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Radiosynthesis and preliminary biodistribution in mice of 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid

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An ascorbate analog labeled with iodine-131, 6-deoxy- 6-[^{131}I]iodo-L-ascorbic acid was prepared for evaluation as an *in vivo* tracer of L-ascorbic acid. The no-carrier-added radiosynthesis was conducted by nucleophilic bromine–iodine exchange between the brominated precursor and sodium [^{131}I]iodide in 2-pentanone at 130–140°C. HPLC purification using a reverse-phase column gave 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid in radiochemical yield of 36–60% with high radiochemical purity and satisfactory-specific radioactivity in a total preparation time of 90 min. Biodistribution studies in fibrosarcoma-bearing mice showed a high uptake in the adrenal glands, accompanied by low activity of tumor accumulation, accumulation properties similar to previous results obtained with ^{14}C-labeled ascorbic acid and 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid, in spite of high level of deiodination.

Keywords: 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid; nucleophilic bromine–iodine exchange; mice; adrenal glands; fibrosarcoma

Introduction

Disruption of redox homeostasis in the living body may lead to oxidative stress capable of inflicting biological damage. Oxidant damage of biological molecules is thought to be of great significance in the development and progress of numerous disease states such as cancer, inflammatory and degenerative diseases.[1] Noninvasive assessment of oxidative stress could therefore be potentially useful in the diagnosis of disease states and in the assessment of treatment response. A variety of intracellular molecules contribute to tissue redox status and, among them, L-ascorbic acid (AsA) plays important physiological roles in cells as a reducing agent, antioxidant, free radical scavenger, and enzyme cofactor.[2] In recent years, it has been demonstrated that AsA is accumulated in mammalian cells by two types of Na^+-dependent transporters, SVCT1 and SVCT2, which show distinct tissue distribution and functional characteristics.[3] The SVCT1 isoform is expressed primarily in the intestine, liver, and kidney, whereas the SVCT2 isoform is found in the adrenal gland, pancreas and choroid plexus. Thus, it is most likely that the activities of these specific membrane transporters crucially influence transport, intracellular accumulation, and recycling of AsA in the living body.[4]

Studies of tissue distribution of the radioactivity of ^{14}C-labeled AsA in animals were initially carried out mainly using whole-body autoradiography, and high levels of radioactivity were observed in the adrenal glands, pituitary glands and parotid glands, depending on the time after injection.[5–7] Colombetti *et al*. proposed that AsA might be useful as a potential imaging agent of the pituitary gland if labeled with a suitable radionuclide.[8] It seems reasonable to anticipate that the development of radiolabeled analogs of AsA with selective transport characteristics and favorable *in vivo* properties may provide a new tool for assessment of tissue redox status induced by oxidative stress, utilizing the nuclear imaging technique.

Among structurally similar AsA analogs, 6-deoxy-6-halo-L-ascorbic acid are very attractive analogs of AsA for the study of the transport and function of the vitamin, without loss of prominent antioxidant activity.[9–15] In earlier studies, we reported the synthesis and *in vivo* biodistribution profile of 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid(6-^{18}FAsA), in which the uptake and distribution pattern of this agent were found to have a remarkable resemblance to that for ^{14}C-labeled AsA, with preferential uptake in the adrenal glands and slow brain uptake kinetics.[16,17] Additional studies in rats have demonstrated that 6-^{18}FAsA increasingly accumulates in damaged regions of the postischemic reperfusion brain,[18] and that tissue glutathione is a factor affecting the *in vivo* accumulation of this agent.[19] On the other hand, the oxidized form of 6-^{18}FAsA, 6-[^{18}F]fluoro-dehydro-L-ascorbic acid, revealed rapid and extensive defluorination in mice.[20] Moreover, it has also been reported by us that the ^{125}I-labeled 2-*O*- and 3-*O*-m-iodobenzyl and 6-*O*-m-iodophenyl derivatives of AsA displayed quite different biodistribution properties from those of ^{14}C-labeled AsA and 6-^{18}FAsA.[21]

