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GADOLINIUM NEUTRON CAPTURE THERAPY: PRECLINICAL STUDIES

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ABSTRACT

Gadolinium neutron capture therapy makes use of photons and electrons produced by nuclear reactions between gadolinium and lower-energy neutrons which occur within the tumor. The results of our studies have shown that its radiation effect is mostly of low LET and that the electrons are the significant component in the over-all dose. The dose from gadolinium neutron capture reactions does not seem to increase in proportion to the gadolinium concentration, and the Gd-157 concentration of about 100 μg/ml appears most optimal for therapy. Close contact between gadolinium and the cell is not necessarily required for cell inactivation, however, the effect of electrons released from intracellular gadolinium may be significant. Experimental studies on tumor-bearing mice and rabbits have shown that this is a very promising modality though further improvements in gadolinium delivery to tumors are needed.

INTRODUCTION

Neutron capture therapy is based on nuclear reactions between low-energy neutrons and an element having a high probability of interacting with them. In 1936 Locher postulated neutron capture therapy with several elements[1]. During the 1950'-1960s this proposal became realized at the Brookhaven National Laboratory in New York where the first clinical trial with boron took place[2]. Renewed interest in this modality emerged recently when a 5-year survival rate of 20% was achieved in patients with grades III-IV gliomas treated with boron neutron capture therapy [3].

Gadolinium, a rare earth element with a strong paramagnetic property, has been widely used as a contrast-enhancing element in magnetic resonance imaging (MRI) in the form of

meglumine gadopentetate (MG). Among its several isotopes, Gd-157 has a very large nuclear cross section to thermal neutrons (67 times larger than that of B-10), and releases photons and electrons with the maximal energy of 7.9 MeV when bombarded with thermal neutrons[4]. Thus, a half a century after Locher suggested[1] investigation begun on Gd-157 as an agent for neutron capture therapy. In this report, we review the results of our preclinical studies to evaluate the feasibilities of gadolinium neutron capture therapy (GNCT) as a potential clinical modality.

IN VITRO STUDIES

These studies were carried out to evaluate the extent and quality of radiation effects caused by gadolinium neutron capture reactions (GNCR) using standard cell survival assays. Attempts have been made to develop a formula to estimate doses resulting from GNCR.

Dose response studies

The first study was aimed at obtaining a cell survival curve to measure dose responses and to speculate the quality of radiations released from GNCR. Chinese hamster (V79) cells in suspensions were placed in Teflon tubes (6 cm long, 0.8 cm in outer diameter) containing medium and 5,000 μg/ml gadolinium (783 μg/ml Gd-157) in the form of meglumine gadopentetate (MG; Magnevist, Schering AG, Berlin, Germany), and were exposed to thermal neutrons generated by a TRIGA II reactor at the Musashi Institute of Technology. Measured dose rates with TLD were 1.05 Gy/h for a Teflon tube filled with 1.7 ml of water; and 10.0 Gy/h for the one filled with 1.7 ml of water containing 783 μg/ml Gd-157. There was a 35% depression of the thermal neutron fluence across the Teflon tube. Cells were plated in petri dishes and colonies were counted after 7 days.

The results show that the survival curve was found to be simple exponential for cells exposed to thermal neutrons only. The survival curve for cells irradiated in the presence of gadolinium exhibited a shoulder at the low neutron fluence region, and when fitted with a linear-quadratic model, the a/ß ratio was 2.17, suggesting that the radiation effect is mainly of low LET. The contribution of neutrons was found to be very small when compared to photons and electrons, though this depends largely on the gadolinium concentration. The enhancement of thermal neutron effects by gadolinium, therefore, was 3.6 fold when measured at 10% survival levels[5].

