AINO JOURNAL, Vol. 6, 2007, pp. 39－44

Original article

Gadolinium neutron capture therapy for malignant brain tumors

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Abstract

Tumoricidal effect on malignant brain tumor of gadolinium neutron capture reactions has been preliminary studied. *In-vitro* C6 37% survival dose (D_{37}) were obtained at 3.55×10^{12} n/cm^2 in 500 ppm ^{n}Gd medium, 1.40×10^{12} n/cm^2 in 2,500 ppm ^{n}Gd containing medium, and 6.80×10^{12} n/cm^2 in Gd-free medium. The relative killing effect of GdNCT against control study was 1.92 fold for 500 ppm ^{n}Gd(+) and 4.86 fold for 2,500 ppm Gd(+). The significant survival prolongation of 9L brain tumor rat was observed by intra-venous injection of GdDTPA. Mean survival via GdNCT was 33.5±3.0 days, and that of control rats was 16.4±0.6 days. The optimal Gd concentration was estimated less than 1,000 ppm ^{157}Gd for various reasons, since neutron fluence rapidly decrease in the deeply seated tumor due to high absorption of neutron by Gd atoms. The maximum contribution of γ rays on tumor absorbed dose was less than 50%. In a spherical tumor model in diameter of 3cm, approximately 25% of absorbed dose was transferred by γ rays and more that 50% of that by internal conversion electrons. The peri-tumoral dose distribution does not decrease sharply like BNCT. The 10% decrease level was estimated to be 2–3 cm from the tumor margin. This blunt dose distribution might be effective to destroy the invading tumor cells in the peri-tumoral lesion manifesting as high density area in T^2-weighted MRI.

Key words: neutron capture therapy, gadolinium, malignant brain tumors

Introduction

Glioblastoma is the most formidable brain tumor and long time struggle against this tumor has been attempted. However long survival from this disease is still very rare and almost no prolongation of its survival rate has been reported for more than 30 years. Since 1960' boron-based neutron capture therapy (BNCT) for glioblasoma has been clinically investigated worldwide (originally, Locher, 1936, Sweet, 1963). In spite of these extensive efforts, 60% 2-year survival and 20% 5-year survival can be barely achieved by the "maximum BNCT" through life threatening radiation necrosis. In BNCT, high LET particles of α and its recoil ^{7}Li particle release 3.3 MeV only within their total trajectory of less than 14μm. Such limited energy transfer within short trajectory in tissue can save serious radiation injury onto the normal brain surrounding the tumor. On the other hand, however, dose distribution in the tumor is sharply dependent on the microdistribution of ^{10}B in tumor that revealed to be heterogeneous carried non-uniform dose distribution in the tumor even aside from a variety of proliferation condition of the tumor cells. Very important point of this therapy is selective destroy of tumor cells invading peri-tumoral parenchyma shown as abnormal T_2 high density area on MRI since 80–90% recurrence occur in this area (Giese, 1996). ^{157}Gd is an fascinating atom for this therapy, since it has a large thermal neutron cross section of 255,000 barn which is 65 times as that of ^{10}B and releases Auger electrons, internal conversion electrons, γ rays and X rays by a single

thermal neutron capture reaction; $^{157}Gd(n, \gamma)^{158}Gd$ reaction sharing among them the total kinetic energy of 7.7 MeV that is almost 2 times as that of $^{10}B(n, \alpha)^{7}Li$ reaction (Greenwood, 1978). Dose distribution of Gd-based NCT (GdNCT) is more uniform than that of BNCT and might be suitable for pathological heterogeneity of malignant tumors and even on the tumor cells invading into this abnormal T^2 high density area on MRI. In this preliminary study, tumoricidal effect of GdNCT on experimental brain tumors in *in-vitro/in-vivo* has been investigated and its problematic clinical future for glioblastoma are discussed.

Materials and methods

$^{157}Gd(n, \gamma)^{158}Gd$ reaction

$^{157}Gd(n, \gamma)^{158}Gd$ reaction is schematically shown in Fig. 1. ^{157}Gd atom, which has 15.6 % natural abundant ratio, releases Auger electron, internal conversion (IC) electron, γ rays and X rays by a thermal neutron capture reaction with a large nuclear cross section of 255,000 barn that is 65 times as that of ^{10}B.

