6-Deoxy-6-[¹⁸F]fluoro-L-ascorbic Acid : Tissue Biodistribution in Ascorbic Acid-deficiency and RG-C6 Glioma Bearing Rats

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A comparative study in Wistar normal rats and ODS rats unable to synthesize ascorbic acid showed that the radioactivity distribution in tissues after i. v. injection of 6-deoxy-6-[¹⁸F]fluoro-L-ascorbic acid (¹⁸F-DFA) was not significantly influenced by the vitamin C status of the animal. Biodistribution studies in Wistar male rats implanted with RG-C6 glioma intracerebrally demonstrated that ¹⁸F-DFA had low tumor localization (0.17% dose/g at 30 min and 0.16% dose/g at 60 min) with very little specific distribution compared with non-target brain tissues. These results indicate that the use of ¹⁸F-DFA may not be a candidate for imaging brain tumor by PET.

Key Words : 6-deoxy-6-[¹⁸F]fluoro-L-ascorbic acid, ODS rat, RG-C6 glioma, brain tumor imaging

1. Introduction

Ascorbic acid, which is thought to contribute to many physiological functions such as an enzyme cofactor, antioxidant and neuromodulator, has also been claimed to be effective in the prevention and treatment of cancer⁽¹⁾⁻⁷⁾. It has been shown from both human and animal studies that the development and progress of cancer evoke increased utilization and requirement for ascorbic acid, although it is not clear how it is utilized by tumor cells⁽⁸⁾⁻¹³⁾.

We have recently developed 6-deoxy-6-[¹⁸F]fluoro-L-ascorbic acid (¹⁸F-DFA, Fig. 1) as a positron labeled analog of ascorbic acid for better understanding events associated with the biochemical functions of ascorbic acid⁽¹⁴⁾. Preliminary tissue distribution with ¹⁸F-DFA in mice bearing 3-methylcholanthrene induced fibrosarcoma demonstrated its ability to accumulate in the tumor,

with a tumor-to-blood ratio of 5.4 at 2 h post injection, suggestive of the feasibility of ¹⁸F-DFA as an imaging agent of tumor biochemistry by PET. Furthermore, the slow and low uptake of radioactivity in the normal brain of mice and rats was also observed between 10 and 120 min after injection. These findings gave rise to a possibility that ¹⁸F-DFA might be a suitable agent for PET studies of brain tumors. This study investigated the *in vivo* uptake characteristics of ¹⁸F-DFA in C6 glial tumor bearing rats, as well as in scurvy-prone ODS rats as an ascorbate-deficient model.

2. Materials and Methods

2.1 Preparation of ¹⁸F-DFA

6-Deoxy-6-[¹⁸F]fluoro-L-ascorbic acid (¹⁸F-DFA) was prepared by a slight modification of the method previously described⁽¹⁴⁾. Briefly, to a TPX (polymethylpenten) vessel containing a solution of tetramethylammonium [¹⁸F]fluoride

Fig. 1 Structure of 6-deoxy-6- ^{18}F fluoro-L-ascorbic acid (^{18}F -DFA).

(37 – 74 MBq) in dry acetonitrile (300 μl) was added methyl 2, 3-*O*-isopropylidene-2-keto-L-gulonate 4, 6-cyclic sulfate (2 mg). After heating the mixture at 80 °C for 15 min, the solvent was removed under argon stream. The residue was dissolved in 40% aqueous H_2SO_4 and heated at 100 °C for 10 min. After cooled at ambient temperature, the reaction mixture was extracted with EtOAc (2 ml), run over a Sep-Pak Florisil cartridge, washed with EtOAc (3 ml), and then eluted with H_2O (3 ml) to give the desired radioactivity. The crude product was further purified by HPLC (Whatman Partisil M9 10/25 ODS-2, 3×10^{-4} M potassium phosphate buffer, pH 6.0, 1.0 ml/min). The radioactive fraction ($t_R = 10.2$ min) corresponding to ^{18}F -DFA was collected. The radiochemical yields were about 17% at end of the synthesis (not corrected for decay).

