Selective accumulation of [^{62}Zn]-labeled glycoconjugated porphyrins as multi-functional positron emission tomography tracers in cancer cells

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Abbreviations

PET: Positron-emission tomography, H₂Glc**1-4**: bisglycoconjugated porphyrins, ZnGlc**1-4**: [^{62}Zn]-labeled bisglycoconjugated porphyrins

Abstract

Positron-emission tomography (PET) can be used to visualize active stage cancer. Fluorine-18 (^{18}F)-labeled 2- ^{18}F 2-deoxy-2-fluoroglucose (^{18}F -FDG), which accumulates in glucose-dependent tissues, is a good cancer-targeting tracer. However, ^{18}F -FDG is obscured in glucose-dependent normal tissues. In this study, we assessed the cancer-selective accumulation of Zinc-labeled glycoconjugated 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (**ZnGlc1-4**), both *in vitro* and *in vivo*. Experiments using both normal and cancer cells confirmed the relationship between cancer cell-selective accumulation and the substitution numbers and orientations of glycoconjugated porphyrins. **ZnGlctrans-2** accumulated at greater levels in cancer cells compared with other glycoconjugated porphyrins. PET imaging showed that **ZnGlctrans-2** accumulated in tumor.

Keywords

Positron emission tomography, ^{62}Zn -labeling PET tracer, ^{62}Zn -zinc porphyrins, cancer selective accumulation

1. Introduction

Positron-emission tomography (PET) can be used to visualize the properties of cancer. PET images can provide important information and are especially helpful in the detection of the active stage of cancer cells and in developing a treatment strategy. The PET tracer fluorine-18 (^{18}F) ($t_{1/2} = 110$ min)-labeled 2- ^{18}F -2-deoxy-2-fluoroglucose (^{18}F -FDG) ¹ is a good cancer-targeting tracer, which accumulates in glucose-dependent tissues. However, ^{18}F -FDG is obscured in glucose-dependent normal tissues such as the brain and, therefore, it is limited to low glucose-dependent tissues. In addition, cancer does not always require glucose. An existing problem is that the kinds of PET tracers cannot be chosen on a case-by-case basis because there are few PET tracers. Thus, different metabolic ^{18}F -labeled PET tracers derived from glucose metabolism have been required and are being developed. For example, cationic charge polymers ², folate ³, and gold-nanoparticles ⁴ are used as PET tracers. Bombesin analogues selectively accumulated in a gastrin-releasing peptide receptor (GRPR)-expressing tumor *in vitro* and *in vivo* ⁵, suggesting that the chemicals of cancer-selective accumulation *in vitro* remain distributed in tissues throughout the body.

Gallium-68 (^{68}Ga) and zinc-62 (^{62}Zn) were used as radiolabeling-nuclides for PET ⁶. ^{18}F -labeled PET tracers are required for fast ^{18}F -labeling methods because of the short half-life of ^{18}F ($t_{1/2} = 110$ min) ⁷. When more than 6 h of half-life is needed to perform PET tracing in a clinical laboratory ⁸, the radioactivity should be eliminated after PET imaging to prevent radiation exposure. Thus, an ideal PET tracer has a short half-life or biological half-life of several hours. It is also better to choose the radioactive metal based on toxicity to avoid unexpected side effects. For example, a ^{68}Ga -labeled PET tracer induces nephrogenic systemic fibrosis ⁹ by blocking ion channels ¹⁰ when the gadolinium ion is released from the tracer or exists as a residue. The toxicity of the ^{62}Zn ion is not problematic for use in clinical PET ^{11,12} and has an adequate half-life of approximately 9 h. Thus, we incorporated the ^{62}Zn ion into a PET tracer.

