Quantitative Bio-imaging of Gadolinium-157 in Tissues
Through Laser-ablation ICP-MS for Neutron Capture Therapy

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Objective: Gadolinium-157 (157Gd) has attracted interest for its use in the preparation containing metal elements of the neutron
capture therapy (NCT), one of the radiation therapies. The study, however, has not developed because of the difficulty to
quantify the concentration of 157Gd in the tissue. Therefore, we established a quantitative imaging technique for 157Gd in bio-tissue
employing laser-ablation inductively coupled mass spectrometry (LA-ICP-MS).

Materials and Methods: 4 female New Zealand white rabbits, which were inoculated with rabbit VX-2 cells participated in this
study. 157Gd in water-in-oil-in-water (WOW) emulsion was injected via the proper hepatic artery into the rabbits and, after 24
or 72 hours, the rabbits were killed, and the liver tissues were harvested. We also prepared 7 standard tissues which were mixed
with gadoteridol solutions, the final amount of 157Gd was 0, 4.4, 22, 44, 220, 440 and 660 μg/g, respectively. The harvested livers
and standards were sectioned on a cryostat at 5 μm intervals and they were analyzed by LA-ICP-MS.

Result: In an experiment on animal cancer tissue, 157Gd was observed to accumulate around the cancer.

Conclusion: The 157Gd concentration in bio tissue can be quantitatively assessed through LA-ICP-MS imaging and it was
expected to contribute the progress of NCT study.

Key words: gadolinium, laser-ablation inductively coupled mass spectrometry (LA-ICP-MS), neutron capture therapy (NCT), bio-imaging

Introduction

Neutron capture therapy (NCT) uses secondary radiation particles emitted by the nuclear neutron capture reaction to kill cancer cells. Locher intro-
duced NCT soon after the discovery of the neutron 1). For therapeutic application of NCT to
malignant melanoma and gliomas, boron-10 (10B) compounds have been used as short-range alpha-
particle–producing agents2) 3).

Recently, gadolinium-157 (157Gd) has attracted attention as an alternative NCT agent because it
has the largest thermal-neutron capture cross section among all stable nuclides (255,000 barns, 66
times as large as that of $^{10}$B) and gamma-rays and Auger electrons are released by the neutron capture reaction $^4\text{He}(n,\gamma)^4\text{Li}$. This property makes it possible to reduce the total neutron fluence needed for the same number of thermal neutron absorptions with $^{10}$B. In addition, because gadolinium has been used as a contrast agent of magnetic resonance imaging diagnosis, $^{157}$Gd-NCT is expected to be used in combination with magnetic resonance imaging. To increase the therapeutic effect of NCT, it is important to enhance the accumulation of $^{157}$Gd in tumor. Although NCT with $^{157}$Gd was first established in the 1980s $^5$, $^{157}$Gd-NCT has not progressed in contrast with $^{10}$B-NCT, because it is difficult to quantitatively measure the amount of $^{157}$Gd accumulated in the tissue.

Laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is an analytical technology that combines the laser ablation sampling technique and ICP-MS. A laser beam is focused on the sample surface to generate fine particles, which are then transported to the ICP-MS instrument for digestion and ionization. The LA-ICP-MS technique is sensitive enough to determine the abundance of trace elements $^6$-$^12$. Gadolinium imaging employing LA-ICP-MS has thus been developed recently $^{13}$-$^{17}$. Although Kostiv et al. has reported the quantitative imaging of nanoparticles containing Gd $^{18}$, the quantitativity was not enough because they did not match the matrix of trace elements in calibration standards with the samples analyzed.

The present study developed a method of quantitative imaging $^{157}$Gd in cancer tissue using the LA-ICP-MS technique, by matching the matrix of trace elements in calibration standards to those of tissue samples.

**Materials and Methods**

1. **Preparation of standard liver tissue for calibration**

To examine the quantitative relationship between the signal intensity obtained by LA-ICP-MS and the $^{157}$Gd concentration in the liver, we prepared a standard tissue in accordance with Hare’s procedure with minor modification $^{19}$: Frozen livers of intact New Zealand white rabbit were purchased from Sankyo Labo Service Corporation and defrosted at 4°C and then washed three times with deionized H$_2$O to remove residual blood. Segments of 3–5 cm$^3$ were sectioned, and blood vessels, fluids and connective tissues were removed using surgical scissors. After homogenization of the liver using a handheld blender (TK-210, Tescom, Tokyo), 53.6 $\mu$g of gadoteridol standard solution in saline (0, 1.88, 9.35, 18.8, 93.5, 186 and 280 gadoteridol-mg/ml) was added to the homogenate of 1 g each to obtain nominal $^{157}$Gd amounts of 0, 4.4, 22, 44, 220, 440 and 660 $\mu$g/g in the homogenized tissue, respectively. A portion of the mixture at each gadolinium concentration was packed into a plastic histology mold, frozen in liquid nitrogen, and stored at −80°C for LA-ICP-MS analysis. The different point to Hare’s method was the matching of the matrix (i.e., the composition of substances in samples) for LA-ICP-MS analysis $^{20}$.

