gadodiamide concentrations ranging from 5 μM to 5 mM induced no stimulation of cell proliferation, compared with the control; however, the 50 mM group showed marked suppression of cell growth compared with the other groups ($p < 0.01$) (Fig. 1, *a*). Also, no morphological changes were observed among the groups other than in the 50 mM group (Fig. 1, *b*, *c*).





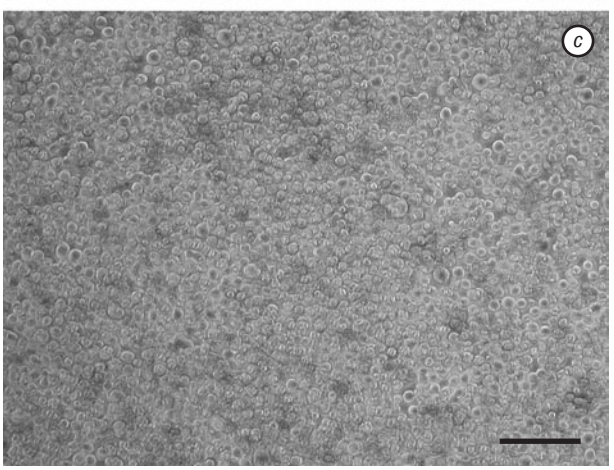


Fig. 1. *a*, The effect of various concentrations of gadodiamide on human myeloma (RPMI8226) cell lines. Cell numbers were measured on day 4. Concentrations of gadodiamide ranging from 5 μM to 5 mM showed no stimulation of cell proliferation compared with the control. The 5 mM group was significantly suppressed compared with the other groups (*$p < 0.01$). Standard deviation (SD) of the mean is shown by vertical bars. The assays were done in quintuple. Morphology of control (*b*) and 500 μM groups (*c*) on day 4 of cell culture. There were no significant morphological changes among the groups, except the 50 mM group

DNA synthesis was suppressed in only the 50 mM group ($p < 0.01$) compared with the other groups. There were no significant differences among the groups except in the 50 mM group (Fig. 2).

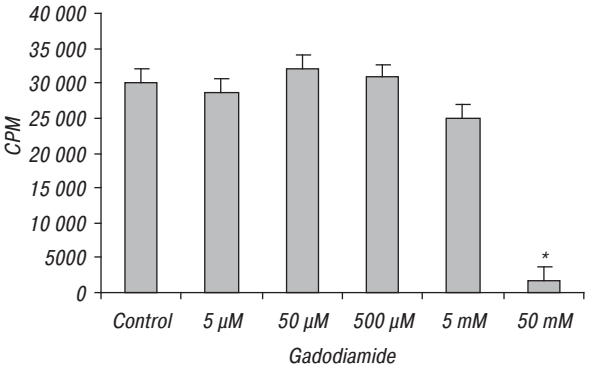


Fig. 2. DNA synthesis after exposure of human myeloma cell line RPMI8226 to gadodiamide for 24 h. Concentrations of gadodiamide ranging from 5 μM to 5 mM did not show a statistical difference in DNA synthesis among the groups. Only the 50 mM group showed significant suppression as compared with the other groups (*$p < 0.01$). Standard deviation (SD) of the mean is shown by vertical bars. Each assay comprised 5 wells

In both the rat (Fig. 3, *a*) and human osteosrcoma (Fig. 3, *b*) cell lines, only the 50 mM groups expressed suppressed cell growth rates compared with the other groups with respect to DNA synthesis. The test groups exposed to gadodiamide concentrations ranging from 5 μM to 50 mM did not show stimulation of cell proliferation in either of the lines. Survey of the cell number showed the same DNA synthesis results for both lines (data not shown).

