Development of a Novel Radioiodinated Glucose Derivative with Interaction to Hexokinase

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SUMMARY

Development of an ^{123}I-labeled glucose derivative that interacted with hexokinase was attempted. By introducing a benzene ring into the glucosamine molecule, we were able to secure a radioiodinated compound, N-iodobenzoyl-D-glucosamine (BGA), with enhanced stability. As a result, a non-competitive inhibitory agent on the hexokinase-regulated phosphorylation reaction was achieved. The inhibitory action and lipophilic property of this novel compound were closely related to an amide bond in its structural configuration. Moreover, on investigating the biodistribution in mice, although this ^{125}I-labeled compound did not display any uptake into the brain, it demonstrated rapid clearance from the blood with high systemic stability. From the above findings, it is highly possible to develop a clinically feasible ^{123}I-labeled radioligand that can monitor the quantitative changes and biodistribution of hexokinase.

Key words; ^{123}I, ^{125}I, Radioiodinated glucose derivative, hexokinase, SPECT.

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Introduction

Recent availability of ^{123}I and the rapid progress in spatial resolution attained by SPECT call for the development of single-photon emitting radiopharmaceuticals. In our attempt to develop a glucose radiopharmaceutical analog, data furnished by the ^{18}F-FDG (1-3) with regard to measurements of both the glucose transport rate across blood-brain barrier (BBB) and hexokinase-regulated phosphorylation rate provide a strategic basis for us to design a clinically feasible radioligand.

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We have recently synthesized a radioiodinated glucose derivative, the 2-O-(p-iodobenzyl)-D-glucose (IBG) (4). This compound, encompassing an iodinated benzene ring, provides the necessary structural configuration to remain stable *in vitro* and *in vivo* reactions. IBG mediates permeability of the glucose carrier across the BBB. However, as this compound is a poor substrate for the enzyme, hexokinase, there exists a potential feasibility to study the glucose transport rate. Next, we designed a radioiodinated glucose derivative to measure parameters related to the ensuing biochemical event, hexokinase-regulated phosphorylation reaction. According to Maley and Lardy (5), various glucosamine derivatives have been reported as competitive inhibitors of hexokinase. Of these, the N-benzoyl-glucosamine analog was considered the most plausible compound to be exploited as a clinical agent. Particularly with the presence of an aromatic ring in its structure, stable radioiodinated derivatives may be made available. In this study, we reported on the synthesis of some N-benzoyl-glucosamine derivatives, and their effects on hexokinase reaction and physical properties were evaluated in comparison with IBG.

Materials and Methods

D-Glucosamine hydrochloride (Nakarai Chemicals Co., Ltd.) was used without recrystallization. Hexokinase and adenosine-5'-triphosphate disodium salt were obtained from SIGMA Chemical Co. and Kohjin Co. Ltd, respectively. Other guarantee-graded reagents were commercially available. Thin layer chromatography (TLC) was carried out with silica gel plates (Kieselgel 60; Art 5553). Radioactive tracer, ^{125}I-NaI (3.7 GBq/mL) in NaOH solution of pH 7-11, was purchased from Amersham, Japan Co. Ltd. The absorbance was determined with a spectrophotometer (330-S, Hitachi Co., Ltd.) and NMR spectra were taken (XL-300; Varian, U.S.A.) with tetramethylsilane as the internal standard. Prior to the experiments, male ddY mice (Shimizu Experimental Supplies, Japan) were acclimatized for at least 1 week, and animal chow and drinking water were allowed ad libitum. Animal experiments were carried out according to guidelines stipulated by the Kyoto University Animal Care and Use Committee.

Synthesis of N-(iodo-benzoyl)-D-glucosamine (BGA)

BGA was synthesized (Fig.1) using glucosamine hydrochloride as the starting material. During the synthetic process, the following intermediates were obtained:

N-(p-methoxy-benzyliden)-glucosamine (1)(6)

Glucosamine hydrochloride (9.0 g; 0.042 mol) was dissolved in 1 N NaOH solution (42.3 mL). After adding 5.76 g of p-anisaldehyde (0.042 mol) into this mixture,

the solution was stirred for 3 h at room temperature and subsequently left standing for 30 min at 0 °C. The collected crude product was first washed with cold water followed by a mixture of ethanol:ether (1:1). The obtained crystals (1), dried in a desicator, were used in the next stage of the synthetic process. The yield was 9.6 g (77 %).