2.2 Preparation of scorbutic model rats

Male ODS (Osteogenic Disorder Shionogi)-od/od rats were purchased from Japan Clea Co., Ltd. For the preparation of scorbutic rats due to deficiency of ascorbic acid, the ODS rats were fed an ascorbic acid free diet CL-2 (Japan Clea) and water containing limited amount of ascorbic acid (0.125 mg/ml) *ad libitem* for between 17 and 24 days as described before^{16)–18)}. All the ODS rats indicated any phenomena, hemorrhage around the eyes and/or nose, and abnormal gait, as the signs of ascorbic acid-deficiency. The rats were

kept at 22 – 25 °C with a 12 h cycle of light and dark.

2.3 Biodistribution of ^{18}F -DFA in scorbutic model rats

The scorbutic rats as an ascorbic acid-deficiency model (135 – 178 g) were injected intravenously with ^{18}F -DFA (214.6 – 695.6 MBq/0.5 ml, 3×10^{-4} M potassium phosphate buffer, pH 6.0). At 30 and 60 min after injection, the rats were killed while under ether anesthesia and the organs were dissected, and weighed. The radioactivity was measured with a Packard auto-gamma 500 scintillation counter and corrected for decay. The results were expressed as percentage injected dose per gram wet tissue (% dose/g).

2.4 Preparation of RG-C6 glioma-bearing rats

The tumor inoculation was performed according to the previously reported procedure¹⁵⁾. The RG-C6 tumor cells were kept frozen until use, then thawed and grown in Eagle's minimum essential medium with 10% fetal calf serum for 72 h. Tumor cells (2.5×10^5 in 5 μl solution) were stereotactically injected into the right cerebral hemisphere of the rats through a pre-bored hole, by means of a Hamilton syringe. The stereotactic coordinates used were 5 mm lateral to the bregma and 5 mm vertically deep to the dual surface. The sham-operated controls were surgically prepared as described above but with the medium solution instead of tumor cells into the right cerebral hemisphere. The tumor-bearing and sham-operated animals were used at 20 – 25 days after inoculation or sham-operation, respectively. The rats were kept at 22 – 25 °C with a 12 h cycle of light and dark. They were allowed free access to water and food *ad libitum*. The intracerebral tumor grew to about 5 mm in diameter and was used for the biodistribution studies.

2.5 Biodistribution of ^{18}F -DFA in RG-C6 glioma-bearing rats

The tumor-bearing and sham-operated rats (270–420 g) were injected intravenously with ^{18}F -DFA (352–925 MBq/0.5 ml, 3×10^{-4} M potassium phosphate buffer, pH 6.0). At 30 and 60 min, the rats were killed while under ether anesthesia and organs were dissected. The brains were then dissected into the following regions: right (or tumor tissue) and left cerebral hemisphere, and cerebellum. The tissue samples were weighed. The radioactivity in each sample was measured with a Packard auto-gamma 500 scintillation counter and corrected for decay. The results were expressed as percentage injected dose per gram wet tissue (% dose/g).

3. Results and Discussion

Rats and mice, which were used as experimental animals in our previous works with ^{18}F -DFA¹⁴, can usually synthesize ascorbic acid in liver and ascorbic acid, therefore, is found in many organs and tissues, including brain¹³. To examine the influence of endogenous ascorbic

acid on uptake and distribution of ^{18}F -DFA, tissue distribution studies were initially undertaken in ODS (Osteogenic Disorder Shionogi) – od/od rats (ODS rats) unable to synthesize ascorbic acid, owing to their lack of hepatic L-gulonolactone oxidase. Ascorbic acid-deficient model rats were prepared by limited diet to the ODS rats^{16)–18)} and the ODS rats used in this study exhibited the signs of typical scorbutic symptoms, such as the discolored skin, hemorrhage around the eyes and nose, and abnormal gait.