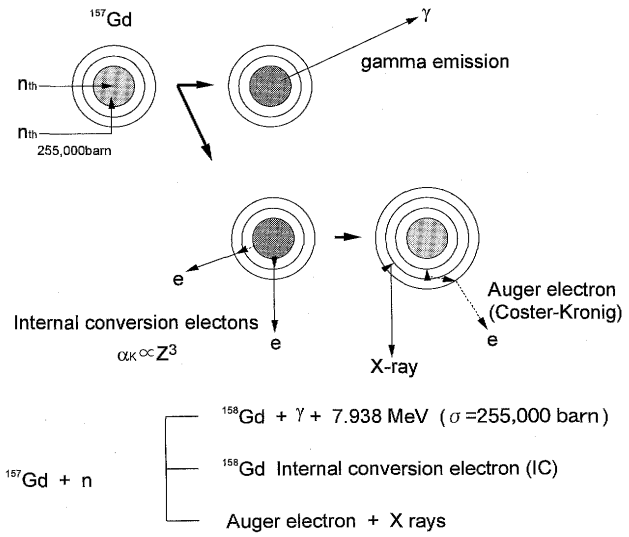

Fig. 1 Schematic drawing of a thermal neutron capture reaction of $^{157}Gd(n, \gamma)^{158}Gd$. Non-radioactive ^{157}Gd atom, which has 15.6% natural abundant ratio, release Auger electron, conversion electron, γ rays and X rays by a thermal neutron capture reaction with a large nuclear cross section of 255,000 barn that is 65 times as that of ^{10}B. The length of Auger electrons is too small, ca.100Å, to be expected tumor killing effect so long as ^{157}Gd does not closely locate in an around the critical target of DNA. Concomitant X rays also shows negligible killing effect on tumor since their kinetic energy is very small among these reaction.

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Tumor cell killing effect

Suspension of 5×10^3/ml C6 gliomsarcoma cells in a logarithmic growth phase was irradiated in 1 ml Eagle's minimum Essential Medium (Nissui, Tokyo, Japan) supplemented with 10 % heat inactivated fetal bovine serum (MEM(FCS+)) containing GdDTPA (Magnevist®, Shelling, USA) at the concentration of 0, 500 and 2,500 ppm ^{n}Gd (=0, 78 and 390 ppm ^{157}Gd). Aliquot of the suspension 5×10^3 cells/ml was contained in a Teflon tube (1 cm diameter and 3 cm high, cylindrical shape) that did not generate any secondary radiation by thermal neutron bombardment. The thermal neutron fluence was determined by averaging the activity of two Au foils symmetrically attached on the Teflon tube surface along the thermal neutron incidence. The thermal neutron fluence ranged 0 to 1×10^{13} nvt. The γ ray dose was monitored by a thermoluminescent dosimeter (TLD) attached on the Teflon tube surface. The γ ray dose ranged 0 to 5 Sv. Immediately after irradiation, 300 and/or 900 irradiated cells were pipetted into the 6 cm petri-dishes (CORNING, NY, USA) and incubated for 10 days to form colonies in a humidified 37 ℃ 5% carbon dioxide atmosphere. The colonies were fixed and stained with formaldehyde 1% toluidine

blue solution and were counted macroscopically.

GdNCT on brain tumor models

Fisher 344 rats were anesthetized by 2 mg/kg intra-peritoneal injection of barbiturate. The head of the rat was placed on the stereotactic flame (Natsume KN-398, Natsume co., Tokyo, Japan) and a burr hole was drilled 2 mm lateral and/or caudal to the mid-point of the coronal suture on the right parietal region. $10^6/10$ μl 9L cell suspension was slowly injected into the right caudate-putamen using 27 gauge Hamilton syringe at the depth of 3 mm from the brain surface under aseptic conditions. The burr hole was closed with bone wax immediately after removal of the syringe.