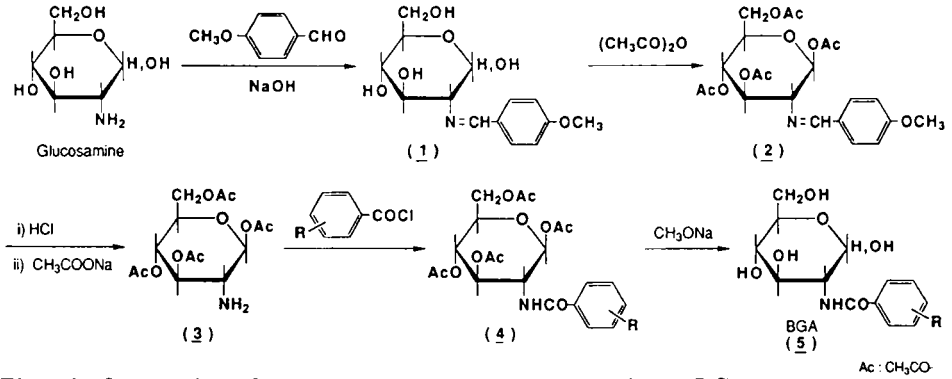

Fig. 1 Synthesis of N-(iodo-benzoyl)-D-glucosamine (BGA) analogues.

N-(p-methoxy-benzyliden)-1,3,4,6-tetra-O-acetyl-glucosamine (2)(6)

Compound (1) (5.0g; 0.017 mol), placed in acetic anhydride (15 mL) containing dry pyridine (27 mL), was stirred for 30 min at 0 °C. After stirring for another 24 h at room temperature, the mixture was poured into ice-cold water and vigorously stirred for 2 h. The crystallized product (2) was collected and washed with cold water and recrystallized from methanol thereafter. The yield was 7.1 g (90 %).

1,3,4,6-tetra-O-acetyl-glucosamine (3)(7)

Having dissolved 5 .0 g (0.01 mol) of compound (2) in acetone (25 mL), concentrated HCl (1 mL) was added prior to standing the mixture for 5 min. The collected crystals were washed with cold ether, resuspended in 2 M sodium acetate solution (50 mL) and thereafter extracted by chloroform (3 x 50 mL). The chloroform layer was decanted, dried with sodium sulfate and evapolated to dryness in vacuo. The obtained crystals (3) were recrystallized from ether. The yield was 2.9 g (77 %).

General procedures for the synthesis of N-(iodo-benzoyl)-1,3,4,6-tetra-O-acetyl-glucosamine (4a-d)(6)

Iodo-benzoic acid (6.45×10^{-3} mol) was stirred in thionyl chloride (10 mL) for 24 h at 65 °C. Non-reacted thionyl chloride in the mixture was removed by evaporating it in vacuo. Iodo-benzoyl chloride obtained was mixed with benzene (2 mL) prior to adding into a mixture of benzene (10 mL) and pyridine (2 mL)

containing dissolved compound (3) (2 g; 5.76×10^{-3} mol). After stirring for 48 h, the mixture was neutralized with 0.1 N HCl solution and the compound (4a-d) was extracted by chloroform. The chloroform layer was decanted, dried and evaporated to dryness in vacuo. The extracted crystals (4a-d) were recrystallized from methanol.