In an attempt to overcome the limitations to PET through safe and photodynamic therapy (PDT) applications, we reported a multi-functional PET tracer of porphyrin-based chemicals based on PDT agents of glycoconjugated porphyrins, namely perfluorophenylporphyrin, which has *S*-glucopyranosyl groups ($\text{H}_2\text{Glc1-4}$) ¹³. While the PDT tracers caused visual side effects *in vitro*, more photocytotoxicity was observed at certain drug concentrations. The cellular uptake of $\text{H}_2\text{Glc1-4}$ differed based on the numbers of *S*-glycosylated groups and orientations (*cis* or *trans*); in order of increasing cellular uptake were $\text{H}_2\text{Glc1}$, $\text{H}_2\text{Glc4}$, $\text{H}_2\text{Glc}^{cis}\text{-2}$, $\text{H}_2\text{Glc3}$, and

H₂Glc*trans*-**2**¹³. The amount of cellular uptake was related to photocytotoxicity¹³. In our previous study, we reported the possibility of [⁶²Zn]-labeled bisglycoconjugated porphyrins, namely [⁶²Zn]-labeled ZnGlc*cis*-**2** ([⁶²Zn]-ZnGlc*cis*-**2**) and [⁶²Zn]-labeled ZnGlc*trans*-**2** ([⁶²Zn]-ZnGlc*trans*-**2**), as PET agents. [⁶²Zn]-labeling containing a porphyrin ring could be achieved within 30 min, and the signals of [⁶²Zn]-ZnGlc*cis*-**2** and [⁶²Zn]-ZnGlc*trans*-**2** were detected by PET in tumor-bearing mice, without any visible side effect *in vivo*¹⁴. In addition, photocytotoxicity of non-radioactive zinc ([Zn])-ZnGlc*cis*-**2** and [Zn]-ZnGlc*trans*-**2** showed cellular uptake similar to that of H₂Glc*cis*-**2** and H₂Glc*trans*-**2**¹⁴.

Cancer-selective accumulation is an important consideration for PET tracers. To clarify the cancer-selective effects of PET tracers, rat gastric mucosal epithelial cells (RGM-1)¹⁵ and oncogenic RGM-1 mutated cells (RGK-1)¹⁶, were used in this study. RGK-1 was established from RGM-1 through exposure to the carcinogenic substance *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine¹⁶. Thus, RGM-1 and RGK-1 have the same genetic background, which can reveal differences between the properties of normal cells and cancerous cells¹⁷. In RGK-1 cells, the expression of heme carrier protein 1 (HCP1), which transports porphyrin-based chemicals into cells¹⁸, is obviously higher than that in RGM-1 cells. As a result, the uptake of porphyrin derivatives in RGK-1 cells is enhanced¹⁸. We have already reported that RGK-1 was preferentially injured by PDT with [Zn]-ZnGlc*cis*-**2** or [Zn]-ZnGlc*trans*-**2** *in vitro*¹⁴. However, cancer cell-selective accumulation and tissue distribution had not been confirmed.

In this study, we assessed the *in vitro* and *in vivo* cancer-selective accumulation of Zn laserphyrin® and ZnGlc**1-4** (Scheme 1). Cell experiments using both RGM-1 and RGK-1 have confirmed the relationship between cancer cell-selective accumulation and the substitution numbers or orientations (*cis* or *trans*) of glycoconjugated porphyrins. Animal studies revealed the tissue distribution of the porphyrins by anatomy and PET/MRI imaging.

2. Materials and Methods

2.1 Materials

Ethanol, methanol, PEG 400, and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A 0.45-μm sterilizing filter (EMD Millipore Co., MA, USA), Gibco DMEM/F12, Gibco FBS, Gibco antibiotic solutions (Life Technologies Co., USA), and Dulbecco's modified EAGLE's medium nutrient mixture F-12 HAM (Sigma-Aldrich Co., LLC., St. Louis, MO) were used. A WST-8 cell proliferation