Cat brain tumor models were prepared in the similar fashion mentioned above. Suspension of 10^6 C6 gliosarcoma cells suspension in 10 μl 10% agar-MEM(CSF-) was implanted into the right parietal region at the depth of 5 mm from the brain surface through the burr hole made on 5 mm lateral and/or caudal from the Blegma. The C6 brain tumor cats without treatment were died around 3 weeks after implantation and the median surviving time was 21 ± 2.6 days ($n=10$). Although this brain tumor model was heterozygous, however the pathological specimens revealed tumor invasion in the peri-tumoral parenchyma and necrotic lesion in the tumor (Fig. 2). Those findings of malignant characters might be suitable for this purpose as a brain tumor model. The subject number of cats was minimum and they were cared according to International Standard Guide for the Care and Use of Laboratory Animal.

The whole brain of 9L brain tumor rats, under general anesthesia after intra-peritoneal injection of 0.2 ml/kg pentbarbiturate, were irradiated with thermal neutrons for 45 minutes with intra-venous bolus administration of GdDTPA 2ml into exposed femoral vein. GdDTPA 1ml was boosted at 22.5 minute during exposure. The γ rays dose was monitored by TLD attached on the scalp surface. The special attention was paid for the protection of their whole body, especially eyes and bucal mucosa from the bombardment of thermal neutrons using thermal neutron absorber ^{6}LiF flexible sheets. The in-situ measurement of Gd concentration in 9L brain tumor rats 2 weeks after the implantation was

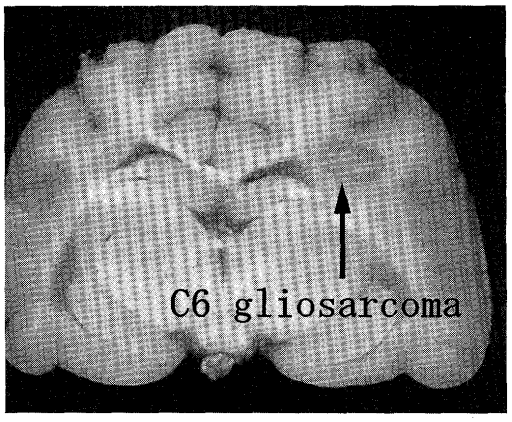

Fig. 2 A coronal section on the cat brain bearing heterograft C6 gliosarcoma tumor in the left parieto-temporal subcortical white matter, Hematoxylin & Eosine stain. This brain tumor model is heterograft, however it is pathologically proven malignant tumor invasion in the peri-tumoral parenchyma and necrotic lesion in the tumor.

carried out by prompt γ ray spectrometry (PGS). Under general anesthesia, 0.2 mg/kg sodium pentbarbiturate, 0.5 ml/kg GdDTPA (Shelling, Co. Ltd., USA) was injected in bolus into a femoral vein during exposure of thermal neutron beam through a burr hole, and prompt γ rays emitted from ^{157}Gd in the tumor was continuously measured by PGS. Two weeks after GdNCT, the brains were removed and underwent pathological examination.

Statistics

Three replication of the *in-vitro* GdNCT were carried out. Values were expressed as mean $\pm$ SE. Significant differences on the survival study were assessed by the student *t*-test. These statistical analysis was performed using Prism® 3.0 of GraphPad Soft Ware Inc., CA, USA.

Results and Discussion

In vitro survival

The surviving fraction of *in-vitro* GdNCT is shown in Fig. 3. The surviving fraction of GdNCT decreased without a sigmoidal shoulder as a function of the thermal neutron dose. 37% survival dose (D_{37}) were obtained at 3.55×10^{12} n/cm^2 for 500 ppm Gd(+) medium, 1.40×10^{12} n/cm^2 for 2500 ppm Gd(+) medium and 6.80×10^{12} n/cm^2 for Gd-free medium. The relative killing effect of GdNCT against control survival was 1.92 fold for 500 ppm ^{n}Gd