N-(o-iodo-benzoyl)-1,3,4,6-tetra-O-acetyl-glucosamine (4a)

Yield 40 %. mp. 234-236 °C. Elemental analysis calculated for $C_{21}H_{24}INO_{10}$, C;43.69%, H;4.19%, N;2.43%, found C;43.58%, H;4.20%, N;2.43%.NMR(DMSO-d6); 1.994(s,3H,$OCOCH_3$), 2.017(s,3H,$OCOCH_3$), 2.024(s,3H,$OCOCH_3$), 2.130(s,3H,$OCOCH_3$), 3.988(ddd,1H,C_5-H), 4.013(dd,1H,C_{6a}-H), 4.144(ddd,1H,C_2-H), 4.215(dd,1H,C_{6b}-H), 4.469(t,1H,C_5'-H), 4.961(t,1H,C_4-H), 5.259(t,1H,C_3-H), 5.793(d,1H,C_1-H), 7.096(dd,1H,C_6'-H), 7.179(dt,1H,C_4'-H), 7.884(d,1H,C_3'-H), 8.607(d,1H,NH).

N-(m-iodo-benzoyl)-1,3,4,6-tetra-O-acetyl-glucosamine (4b)

Yield 45 %. mp. 243-245.5 °C. Elemental analysis calculated for $C_{21}H_{24}INO_{10}$, C;43.69%, H;4.19%, N;2.43%, found C;43.67%, H;4.21%, N;2.33%. NMR($CDCl_3$); 2.037(s,3H,$OCOCH_3$), 2.083(s,6H,$OCOCH_3$), 2.110(s,3H,$OCOCH_3$), 3.900(ddd,1H,C_5-H), 4.173(dd,1H,C_{6a}-H), 4.303(dd,1H,C_{6b}-H), 4.577(ddd,1H,C_2-H), 5.222(t,1H,C_4-H), 5.355(dd,1H,C_3-H), 5.795(d,1H,C_1-H), 6.572(d,1H,NH), 7.129(t,1H,C_5'-H), 7.645(dt,1H,C_6'-H), 7.825(dt,1H,C_4'-H), 8.056(t,1H,C_2'-H).

N-(p-iodo-benzoyl)-1,3,4,6-tetra-O-acetyl-glucosamine (4c)

Yield 55 %. mp. 252-254 °C. Elemental analysis calculated for $C_{21}H_{24}INO_{10}$, C;43.69%, H;4.19%, N;2.43%, found C;43.75%, H;4.11%, N;2.45%. NMR($CDCl_3$); 1.998(s,3H,$OCOCH_3$), 2.067(s,3H,$OCOCH_3$), 2.071(s,3H,$OCOCH_3$), 2.108(s,3H,$OCOCH_3$), 3.871(ddd,1H,C_5-H), 4.166(dd,1H,C_{6a}-H), 4.299(dd,1H,C_{6b}-H), 4.556(ddd,1H,C_2-H), 5.215(t,1H,C_4-H), 5.308(t,1H,C_3-H), 5.795(d,1H,C_1-H), 6.481(d,1H,NH), 7.414(t,2H,C_2',$_6$'-H), 7.749(d,2H,C_3',$_5$'-H).

N-(p-iodo-m-methyl-benzoyl)-1,3,4,6-tetra-O-acetyl-glucosamine (4d)

Yield 35 %. mp. 222.5-224 °C. Elemental analysis calculated for $C_{22}H_{26}INO_{10}$, C;44.68%, H;4.43%, N;2.37%, found C;44.78%, H;4.43%, N;2.01%. NMR($CDCl_3$); 2.000(s,3H,$OCOCH_3$), 2.066(s,3H,$OCOCH_3$), 2.073(s,3H,$OCOCH_3$), 2.108(s,3H,$OCOCH_3$), 2.412(s,3H,C_3'-H), 3.881(ddd,1H,C_5-H), 4.167(dd,1H,C_{6a}-H), 4.303(dd,1H,C_{6b}-H), 4.572(ddd,1H,C_2-H), 5.221(t,1H,C_4'-H), 5.326(dd,1H,C_3-H), 5.799(d,1H,C_1-H), 6.500(d,1H,NH), 7.139(dd,1H,C_6'-H), 7.580(d,1H,C_2'-H), 7.811(d,1H,C_5'-H).