assay kit was purchased from Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Laserphyrin[®] was kindly provided by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). 5-[4-(β-D-Glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-10,15,20-tris(pentafluorophenyl)porphyrin (H₂Glc**1**), 5,10-bis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-15,20-bis(pentafluorophenyl)porphyrin (H₂Glc_{cis}-**2**), 5,15-bis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-10,20-bis(pentafluorophenyl)porphyrin (H₂Glc_{trans}-**2**), 5,10,15-tris[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-20-pentafluorophenylporphyrin (H₂Glc**3**), 5,10,15,20-tetrakis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]porphyrin (H₂Glc**4**), 5-[4-(β-D-Glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-10,15,20-tris(pentafluorophenyl)porphyrin zinc complex (ZnGlc**1**), 5,10-bis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-15,20-bis(pentafluorophenyl)porphyrin zinc complex (ZnGlc_{cis}-**2**), 5,15-bis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-10,20-bis(pentafluorophenyl)porphyrin zinc complex (ZnGlc_{trans}-**2**), 5,10,15-tris[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-20-pentafluorophenylporphyrin zinc complex (ZnGlc**3**), and 5,10,15,20-tetrakis[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]porphyrin zinc complex (ZnGlc**4**) were prepared according to our previously described methods (Figure 1, Supporting information Figure S1)^{13,19}. Solvents of samples were prepared by mixing 20% vol ethanol, 30% vol PEG 400, and 50% vol distilled water. [⁶²Zn] solution and zinc-65 ([⁶⁵Zn]) solution were generated by an online preparation system for multitracer solutions at the Research Center for Nuclear Physics (RCNP, Osaka, Japan)⁶. Here, we refer to non-radioactive zinc as [Zn].

2.2 Synthesis of [⁶²Zn]- and [⁶⁵Zn]-bisglycoconjugated porphyrin zinc complexes

[⁶⁵Zn]-ZnCl₂ (5 MBq) or [⁶²Zn]-ZnCl₂ (5 MBq) was added to bisglycoconjugated porphyrins, namely H₂Glc_{cis}-**2** and H₂Glc_{trans}-**2** in methanol (1.5 mM). The reaction mixture was stirred and heated (~60°C) for about 10 min, until the color changed from brownish-red to pinkish-red, indicating that H₂Glc**1-4** had reacted with [⁶²Zn] or [⁶⁵Zn].

[⁶²Zn]-ZnGlc**1-4** was concentrated under reduced pressure, and then 300 μL of [⁶²Zn]-ZnGlc**1-4**-solvent was added. These preparations were passed through a 0.45-μm sterilizing filter before use.

2.3 Synthesis of [⁶²Zn]- and [⁶⁵Zn]-Zn laserphyrin[®] zinc complex

A procedure similar to that described for [⁶²Zn]- or [⁶⁵Zn]-glycoconjugated zinc complexes was applied to laserphyrin[®] to yield [⁶²Zn]- and [⁶⁵Zn]-Zn laserphyrin[®].

2.4 Cell culture

RGM-1 and RGK-1 cells were cultured in DMEM/F12 and DMEM/F-12 HAM, respectively. The culture media contained 10% inactivated fetal bovine serum (FBS) and 1% antibiotic solutions. Both cell lines were cultured in a 5%-CO₂ cell culture incubator at 37°C.

2.5 Cellular uptake of non-radioactive samples by RGM-1 and RGK-1 cells

Cells were incubated in 6-well dishes at a density of 1×10^6 cells/well for 24 h. Then, 0.5 μ M laserphyrin[®], H₂Glc*trans*-**2**, [Zn]-Zn laserphyrin[®], or [Zn]-ZnGlc**1-4** in 1% DMSO/culture medium were exposed to cells for 0–24 h. Samples were extracted from cells by the addition of 100 μ L of DMSO after washing with phosphate-buffered saline (PBS) twice. The extracts were centrifuged for 10 min at 10,000 rpm. Fluorescence intensities of supernatants (50 μ L) in 96-well dishes were measured using a plate reader (VarioSkan, Thermo Fisher Scientific K. K., Kanagawa, Japan) at the following wavelengths: Laserphyrin[®], Ex. 410 nm and Em. 660 nm; [Zn]-Zn laserphyrin[®], Ex. 410 nm and Em. 650 nm; [Zn]-ZnGlc**1**, [Zn]-ZnGlc*cis*-**2**, [Zn]-ZnGlc**3**, Ex. 410 nm and Em. 645 nm; H₂Glc*trans*-**2**, Ex. 400 nm and Em. 701 nm; [Zn]-ZnGlc*trans*-**2**, Ex. 410 nm and Em. 646 nm, and [Zn]-ZnGlc**4**, Ex. 410 nm and Em. 650 nm.

