Zinc-65 Imaging of Rat Brain Tumors

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ABSTRACT

The uptake of zinc, an essential nutrient, is critical for cell proliferation. On the basis of the idea that zinc uptake can be an index of viability in proliferating cells, tumor imaging with 65Zn was performed using autoradiography. After s.c. implantation of ascites hepatoma (AH7974F) cells into the dorsum, 1 h after i.v. injection of 65ZnCl2, 65Zn uptake in the tumor was higher than in the brain tissue but lower than in the liver, which suggests that brain tumors can be positively imaged with 65Zn. After implantation of AH7974F cells into the periaqueductal gray, 1 h after i.v. injection of 65ZnCl2, 65Zn uptake in the tumor was 10 times higher than in other brain regions. After implantation of C6 glioma cells into the hippocampus, 65Zn uptake in the tumor was also much higher than in other brain regions. The present findings demonstrate that brain tumors can be imaged with radiolabeled zinc. To compare brain tumor imaging with 65Zn with that of 18F-fluorodeoxyglucose (FDG), which is widely used for the diagnosis of brain tumors, 14C-FDG imaging of the C6 glioma was performed in the same manner. 14C-FDG uptake in the tumor was 1.5 times higher than in the contralateral region in which 14C-FDG uptake was relatively high. It is likely that zinc uptake is more specific for brain tumors than FDG uptake, which suggests that there is great potential for the use of 65Zn, a short half-life γ emitter, in the diagnosis of brain tumors.

INTRODUCTION

Noninvasive nuclear medicine techniques are important for the diagnosis of brain tumors. Unlike morphological imaging, such as X-ray CT, and magnetic resonance imaging, nuclear medicine techniques can detect tumors by imaging biochemical and metabolic changes of tumors (1). 18F-FDG PET is widely used for the diagnosis of brain tumors (2–5); 18F-FDG uptake in primary brain tumors is relatively high. After injection of 65ZnCl2, 60Zn uptake in the tumor was higher than in the brain tissue but lower than in the liver, which suggests that brain tumors can be positively imaged with 65Zn. After implantation of AH7974F cells into the periaqueductal gray, 1 h after i.v. injection of 65ZnCl2, 65Zn uptake in the tumor was 10 times higher than in other brain regions. After implantation of C6 glioma cells into the hippocampus, 65Zn uptake in the tumor was also much higher than in other brain regions. The present findings demonstrate that brain tumors can be imaged with radiolabeled zinc. To compare brain tumor imaging with 65Zn with that of 18F-fluorodeoxyglucose (FDG), which is widely used for the diagnosis of brain tumors, 14C-FDG imaging of the C6 glioma was performed in the same manner. 14C-FDG uptake in the tumor was 1.5 times higher than in the contralateral region in which 14C-FDG uptake was relatively high. It is likely that zinc uptake is more specific for brain tumors than FDG uptake, which suggests that there is great potential for the use of 65Zn, a short half-life γ emitter, in the diagnosis of brain tumors.

Zinc is also involved in the metabolism and interaction of tumor cells. Dietary zinc deprivation effectively suppresses the proliferation of transplanted tumors in tumor-bearing animals (14–18). Fong et al. (19) demonstrated that dietary zinc deprivation enhances the carcinogenic effects of methylbenzyl nitrosamine. Dimethylbenz[a]anthracene-induced carcinoma is inhibited by dietary zinc loading (20). The response of neoplastic tumors to zinc deficiency indicates a potential for therapeutic opportunities, although there is no known role for zinc in oncogenesis (21). On the other hand, zinc metabolism in tumor cells may be associated with their malignancy. Both normal and malignant cells secrete matrix metalloproteinases, which are important for invasion and metastases. The amount of the enzymes secreted by malignant cells is reported to exceed that of normal cells (22). In tumor cells, the functions of zinc are closely related to metastasis and proliferation. Thus, zinc uptake appears to be an index of tumor viability. When tumors are imaged with radiolabeled zinc, the information obtained is considered to be unique and important for diagnosis and therapy.

In the present paper, imaging of 65Zn uptake in tumors was examined using autoradiography. Brain tumors may be positively imaged with 65Zn because of the slow turnover of zinc in the brain (23, 24).

