Suppression of Rabbit VX-2 Subcutaneous Tumor Growth by Gadolinium Neutron Capture Therapy

Yasuyuki Akine,[1,5] Nobuhiko Tokita,[1,6] Koichi Tokuuye,[2] Michinao Satoh,[2] Hisahiko Churei,[2] Cécile Le Pechoux,[1,3] Tooru Kobayashi[4] and Keiji Kanda[4]

[1]Outpatient Clinic and [2]Department of Radiation Therapy, The National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan, [3]Henri Mondor Hospital, 51 av du Mal de Lattre de Tassigny, Creteil, France and [4]Research Reactor Institute, Kyoto University, Noda, Kumatori, Sennan-gun, Osaka 590-04, Japan

VX-2 tumors growing in hind legs of New Zealand White rabbits (n=4) were exposed to thermal neutrons for 40 min (2.1×10^{12} neutrons cm^{-2}) while one of two hind leg tumors of each rabbit was infused continuously with meglumine gadopentetate through a branch of the left femoral artery. The contralateral (uninfused) tumors served as controls. Although no differential distribution of gadolinium was achieved between the tumor and its adjacent normal tissue, the gadolinium concentration in the infused tumor was approximately 5–6 fold higher than that in the contralateral tumor. Growth of gadolinium-infused tumors was significantly inhibited compared to that of control tumors ($P<0.05$) between the 16th and 23rd days after treatment.

Key words: Neutron capture therapy — Gadolinium — Rabbit — VX-2 tumor — Intraarterial infusion

Gadolinium neutron capture therapy (GNCT) takes advantage of nuclear reactions as a result of thermal neutron capture by Gd-157 atoms distributed throughout a tumor. The interactions result in emission of photons and electrons with broad energy levels up to 7.9 MeV[1] which are considered to be mostly of low linear energy transfer (LET).[2] We have previously shown that cytotoxic effects of thermal neutrons are enhanced in the presence of gadolinium in Chinese hamster cells[2] and in mouse tumor models.[3] In another study, mice inoculated intraperitoneally with Ehrlich ascites cells survived considerably longer if gadolinium in microcapsules was given prior to thermal neutron irradiation.[4] This study suggests that close contact between the cell and gadolinium is not necessarily required for cell inactivation, although the electrons are the most important component in GNCT.[5] In our previous *in vivo* studies Gd-157 in the form of meglumine gadopentetate (MG) was directly introduced to the tumor cell milieu. However, in the present study, Gd-157 was delivered in VX-2 tumors[6] growing in rabbits' hind legs through a branch of the femoral artery. To overcome its rapid clearance from the tumor, Gd-157 was infused continuously during neutron irradiation.

Four New Zealand White rabbits (10- to 20-week-old males weighing 2.3–3.1 kg) were used for GNCT, and two for gadolinium concentration studies. Cell suspensions made after mincing excised tumor fragments and filtering them through a mesh were injected subcutaneously in the posterior aspect of the lower half of the hind leg eight days prior to irradiation. Three to four days prior to irradiation, under general anesthesia (5 mg/kg ketamine hydrochloride, i.m. and 15 mg/kg sodium pentobarbital, i.v.), the rabbits underwent catheterization. After a cut down, a 0.67 mm plastic catheter was inserted in a retrograde manner into a branch of the left femoral artery (the saphenous artery).[7] The flow from the catheter to the tumor via the femoral artery was confirmed radiographically using an iodine contrast medium. After filling the catheter with heparin sodium (1000 units/ml), its tip was sealed with a rubber cap, and it was stitched to the subcutaneous tissue before closing the skin. On the 8th day after inoculation the rabbits were transported to the reactor site for irradiation.

The biomedical facility of the Heavy Water Facility of the Kyoto University Reactor (KUR, 5 MW), equipped with a 15-cm-thick bismuth screen having a 60-cm circular window, provides thermal neutrons with minimal contamination with epithermal or fast neutrons.[8] The thermal neutron flux and gamma ray dose rate at the irradiation field (without samples) were 3×10^9 cm^{-2} s^{-1} and 1 Gy/h, respectively.[9] For irradiation, the rabbits were anesthetized as described above and immobilized on a specially fabricated table with the posterior aspects of the hind legs facing the bismuth window without body shielding. A 27-guage needle was inserted into the rubber cap of the catheter through the skin, and just prior to

neutron irradiation, MG infusion was started at a flow rate of 0.48 ml/min. Each rabbit, without shielding, was exposed to thermal neutrons for 40 min while receiving a total volume of 19 ml of MG fluid (334 mg MG). The total neutron fluence given was based on the fact that this tumor model appears very radiosensitive (Akine, unpublished data). Dosimetry for both photons and thermal neutrons was done with thermoluminescent dosimeters (1.2 mm TLD: BeO) and 3 mm gold foils placed on the hind leg skin. The average neutron fluence measured was $(2.1 \pm 0.47) \times 10^{12}$ neutrons cm^{-2}, and the average photon doses on the skin were 4.4 ± 3.1 Gy for gadolinium-infused legs, and 3.7 ± 1.1 Gy for control legs. Photon doses measured with TLDs were found to vary considerably depending on the location of TLDs in the leg.