General procedures for the synthesis of N-(iodo-benzoyl)-D-glucosamine (BGA)(5a-d)

Sodium ethoxide (1 N; 1 mL) was added into a suspension containing compound (4a-d) and absolute methanol. After stirring for 24 h, the crystallized compound was collected and recrystallized from ethanol. The yields, decomposition point, NMR/elemental compositions of these BGA species were evaluated, and their results are as follows.

N-(o-iodo-benzoyl)-D-glucosamine (o-BGA)(5a)

Yield 70 %. mp. 202-205 °C(decomp.). Elemental analysis calculated for $C_{13}H_{16}INO_6$, C;38.16%, H;3.94%, N;3.42%, found C;37.94%, H;3.87%, N;3.21%. NMR(CDCl3,ppm); 3.080-3.775(m,$C_{2,3,4,5,6a,6b}$-H), 4.438(t,C_6-OH_α), 4.539(t,C_1-H_β,C_6-OH_β), 4.641(d,C_3-OH_α), 4.836(d,1H,C_3-OH_β), 4.927(d,C_4-OH_α), 4.954(d,C_4-OH_β), 5.115(t,C_1-H_α), 6.460(d,C_1-OH_α), 6.582(d,C_1-OH_β), 7.141(dd,C_6'-H_β), 7.166(dd,C_6'-H_β), 7.475-7.360(m,C_4'-$H_{\alpha,\beta}$,C_5'-$H_{\alpha,\beta}$), 7.870(dd,C_3'-H_α), 7.884(dd,C_3'-H_β), 8.070(d,NH_α), 8.164(d,NH_β).

N-(m-iodo-benzoyl)-D-glucosamine (m-BGA)(5b)

Yield 89 %. mp. 195-198 °C(decomp.). Elemental analysis calculated for $C_{13}H_{16}INO_6$, C;38.16%, H;3.94%, N;3.42%, found C;37.83%, H;3.92%, N;3.14%. NMR(CDCl3,ppm); 3.090-3.835(m,$C_{2,3,4,5,6a,6b}$-H), 4.443(t,C_6-OH_α), 4.541(t,C_6-OH_β), 4.595(t,C_1-H_β), 4.596(d,C_3-OH_β), 4.701(d,1H,C_3-OH_α), 4.958(d,C_4-OH_α), 4.960-4.995(d,C_4-OH_β), 5.055(t,C_1-H_α), 6.456(d,C_1-OH_α), 6.570(d,C_1-OH_β), 7.267(t,C_5'-H_α), 7.280(t,C_5'-H_β), 7.840-7.900(m,C_4'-$H_{\alpha,\beta}$,C_6'-$H_{\alpha,\beta}$), 8.219(t,C_2'-H_β), 8.205-8.330(d,$NH_{\alpha,\beta}$), 8.287(t,C_2'-H_α).

N-(p-iodo-benzoyl)-D-glucosamine (p-BGA)(5c)

Yield 80 %. mp. 198-201 °C(decomp.). Elemental analysis calculated for $C_{13}H_{16}INO_6$, C;38.16%, H;3.94%, N;3.42%, found C;38.03%, H;3.88%, N;3.24%. NMR(DMSO-d6,ppm); 3.060-3.825(m,$C_{2,3,4,5,6}$-H), 4.294-4.564(br,C_6-OH), 4.605(d,C_1-H_β), 4.675-5.041(br,$C_{3,4}$-OH), 5.066(d,C_1-H_α), 6.264-6.687(br,C_1-OH), 7.649(d,C_2'-H_β,C_6'-H_β), 7.692(d,C_2'-H_α,C_6'-H_α), 7.842(d,C_3'-H_α,C_5'-H_α), 7.855(d,C_3'-H_β,C_5'-H_β), 8.152(d,NH_α), 8.252(d,NH_β).