6-Deoxy-6-iodo-L-ascorbic acid (6-IAsA) has already been described in the literature[9] and its ^{125}I-labeled form was utilized

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for studying the transport properties in human fibroblasts.[10] It has been proved that 6-IAsA is an effective inhibitor of AsA transport, with transport affinity similar to that of AsA, and is taken up into cells by a Na^+-dependent AsA transporter.[11] Thus, 6-IAsA appeared to be a potential candidate to be developed into an *in vivo* tracer for the study of the uptake and accumulation of AsA due to its distinctive transport characteristics. This paper describes the synthesis and radiochemical synthesis of 6-IAsA, and the preliminary biodistribution in tumor-bearing mice was also evaluated for its ^{131}I-labeled analog.

Results and discussion

Chemistry

In order to use it as an authentic standard, as well as the aim of finding a procedure applicable for labeling with iodine-131, the synthesis of 6-deoxy-6-iodo-L-ascorbic acid (6-IAsA) was initially investigated by using three different approaches (Scheme 1), one of which was employed for the preparation of 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA) in this study. The synthesis of 6-IAsA was reported by Kiss *et al.*[9] and this procedure involved nucleophilic substitution of methyl 2,3-isopropylidene-6-*O*-(toluene-*p*-sulfonyl)-L-gulonate with iodide ion in 2-pentanone, followed by acid hydrolysis. However, in our experience, this two-step procedure gave only 26% of the desired compound. We found that the acid-treatment step proceeded with the great difficulty and was always an obvious complication. Likewise, an alternate attempt to prepare 6-IAsA by involving the nucleophilic opening of the cyclic sulfate compound with iodide ion, on analogy with our previous synthesis of 6-deoxy-6-fluoro-L-ascorbic acid (6-FAsA),[16] was also fruitless (23%), although it was obtained with high purity. It is important to note that 6-IAsA has low stability in solution, like ascorbate itself, and care must be taken to avoid unfavorable oxidative decomposition and byproduct formation, thus limiting the repeated or time-consuming use of HPLC or column chromatography runs. In seeking an additional approach to 6-IAsA, we were prompted to examine the response toward halogen exchange. According to the procedure described by Bock *et al.*,[22] 6-bromo-6-deoxy-L-ascorbic acid (6-BrAsA) was prepared in 56% yield by the reaction of L-ascorbic acid with HBr in AcOH. Subsequent refluxing of 6-BrAsA with NaI in 2-pentanone for 6 h (oil bath: 130°C) resulted in the isolation of 6-IAsA in 73% yield, with slight contamination (less than 5%) of 6-BrAsA. The required heating for a long time reflects the stability of the carbon–bromine bond in regard to the displacement reaction. The ^{1}H-NMR, mass spectra, and the melting point of the compounds obtained by the three procedures described above were in agreement with those reported in the literature. Additional proof of the structure was proved by X-ray crystallography, as shown in Figure 1. Crystals suitable for X-ray analysis were grown from CH_3NO_2 solution as a precipitant at 4°C for one day [23].

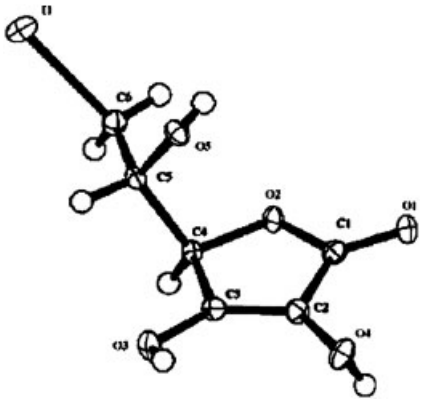
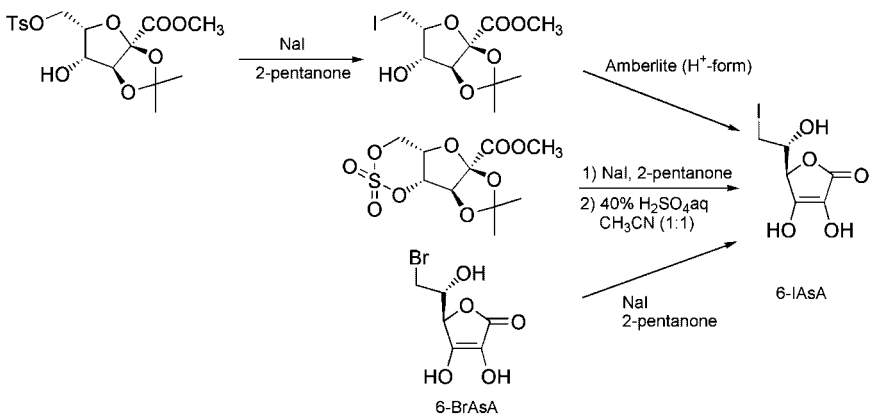