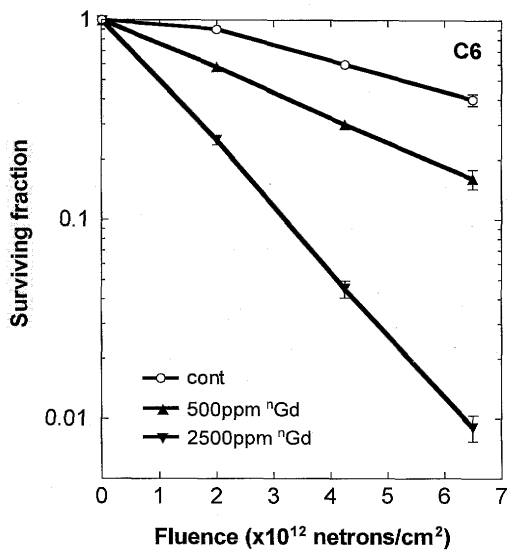

Fig. 3 The surviving fraction of *in-vitro* GdNCT. The surviving fraction of GdNCT decreased without a sigmoid shoulder as a function of the thermal neutron dose. 37% survival dose (D_{37}) were 3.55 $\times 10^{12}$ n/cm^2 for 500 ppm Gd(+) medium, 1.40$\times$ 10^{12} n/cm^2 for 2500 ppm Gd(+) medium and 6.80$\times 10^{12}$ n/cm^2 for Gd-free medium. The relative killing effect of GdNCT against control survival was 1.92 fold for 500 ppm ^{n}Gd(+) and 4.86 fold for 2,500 ppm ^{n}Gd(+). The plating efficiency was reliable, c. a. 60%.

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Pharmacokinetics of Gd in tumors

The time course of the Gd concentration in 9 L brain tumor is shown in Fig. 4. The concentration in the tumor rapidly decreased with two-component decay. The half-life time of the early decay was approximately 19 minutes. The peak concentration of Gd in the tumor was almost proportional to the total amount of intravenous injection. Average concentration of Gd in the tumor in these experimental conditions was 155 ppm ^{157}Gd (= 993 ppm ^{n}Gd). The Gd concentrations in rat blood were not well studied for the limited number of the subject. If the T/B ratio of 0.54 for human was assumed in rat, Gd average concentration in blood during GdNCT was estimated to be approximately 287 ppm ^{157}Gd (=1840 ppm ^{n}Gd).

Rat GdNCT

A Kaplan-Meier plot of GdNCT for rat brain tumor was shown in Fig. 5. 9L rats received no any treatment died uniformly at 16.4$\pm$0.6 days. There was no significant deference in

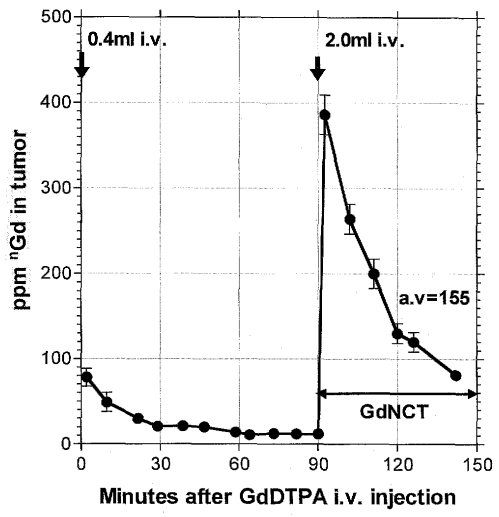

Fig. 4 The time course of the Gd concentration in 9L brain tumor. The half-life time of the early decay was approximately 19 minutes after intra-venous bolus injection of GdDTPA. The peak concentration of Gd in the tumor was almost proportional to the total amount of intravenous injection. Average concentration of Gd in rat brain tumor in these experimental conditions was 155 ppm ^{157}Gd (=993 ppm ^{n}Gd) (n=10).