MATERIALS AND METHODS

Chemicals. 65ZnCl2 [85.1 MBq (2.30 mCi)/mg] in 0.5% HCl and 14C-FDG [11.1 GBq (300 mCi/ml)] in saline were obtained from NEN Life Science Products, Inc. (Boston, MA) and American Radiolabeled Chemicals Inc. (St. Louis, MO), respectively.

Cell Culture. Ascites hepatoma AH7974F cells, originally obtained from a hepatoma induced with 4-dimethylaminoazobenzene, were maintained in DMEM (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), in a humidified atmosphere of 5% CO2 and 95% air at 37°C. Astrocytoma C6, glioma cells, originally obtained from a glioma induced with N-nitrosomethylurea, were maintained in MEM (Nissui Pharmaceutical Co., Ltd), containing 10% heat-inactivated fetal bovine serum, penicillin G (100 units/ml), and streptomycin (100 μg/ml), under the conditions described above.

Experimental Animals. Male Donryu rats (4 and 6 weeks of age) and male Fischer rats (4 and 8 weeks of age) were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The animals were housed under standard laboratory conditions (23 ± 1°C, 55 ± 5% humidity). The animals had access to tap water and were fed a conventional chow diet (Oriental Yeast Co., Ltd, Yokohama, Japan) ad libitum. The lights were on from 8 a.m. to 8 p.m. All of the experiments were performed in accordance with the Principles of Laboratory Animal Care of the NIH and University of Shizuoka.

Whole-Body Autoradiography. Male Donryu rats (5 weeks of age) were given s.c. injections of AH7974F cells (1 × 105 cells/ml) or 65ZnCl2 (10 μCi/ml) of 0.1 ml acetate buffer (pH 4.0)/rat was injected into the tail vein of control and tumor-bearing rats (n = 2 per group). The rats were killed under deep diethyl ether anesthesia 1 h after injection of 65ZnCl2, frozen immediately, with ice-cold 8% carbomyethyl cellulose, frozen on the specimen stage at −20°C, and sliced at 100-μm thickness at −20°C with a microtome (Cryotome CR-502; Nakagawa Co., Ltd, Tokyo, Japan). The serial sagittal slices were dried in a Cryotome at −20°C. The distribution of radioactivity in each area of the selected slices was determined using autoradiography (Bio-Imaging Analyzer BAS 2000; Fuji Photo Film, Tokyo, Japan) after exposure to the imaging plates (Fuji imaging plate, 20 × 40 cm; Fuji Photo Film Co. Ltd) for 7 days.
In Vivo Distribution of 65Zn. Four Donryu rats (5 weeks of age) were given s.c. injections of AH 7974F cells (1 × 10^7 cells/0.2 ml of culture media/rat) into the inguinal region. Ten days after implantation, 65ZnCl2 [4 μCi/0.2 ml 0.1 M acetate buffer (pH 4.0)/rat] was injected into the tail vein of tumor-bearing rats. One hour after the injection of 65ZnCl2, the rats were killed after collecting the blood under deep diethyl ether anesthesia. The brain and tumor were excised from the rats, weighed, and counted for the radioactivity in a gamma-counter (Packard 5530; Packard Instrument Co., Inc., Meriden, CT). Four Fischer rats (5 weeks of age) were given s.c. injections of C6 glioma cells (2 × 10^7 cells/0.25 ml of culture media/rat) into the inguinal region. Seven days after implantation, tumor-bearing rats were treated in the same manner as described above.