A portion of the above mixture at each concentration of $^{157}$Gd was weighed and put into a perfluoroethylene bottle with 0.4 ml of 68% HNO$_3$, left overnight at room temperature (25°C), and then digested with 0.2 ml of H$_2$O$_2$ in a microwave oven (ETHOS PLUS, Milestone General, Bergamo, Italy). These digested liver samples were stored at room temperature until conventional ICP-MS analysis was performed.

2. **Preparation of VX–2 cancer liver from rabbits administrated with $^{157}$Gd**

All animal experiments in this study were conducted in accordance with the guidelines of Meiji Pharmaceutical University’s animal ethics committee (Approval number: 2612) and the Declaration of Helsinki. To examine the distribution in vivo, $^{157}$Gd in water–in–oil–in–water (WOW) emulsion was prepared as reported previously $^{21}$: gadoteridol solution (1,396.5 mg/5 ml) was filtered using a controlled porous glass membrane and added to 5 ml of iodized poppy-seed oil (lipiodol) containing surfactant to form a water–in–oil (WO) emulsion. The WO emulsion was emulsified again with aqueous phase containing 5 ml of saline and surfactant (to form another WOW emulsion). The $^{157}$Gd WOW emulsion was then injected via the proper hepatic artery into female New Zealand white rabbits, which were inoculated with rabbit VX–2 cells (Shope virus induced squamous carcinoma cell line, skin origin) to the left lobe of the liver two weeks before $^{22}$. At 24 or 72
hours after injection, the rabbits were killed and the liver tissues harvested, frozen in liquid nitrogen, and stored at −80°C until use.

3. Analysis of standard liver and VX-2 cancer in liver tissues

The standard liver and VX-2 cancer in liver tissues were sectioned on a CM3050S cryostat (Leica Microsystems, Bensheim, Germany) at 5 μm intervals. Cut sections were mounted on glass microscope slides, air-dried and stored at −80°C. The sections were inserted into a cell and ablated line by line using a commercial laser ablation instrument, an NWR213 (ESI New Wave Research, Oregon, USA), coupled to an Agilent 8800 Triple Quad ICP mass spectrometer (Agilent Technologies, Australia). The distribution of $^{157}$Gd and $^{63}$Cu were visualized using with the iQuant2 software 23). Additionally, $^{157}$Gd concentrations in the digested liver samples were measured using the conventional ICP–MS method as previously reported 24)-33).

As evidence has been given that copper concentrations are higher in the liver than in the skin 34)-38), we expected that the distribution of copper could be distinguished between the liver and the VX2 inoculation cells. LA–ICP–MS analysis was thus also conducted on the copper–63 ($^{63}$Cu) distribution in the VX–2 cancer in the liver sample to visualize the location of cancer tissue. Overlaying the distributions of $^{63}$Cu and $^{157}$Gd could help confirm the distribution of $^{157}$Gd in the liver and cancer tissues, because $^{157}$Gd accumulated in tissues is washed out in usual histological techniques, such as hematoxylin and eosin staining. Instrumental parameters and analytical conditions are summarized in Table–1.

4. Chemicals

Stock solution of 0.5% HNO$_3$ was prepared daily from 68% HNO$_3$ (ultrapure grade, Tama Chemicals Co., Kawasaki, Japan) and deionized H$_2$O. Thirty-five percent H$_2$O$_2$ (ultrapure grade, Tama Chemicals Co., Kawasaki, Japan) was used for all digestions. Gadoteridol was purchased from BRACCO–Eisai Co. (ProHance, Tokyo, Japan).

### Results

Figure–1 shows the standard curves calculated from the signal intensity of LA–ICP–MS on sections of the standard tissues and $^{157}$Gd concentrations in the corresponding digested liver obtained by conventional ICP–MS measurement. The homogeneity of the $^{157}$Gd distribution in the standard section was assessed for 10 repeated measurements; the percentage relative standard deviation was less than 30% for concentrations over 5.5 μg/g. The linearity of calibration curve was good, ranging from 0–760 μg/g $^{157}$Gd concentration ($r^2$ = 0.9977). Calculated limits of detection and limits of quantification of $^{157}$Gd determined from the blank tissue were respectively 0.0135 and 0.0409 μg/g, which were calculated as Matsukawa et al. 39).