N-(p-iodo-m-methyl-benzoyl)-D-glucosamine (pm-BGA)(5d)

Yield 55 %. mp. 227-230 °C(decomp.). Elemental analysis calculated for $C_{14}H_{18}INO_6$, C;39.73%, H;4.29%, N;3.31%, found C;39.66%, H;4.22%, N;3.26%. NMR(DMSO-d6,ppm); 2.420(s,CH_3), 3.085-3.830(m,$C_{2,3,4,5,6a,6b}$-H), 4.446(t,C_6-OH_α), 4.544(t,C_6-OH_β), 4.598(dd,C_1-H_β), 4.693(d,C_3-OH_α), 4.905(d,1H,C_3-OH_β), 4.959(d,C_4-OH_α), 4.977(d,C_4-OH_β), 5.059(dd,C_1-H_α), 6.454(d,C_1-OH_α), 6.554(d,C_1-OH_β), 7.403(dd,C_6'-H_β), 7.447(dd,C_6'-H_α), 7.797(d,C_2'-H_β), 7.861(d,C_2'-H_α), 7.907(d,C_5'-H_α), 7.921(d,C_5'-H_β), 8.095(d,NH_α), 8.201(d,NH_β).

Assay on phosphorylation rate

The phosphorylation rate of BGA by hexokinase was assayed according to the method described previously (4). Into 2.5 mL assay solution (pH 8.5 with glycylglycine and $MgCl_2$), substrate solution (0.4 mL) and 0.5 mL hexokinase solution (16U/mg/2 mL) were added and mixed well. Changes in absorbance at 560 nm were recorded between 15 and 75 s after mixing.

Effects of BGA on hexokinase-regulated phosphorylation rate

The effects of BGA on hexokinase-regulated phosphorylation were assayed. Glucose ($1.0x10^{-3}$ - $3.0x10^{-3}$ M) or ATP ($3.0x10^{-3}$ - $2.0x10^{-2}$ M) solution (0.2 mL) and BGA solution (0.2 mL; concentration range: $2.0x10^{-3}$ and $4.0x10^{-3}$ M) were added and mixed well with the assay solution (2.5 mL). After mixing this glucose/BGA or ATP/BGA mixture with a hexokinase solution (0.1 mL; 13-16 U/mg/2mL), changes in absorbance were recorded at the post-mixing time mentioned above. As a control, 0.2 mL glucose or ATP solution and distilled water (0.2 mL) were mixed with the assay solution (2.5 mL) and hexokinase enzyme. Changes in absorbance were recorded after mixing. The results were analyzed with reciprocal plots and their respective inhibition constants, Ki, were calculated, accordingly.

Labelling by radioisotopic exchange

Radioisotopic exchange reaction with radionuclide ^{125}I (supplied as NaI) was carried out using divalent Cu as the catalyst (4). BGA (4 mg; 0.01 mmol) was dissolved in a mixture of ethanol (0.5 mL) and water (0.5 mL). Into this mixture, 20 uL CuSO4 ($4x10^{-3}$ M), 10 uL $(NH_4)_2SO_4$ ($1.5x10^{-1}$ M) and ^{125}I-NaI alkaline solution of 37-148 MBq were added. The mixture was heated at 85 °C for 3 h. In order to remove the non-reacted excessive ^{125}I, the mixture was subjected to the silica gel column chromatography after cooling, and eluted further with a chloroform:methanol (8:2) mixture. Radiochemical purity was assayed by TLC with silica gel plates. A chloroform:methanol (7:3) mixture was used as the solvent.

Partition ratio between n-octanol and water

The partition ratio was measured by mixing 20 uL of ^{125}I-BGA (20 KBq) in a previously mixed and saturated solution of 0.1 M phosphate buffer (2.0 mL, pH 7.4) and n-octanol (2.0 mL). The mixture was vortexed for 30 s and incubated for 30 min at 37 °C. After centrifugation for 10 min at 3000 rpm(700 G), aliquots (0.1 mL) of each phase were taken, and the radioactivity was determined with a well type scintilation counter. The partition ratio was determined by dividing the radioactivity in n-octanol phase with that in the buffer phase.

Biodistribution in normal mice

A dose of 20 KBq of each ^{125}I-BGA analog was injected into the tail vein of male ddY mice weighing about 30 g. At appropriately specified time intervals, the animals were sacrificed and their organs isolated. Blood was sampled by cardiac puncture and organs were weighed prior to recording their radioactivity counts with a well counter. Results were expressed as %dose/g organ weight.