Figure 1. ORTEP drawing of the molecule (Crystal structure of 6-deoxy-6-iodo-L-ascorbic acid).

Radiochemistry

A previous report by Rumsey *et al.*[11] on the preparation of ^{125}I-labeled form of 6-IAsA involved a halogen exchange between methyl 2,3-isopropylidene-6-deoxy-6-iodo-L-gulonate and $Na^{125}I$ in boiling acetone for three days followed by HCl-catalyzed hydrolysis at 60°C for 2 h. A major disadvantage of this radioiodination procedure is the requirement of a prolonged reaction time, although further improvement may be possible, and thus we preferred to develop a single-step labeling procedure, based on the exchange of radioiodine for bromide, because of its simplicity and potential for greater practical yield.

Radioiodination was carried out by heating a solution of 6-BrAsA in 2-pentanone with no-carrier-added $Na^{131}I$, which was dried beforehand (Scheme 2). Because in preliminary experiments the direct use of an aqueous radioiodide dispensing solution (0.1 M NaOH) provided poor incorporation of ^{131}I as had



Scheme 1. Synthetic route for 6-deoxy-6-iodo-L-ascorbic acid (6-IAsA).

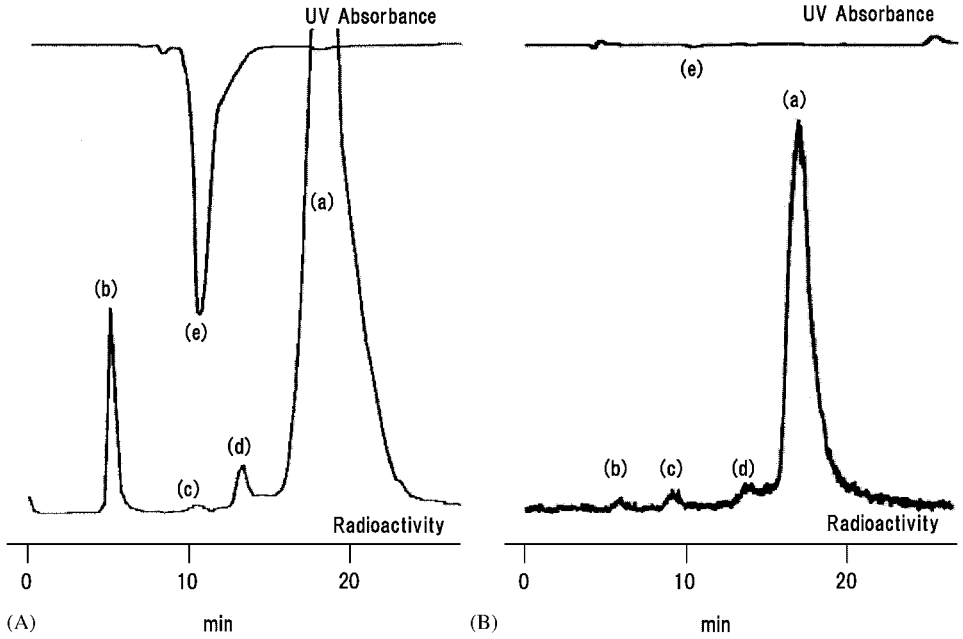
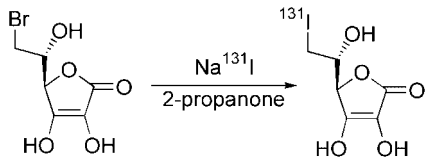