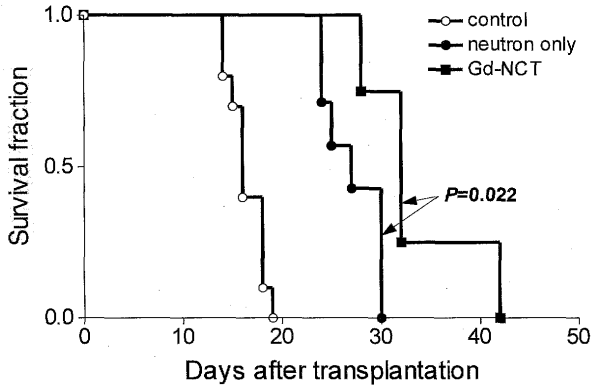

Fig. 5 Kaplan-Meier plots of 9L brain tumor rats after GdNCT. 9L rats received no any treatment died uniformly at 16.4$\pm$0.6 days. There was no significant deference in the survival length between the neutron only and/or 0.4ml GdDTPA i.v., 27.0 days. (p=0.5000). The significant prolongation of their life was observed on the 2 +1 ml GdDTPA i. v., 32.0 days ($p<0.0001$).

the survival length between the neutron only (27.0$\pm$0.9 days) and 0.4 ml ^{n}GdDTPA (27.1$\pm$ 1.0 days), p=0.134. The significant prolongation of their survival was observed on the 2+ 1 ml ^{n}GdDTPA, 33.5$\pm$3.0 days. The rats were sacrificed and brain tumors were pathologically investigated. A large number of lymphocyte aggregates in tumors were observed after

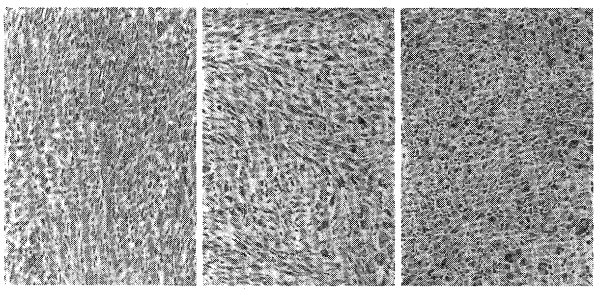

Fig. 6 The rats were sacrificed and removed brain were pathologically investigated 2 weeks after GdNCT. A large number of lymphocyte aggregates surrounding tumors were observed after GdNCT. However vial tumor cuffing remained. These vial tumor cells are suspected to be highly radiation-resistant and/or sublethal. No significant damage was observed in the normal brain after GdNCT even on a neutron bombarded parietal-parenchyma surrounding a superior sagittal sinus.

GdNCT as shown in Fig. 6. However vial tumor cuffing remained. These vial tumor cells might be highly radiation-resistant and/or surviving in sublethal damage. No pathological damage was observed on the normal brain after GdNCT even on a parietal parenchyma surrounding a superior sagittal sinus.

Average Gd concentrations in brain tumor as confirmed in this study, were around 150 ppm ^{157}Gd. Using ^{156}Gd-enriched DTPA, the concentration in tumor is theoretically elevated to six fold. Gd-NCT loaded with a high-dose administration of GdDTPA, almost ten fold amount of the clinical dose for MRI enhancement study, showed a tumoricidal effect in 9L brain tumor without serious injury to normal brain and vessels after a thermal neutron exposure of 1.8×10^{13} n/cm^2. If we assume the goal of ^{n}Gd concentration in tumor to be several thousand ppm, more than a ten fold specific accumulation in tumor can be achieved. Furthermore, It is essential for GdNCT to increase the retentions period of Gd in tumor. Although *in-vitro* study for GdNCT has been investigated successfully (Stasio, 2001, Takagaki, 1991, Akine, 1992), however *in-vivo* study for GdNCT is quite difficult since it is hardly to keep Gd concentration in tumor during GdNCT. Akine reported excellent *in-vivo* data using murine ascites tumor with intra-peritoneal administration of Gd(Akine, 1992). Ichikawa has been investigated gadolinium-containing microcapsules to keep Gd concentration in tumor to be constant during GdNCT (Le, 2006, Ichikawa, 2007). In our study, in order to keep concentration of Gd in tumor to be constant during GdNCT, GdDTPA has been continuously administrated intravenously without lethal side effect. Pathological examination after GdNCT, good response has been obtained without any evidence of vascular damage, especially peri-sinus region, although Gd concentration in vessel has been large, c. a. 5,000 ppm. Another potential modality of GdDTPA, intraventricular and/or intrathecal high dose injection can show a supplemental killing effect in cases of subependymal or intraventricular tumor disseminations by the simultaneously combined treatment with BNCT.