Brain Autoradiography. Donryu rats (7 weeks of age) were anesthetized with chloral hydrate in physiological saline and placed in a stereotaxic apparatus. AH7974F cells (2 × 10^7/10 μl of culture media/rat) or vehicle (10 μl of culture media/rat) were injected at a rate of 0.7 μl/min into the periaqueductal gray of the rats (−4.9 mm posterior to bregma, ±0 mm lateral to the midline suture, and −5.1 mm from the dura) via a microdialysis probe without a dialyzing membrane using a microinjection pump (CMA/100; CMA Microdialysis, Solna, Sweden; 4 rats per group). Fourteen days after injection, 65ZnCl2 [20 μCi/0.3 ml of 0.1 M acetate buffer (pH 4.0)/300 g of body weight] was injected into the tail vein of the rats. The rats were killed under deep diethyl ether anesthesia 1 h after injection of 65ZnCl2. The brains were excised from the rats and frozen immediately, fixed with ice-cold 4% sodium carboxymethyl cellulose on the specimen stage, frozen at 20°C, and sliced at 300-μm thickness at −20°C with a microtome (Cryostat HM505E; Microm Laborgerate GmbH, Heidelberg, Germany). The serial coronal slices were dried in a Cryostat at −20°C. The distribution of radioactivity in each area of the slices was determined by autoradiography after exposure to the imaging plates for 7 days as described above. The exact time of exposure was measured by taking into account the physical decay. Radioactivity (PSL/mm^2) in each area of the autoradiograms of the selected slices was measured quantitatively using a Bio-imaging analyzer and was corrected according to PSL/mm^2 internal standards in each autoradiogram. There was a linear correlation between PSL/mm^2 and cpm obtained by the gamma counter.

RESULTS

65Zn Distribution in Rats s.c. Implanted with Tumor Cells. One hour after i.v. injection of 65ZnCl2, 65Zn was concentrated in the tumor. 65Zn uptake in the tumor was higher than in the brain tissue (Fig. 1). 65Zn uptake in the tumor, however, was lower than in the liver. 65Zn uptake in the liver (in which zinc-metallothionein preferentially increases with the growth of tumors) of AH7974F-bearing rats was higher than in control rats, as reported previously (25, 26).

When the 65Zn concentration in the tumor was quantitatively compared with that in the brain and the blood, the 65Zn concentration in the AH7974F tumor was approximately three times higher than in the brain tissue and was approximately twice that in the blood (Table 1). In the C6 glioma-bearing rats, the 65Zn concentration in the C6 glioma was also relatively high. The tumor:brain and tumor:blood ratios were approximately 6 and 2, respectively (Table 1).
**65Zn Distribution in the Brain of Rats Intracerebrally Implanted with Tumor Cells.** Brain autoradiography of normal rats demonstrated that 65Zn is concentrated in the choroid plexus 1 h after i.v. injection of 65ZnCl2 and that 65Zn concentration in the brain parenchyma is increased with a decrease in choroidal 65Zn (23). The maximum uptake of 65Zn in the brain parenchyma occurs ~6 days after the injection (24). To image brain tumors with radioactive zinc, brain autoradiography with 65Zn was performed using rats intracerebrally implanted with AH7974F or C6 glioma cells. After implantation of AH7974F cells into the periaqueductal gray, 65Zn was highly concentrated in the tumor 1 h after injection of 65ZnCl2, whereas 65Zn concentration in other brain regions was remarkably low (Fig. 2A). When the 65Zn concentration in the brains was quantitatively determined using a Bioimaging analyzer, 65Zn uptake in the tumor was ~10 times higher than in other brain regions and was approximately twice that in the choroid plexus (Fig. 3). 65Zn concentration in the brains of AH7974F tumor-bearing rats compared with control rats was significantly higher in the cerebral cortex, hippocampus, thalamus, hypothalamic nuclei, substantia nigra, and cerebellar lobules. Rats intrahippocampally implanted with C6 glioma cells are used as a primary brain tumor model. One hour after injection of

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Tumor % dose/g wet weight</th>
<th>Brain % dose/g wet weight</th>
<th>Blood % dose/g wet weight</th>
<th>Tumor:Brain Ratio</th>
<th>Tumor:Blood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH7974F</td>
<td>0.25 ± 0.08</td>
<td>0.08 ± 0.01</td>
<td>0.15 ± 0.05</td>
<td>3.1</td>
<td>1.7</td>
</tr>
<tr>
<td>C6 glioma</td>
<td>0.75 ± 0.18</td>
<td>0.12 ± 0.02</td>
<td>0.46 ± 0.07</td>
<td>6.3</td>
<td>1.6</td>
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Fig. 2. Brain tumor imaging. 65ZnCl2 was i.v. injected into rats 14 d after injection of vehicle (control) or AH7974F cells into the periaqueductal gray (A; n = 4). 65ZnCl2 was i.v. injected into rats 14 d after injection of vehicle or C6 glioma cells into the hippocampus (B; n = 4). 14C-FDG was i.v. injected into rats 14 d after injection of C6 glioma cells into the hippocampus (C; n = 4). Autoradiography was performed on selected coronal slices 1 h after injection of 65ZnCl2 or 14C-FDG. Each experiment was performed four times, and the autoradiograms obtained were almost identical. The schemes (left side) show maps of the rat brain. LV, lateral ventricle; 3V, third ventricle; Aq, aqueduct; HIP, hippocampus.
DISCUSSION