Figure 2. (A) Preparative HPLC-chromatogram of the 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid mixture. HPLC conditions: Nacalai tesque COSMOSIL 5C18 AR-II, 10 × 250 mm); Eluent:0.04 M Na_2HPO_4 with 0.1% triethylamine (pH = 5.65); Flow rate: 2.8 mL/min. HPLC analysis shows (a) the product, 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA), (b) free [^{131}I]iodide, (c) and (d) unknown byproducts, and (e) the precursor, 6-deoxy-6-bromo-L-ascorbic acid. (B) Analytical HPLC chromatogram of the isolated 6-^{131}IAsA fraction.



Scheme 2. Radiochemical synthesis of 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA) by exchange of radioiodine for bromide.

been noted earlier for some other radiolabelings,[24] the water was removed prior to the reaction. The reaction was allowed to proceed by heating in a closed vessel. The incorporation of radioiodine was dependent on the reaction time, temperature and amount of precursor used, and the optimum results were obtained when a concentration of 30 μg–1 mg of 6-BrAsA/300 μL was allowed to react at temperatures approaching 130–140°C for 20–40 min. Neither prolonged heating nor temperatures higher than 140°C significantly led to improved radiolabeling yields. The product was purified by reverse-phase HPLC, the conditions of which were selected to ensure good separation of the precursor, 6-BrAsA, from the radioiodinated product. A solvent system of 0.04 M Na_2HPO_4 with 0.1% triethylamine (pH = 5.65),[25] which was bubbled with nitrogen gas beforehand, was used as the HPLC eluent in order to eliminate solvent evaporation in isolation and also to serve as a formulation medium for intravenous injection. A typical HPLC chromatogram of the hot reaction mixture (Figure 2(A)) had two major radioactive peaks, one due to free radioiodide (peak b) and the other consistent with the required product (peak a) plus two additional very small peaks (c and d). Unreacted 6-BrAsA (peak e) appeared at a retention time of 10 min. Isolated radiochemical yields of 6-^{131}IAsA after HPLC purification ranged from 36 to 60% with a radiochemical purity of 95% in a total preparation time of 90 min. The radioactive impurities were still found in the final sample after HPLC purification, as shown in Figure 2(B). The specific activities of the obtained 6-^{131}IAsA varied from 18 to 74 GBq/μmol as determined by direct measurement of the HPLC eluate using a UV detector. The preparations were chemically pure except for occasionally low-level contamination from 6-BrAsA (3–6 nmol in the final solution) due to tailing during HPLC isolation, depending on the amount of the precursor used. We found that complete removal of the precursor from the final product could be attained when less than 30 μg of the precursor was used for the exchange reaction. When the HPLC-collected fraction containing 6-^{131}IAsA was allowed to stand at 0°C, radiochemical purity was maintained for up to 30 min, after which it dropped to about 85% at 4 h, due to increased formation of radioactive impurities eluted prior to 6-^{131}IAsA as assessed by HPLC.

Biodistribution

Table 1 summarizes the results of the biodistribution of 6-^{131}IAsA to a range of tissues in C3H/He mice bearing fibrosarcoma, which were used as a tumor model due to our additional interest in ascorbate transport and uptake in tumors. There was a somewhat slower clearance of the radioactivity from the blood pool in comparison with 6-^{18}FAsA, but, as expected, the highest concentration of radioactivity was found in the adrenal glands, with a maximum uptake (109.37 ± 28.18%ID/g) at 30 min after injection, which gradually decreased with time. Thus, the results indicate that the preferential *in vivo* uptake in the adrenal glands does not change significantly when iodine atom is substituted for the primary 6-hydroxyl group of the ascorbate molecule.