The distribution of ^{18}F activity in various tissues in the ascorbic acid-deficient rats following tail-vein injection of ^{18}F -DFA is shown in Table 1, including the data in normal Wistar rats for comparison. High uptake of radioactivity occurred in the adrenals, kidney, liver and small intestine, very similar to that observed in normal Wistar rats. The ^{18}F concentration in the adrenals varied considerably between individual animals for reasons possibly connected with stress.

As is evident from these data, there was no statistically significant differences in ^{18}F concentration of most tissues between the ODS rats and

Table 1 Distribution of radioactivity after i. v. injection of 6-deoxy-6-[^{18}F] fluoro-L-ascorbic acid in normal Wistar rats and scorbutic ODS rats

Tissue	Uptake (% dose/g)*			
	Scorbutic ODS		Normal**	
	30 min (n=4)	60 min (n=3)	30 min (n=4)	60 min (n=3)
Blood	0.26±0.18	0.09±0.02***	0.31±0.14	0.16±0.02
Lung	1.73±0.90	1.51±1.27	1.00±0.62	1.49±0.68
Liver	1.15±0.43	0.71±0.50	1.15±0.72	1.03±0.27
Small intestine	1.62±0.69	1.48±1.24	1.24±1.03	1.51±0.72
Kidneys	2.66±1.53	1.61±0.14	2.30±1.00	1.39±0.17
Adrenals	4.94±3.13	4.57±3.96	4.55±5.49	6.95±2.90
Heart	0.55±0.21	0.46±0.34	0.24±0.05	0.28±0.08
Cerebellum	0.10±0.04	0.08±0.06	0.09±0.04	0.12±0.03
Cerebrum	0.06±0.01	0.06±0.05	0.08±0.03	0.10±0.03
Bone	0.31±0.04	0.21±0.12	0.36±0.13	0.32±0.08

* Means±SD

** Taken from literature (Ref. 14)

*** p<0.05 compared to normal rats

normal Wistar rats, with the exception of the blood circulation where the ODS rats had a slight lower radioactivity when compared with the normal rats. Therefore, it can be considered that endogenous ascorbic acid dose not affect uptake behavior and the resulting tissue distribution of exogenously administered ^{18}F -DFA. Similar result has been obtained with ^{14}C -labeled ascorbic acid, in which as determined by whole body autoradiographic studies, no marked differences in the distribution of radioactivity in the tissues of normally fed and vitamin C-depleted guinea pigs after intraperitoneal injection of [^{14}C] ascorbic acid have been observed¹⁹⁾. Thus, this result suggests that the nutritional state of the test animal is not necessary to be considered when doing ^{18}F -DFA studies.

An animal model of a malignant brain tumor was prepared by implanting C6 glioma cells intracerebrally into right hemisphere of male Wistar rats¹⁵⁾, which had free access to food and water

throughout the duration of the experiments. The rats, with intracerebral tumors greater than 5 mm in diameter, received ^{18}F -DFA 20 to 25 days after tumor cell implantation. The sham-operated controls were subjected to the identical surgical procedure used to implant the tumor cells intracerebrally. Table 2 shows that tumor load did not greatly influence the uptake of ^{18}F -DFA in normal organs. At 30 min post injection, the accumulation of radioactivity in the intracerebral tumor region was only 0.17% dose/g, with tumor-to-contralateral brain tissue (left hemisphere) and tumor-to-blood ratios of 3.4 : 1 and 1.3 : 1, respectively. The radioactivity concentration in the tumor persisted during the time of observation (60 min), but with a seemingly slight increase of radioactivity in the surrounding nontumor brain regions. Animal experiments also showed that ^{18}F -DFA was taken up by the region of the right hemisphere in the sham-operated controls. This area corresponds to approximately the same loca-

Table 2 Distribution of radioactivity after i. v. injection of 6-deoxy-6- ^{18}F fluoro-L-ascorbic acid in transplanted RG-C6 tumors rats ($n=3$)

