論 文 の 内 容 の 要 旨

論文題目　Study on Nanoparticles-based Neutron Capture Therapy
（ナノ粒子を用いた中性子捕捉療法の研究）

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Glioblastoma multiforme is one type of brain cancer, which is the most aggressive diffuse glioma of astrocytic lineage and is classified into grade 4 based on WHO classification. With the average incidence rate of 3.19/100,000 populations, median survival of 15 months, and median age of diagnosis is 64 years; GBM remains an incurable disease. The incidence and mortality are almost equal, indicating the fatal outcome of this brain malignancy, and the need for new therapeutic approaches. Although molecular biology-based therapeutic approaches are the major focus of current clinical research efforts, a more immediate impact might be obtained by developing a safe radiation dose-escalation strategy, and NCT represents such a novel approach.

Neutron capture therapy is a form of cancer treatment that combines neutron irradiation and the preferential loading of target tissues or cells with particular compounds. In NCT, neutron beam is applied to the region of interest where the target tissue contains a relatively high concentration of neutron absorber (generally stable isotope) compounds compared to the surrounding normal tissues. Reaction between these compounds with neutron creates secondary products that deposit most of their dose locally, sparing the surrounding normal tissues. Thermal neutrons are preferable in NCT treatment, considering that slow neutrons generally spend more time around a nucleus and are more likely to be captured. To ensure maximum cell damage on target tissue, the neutron absorber nuclides used as NCT agent need to have very high abilities to absorb thermal neutron (e.g. boron and gadolinium) compared to other elements present in bulk of biological tissues.

Boron and gadolinium are taken into consideration as NCT agent due to their high neutron cross section and the characteristics of secondary particles they produce after neutron capture reaction. Even though currently used compound in clinical trials is boron-based compound, investigations of gadolinium as NCT agent have been carried out by many researchers interested in several advantages that gadolinium offers. Isotope ^{10}B splits into high linear energy transfer α particle and ^{7}Li nucleus upon neutron absorption. Alpha particle and lithium ion emitted by the boron neutron capture reaction (BNCR) have combined path length of approximately one cell diameter i.e. about 12 microns, which theoretically

limiting the radiation effect to those tumor cells that have taken up a sufficient amount of ^{10}B. However, this short flight range of alpha particles and recoil lithium released after BNCR might also bring an inherent problem that it is necessary to deposit boron homogenously intracellularly to destroy the cell.

Gadolinium-157 has been considered as an alternative or complimentary to boron in NCT because of its highest neutron cross section (255,000 barns) which is around 66 times larger compared to ^{10}B thermal neutron cross section. The advantage of gadolinium's higher neutron cross section is that the same number of neutron capture reactions with ^{10}B, can be produced with significantly lower neutron fluence. Gadolinium neutron capture reaction (GdNCR) also produces γ-rays, internal conversion electrons, X-rays and Auger electrons with total kinetic energy about 3 times of that produced by boron in BNCR. Gadolinium neutron capture reaction results in release of gamma rays which is considered as a drawback as well as favorable characteristic, because it might cause ionizing effect to outer normal tissue while at the same time providing the advantage that the location of the element is not critical with regard to target cell due to their longer flight ranges. Gadolinium neutron capture reaction also produces a series of low-energy conversion and Auger electrons, which are high linear energy transfer (LET) secondary particles effective in breaking DNA double strand.

Current clinical trials and experimental investigation of boron and gadolinium as NCT agent have been showing promising results for the application of this therapy modality in cancer treatment. Many research groups in the world have been performing BNCT clinical trials on several types of cancer including those that is reported to be difficult to treat. The investigation on GdNCT have been limited to *in vitro* and *in vivo* experiments due to the lack of efficient drug delivery system of gadolinium compound, and also because of the longer range gamma-rays it produces after neutron capture reaction, which might reduce the localization effect of cancer cells killing. However, quite a lot of reports have been presented suggesting the promising application of gadolinium as NCT agent. Recent development of accelerator-based neutron source for a more hospital-friendly installment of NCT treatment facility has been encouraging more researchers to work on the optimization of NCT treatment toward its wider application in cancer therapy.

In this thesis we evaluated the feasibility of two gadolinium-based nanoparticles as NCT agent by performing neutron irradiation to colon-26 tumor-bearing mouse injected intravenously by gadolinium-based compounds as an effort to investigate the effectivity of cancer cells killing after NCT treatment by carrying out neutron irradiation at nuclear reactor facility of Kyoto University Research Reactor Institute. The first compound is the continued work of feasibility evaluation of radiochemotherapy-combined effect on Gd-DTPA/DACHPt-loaded nanomicelles, and the second is a calcium phosphate based nanoparticles previously developed to enhance image contrast in MRI. Preliminary investigation of NCT dose calculation was also performed mainly by using PHITS (Particle and Heavy Ion Transport code System) calculation method as an independent approach for evaluating energy deposition for each of BNCT and GdNCT as well as their combined effect. This early stage of

dose deposition investigation was aimed as an early stage before going into real calculation of dose deposition for both boron and gadolinium based NCT. Investigation of possible neutron depression effect was also conducted by setting up high neutron agent concentration on simple cylindrical phantom as well as the assessment of combined effect feasibility evaluation was also carried out for the two compounds.