Electron contributions

The second study was to assess contributions of each radiation component of GNCR to the total dose. The survival data described above were plotted based on the available dosimetry data which consisted of doses (in Gy) from neutrons, gamma rays from both GNCR and the reactor core, excluding electrons. For comparison, an x-ray survival curve, derived from 6 separate experiments, was obtained using 250 KVp x-rays (General Electric Maxitron, 2 mm

Cu HVL) at a dose rate of 1.2-1.4 Gy/min.

When the two survival curves were plotted as a function of dose (Gy), there was a significant difference in the 10% survival dose; 1.9 Gy for cells received GNCT and 9.1 Gy for cells irradiated with x rays. Thus, the difference of 7.2 Gy must have come from the electrons. The results indicated that the electrons were the significant component of the radiation effect caused by GNCR[6].

Biological dosimetry

The third study employed biological dosimetry to estimate the dose from GNCR since physical dosimetry has not been established for the electron component. To accomplish this, several survival curves were generated after cells suspended in medium containing MG (0 to 3132 μg/ml Gd-157) were exposed to thermal neutrons at the Heavy Water Facility of the Kyoto University Reactor (KUR, 5MW, $3x10^8$ thermal neutrons/cm^2/sec.; a gamma dose rate of 1 Gy/hr). For comparison, the x-ray survival curve as described above was used (Fig. 1a). The survival curves for cells irradiated in the presence of gadolinium exhibited a shoulder at the low-neutron fluence region, therefore, they were fit with a linear-quadratic model. The neutron fluence for any given survival level was calculated accordingly (Fig. 1b).

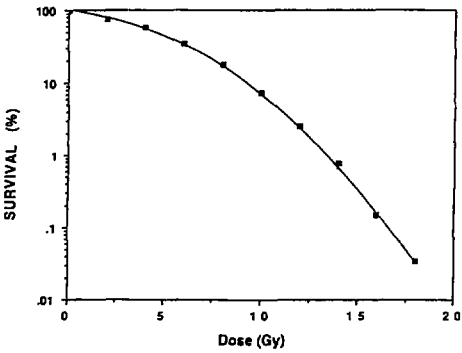

Fig.1 (a)

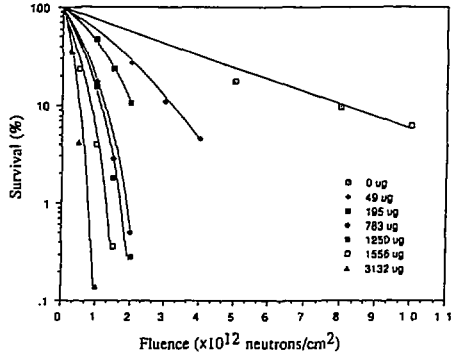

Fig.1 (b)

Fig.1 (a) Survival of cells exposed to 250 KVp x rays. (b) Survival curves for cells suspended in MG (0-3132 μg/ml Gd-157) and exposed to thermal neutrons. Note the presence of shoulders on the survival curves except for the one with 0 μg/ml Gd-157 (neutrons only).

Based on the results, x-ray equivalent doses for any effects resulting from GNCR can be estimated by matching the survival levels. For example, if GNCR given under a condition resulted in 10% survival, this survival level corresponds to 9.1 Gy with x rays, hence, the dose from the GNCR was equivalent to 9.1 Gy x rays. After analyzing the survival curves, an x-ray equivalent dose has been tentatively estimated as: Deq (Gy) = $0.56\ N^{1.36}G^{m}$, where m

$= 0.407N^{-0.267}$; Deq is the x-ray equivalent dose in Gy; N, the value obtained by neutron fluence /10^{12}/cm^2; and G, the Gd-157 concentration (μg/ml)[7]. The equation provides only approximate values and is valid within the data ranges shown in Fig. 1b.

To evaluate the extent of MG enhancement of the thermal neutron effect, the thermal neutron fluence required for the 10% survival was plotted as a function of Gd-157 concentration. The results showed that the required fluence decreases rapidly between 0 to about 100 μg/ml Gd-157, indicating a high rate of enhancement of the thermal neutron effect in this region[7].