RESULTS

Synthesis and chemical stability of BGA derivatives:

The respective BGA derivatives (Fig. 1) were synthesized through a 5-step reaction. Synthesized BGA derivatives remained stable even when these compounds were stored for 3 months in a dessicator at room temperature.

Effects on hexokinase-regulated glucose phosphorylation:

Regarding the action of BGA on hexokinase-regulated glucose phosphorylation, effects of a typical example with radioiodination performed at the m-position (m-BGA) on this reaction indicated by the double reciprocal plot in Fig. 2. The inhibitory effect of m-BGA on hexokinase-regulated phosphorylation reaction was non-competitive against glucose (Fig. 2A), and the inhibition constant value, Ki, was evaluated as 1.98 x 10^{-4} M (Fig. 2A). Further, the effects of m-BGA on the action of ATP on phosphorylation reaction were investigated (Fig. 2B). This BGA derivative indicated a competitive inhibitory effect with a Ki

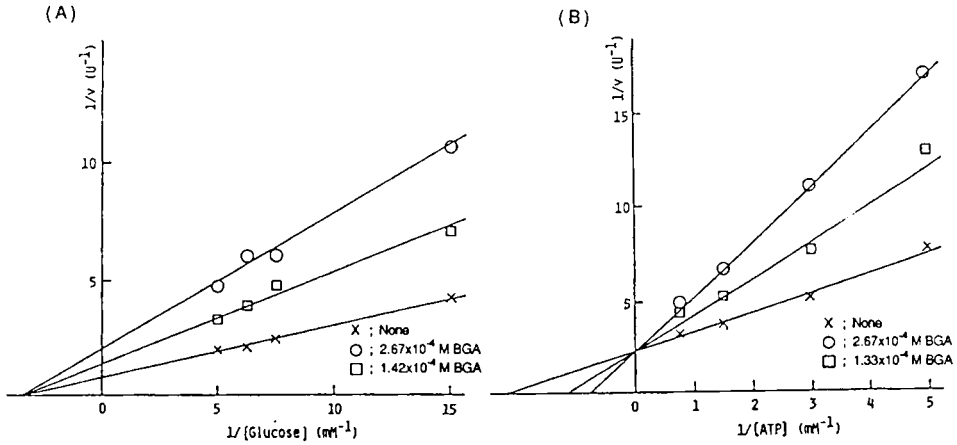

Fig. 2 Effects of N-(m-iodo-benzoyl)-D-glucosamine (m-BGA) on the hexokinase activity.

Assay procedure is discribed in the method section. (A) : assayed under a constant concentration of ATP, (B) : assayed under a constant concentration of glucose.

value of 1.39 x 10^{-4} M on hexokinase-regulated phosphorylation reaction against ATP. The respective Km and Vmax values of hexokinase on reactions associated with glucose or ATP coincided well with previous findings (8-10). Moreover, the respective Ki values in hexokinase reactions involving other derivatives are indicated in Table 1. As o-BGA elicited a meager inhibitory effect on glucose reaction, its inhibitory action was unable to be determined. Unlike o-BGA, p-BGA and pm-BGA displayed an inhibitory action in a manner similar to that of m-BGA.

Table 1. Inhibition to phosphorylation reaction of hexokinase and partition ratio of iodinated glucose derivatives.

Compound	Ki value* (M) (vs glucose)	Ki value** (M) (vs ATP)	Partition ratio#
o-BGA	n.d.	$3.07X10^{-3}$	n.d.
m-BGA	$1.98X10^{-4}$	$1.39X10^{-4}$	0.47±0.05
p-BGA	$1.16X10^{-4}$	$1.05X10^{-4}$	0.55±0.06
pm-BGA	$5.07X10^{-5}$	$1.05X10^{-4}$	1.14±0.12
IBG(4)	no inhibition	no inhibition	1.42±0.11

* Km(glucose) : $2.89X10^{-4}$ M, Vmax(glucose) : 0.77 U^{-1}.
** Km(ATP) : $4.65X10^{-4}$ M, Vmax(ATP) : 2.26 U^{-1}.
# Octanol/Phosphate buffer (pH 7.4).
n.d. : not determined.