The distribution of $^{157}$Gd in the VX–2 cancer in

| Table–1 Instrumentation and operational settings |
|-----------------------------------------------|
| **ICP–MS (Agilent 8800)**                      |
| RF incident power | 1,550 W | 1,550 W |
| Plasma gas flow rate | 15.0 l/min | 15.0 l/min |
| He flow rate | 0.8 l/min | not used |
| Carrier Ar gas flow rate | 0.95 l/min | 1.07 l/min |
| Monitored isotope | $^{13}$C, $^{63}$Cu, $^{156}$Gd, $^{157}$Gd, $^{159}$Gd | $^{13}$C, $^{156}$Gd, $^{157}$Gd, $^{159}$Gd, $^{160}$Gd |
| Date acquisition mode | Time–resolved analysis | spectra |
| Number of sweeps | 64 for 24 hours sample | 78 for 72 hours sample |

**Laser (New Wave Research NWR213)**

| Parameter | Value |
|-----------|-------|
| Wavelength | 213 nm |
| Pulse energy | 1.8 % |
| Fluence | 2.4 J cm$^{-2}$ |
| Repetition rate | 10 Hz |
| Spot size | 100 x 100 μm (square) |
| Scan speed | 80 μm s$^{-1}$ |

LA: analytical mode for laser ablation, RF: radio frequency
the liver model determined by LA-ICP-MS is shown in Figure-2 (left); the concentration was estimated using the standard curve in Figure-1. The middle panels show that $^{63}$Cu accumulated only in the normal tissue and not in the cancer. Right panels show the merged images of distributions of $^{157}$Gd and $^{63}$Cu. $^{157}$Gd was distributed along the boundary between the cancer and normal tissues 24 hours after injection, with the highest concentration being 1,066.3 $\mu$g/g. Meanwhile, 72 hours after injection, $^{157}$Gd accumulated in the cancer with a maximum concentration of 376.0 $\mu$g/g.

**Discussion**

We showed the linear correlation between the signal intensity of imaging and actual tissue concentration of $^{157}$Gd in the liver. Even 24 hours after the injection of $^{157}$Gd in WOW emulsion, almost all data points (99.95%) of the $^{157}$Gd signal intensity fell within the range of the standard curve. This method can therefore be used to estimate the amount of $^{157}$Gd accumulated in the tissue by varying the dose administered for NCT. As expected, the location of cancer tissue was identified by the $^{63}$Cu distribution. In this study, we utilized the low concentration of copper in VX-2 cells to distinguish the normal and cancer tissues. This observation, together with those of higher concentration of copper in lymphoma, breast cancer and gastrointestinal tract cancer $^{40}$, as well as lower concentration of zinc in liver and pancreatic carcinomas $^{31}$, suggests that copper and zinc distributions are available for the determination of the cancer area.

As identified from the imaging results, $^{157}$Gd was located at the tumor surface lesion including feeding vessels 24 hours after administration. Meanwhile, gadolinium was found in deeper lesions in the cancer tissue and not only border lesions with the feeding vessels 72 hours after administration. In NCT, differences in $^{157}$Gd concentrations between the surrounding normal tissue and cancer tissue were important for the evaluation of the preparation. For a therapeutic option to be viable, the radiation dose delivered to the cancer must exceed the background radiation that normal tissue receives from nonspecific neutron absorption. Generally, the selective tumor/normal tissue concentration ratios must be above unity and preferably 3:1 or higher $^{42}$. The results suggest that the concentration of $^{157}$Gd can be estimated by considering the distribution over time.
Conclusions

There were two device points in the present study. First, because the matching of the matrix (i.e., the composition of substances in samples) was important for LA-ICP-MS analysis, we prepared standard samples and liver tissue samples following the method of Hare et al. The volume of liquid added was the same for all analyzed samples, providing an excellent decision coefficient. Second, we imaged $^{63}$Cu as a marker to determine the area of cancer in the sample, overlaying the $^{63}$Cu image on the $^{157}$Gd image. The present study thus showed that the $^{157}$Gd concentration in bio tissue can be quantitatively assessed through LA-ICP-MS imaging. We thus expect rapid progress in NCT using $^{157}$Gd agents.

Conflicts of interest

There are no conflicts to declare.

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