On the other hand, the thyroid uptake for 6-^{131}IAsA increased from 15.01 %ID/g at 2 min postinjection to 30.39%ID/g

Table 1. Distribution of radioactivity in tissues of fibrosarcoma-bearing mice following intravenous administration of 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA)

Organ	Uptake (%dose/g)[a]					
	6-^{131}IAsA				6-^{18}FAsA[b]	
	2 min	10 min	30 min	60 min	2 min	60 min
Blood	16.37 ± 5.30	6.68 ± 3.33	4.38 ± 0.59	2.96 ± 0.18	4.01 ± 0.44	1.90 ± 1.11
Lung	44.26 ± 24.74	22.12 ± 5.29	22.37 ± 2.39	15.09 ± 4.27	30.10 ± 2.69	7.27 ± 2.49
Liver	10.98 ± 5.65	11.35 ± 1.46	5.66 ± 0.78	3.26 ± 0.37	21.21 ± 1.82	6.23 ± 2.20
Small intestine	24.92 ± 6.77	17.01 ± 2.39	17.45 ± 2.66	9.20 ± 2.55	29.88 ± 2.53	9.71 ± 4.34
Adrenals	47.39 ± 18.19	48.34 ± 16.94	109.37 ± 28.18	56.82 ± 20.70	23.43 ± 8.20	12.90 ± 7.83
Kidneys	28.43 ± 8.88	17.50 ± 2.83	10.87 ± 0.25	5.71 ± 1.00	41.42 ± 6.64	8.31 ± 2.55
Heart	13.78 ± 6.30	6.32 ± 1.13	5.19 ± 0.34	3.75 ± 0.56	4.78 ± 0.17	1.73 ± 0.39
Muscle	3.46 ± 0.07	3.45 ± 0.70	2.42 ± 0.16	1.99 ± 0.50	1.86 ± 0.41	0.76 ± 0.17
Bone	5.60 ± 0.87	5.42 ± 1.81	3.73 ± 0.25	4.03 ± 1.34	2.04 ± 0.18	1.62 ± 1.06
Brain	1.04 ± 0.29	0.66 ± 0.04	0.68 ± 0.03	0.64 ± 0.10	0.73 ± 0.15	0.49 ± 0.08
Thyroid	15.01 ± 4.17	12.33 ± 5.38	19.89 ± 2.47	30.39 ± 9.78	—	—
Tumor	3.55 ± 0.31	6.09 ± 2.61	5.36 ± 0.74	2.97 ± 1.05	4.31 ± 0.05	3.21 ± 0.70

[a]All data were shown as means ± SD ($n = 3$).
[b]Data from References 16, 20 for comparison.

at 60 min, suggesting a high level of *in vivo* deiodination for 6-^{131}IAsA. Compared with the previous results of 6-^{18}FAsA, the tissues that exhibited lower uptake of radioactivity were the liver and kidneys while the lungs had much higher uptake. The accumulation of the radioactivity of 6-^{131}IAsA in the brain was low throughout the course of the study, demonstrating the difficulty to penetrate the blood–brain barrier, as for 6-^{18}FAsA. Moreover, tumor accumulation in the fibrosarcoma was not significant, similar to that of 6-^{18}FAsA. Considering the reported similar transport affinities (Michaelis constant) of 6-BrAsA ($Km = 15.8\,\mu M$ for SVCT2)[15] and 6-IAsA ($Km = 5.2\,\mu M$)[11] having better affinities than AsA itself, the presence of a small amount (1 nmol/mouse) of the precursor (6-BrAsA) in the injectable solution may have caused no little influence on the overall biodistribution of 6-^{131}IAsA, although there is no evidence of this. Nevertheless, the tumor-bearing mouse biodistribution of 6-^{131}IAsA reflected that of 6-^{18}FAsA or ^{14}C-labeled ascorbic acid in that it is rapidly taken up in the adrenal glands with a high level of the vitamin C transporter, SVCT2.