In GdNCT, macroscopic dose distribution in tumor is almost uniform. Theoretically, in a spherical tumor with radius of R, dose intensity: $\phi(r)$ uniformly caused by ^{157}Gd(n, γ and/or e) ^{158}Gd nuclear reaction in tumor is expresses as follows:

$$d^2\phi(r)/d^2r+2d\phi(r)/rdr+B^2\phi(r)=0$$
$$\phi(r)=(pN\sigma\Phi/r)\sin(\pi r/R)$$

where, r is the distance from the tumor center, N: ^{157}Gd concentration, s: 255,000 barn, Φ is the thermal neutron flux, and B is the constant depending up on the size of the spheroid. Total dose distribution is estimated as follows,

$$\gamma^*+{}^{157}\mathrm{Gd(n,}\ \gamma\ \text{and/or e)}^{158}\mathrm{Gd}+{}^{14}\mathrm{N(n,\ p)}^{14}\mathrm{C}$$
$$=\gamma^*+[\mathrm{N(Gd)}\sigma\mathrm{(Gd)RBE}(\gamma\ \text{and/or e})+\mathrm{N(N)}\sigma\mathrm{(N)RBE(p)}]\phi(r)$$

where, γ^* is caused by reactor core and structural materials of the reactor as contamination, RBE is the relative biological effectiveness, and N indicates concentration of ^{157}Gd and/or ^{14}N in tissue. The maximum contribution of γ rays on tumor absorbed dose was less than 50%. In a spherical tumor model in diameter of 3cm, approximately 25% of absorbed dose was caused by γ rays and more that 50% of absorbed by Internal Conversion electrons. Selective high tumor dose is owing to IC electrons of which trajectory is approximately 100 μm in tissue. The peri-tumoral dose distribution does not decrease sharply like BNCT. The 10% decrease level was estimated to be 2–3 cm from the tumor margin. This blunt dose distribution might be effective

to destroy the invading tumor cells in the peri-tumoral lesion manifesting as high density area in T_2-weighted MRI. Dose distribution onto tumor surrounding tissues caused by high energy γ rays and electrons might be valid for tumors of an infiltrating type. Because of spatial γ^* and proton caused by ^{14}N are inevitably exposed, they does not allow long irradiation period for safety reasons. This frustrating situation can be alleviated by combination of GdNCT+BNCT especially for deeply seated tumors. The optimal ^{157}Gd concentration was estimated less than c. a. 1,000 ppm ^{157}Gd. This optimal concentration is well coincident with the theological results using a two-dimensional neutron-coupled γ ray transport code (DOT 3.5) for GdNCT (Matsumoto, 1992). GdNCT is largely dependent on a balance of Gd concentration in tumor and decrease of thermal neutron fluence. Decrease of thermal neutron fluence for large absorbed cross section of ^{157}Gd atom is the most problematic intrinsic factor of GdNCT. Our study revealed that decrease of thermal neutron in deeply seated tumor was intolerable when ^{157}Gd concentration in tumor becomes more than 1,000 ppm. On the other hand, tumor absorbed dose is slightly depending to the tumor volume it self. This "volume effect" is owing to γ ray dose of $^{157}Gd(n, \gamma)$ ^{158}Gd generated in the tumor itself. This is the reason why large tumor model is necessary to evaluate the precise effectiveness of GdNCT. However this effect might not be so large to influence killing effect since the trajectory of their γ rays are very large. We have been under evaluation using cat C6 brain tumor model.

Acknowledgments

Dose calculation of GdNCT was owing to Dr. Yoshinori Sakurai, Associated Professor, Department of Sapporo Medical College. Research Grants from the Japanese Ministry of Education, Culture, and Sports, Science, and Technology, and also from BNCT Research Laboratory, Kyoto Japan, has financially supported this study. Neutron sources for this study have been applied in visiting research programs of Research Reactor Institutes, Kyoto University (KURRI), #19020, 2007, and Tokyo University (JRR4), #7127, 2007.

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