To estimate gadolinium concentrations both in tumors and the surrounding normal tissue of infused and contralateral legs, the tumors were excised along with the adjacent normal tissues from both legs of two rabbits after a 10 min MG infusion at 0.48 ml/min through the catheter. Based on inductively-coupled plasma spectrometry methods[10] the average gadolinium concentrations in the tumors and the adjacent normal tissues were 1.55 μmol/g (2.32, 0.774) and 3.56 μmol/g (5.48, 1.62), respectively, for the MG-infused leg; and 0.28 μmol/g (0.40, 0.166) and 0.17 μmol/g (0.25, 0.09) respectively, for the contralateral leg. Thus, there was no difference in gadolinium concentrations between the tumor and its adjacent normal tissue, but the gadolinium concentration was 5–6 fold higher in the infused tumor than in the contralateral tumor.

Growths of individual tumors were periodically measured with a caliper, and the size of a tumor was defined as the product of its two perpendicular diameters. Differences in growth patterns of tumors after irradiation with or without gadolinium infusion were analyzed according to null hypothesis tests.[11] The results revealed that the growth of those infused with gadolinium was significantly inhibited compared to the control ($P < 0.05$) between the 16th and 23rd days after treatment (Fig. 1). There were no changes noted macroscopically or histologically in any of the normal tissue samples removed at approximately a month after the treatment.

We have previously shown that estimation of equivalent 250 kVp X-ray doses is possible for various combinations of gadolinium concentrations and neutron fluence over limited ranges.[12] Based on this method, X-ray equivalent doses were estimated: 5.2 Gy for the tumor infused with MG; 6.9 Gy for its adjacent normal tissue; 2.9 Gy for the tumor on the contralateral leg, and 2.5 Gy for its adjacent normal tissue.

No selective gadolinium delivery to tumors has been achieved with continuous intraarterial infusion, and the obtained Gd-157 concentration in the tumor and/or the
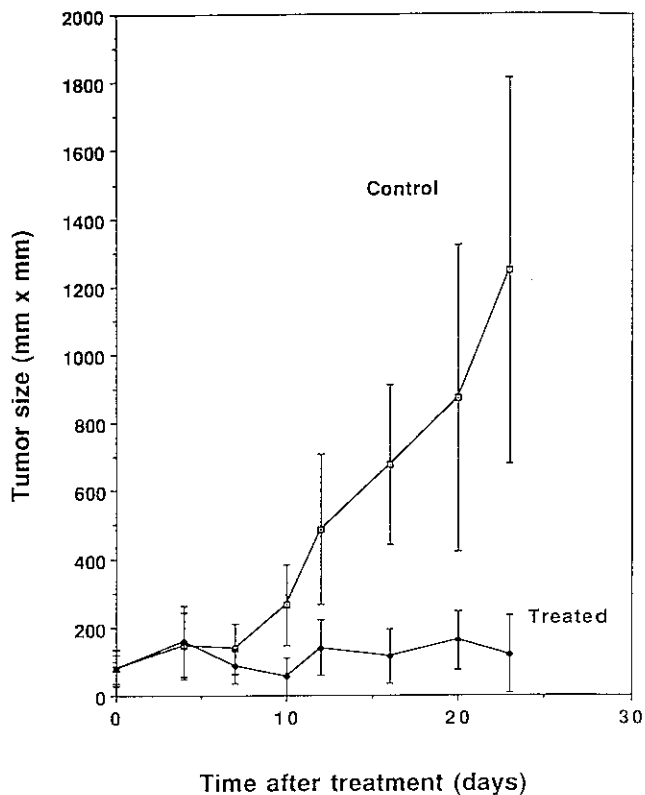

Fig. 1. Average tumor sizes with standard errors are plotted as a function of time (days) after treatment. "Treated" corresponds to the tumors infused with meglumine gadopentetate and irradiated with neutrons. "Control" corresponds to those uninfused and irradiated with neutrons.

surrounding normal tissues was approximately 40–90 ppm (1.6–3.6 μmol/g). In our cell survival assays, the optimal Gd-157 concentration in the tumor was predicted to be around 100 ppm.[12] At higher gadolinium concentrations the depth-dose distributions are significantly reduced as a result of absorption of thermal neutrons in the medium.[13] It appears that gadolinium concentrations in tumors should be kept low and that the neutron fluence must be adjusted to obtain the desired effect.

It was not possible to obtain a dose-response relationship for the tumor control or normal tissue responses because of the limited beam time allocated to this study, which required the reactor to be turned on and off for each exposure.

The dose distributions of released photons in GNCT are reported to be comparable to those obtained in boron neutron capture therapy.[13, 14] A recent computation study has shown the significance of contributions of electrons released from gadolinium located outside the cells.[15] Our *in vitro* study indicates that the electrons

contribute significantly to the overall radiation effect in GNCT.[5] Among the released electrons of a broad energy spectrum,[1] Auger electrons are known to be of high LET.[16] As the range of these electrons is extremely limited in tissue, gadolinium distributions in the cell, particularly with respect to the target genome, are crucial in determining the degree of biological effects inflicted by GNCT. This effect has been shown by double-strand break studies.[17] Our preliminary data indicate that MG may enter the cell freely (Akine et al., unpublished data 1993), and thus the contribution of low-energy electrons could be significant.