Zinc serves as an endogenous neuromodulator in the brain (27). Zinc homeostasis in the brain is closely related to brain functions such as learning and memory and also to neurological disorders, such as epilepsy (28, 29). It is estimated that the turnover of functioning zinc in the brain, however, is much slower than in peripheral tissues, such as liver (27). Thus, there is a possibility that the movement of zinc related to proliferation and/or functions in malignant cells can be positively imaged with radioactive zinc in the brain. The whole-body autoradiography of AH7974F tumor-bearing rats demonstrated that 65Zn uptake in the tumor is higher than in the brain tissue 1 h after i.v. injection of 65ZnCl2, whereas 65Zn uptake in the tumor was lower than in the liver. The liver is an important organ for zinc metabolism in the body (30). The turnover of functioning zinc in the liver may be faster than in the tumor. When 65Zn uptake in the heptoma and glioma, which were s.c. implanted into the inguinal region, was quantitatively compared to that in the brain, 65Zn uptake in both tumors was several times higher than in the brain. The present findings suggest that brain tumors can be positively imaged with radioactive zinc in the brain tissue.

Various nutrients are supplied to the brain parenchyma across the blood-brain and the blood-cerebrospinal fluid barriers. Glucose is a critical nutrient as the primary energy source for the brain. Although zinc is also a critical nutrient for brain functions, the transport of zinc, unlike glucose, is tightly restricted by the brain barrier systems (27). Angiogenesis is characteristic of tumors, including brain tumors, for acquisition of nutrients. The blood-brain barrier does not usually exist in brain tumors (31). Therefore, when brain tumors are positively imaged with radioactive zinc in the brain, it is expected that information relating to the viability of tumor cells will be obtained from the zinc image.

After implantation of AH7974F cells into the periaqueductal gray of rats, 65Zn uptake in the tumor was ~10 times higher than in other brain regions 1 h after i.v. injection of 65ZnCl2. After implantation of C6 glioma cells into the hippocampus of rats, 65Zn uptake in the tumor was also much higher than in other brain regions. To compare the potential of 65Zn with that of [18F]FDG for the imaging of brain tumors, 14C-FDG imaging of the C6 glioma was performed in the same manner. Because a considerable amount of 14C-FDG was taken up in the brain, 14C-FDG uptake in the tumor was ~1.5 times higher than in the contralateral region. The present findings demonstrate that 65Zn uptake may be more specific for brain tumors than is 14C-FDG uptake, suggesting the potential for 60mZn, a short-half-life γ emitter (t1/2, 13.76 h; energy, 439 keV), in the diagnosis of brain tumors by
Brain tumors. 201 Tl transported into the brain extracellular fluid is taken up by tumor cells in proportion to the activity of the Na+/K+ pump, which somewhat reflects the metabolic activity of the tumors (1, 33). 99mTc and 67Ga, in addition to 201 Tl, are used for brain tumor detection by SPECT. Because these elements are unnecessary for cell functions, however, the movement of these elements does not always reflect physiological response. On the other hand, 65Zn is concentrated in viable regions in peripheral tumor tissues (34). The net zinc uptake in C6 glioma cells occurs only above certain thresholds in time and concentration in vitro, which suggests that excessive zinc is not taken up in C6 glioma cells under physiological conditions (35). Therefore, when radioactive zinc is administered to the patients, it is likely that radioactive zinc transported into brain extracellular space is actively taken up in tumor cells and is used for the functions of the proliferating cells.

In conclusion, the present study demonstrates the potential for brain tumor imaging with 65Zn. Further investigation on prediction of the histological grade of brain tumors, prediction of prognosis, and evaluation of response to treatment is required to determine the extent of its usefulness.

REFERENCES

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