Experimental

General

Chemical reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. Nitromethane from $CaCl_2$ and CH_3CN from CaH_2 was distilled before use. 2-Pentanone (methyl n-propyl ketone) was refluxed with a small amount of $KMnO_4$, then filtered and dried with Na_2SO_4, followed by distillation before use. ^{1}H NMR spectra were obtained on a Varian Inova 400 (400 MHz) and are referenced to TMS ($\delta = 0$ ppm). Infrared (IR) spectra were recorded with a Shimadzu FTIR-8400 spectrometer and the mass spectra were obtained with a JEOL JMS DX-610 (FAB Mass). All melting points were determined on a Yanaco melting point apparatus (Yanagimoto Ind. Co. Japan) and are uncorrected. Column chromatography was performed on Silica gel 60 N (63–210mesh, Kanto Chemical Co. Inc., Japan), the progress of the reaction was monitored by TLC on Silica gel 60F 254 plates (Merck, Germany), and the spots were visualized with UV light or by spraying with 5% alcoholic molybdophosphoric acid. In the synthetic procedures, the organic extracts were routinely dried over anhydrous Na_2SO_4 and evaporated with a rotary evaporator under reduced pressure. Elemental analyses were performed by the staff of the micro-analysis department of Kyushu University. Analysis of the radioactivity of the TLC plates was performed with an Aloka radio chromatogram scanner. HPLC was done by using a Shimadzu Liquid Chromatograph system (SCL/SIL/SPD-6A) (Cosmosil 5C18-AR-II Packed column, 250 × 10 mm, Nacalai Tesque, Japan) and by monitoring the radioactivity (NaI (Tl) detector) as well as the UV absorption (at 254 nm). The radioactivity was quantified with an auto-well gamma counter (ARC-370, Aloka, Japan). Identity of the labeled compound was confirmed from co-injection with authentic samples by HPLC under the same conditions and also by TLC. Specific radioactivity and radiochemical purity were determined by the same HPLC system. No-carrier-added sodium [^{131}I]iodide (0.1 M NaOH solution; ~100 GBq/μmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Animal experiments were carried out in accordance with our institutional guidelines and were approved by the Animal Care and Use Committee, Kyushu University.

Chemistry

6-Deoxy-6-iodo-L-ascorbic acid (6-IAsA)

(a) 6-IAsA was prepared in 26% yield as a white solid by nucleophilic substitution of methyl 2,3-isopropylidene-6-*O*-(toluene-*p*-sulfonyl)-L-gulonate with NaI in 2-pentanone, followed by acid hydrolysis with Amberlite IR-120 (plus) ion exchange resin (Aldrich) according to the literature procedure.[9] Mp = 201–202°C (lit.[9] 203–205°C). IR(KBr) cm^{-1}: 3366, 1744, 1666. FAB-MS (*m/z*): 287($M^+ + 1$). 1H NMR(400 MHz, D_2O):δppm 5.06(d, 1H, $J_{4,5} = 2.0$ Hz); 4.11(ddd, 1H, $J_{5,6} = 5.7$ Hz); 3.43(dd, 1 H, $J_{6,6'} = 10.5$ Hz); 3.36(dd, 1 H, $J_{6',5} = 8.0$ Hz).

(b) A mixture of methyl 2,3-isopropylidene-2-keto-L-gulonate 4,6-cyclic sulfate[16] (1 g) and dried NaI (2.4 g) in 2-pentanone (20 mL) was refluxed for 6 h under argon. After cooling to room temperature, the solvent was evaporated under reduced pressure to give a crude mass, which was chromatographed on silica gel (CH_2Cl_2:MeOH = 1:1) to afford a crude product. A mixture of the above crude iodinated compound and 40% aqueous H_2SO_4 (10 mL) in CH_3CN (10 mL) was heated at 80°C for 1 h. After cooling to room temperature and adding water (20 mL), the resulting mixture was extracted with AcOEt (4 × 5 mL) and dried. Evaporation under reduced pressure of the combined organic layers gave 6-IAsA (215 mg, 23%) as a pale yellow solid, which was recrystallized from CH_3NO_2 to give colorless needles. Mp = 204–205°C (lit.[9] 203–205°C). IR(KBr) cm^{-1}:3366,1744,1666. FAB-MS(*m/z*): 287(M^++1). 1H NMR(400 MHz, D_2O):δppm 5.08(d, 1H, $J_{4,5}$ = 2.1 Hz); 4.12(ddd, 1 H, $J_{5,6}$ = 5.6 Hz);3.44(dd, 1 H, $J_{6,6'}$ = 10.5 Hz); 3.34(dd, 1 H, $J_{6',5}$ = 8.1 Hz). *Anal.* Calcd for $C_6H_7IO_5$: C, 25.20; H, 2.47. Found: C, 25.20; H, 2.49.