The *in vivo* experiment on Gd-DTPA/DACHPt-loaded nanomicelles for NCT agent has shown that gadolinium accumulation is more effective compared to the mice injected with bare Gd-DTPA from the observation of MRI contrast enhancement and ICP-MS measurement. Previous experiment at KURRI with neutron collimator, various neutron fluences, including neutron-shielded condition, still showed quite high toxicity of gadolinium-platinum nanomicelles on irradiated tumor-bearing mice. On this experiment with various neutron fluences, we observed significant prolongation of survival rate for Gd-DTPA/DACHPt-loaded nanomicelles-injected group irradiated for 17.5 minutes (1×10^{12} n/cm^2 neutron fluence). Even though only 60% survived until 27 days after neutron irradiation, this result shown that irradiation time affect deposited dose quite significantly into mouse organs that might cause mouse death.

Continued experiment of neutron irradiation on mice injected with Gd-DTPA/DACHPt-loaded micelles and DACHPt micelles only, have revealed that even with current experimental procedure, high toxicity was still observed on all irradiated mice for both group. We have performed neutron irradiation on 12 hours and 24 hours after compound injection considering different platinum amount in blood plasma at those times. However, we still observed severe toxicity, which caused death to all irradiated mice of both Gd-DTPA/DACHPt-loaded micelles and DACHPt micelles only injected group. Even though we could observe tumor growth suppression compared to the non-irradiated group, the high toxicity results suggested that the feasibility of combined radiochemotherapy for Gd-Pt nanomicelles is difficult to achieve within tolerable toxicity with current experimental setup of injected dose and neutron irradiation dose.

For the evaluation of Gd-DTPA/CaP nanoparticles as NCT agent, the experimental results have shown that Gd-DTPA/CaP nanoparticles significantly enhanced gadolinium accumulation compared to those of free Gd-DTPA proven from the results of previously reported MRI image along with the ICP-MS measurement, where the gadolinium concentration in tumor site is significantly higher for Gd-DTPA/CaP nanoparticles. Several experimental setups were performed for mice group injected with this compound and the results have demonstrated the possible effectivity of it as GdNCT agent. Tumor growth suppression was obtained up to more than four times higher compared to the non-treated group for a single injection of Gd-DTPA/CaP nanoparticles, supported by the morphological and histopathological results after neutron irradiation.

It has also been shown that Gd-DTPA/CaP nanoparticles accumulate gadolinium in tumor site more effectively after comparing the results of our previous work on gadolinium-entrapping liposome. Gd-DTPA/CaP nanoparticles' smaller particle size is proven to be capable of accumulating gadolinium in higher percentage from initial injected dose. This smaller size has also been proven to be efficient in

escaping the renal filtration demonstrated by the ratio of gadolinium concentration in tumor to kidney, which is significantly lower in Gd-DTPA/CaP nanoparticles compared to those in gadolinium-entrapped liposome from our previous work.

Higher gadolinium accumulation in tumor site was also successfully achieved for multiple injections of Gd-DTPA/CaP nanoparticles as well as significant increase of gadolinium concentration in blood plasma at 30 hours after the first injection, indicating prolonged blood circulation of Gd-DTPA/CaP nanoparticles. However, the multiple-injected mice group did not reveal better tumor growth suppression even though gadolinium concentration accumulated in tumor site is much higher compared to the single-injected mice group. There is a possibility that neutron depression occurred in this group where neutron were being absorbed and could not reach deeper site of the tumor. Nevertheless, we could still observe tumor growth suppression after neutron irradiation, which suggests the possibility of cancer cells killing from secondary particles of GdNCR. Low toxicity of Gd-DTPA/CaP nanoparticles has also been proven from the observation of no significant mice weight loss during 27 days after neutron irradiation, which is also supported by the results on multiple-injected group, where we observed mice dead on multiple injections of bare Gd-DTPA while all mice injected with Gd-DTPA/CaP nanoparticles survived until the end of observation. These results let us come into conclusion that Gd-DTPA/CaP nanoparticles might be a promising delivery device for GdNCT.

Preliminary investigation of dose deposition intended to be an early stage of independent approach before going into real calculation of dose deposition from all possible contributing elements in BNCT and GdNCT dose deposition. The calculation was designed by referring to the experimental condition of neutron irradiation for gadolinium-based compounds-injected group. Dose calculation approach performed in this study was performed on a simple mouse phantom designed based on the tumor-bearing mice irradiated at KUR along with a simple cylindrical phantom with the materials of GI tract tissue to represent colon cancer cells. For the calculation results on simple mouse phantom, higher heat deposition in tumor site was observed for GdNCT compared to those in BNCT, which might come from the higher neutron cross section of gadolinium. Heat deposition on tumor site for both BNCT and GdNCT is also shown to be higher compared to other mice organs indicating the effect of neutron capture reaction by such neutron absorber compound considering that the percentage of boron or gadolinium in tumor is much lower compared to the other elements composing the other organs.

As for the cylindrical phantom dose calculation, there is a possibility of higher neutron depression for gadolinium from the observation of its higher decrease rate in heat deposition for both concentration being considered in the calculation, even though the ratio is not linear compared to its ratio of neutron cross section when compared to boron. Nevertheless, since the result of dose calculation in current study is still a preliminary investigation, we can not come into conclusion yet before performing further advanced calculation especially the necessary to perform microdosimetry of dose deposition from very short range secondary particles such as Auger and Coster-Kronig electrons produced after GdNCR.