Labelling reaction (Iodination) :

Using Cu^{2+} as the catalyst, BGA analogs were labeled with ^{125}I according to the radioisotopic exchange reaction in a manner similar to IBG iodination (4). By developing TLC with a mixture of chloroform:methanol (7:3), the labelling yield was evaluated. The Rf value for the radioiodinated glucosamine derivative ranged from 0.52-0.62, whereas ^{125}I-NaI indicated a value of 0.29-0.38. By heating the mixture at a temperature of 85 °C for 3 h, traces of the non-reacted ^{125}I-NaI were no longer detected, and a peak representing the dissociated compound(s) of Rf value 0.61-0.71 was obtained. On using a solvent system of chloroform:methanol (8:2) in a silica gel column chromatography, the dissociated compound(s) can be easily removed. The radiochemical yields for o-, m-, p- and pm-BGA were 3.0, 91.0, 83.2 and 78.7%, respectively. In the case of o-BGA, despite a reaction time of 10 h, the maximum yield of the labeled radioligand was only 5.5%, rendering labelling of this derivative infeasible. As for ^{125}I-labeled compounds in our study, the radiochemical purity of all radioiodinated compounds was more than 97%. Moreover, the position of the radioactive peak in the TLC analysis converged with that depicted by coloration of the qualitative assay with naphthoresorcinol(11). The radioiodinated compounds were stable when stored at 4 °C for 3 months.

Partition ratios of the labeled compounds between octanol and buffer

Partition ratios between octanol and buffer for the labeled compounds were determined, and the values at pH 7.4 are indicated in Table 1. The lipophilicity increased in the following rank order: m- < p- < pm-BGA. The partition ratio of pm-BGA was approximately equivalent to that of IBG.

Biodistribution in mice:

Tables 2-4 illustrate the results of biodistribution of the various ^{125}I-BGA derivatives in normal mice. In all these radioligands, clearance from blood manifested a biphasic pattern, and m-BGA indicated the most rapid clearance from blood. Further, relatively high uptakes in the kidneys and liver were observed from the early stage post-administration. Thereafter clearance from these organs was prompt as well. In all labeled compounds, uptake in the stomach registered a consistent value of about 1% from the point of administration to the 3-hr period that ensued. In addition, m-BGA indicated a peak uptake in the pancreas, especially at 20 min post-administration. With regards to other organs, the clearance was observed to be rapid equivalently to that from blood.

Table 2. Biodistribution of N-(m-^{125}I-iodo-benzoyl)-D-glucosamine (m-BGA) in normal mice.

	Time after i.v. injection (min)				
Organ	2	5	20	60	180
Blood	14.19(3.00)*	6.23(0.50)	2.83(0.39)	0.94(0.19)	0.26(0.05)
Liver	8.17(0.51)	12.52(0.36)	10.38(2.44)	2.75(0.44)	0.68(0.18)
Kidney	13.25(1.54)	21.35(3.04)	13.86(3.44)	6.17(1.51)	0.53(0.13)
Stomach	1.35(0.53)	1.43(0.05)	1.29(0.21)	1.09(0.61)	1.04(0.24)
Intestine	1.59(0.38)	2.46(0.54)	2.42(0.69)	1.74(0.78)	0.94(0.25)
Pancreas	4.42(1.03)	6.82(1.06)	7.80(1.57)	4.78(0.68)	1.88(0.40)
Heart	5.28(1.09)	2.62(0.30)	1.22(0.21)	0.72(0.08)	0.29(0.03)
Brain	0.39(0.04)	0.18(0.03)	0.09(0.02)	0.04(0.02)	0.01(0.01)

* Each value is mean(s.d.) for 4 animals (%dose/g of organ).

Table 3. Biodistribution of N-(p-^{125}I-iodo-benzoyl)-D-glucosamine (p-BGA) in normal mice.