IN VIVO STUDIES

Three separate studies, two with mouse subcutaneous and ascites tumor models, and one with rabbit tumors, have been carried out to evaluate the efficacy of different methods of gadolinium delivery to tumors.

Subcutaneous tumors in mice

The first study was aimed at evaluating (1) the dynamics of MG given subcutaneously in mice; (2) the response of tumors after GNCT, and (3) estimation of the tumor dose.

Three-week-old ICR male mice were inoculated with Ehrlich ascites tumor cells (3 x 10^6 cells/0.1 ml PBS) with 0.1 ml PBS or 0.1 ml MG (37.1 mg) at one or two subcutaneous sites on the dorsum. The mice were then individually exposed to thermal neutrons (~1.1 x 10^{12} neutrons cm^{-2}) at KUR for 8 minutes. The biological endpoint was the time period required for mice to form tumors with an arbitrary size of 100 mm^2 (a product of two perpendicular axes). In a separate study three groups (n = 10 each) were similarly inoculated and irradiated with 3 MeV electrons receiving a total dose of 5, 12 or 20 Gy at a dose rate of approximately 2 Gy/min.

The dynamics of MG after subcutaneous injection, measured with Gd-153-MG (0.5 mmol MG/50μCi /ml) by radioactivity counting, revealed that the residual MG concentration at the inoculation site was reduced to 78% (4.7 mg Gd-157/ml) at the beginning and 61% (3.6 mg Gd-157/ml) at the completion of neutron irradiation.

The tumor responses showed that it took 28.7 ± 2.3 days for mice treated with neutrons and MG (N+, Gd+) to form 100 mm^2 tumors; 15.2 ± 0.2 days for those treated with neutrons without MG (N+, Gd-); and 7.3 ± 0.4 days for those unirradiated with (N-, Gd+) or without (N-, Gd-) MG.

Time periods required for mice irradiated 5, 12 or 20 Gy to form 100 mm^2 tumors were 10.9 ± 2.4, 21.4 ± 5.4, and 35.0 ± 7.1 days, respectively. Thus, the corresponding electron doses for groups (N+, Gd-) and (N+, Gd+) are 7.8 and 16.2 Gy, respectively, and a 2.1 fold (16.2/7.8) enhancement was obtained by the presence of MG[8].

MG-containing microcapsules in murine ascites tumors

The second study was to evaluate MG-containing microcapsules as a gadolinium carrier. The microcapsules (MG content 31% W/W) release MG slowly (14% of the total MG released in 20 min) when suspended in saline[9]. ICR mice were anesthetized and inoculated intraperitoneally (ip) with 10^7 Ehrlich ascites cells in 0.2 ml PBS. MG or placebo microcapsules (220 mg) were suspended in 0.5 ml dextran 40 just before use and were administered ip. The mice were individually exposed to ~2.16 x 10^{12} thermal neutrons cm^{-2} at KUR. Nine mice received MG microcapsules and neutron irradiation (Gd+, N+), and ten were given placebo microcapsules and neutron irradiation (Gd-, N+). Control consisted of two groups of 14 mice each receiving 10^7 tumor cells and MG (Gd+, N-), or placebo (Gd-, N-) microcapsules without neutron irradiation.

The results show that mice given MG microcapsules survived significantly longer than those given placebo microcapsules or control at 60 days ($p<0.05$) with two of the nine mice surviving without disease at 180 days. All mice in other groups died within 17 days after treatments a result of progressive accumulation of tumor cells. None of the mice given MG or placebo microcapsules and neutron irradiation showed any sign of intestinal injury such as diarrhea. The estimated Gd-157 concentration in microcapsules was 2.5 mg/ml peritoneal fluid, and the dissolved Gd-157 in the peritoneal fluid was estimated to be 370 μg/ml or less. At this concentration and the neutron fluence given, the radiation effect from the extramicrocapsular Gd-157 dissolved in the peritoneal fluid was expected to result in in 20% survival levels. Hence, the cytocidal effect on the ascites tumor cells was mainly due to radiations produced from MG in the microcapsules rather than those from dissolved MG. The results show that microcapsules improved both the Gd-157 retention periods and survival[10].