2.6 Cellular uptake of radioactive samples by RGM-1 and RGK-1 cells

Cells were incubated in 6-well dishes at a density of 1×10^6 cells/well for 24 h. Cells were then exposed to 0.5 μ M [⁶²Zn]- or [⁶⁵Zn]-Zn laserphyrin[®], ZnGlc*cis*-**2**, and ZnGlc*trans*-**2** for 0–12 h. After being washed with PBS twice, cells were detached with 500 μ L of Trypsin/ethylenediaminetetraacetic acid (EDTA) and lysed with 500 μ L of 1% Triton X-100. The radioactivity of the extracts was measured by using a γ -counter (TDC-521, Aloka, Tokyo, Japan)

2.7 Tissue distribution in mice with tumors

Five-week-old BALBc/Slc-nu/nu mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). RGK cells (1×10^6 cells/100 μ L) were administrated subcutaneously to the outside of the left thigh. The tumor-bearing mice were established 1–2 weeks later. [⁶⁵Zn]-ZnCl₂ or [⁶⁵Zn]-labeled sample (5×10^4 cpm, 200 μ L) was injected

through the tail veins. After a prescribed period, the mice were anesthetized with pentobarbital. Blood was then collected via cardiac puncture. The major organs (tumor, brain, heart, lung, liver, kidney, and spleen) were collected and their radioactivity measured using a γ -counter (AccuFlex γ 7001, Aloka). The data were normalized to the radioactivity of the injected dose per gram (ID%/g).

2.8 PET/MRI imaging with [^{62}Zn]-labeled samples

PET/MRI imaging was performed as previously described²⁰. Briefly, [^{62}Zn]-Zn laserphyrin[®], [^{62}Zn]-ZnGlcis-**2**, and [^{62}Zn]-ZnGlctrans-**2** (100 μL , 75 μM , 0.5 MBq) were injected into tumor-bearing mice through the tail vein. At 1, 3, and 8 h after the injection, PET/MRI images were captured, with the mice in a prone position and under anesthesia (inhalation of 2% isoflurane through a tube). We performed simultaneous PET/MRI acquisition using iPET/MRI scanner²¹. This scanner has 0.3 T permanent magnet and MRI image was acquired with a fast low angle shot T1-weighted sequence. The following imaging parameters were set; 13.33 MHz NMR frequency, 40 mm field of view (FOV) and 128/256 phase and frequency encodes. The matrix size of MRI image was 256 x 128 with 1.25 mm thickness of slices. PET scanner has 40 mm transaxial FOV and 18.2 mm axial FOV. To cover whole mouse body, five PET acquisitions with different positions were performed. PET data were list-mode data and we first rebinned the list-mode data to two dimensional sinogram data by single slice rebinning technique. The image reconstruction was performed using order subset expectation maximization (OSEM) algorithm with iteration of 3 and subset of 5. No attenuation and scatter corrections were performed due to small size of object.