(c) A mixture of 6-bromo-6-deoxy-L-ascorbic acid (6-BrAsA, 478 mg), which was obtained by the reaction of L-ascorbic acid with HBr in AcOH according to the literature procedure[22] and dried NaI (370 mg) in 2-pentanone (15 mL), was refluxed for 6 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and water (10 mL) was added to the residue. The mixture was extracted with AcOEt (3 × 10 mL). The organic layer was dried and evaporated to give a crude mass. Addition of CH_2Cl_2 (20 mL) to the mass allowed crystallization. Filtration gave a pale yellow crystal (417 mg, 73%). Recrystallization from CH_3NO_2 gave a colorless crystal. Mp = 206–208°C (lit.[9] 203–205°C). IR(KBr) cm^{-1}: 3366, 1744, 1666. FAB-MS(*m/z*): 287(M^++1). 1H NMR(400 MHz, D_2O):δppm 5.06(d, 1H, $J_{4,5}$ = 2.0 Hz); 4.11(ddd, 1H, $J_{5,6}$ = 5.6 Hz); 3.42(dd, 1H, $J_{6,6'}$ = 10.5 Hz); 3.36(dd, 1H, $J_{6',5}$ = 8.0 Hz). 1H NMR and HPLC analysis showed that the recrystallized sample contained a small amount of 6-BrAsA (less than 5%).

Radiochemistry

6-Deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA)

No-carrier-added $Na^{131}I$(3.7–37 MBq) was added to a vial containing a solution of 6-bromo-6-deoxy-L-ascorbic acid (6-BrAsA, 30 μg-1 mg) in CH_3CN (300 μL). The solvent was evaporated to dryness under a stream of nitrogen at 60°C. Residual water was further removed by azeotropic distillation with additional CH_3CN (2 × 300 μL). To this residue was added anhydrous 2-pentanone (300 μL), and the vial was sealed and then heated at 130–140°C for 20–40 min. After evaporation of the solvent, the residue was reconstituted in the HPLC mobile phase and injected into the HPLC column (Nacalai Tesque COSMOSIL 5C18 AR-II, 10 × 250 mm, 0.04 M Na_2HPO_4 with 0.1% triethylamine (pH = 5.65),[25] flow rate 2.8 mL/min). The product fraction was collected after a retention time of 17 min. The isolated radiochemical yield of 6-^{131}IAsA ranged from 36 to 60% with a radiochemical purity of 95% in a total preparation time of 90 min. The specific activities of the obtained 6-^{131}IAsA varied from 18 to 74 GBq/μmol as determined by direct measurement of the HPLC eluate using a UV detector. The HPLC-collected fraction containing the radiotracer occasionally had low-level contamination from 6-BrAsA (3–6 nmol in the final solution). Complete removal of the precursor from the final product could be attained when less than 30 μg of the precursor was used for the exchange reaction. The radiochemical purity was maintained for up to 30 min at 0°C, after which it dropped to about 85% at 4 h, due to increased formation of radioactive impurities eluted prior to 6-^{131}IAsA as assessed by HPLC.

Biodistribution

NFSa-fibrosarcoma was inoculated s.c. into the right hind leg muscle of female C3H/He mice (5 weeks old, 18–20 g). These mice, which developed tumors with a diameter of about 1 cm at 9–14 days after inoculation, were used for *in vivo* biodistribution studies. The animals were given free access to food and water during the investigation.