In conclusion, we evaluated the use of MG and its delivery by continuous intraarterial infusion as a step toward clinical applications. The results are still preliminary but encouraging enough to justify further investigation of this modality.

REFERENCES

1) Greenwood, R. C., Reich, C. W., Baader, H. A., Koch, H. R., Breitig, D., Schult, O. W. B., Fogelberg, B., Backlin, A., Mampe, W., von Egidy, T. and Schreckenbach, K. Collective and two-quasiparticle states in ^{158}Gd observed through study of radiative neutron capture in ^{157}Gd. Nucl. Phys., A304, 327–428 (1978).

2) Akine, Y., Tokita, N., Matsumoto, T., Oyama, H., Egawa, S. and Aizawa, O. Radiation effect of gadolinium-neutron capture reactions on the survival of Chinese hamster cells. Strahlenther. Onkol., 166, 831–833 (1990).

3) Akine, Y., Tokita, N., Tokuuye, K., Satoh, M., Kobayashi, T. and Kanda, K. Electron-equivalent dose for the effect of gadolinium neutron capture therapy on the growth of subcutaneously-implanted Ehrlich tumor cells in mice. Jpn. J. Clin. Oncol., in press.

4) Akine, Y., Tokita, N., Tokuuye, K., Satoh, M., Fukumori, Y., Tokumitsu, H., Kanamori, R., Kobayashi, T. and Kanda, K. Neutron-capture therapy of murine ascites tumor with gadolinium-containing microcapsules. J. Cancer Res. Clin. Oncol., 119, 71–73 (1992).

5) Akine, Y., Tokita, N., Matsumoto, T., Oyama, H. and Aizawa, O. Gadolinium-neutron capture reactions: a radiobiological assay. In "Progress in Neutron Capture Therapy for Cancer," ed. B. J. Allen, D. E. Moore and B. V. Harrington, pp. 361–363 (1992). Plenum Press, New York.

6) Rous, P. and Beard, J. W. The progression to carcinoma of virus-induced rabbit papillomas (Shope). J. Exp. Med., 62, 523–548 (1935).

7) Honma, Y., Suda, H., Ebe, K., Hatanaka, M. and Nakanishi, T. Simplified intraarterial infusion and angiographic method for rabbit VX-2 hind limb tumor via the saphenous artery. Nippon Acta Radiol., 50, 426–428 (1990) (in Japanese).

8) Kanda, K., Kobayashi, K., Okamoto, S. and Shibata, T. Thermal neutron standard field with a Maxwellian distribution using the KUR heavy water facility. Nucl. Instrum.

Methods, 148, 535–541 (1978).

9) Kobayashi, T., Kanda, K., Ujeno, Y., and Ishida, M. R. Biomedical irradiation system for boron neutron capture therapy at the Kyoto University Reactor. In "Neutron Beam Design, Development, and Performance for Neutron Capture Therapy," ed. O. K. Harling, J. A. Bernard and R. G. Zamenhof, pp. 321–339 (1990). Plenum Press, New York.

10) Nomura, S. and Azuma, H. The quantitative determination of Gd-DTPA in biological samples by inductively-coupled plasma (ICP) spectrometry. Yakubutsu Doutai, 3, 175–179 (1988) (in Japanese).

11) Brown, B. W. and Hollander, M. "Statistics: a Biomedical Introduction," pp. 109–129 (1977). John Wiley & Sons, Inc., New York.

12) Tokuuye, K., Tokita, N., Akine, Y., Kobayashi, T. and Kanda, K. Biological dosimetry for gadolinium neutron capture reaction. Proc. Fifth Int. Symp. Neutron Capture Therapy, in press.

13) Matsumoto, T. Transport calculations of depth-dose distributions for gadolinium neutron capture therapy. Phys. Med. Biol., 37, 155–162 (1992).

14) Brugger, R. M. and Shih, J. A. Evaluation of gadolinium-157 as a neutron capture therapy agent. Strahlenther. Onkol., 165, 153–156 (1989).

15) Masiakowski, J. T., Horton, J. L. and Peters, L. J. Gadolinium neutron capture therapy for brain tumors: a computer study. Med. Phys., 19, 1–8 (1992).

16) Burki, H. J., Roots, R., Feinendegen, L. E. and Bond, V. P. Inactivation of mammalian cells after disintegration of ^{3}H or ^{125}I in cell DNA at $-196°C$. Int. J. Radiat. Biol., 24, 363–375 (1973).

17) Martin, R. F., D'Cunha, G., Pardee, M. and Allen, B. J. Induction of double-strand breaks following neutron capture by DNA-bound ^{157}Gd. Int. J. Radiat. Biol., 54, 205–208 (1988).