2.9 Photocytotoxicity of non-radioactive samples into RGM-1 and RGK-1 cells

Cells were incubated for 24 h in 96-well dishes at a density of 5×10^3 cells/well. Cells were then exposed to 2.0 μM [Zn]-Zn laserphyrin[®] or 1.0 μM [Zn]-ZnGlcis-**2** or 0.5 μM [Zn]-ZnGlctrans-**2** for 0–24 h. After cells were washed twice with PBS, fresh culture medium (100 μL) was added. The cells were exposed to light from a 100 W halogen lamp equipped with a water jacket and a Y-50 cut-off filter ($\lambda > 500$ nm). The light intensity was measured using a UV-vis power meter (ORION/TH, Ophir Optronic Ltd., Jerusalem, Israel). The irradiation time was adjusted to obtain the desired light dose of $16 \text{ J} \cdot \text{cm}^{-2}$. The mitochondrial NADH dehydrogenase activity of the cells after 24 h of photoirradiation was measured using a WST-8 Cell Proliferation Assay kit according to the

manufacturer's instructions. The absorbance at 450 nm was measured using a plate reader. The percentage of cell survival was calculated by normalization, with respect to the absorbance for untreated cells (vehicle).

2.10 Statistical analysis

All statistical evaluations were performed using the Student's *t*-test. All values for cellular uptake were expressed as mean $\pm$ standard deviation.

3. Results

3.1 Cancer cell selective uptake of Zn-labeled PET agents

The chemical structures of [Zn]-Zn laserphyrin[®], [Zn]-ZnGlc1, [Zn]-ZnGlc*cis*-2, [Zn]-ZnGlc*trans*-2, [Zn]-ZnGlc3, and [Zn]-ZnGlc4 are shown in Chart 1. For determining cancer cell-selective accumulation, normal rat gastric mucosa cells (RGM-1) and cancerous mutant RGM-1 cells (RGK-1) were used¹⁷. Comparison of the amounts of cellular uptake between normal cells and cancerous cells can easily provide confirmation of cancer selective properties of PET agents *in vitro*. Figure 1 shows the relative cellular uptake of [Zn]-labeled PET agents, based on autofluorescence. Cellular uptake did not depend on the numbers of modified glucose in glucose-modified PET agents, [Zn]-ZnGlc*trans*-2, which showed the maximum amounts of cellular uptake. Although the *cis* isomers had the same numbers of modified glucose as the *trans* isomers, the level of accumulation showed the same tendency as that of other PET agents, except for *trans* isomers. The *trans* isomer also showed cancer cell-selective accumulation, as demonstrated by RGK-1 accumulation of [Zn]-ZnGlc*trans*-2 being around 4 times more than that of RGM-1 after 24 h. [Zn]-ZnGlc*trans*-2 appeared to be the best PDT agent in this study, showing properties of cancer cell-selective accumulation.

We also confirmed that Zn-labeling caused a change in cellular uptake (Figure 2) by seemingly enhancing cancer cell-selective accumulation. Laserphyrin[®] has greater cellular uptake by Zn-labeling. However, this enhancement was not significant in cancer cell-selective accumulation. Zn-labeled H₂Glc*trans*-2 clearly showed enhanced cancer cell-selective accumulation after 24 h, with high cellular uptake by 12 h.

Supporting information Figure S2 shows that radioactive zinc-labeled PET reagents, with [⁶²Zn] ion and [⁶⁵Zn] ion, had the same cellular uptake tendencies as the results with [Zn] ion, shown in Figure 1 and Figure 2.

3.2 Tissue distributions and PET/MRI imaging

Because [Zn]-ZnGlc*trans*-**2** showed high cellular uptake and cancer cell-selective accumulation, we choose [Zn]-ZnGlc*trans*-**2** from [Zn]-ZnGlc**1-4** to use in animal experiments. In a tissue distribution study, laserphyrin[®] and H₂Glc*trans*-**2** labeled with [⁶⁵Zn] showed half-lives of around 244 days; the half-life of [⁶²Zn] is too short for use in this study. Figure 3 shows the tissue distributions of ⁶⁵ZnCl₂, [⁶⁵Zn]-Zn laserphyrin[®], and [⁶⁵Zn]-ZnGlc*trans*-**2**. [⁶⁵Zn]-Zn laserphyrin[®] in the blood decreased in a time-dependent manner, with almost complete elimination within 24 h, while it was still detectable in the liver, kidney, and spleen. Blood levels of ⁶⁵ZnCl₂ and [⁶⁵Zn]-ZnGlc*trans*-**2** did not diminish, and [⁶⁵Zn]-ZnGlc*trans*-**2** was also detected in the liver, kidney, and spleen, at higher amounts than those of ⁶⁵ZnCl₂ and [⁶⁵Zn]-Zn laserphyrin[®].