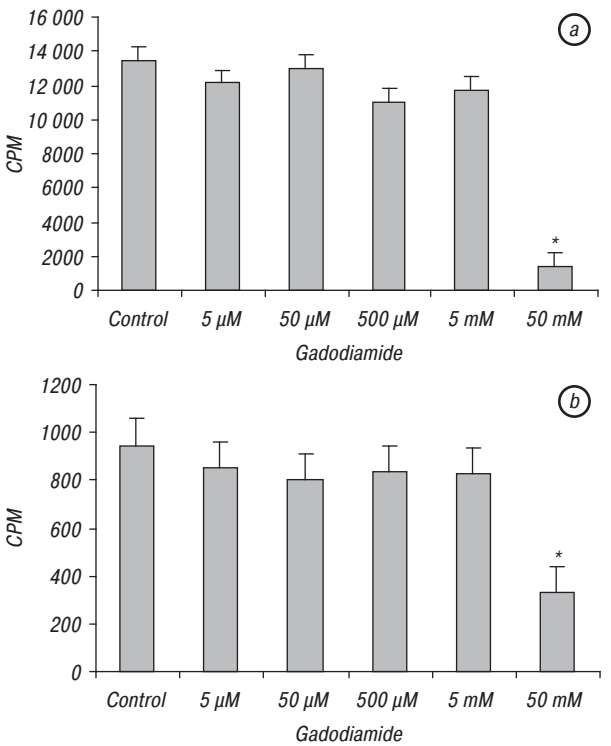


Fig. 3. DNA synthesis of rat osteosarcoma cell line UMR-106 (*a*) and of human sarcoma cell line Saos-2 (*b*) was estimated by exposure of the cells to various concentrations of gadodiamide for 24 h. Proliferation rates of only cells exposed to 50 mM gadodiamide were suppressed compared with the other groups (*$p < 0.01$). There was no significant difference among the groups, except the 50 mM group. Standard deviation (SD) of the mean is shown by vertical bars. Each assay comprised 5 wells

Although administration of an MRI contrast agent is very useful in the diagnosis of oncological diseases, there are few reports on the effect of contrast agents on cancer cells. This may be due to the previous concept that gadolinium is toxic only to normal cells [3, 4]. Studies indicate, however, that some cells are activated by polyvalent agonists such as Gd^{3+} binding to their cation sensing receptor [7–9]. Myeloma cells being one such type [4], a hypothesis has been that MRI contrast agents probably stimulate myeloma cell proliferation. Since 25 μM of Gd^{3+} has been shown to stimulate the proliferation of myeloma cells [12], if gadodiamide releases Gd^{3+} almost completely, then it would be reasonable to assumue that either 5 μM or 50 μM gadodiamide stimulates proliferation of myeloma cells. Our study, however, showed that the cultured myeloma cell lines were not stimulated by the MRI contrast agent as expected. Since cell growth was very high in our assay of cell number, the statistical difference among the groups except the 50 mM group could not be determined by only counting the number of myeloma cells. Therefore, although measuring DNA synthesis was carried out for the assessment of cell proliferation, the short exposure time (24 h) to gadolinium could not show evidence of cell proliferation of this cell line. Further study is warranted on exposure time of cells to contrast agents. Both human and rat osteosarcoma cell lines, which possess the same cation receptor as myeloma [11], were also not stimulated by gadodiamide. These observations suggest that MRI contrast agents are safe for use on patients with these types of cancer cells. While all cell growth was suppressed by a 50 mM concentration, intravenously injected gadodiamide was diluted to approximately 500 μM to 1 mM by blood. Thus, the 50 mM study group falls well outside the scope of reliable analysis. On the other hand, in examinations by MRI angiography, which normally do not relate to the diagnosis of tumorigenic disease, a high dose of MRI contrast agent is usually required. That 5 mM dilution also had no proliferative effect on any cell line tested, suggests that doses of up to 5 times normally used may not induce significant adverse effects. Furthermore, given that 95–98% of gadodiamide is cleared away by 24 h after injection (according to manufacturer data), there is probably only a very small amount remaining thereafter. This study also showed that proliferation rates of cancer cell lines were not influenced by exposure to very small amounts of gadodiamide for a short period of time.

Finally, our results show that there is little evidence to suggest that commercially available MRI contrast agents stimulate proliferation of the tested cancer cell lines.

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