Tissue	Uptake (% dose/g)*			
	Bearing tumor in cerebrum		Sham-operated	
	30 min	60 min	30 min	60 min
Blood	0.13 $\pm$ 0.03	0.07 $\pm$ 0.02	0.10 $\pm$ 0.02	0.08 $\pm$ 0.01
Liver	1.17 $\pm$ 0.59	0.90 $\pm$ 0.24	1.53 $\pm$ 0.22	1.00 $\pm$ 0.26
Kidneys	1.40 $\pm$ 0.52	0.96 $\pm$ 0.09	1.14 $\pm$ 0.24	0.87 $\pm$ 0.13
Adrenals	3.16 $\pm$ 0.89	6.30 $\pm$ 2.90	6.00 $\pm$ 2.06	6.08 $\pm$ 0.22
Heart	0.20 $\pm$ 0.07	0.24 $\pm$ 0.04	0.25 $\pm$ 0.03	0.24 $\pm$ 0.06
Bone	0.14 $\pm$ 0.05	0.14 $\pm$ 0.01	0.10 $\pm$ 0.03	0.12 $\pm$ 0.05
Cerebellum	0.06 $\pm$ 0.03	0.10 $\pm$ 0.05	0.07 $\pm$ 0.01	0.09 $\pm$ 0.03
Brain				
Tumor**	0.17 $\pm$ 0.08	0.16 $\pm$ 0.02 [†]	—	—
Right hemisphere***	—	—	0.08 $\pm$ 0.01	0.12 $\pm$ 0.02
Left hemisphere	0.05 $\pm$ 0.01	0.08 $\pm$ 0.03	0.07 $\pm$ 0.01	0.08 $\pm$ 0.02
(Tumor/Left hemisphere)	3.44 $\pm$ 0.81	1.95 $\pm$ 0.35	—	—
(Tumor/Blood)	1.31 $\pm$ 0.75	2.22 $\pm$ 0.69	—	—

* Means $\pm$ SD

** Implanted intracerebrally into right hemisphere of Wistar male rats with C6 glioma cells

*** Correspond to approximately the same location as tumor in the C6 glioma bearing rats

[†] $p < 0.01$ versus left hemisphere

tion as tumor in the C6 glioma bearing rats. Furthermore, no statistically significant differences in the uptake were seen between the C6 glioma area and right hemisphere area of the sham-operated rats, indicating poor specificity of ¹⁸F-DFA for intracerebral tumor. ¹⁸F-DFA may have a propensity to accumulate in an inflammatory tissue that might be caused by surgical procedure used here, although there were no significant differences in radioactivity concentration between the left and right hemisphere areas in the sham-operated rats.

It is reported that the blood-brain-barrier (B.B.B) is broken in rats with an intracerebrally transplanted RG-C6 tumor¹⁵⁾. However, in this study the uptake of ¹⁸F-DFA in the tumor-bearing brain was not significantly increased compared to the normal brain, in spite of the probable disruption of the B.B.B. The uptake of ¹⁸F-DFA into the rat brain appears to be *via* the process analogous observed with ascorbic acid, which centers into the cerebrospinal fluid and then into brain²⁰⁾.

In conclusion, ¹⁸F-DFA showed only little capacity to accumulate in C6 cerebral glioma following administration with low contrast between the tumor and surrounding brain tissue and, therefore, it may not be suitable for characterizing brain tumors *in vivo* by PET. Further studies will involve the evaluation of ¹⁸F-DFA in a rat model of cerebral ischemia.

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要 旨

**6-デオキシ-6-[^{18}F]フルオロ-L-アスコルビン酸：アスコルビン酸欠乏症ラット
および RG-C6 グリオーマ移植ラットにおける生体内分布**

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正常ラットと遺伝性アスコルビン酸合成不能ラット (ODS ラット) との比較研究により, 6-デオキシ-6-[^{18}F]フルオロ-L-アスコルビン酸 (^{18}F -DFA) 投与後の生体内放射能分布は, 動物生体内のアスコルビン酸による顕著な影響を受けないことを明らかにした。RG-C6 グリオーマを脳内に移植したラットにおける生体内分布研究の結果, ^{18}F -DFA は腫瘍への取込みが低く腫瘍特異性も低かった。したがって, ^{18}F -DFA は PET による脳腫瘍イメージング剤としては有用性が低いことが示唆された。