Detailed tissue distributions were determined. ⁶⁵ZnCl₂ and [⁶⁵Zn]-Zn laserphyrin[®] diminished from the blood and decreased in tissues over time, whereas [⁶⁵Zn]-ZnGlc*trans*-**2** remained in the blood and slightly decreased in tissues over time. For PET/MRI imaging with [⁶²Zn]-labeled PET agents, 100 μL of [⁶²Zn]-Zn laserphyrin[®] and [⁶²Zn]-ZnGlc*trans*-**2** (0.5 MBq) were administered to mice through the tail vein. Figure 4 shows the results of PET/MRI imaging. PET successfully detected [⁶²Zn]-Zn laserphyrin[®] and [⁶²Zn]-ZnGlc*trans*-**2**, at locations that correspond to our tissue distribution results. Whole body PET/MRI images are shown in Supporting Information Movie S1-6.

4. Discussion

Our *in vitro* study revealed a high level of cellular uptake of [Zn]-ZnGlc*trans*-**2**, with cancer cell-selective accumulation (Figure 1). This finding was consistent with previous studies of cellular uptake of PET agents without Zn-labeling¹³ and those of cancer cells-selective photocytotoxic effects¹⁴. The substitution numbers were not significant for cellular uptake (Figure 1). Of note, [Zn]-ZnGlc*trans*-**2** accumulated in cancer cells specifically. It indicated that orientations of glycoconjugated porphyrins are more important for cancer cell-selective accumulation than the substitution numbers. Hydrophobicity parameters (Log *P*), which is an index for suggesting an affiliation of cellular membrane, showed in ascending order ZnGlc**1** (Log *P* = 5.7), ZnGlc*cis*-**2** (Log *P* = 4.8), ZnGlc*trans*-**2** (Log *P* = 4.3), ZnGlc**3** (Log *P* = 3.4) and ZnGlc**4** (Log *P* = 2.2) in previous study¹³. The order of cellular uptake was the same except for [Zn]-ZnGlc*trans*-**2** in figure 1. Therefore, why [Zn]-ZnGlc*trans*-**2** showed cancer cell-selective accumulation is difficult to confirm by Log *P*. In this study, cell viability after PDT was decreased an exposure

time-dependently (Supporting Information Figure S3), indicating that photocytotoxic effects depend on cellular accumulation. Zn labeling also contributes to cancer-selective accumulation, the extent of which varied from structure to structure (Figure 2 and Supporting Information Figure S1). Zinc is necessary for the maintenance of homeostasis, playing a role in cellular responses, including cellular uptake^{22,23}. Intracellular zinc concentrations in normal cells are regulated and balanced²⁴, whereas cancer cells accumulate zinc for proliferation and differentiation^{25,26}. Although the expression of zinc transporter in cancer cells is greater than that in normal cells²³, an exposure of zinc preferentially should enhance endocytosis in cancer cells preferentially. Zinc may be released from Zn-labeled PET agents by metabolism of Zn-labeled samples, and the cellular uptake should be enhanced, especially in cancer cells. Furthermore, porphyrin derivatives also accumulated in cancer cells, specifically through HCP-1^{18,27}. The structures of Zn-labeled PET agents used in this study satisfy the requirements of cancer cells and as a result, show cancer cell-selective accumulation.