The fibrosarcoma-bearing mice were intravenously injected through the tail vein with a solution of 6-^{131}IAsA (10 kBq/g of body weight) in an HPLC eluent (0.3 mL), immediately after HPLC purification. The maximum amount of the precursor (6-BrAsA) in the 6-^{131}IAsA samples was estimated to be about 1 nmol. The mice were sacrificed by exsanguination under anesthesia at a predetermined time after injection of 6-^{131}IAsA. A sample (0.2–0.5 mL) of blood was collected at the time of euthanasia. Samples of the tissues and tumors were excised immediately and weighed. The tissue radioactivity was counted by a gamma counter (Aloka, ARC-370). The tissue radioactivity levels were calculated as a percentage of the injected dose per gram of tissue (%ID/g of tissue).

Conclusion

We prepared 6-deoxy-6-[^{131}I]iodo-L-ascorbic acid (6-^{131}IAsA) by exchanging the corresponding brominated precursor with $Na^{131}I$ in 2-pentanone at 130–140°C. Isolated radiochemical yields of 36–60% with a radiochemical purity of 95% was obtained after HPLC purification in 90 min, and the specific activity (18–74 GBq/μmol) was adequate for *in vivo* studies in small animals. Tissue distribution from tumor-bearing mice with 6-^{131}IAsA is similar to the reported distribution of ascorbic acid and 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid (6-^{18}FAsA), particularly in light of the significant accumulation of radioactivity in adrenal glands, in spite of high level of *in vivo* deiodination. It appeared that 6-^{131}IAsA has limited *in vitro* stability even in the buffer solution used for injection. However, 6-^{131}IAsA showed an increase in the adrenal radioactivity levels up to 30 min post-injection in the biodistribution study. The presence of carrier molecule, as well as 6-BrAsA in the injection, or endogenous molecules in the body might have contributed partially as antioxidants to the *in vivo* stability for 6-^{131}IAsA. It may be also necessary to develop a suitable method for stabilization of the 6-^{131}IAsA post preparation. Further studies in animal models are in progress to determine whether uptake and accumulation of radioactivity of this agent in the adrenal glands is associated with adrenal function.

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[23] X-ray Crystallographic analysis data: Crystal data for 6-IAsA: Crystal dimensions 0.3 mm × 0.2 mm × 0.1 mm, $C_6H_7O_5I$, MW = 286.02, monoclinic, space group $P2_12_12_1$ (no. 19), $a = 8.146(4)$ Å, $b = 8.525(5)$ Å, $c = 12.243(8)$ Å, $V = 850.2(8)$ Å^3, $Z = 4$, $T = 123$ *K*, 1924 reflections measured, 1924 unique reflections ($R_{int} = 0.000$), refinement with 135 parameters converged with agreement factors R_1 ($I > 2s$) = 0.0225, wR_2 (all data) = 0.0581, GOF = 1.31. X-ray data were collected on a Rigaku Radix-Rapid diffractmeter with graphite monochromated MoKa radiation ($l = 0.71069$ Å). The molecular structures were solved by direct method (SIR program)[23–1] and expanded using Fourier techniques (DIRDF99)[23–2] . The refinements were converged using the full-matrix least-squares method from the Crystal Structure software package[23–3] to give the $P2_12_12_1$ (no. 19) space group. All non-hydrogen atoms were refined anisotropically; hydrogen atoms were included at standard positions (C–H = 0.96Å, C–C–H = 120Å) and refined isotropically using a rigid model.23-1) a) SIR92: A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst*. **1994**, *27*, 435; b) SIR97: A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Cryst*. **1999**, *32*, 115–119; 23-2) DIRDIF99: P. T. Beurskens, G. Admiraak, G. Beurskens, W. P. Bosman, R. de Gelder, R. Israel, J. M. M. Smits, **1999**, The DIRDIF-99 program system, *Technical Report of the Crystallography Laboratory*, University of Nijmegen, the Netherlands; 23-3) Crystal Structure 3.8: Crystal Structure Analysis Package, Rigaku and Rigaku/MSC (2000–2006). 9009 New Trails Dr The Woodlands TX 77381 USA.

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