Intraarterial infusion of MG to rabbit tumors

The third study was aimed at examining the efficacy of gadolinium delivery to tumors by intraarterial infusion. Four New Zealand White rabbits (10-20-week-old male weighing 2.3-3.1kg) bearing VX-2 tumors in both hind legs were used for GNCT, and two for gadolinium concentration studies. During neutron irradiation MG was infused through a catheter preinserted into a branch of the left femoral artery at a flow rate of 0.48 ml/min. Under general anesthesia the rabbits were exposed individually to KUR thermal neutrons for 40 min while a total volume of 19 ml of MG fluid (334 mg MG) had been infused . The average neutron fluence measured was (2.1 ± 0.47) x 10^{12} neutrons cm^{-2} s^{-1}, and the photon dose was 4.4 ± 3.1 Gy for gadolinium-infused legs, and 3.7 ± 1.1 Gy for control legs. Gadolinium concentrations in the tumor and the surrounding normal tissue [11] after infusing MG for ten minutes (0.48 ml/min) were 1.55 μmol/g (2.32 and 0.774) for the tumors, and 3.56 μmol/g (5.48 and 1.62) for the adjacent normal tissue of the MG-infused legs; 0.29 μmol/g (0.40 and 0.17) for the tumors, and 0.17 μmol/g (0.25 and 0.09) for the normal tissue of the contralateral legs.

The results revealed that the average tumor size of the infused legs was smaller than that of the contralateral legs with a statistically significant difference ($p < 0.05$) from the 16th day to

the end of observation (the 23rd day)[12]. There were no changes noted macroscopically or histologically in any of the normal tissue samples reviewed. No selective gadolinium delivery to tumors has been achieved with this approach, however, the obtained Gd-157 concentration in the tumor was approximately 40 - 90 μg/ml which was high enough for neutron capture therapy[7].

DISCUSSION AND CONCLUSIONS

The *in vitro* studies have shown that the radiation effect resulting from GNCR is mostly of low LET which differs itself from boron neutron capture therapy where released radiations are of high-LET particles. The dose distributions of released photons alone in GNCT are reported to be comparable to those obtained in BNCT[13),14]. Considering the broad energy range of released electrons, and that Auger, and other low-energy electrons are known to have a very limited range in tissue and are of high LET[15], the location of gadolinium in the cell, particularly with respect to cellular DNA is crucial in determining the degree of biological effects inflicted upon by GNCR. This has been shown by studies which revealed that double strand breaks caused by neutron irradiation were enhanced in the presence of gadolinium and that the effects were markedly reduced when gadolinium was sequestered from the nucleus[16]. Our preliminary study indicates that MG enters both human and animal cells freely (Akine et al, unpublished data 1993) which suggests that the contribution of low-energy electrons may be significant. Recently, contributions of electrons and photons have been estimated by Monte Carlo calculations[17].

For GNCT to be effective, it is important to maintain gadolinium concentrations in the surrounding normal tissue as low as possible. The *in vivo* studies have shown no adverse effect on normal tissue while there has been significant tumor growth suppression. It is still premature to determine the extent of normal tissue damage after GNCT. We are presently looking into other gadolinium carriers such as porphyrin-gadolinium complex. As a delivery system, we are investigating transcatheter arterial embolization as well as direct intratumoral administration.

In the future, other developments are needed in nuclear engineering to provide epithermal rather than thermal neutrons so that deep-seated tumors can be treated. Furthermore, to make neutron capture therapy practical, it is desirable to develop a hospital-based slow-neutron accelerator. In conclusion, the results are still preliminary but very encouraging enough to justify further investigation of this modality.

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