In our *in vivo* study, cancer-specific accumulation was consistent with the results of cellular uptake, with [⁶⁵Zn]-ZnGlc*trans*-**2** more detectable in tumors than [⁶⁵Zn]-Zn laserphyrin® (Figure 3). The major difference in tissue distributions was related to the blood circulation time, which probably caused the difference in accumulation in the tumor. The actual shapes of the compounds in the blood were not determined. However, the results suggest 2 possibilities of the structure in the blood: the biologically inactive form or a formulation of complexes, in conjunction with blood components, such as albumin. For example, biologically inactive nanocarriers²⁸ and blood components²⁹ show long blood-circulation periods. Nanocarriers were designed, by modifying polyethylene glycol³⁰ and maintaining the proper size for blood circulation³¹, to avoid rapid clearance by the immune system, and as a result, nanocarriers are mainly detected in the blood and blood-rich tissues, such as the liver, spleen, and kidney²⁸. These nanocarriers also have enhanced permeability and retention (EPR) effects³², without interacting with blood components. On the other hand, some blood components permit chemicals to circulate in the blood for a long time by forming complexes³³. For example, albumin, with a plasma half-life of 3-4 days *in vivo*, forms complexes of cationic chemicals, and the complexes show long blood circulation periods. The ζ potential of the albumin-modified complex decreased in a concentration-dependent manner³⁴. A ζ potential between neutral and negative is adequate for blood circulation³⁰, which is one of the reasons that an albumin complex enhances blood circulation. Modifications of other blood components also enhance the hemocompatibility of chemicals³³. [⁶⁵Zn]-ZnGlc*trans*-**2**

likely circulates in the blood after forming complexes with blood components because the tissue distributions are close to the results observed with [^{65}Zn]- ZnCl_2 , which mainly forms complexes with albumin in the blood ³⁵. This may contribute to tissue distribution.

Although PET/MRI imaging takes 25 min per one whole body image, PET/MRI images may include the change of tissue distribution. According to figure 3, the tendency of tissue distribution from 1 h to 3 h was not significant. Therefore we suggested that PET/MRI images should not be changed significantly within the duration time. However, we are difficult to discuss about the detail of pharmacokinetics from only PET/MRI images.

Long blood circulation time is favorable for cancer-selective accumulation; this was especially true for [^{65}Zn]- $\text{ZnGlc}trans\text{-2}$ in this study, which obscured the cancer in the PET image (Figure 4). In order to visualize the cancer clearly, a difference in signal contrast between the tumor and surrounding tissues is required. In fact, the accumulation in cancer at left leg is difficult to see in PET/MRI images (Figure 4) because an accumulation in liver was too high compared to in tumor (Figure 3). For resolving this problem, we have been determining the conjugate points between chemical properties and biological reactions to create a further multi-functional PET agent.

ZnGlc1-4 should be applied as an antioxidant, and not only a PET agent, for inhibiting the side effects of PDT through suppression of inflammation. Zinc is a basic component of antioxidants in the blood ³⁶, and zinc porphyrin is well known as a scavenger of reactive oxygen species (ROS) ³⁷. ROS scavengers are expected to suppress cancerous growth, invasion, and metastasis ^{17,38,39} and inhibit inflammation in the intestine ⁴⁰. We showed cancer-selective PDT effects in Supporting Information Figure S3 and in a previous report ¹⁴, which suggest that ZnGlc1-4 may become an ideal therapeutic and diagnostic agent in PDT and PET, respectively.

5. Conclusions

[^{62}Zn]- $\text{ZnGlc}trans\text{-2}$ showed cancer-selective accumulation, and the amount of cellular uptake in cancerous gastric cells was around 4-times more than that in normal gastric cells. The cellular uptake by cancer cells nearly coincides with the results of cancer accumulation *in vivo*. [^{62}Zn]- $\text{ZnGlc}trans\text{-2}$ circulated in the blood for over 24 h without decreasing and showed greater accumulation in cancer compared to other PET agents used in this study.

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Figure captions

Scheme 1. Schematic diagram of the studies for confirming cancer-selective accumulation by [^{62}Zn]-labeled laserphyrin[®] and [^{62}Zn]-ZnGlc1-4.

Chart 1. Structures of [^{62}Zn]-Zn laserphyrin[®] and [^{62}Zn]-ZnGlc1-4.

Figure 1. Relative cellular uptake amount of [Zn]-Zn laserphyrin[®] and [Zn]-ZnGlc1-4 by rat gastric mucosal epithelial cells (RGM-1) and oncogenic RGM-1 mutated cells (RGK-1) cells. RGM-1 and RGK-1 cells (1×10^6 cells/well) were incubated with each chemical (0.5 M) at 37°C for 1, 6, 12, or 24 h. Cellular uptake was measured by fluorescence intensity. The cellular uptake amount represents the percentage of the total amount of photosensitizer added. The concentration of the photosensitizers was 0.5 M. Values are the mean $\pm$ standard deviation of three replicate experiments. Significant differences are denoted as: * $p < 0.05$, ** $p < 0.01$.

Figure 2. Relative cellular uptake amount by rat gastric mucosal epithelial cells (RGM-1) and oncogenic RGM-1 mutated cells (RGK-1) cells of laserphyrin[®] and H₂Glc*trans*-2 with or without Zn-labeling. RGM-1 and RGK-1 cells (1×10^6 cells/well) were incubated with of each chemical (0.5 M) at 37°C for 1, 6, 12, or 24 h. Cellular uptake was measured by fluorescence intensity. The cellular uptake amount represents the percentage of the total amount of photosensitizer added. Values are the mean $\pm$ standard deviation of three replicate experiments. Significant differences were denoted as: * $p < 0.05$, ** $p < 0.01$.

Figure 3. Tissue distribution after administration through the tail vein of $^{65}\text{ZnCl}_2$, [^{65}Zn]-Zn laserphyrin[®], or [^{65}Zn]-ZnGlc*trans*-2 in oncogenic rat gastric mucosal epithelial mutated cells (RGK-1) tumor model mice. 200 μL of $^{65}\text{ZnCl}_2$, [^{65}Zn]-Zn laserphyrin[®], or [^{65}Zn]-ZnGlc*trans*-2 (5×10^4 cpm) was injected. The mice were anesthetized, tissues were dissected, and radioactivity of [^{65}Zn] from dissected tissues was detected by using a γ counter. (a)

Injected dose (ID) % of 1 mL of blood. (b) ID% of 1 g of tissue (tumor, brain, heart, lung, liver, kidney, and spleen).
Values are the mean $\pm$ standard deviation of three replicate experiments.

Figure 4. PET/MRI imaging of [^{62}Zn]-Zn laserphyrin[®] and [^{62}Zn]-ZnGlc*trans*-**2**. These images were acquired after intravenous administration of [^{62}Zn]-Zn laserphyrin[®] and [^{62}Zn]-ZnGlc*trans*-**2** (0.5 MBq, 100 μL , 75 μM) through the tail vein. Whole body PET/MRI images are shown in Supporting Information Movie S1-6.

[⁶²Zn]-Zn laserphyrin

[⁶²Zn]-ZnGlc 1

[⁶²Zn]-ZnGlc *cis*-2

[⁶²Zn]-ZnGlc *trans*-2

[⁶²Zn]-ZnGlc 3

[⁶²Zn]-ZnGlc 4

Figure 1

Figure 2

(a) Without Zn-labeling

(b) With Zn-labeling

Figure 3

(a) Blood circulation

(b) Tissue distribution ($^{65}\text{ZnCl}_2$)

(b) Tissue distribution ([^{65}Zn]-Zn laserphyrin)

(b) Tissue distribution ([^{65}Zn]-ZnGlctrans-2)

Figure 4

Study for determining
cancer-selective accumulation

[⁶²Zn]-Zn laserphyrin
or [⁶²Zn]-ZnGlc1-4

injection

i.v.

*Cellular uptake
using normal and cancer cells

*Tissue distribution
*PET/MRI imaging