	Time after i.v. injection (min)				
Organ	2	5	20	60	180
Blood	6.78(1.38)*	3.72(0.29)	2.19(0.14)	1.47(0.20)	0.90(0.12)
Liver	13.30(2.18)	17.98(2.24)	21.63(1.59)	19.44(2.76)	11.35(1.82)
Kidney	21.44(4.01)	23.85(3.67)	17.66(1.29)	9.85(1.20)	3.62(0.33)
Stomach	1.45(0.22)	1.73(0.19)	2.03(0.63)	1.75(0.57)	1.08(0.30)
Intestine	3.35(0.51)	3.55(0.65)	4.20(0.16)	4.40(0.41)	3.35(0.49)
Pancreas	3.98(1.24)	4.95(1.17)	4.43(0.16)	4.63(0.16)	3.44(0.24)
Heart	3.39(0.67)	2.14(0.20)	1.73(0.10)	1.45(0.13)	1.19(0.11)
Brain	0.18(0.05)	0.11(0.01)	0.08(0.01)	0.04(0.01)	0.05(0.02)

* Each value is mean(s.d.) for 4 animals (%dose/g of organ).

DISCUSSSION

In an attempt to develop a clinical radioligand to study the glucose pharmacokinetics by SPECT diagnosis, we employed ^{123}I as the labelling nuclide for glucose. Previous studies on the radioiodinated glucose derivative, 2-(p-iodobenzyl)-glucose(IBG), have been investigated (4). The use of a radiopharmaceutical that can selectively evaluate systemic glucose transport was deemed possible. In our present study, ^{123}I-labeled agents were developed with the objective to possibly extract information related to hexokinase activities. As such, we focused on the N-acyl derivative of glucosamine, a compound that has interaction to the hexokinase reaction. Further, based on investigations on IBG, we considered that by introducing a benzene ring into the glucosamine, we could effectively achieve chemical stabilization of the radioiodinated compounds. We therefore designed the agent, N-(iodo-benzoyl)-D-glucosamine (BGA), in our present study.

Table 4. Biodistribution of N-(p-^{125}I-iodo-m-methyl-benzoyl)-D-glucosamine (pm-BGA) in normal mice.

	Time after i.v. injection (min)				
Organ	2	5	20	60	180
Blood	6.39(1.06)*	3.54(0.36)	1.94(0.28)	1.12(0.23)	0.69(0.04)
Liver	14.12(2.06)	19.26(2.24)	19.62(1.39)	14.13(1.78)	8.44(1.60)
Kidney	21.92(2.82)	30.45(3.38)	18.81(4.57)	8.16(0.96)	3.74(0.68)
Stomach	0.99(0.26)	1.18(0.42)	1.64(0.30)	1.47(0.48)	2.05(1.41)
Intestine	1.77(0.68)	2.67(0.24)	1.92(0.33)	2.39(0.69)	3.96(0.52)
Pancreas	4.83(1.43)	4.53(1.36)	5.06(0.90)	5.04(0.74)	5.05(1.06)
Heart	3.51(0.63)	2.23(0.64)	1.47(0.23)	1.10(0.23)	1.17(0.07)
Brain	0.28(0.06)	0.16(0.03)	0.09(0.03)	0.11(0.08)	0.05(0.01)

* Each value is mean(s.d.) for 4 animals (%dose/g of organ).

Through a 5-step reaction with glucosamine as the starting material, BGA was synthesized (Fig. 1). Structures of the respective compounds obtained were evaluated based on results of qualitative analyses. Besides, introducing a benzene ring into the glucosamine in a manner similar to IBG synthesis enabled the synthesized BGA derivatives to remain, as expected, stable for a period of 3 months when stored at 4 °C. This implicates that the radiochemical stability of such a synthesized BGA derivative is relatively high.

On investigating the effects of BGA on hexokinase-regulated phosphorylation, the glucosamine derivative, BGA, was not phosphorylated in this reaction. Although BGA indicated a non-competitive inhibitory effect on this glucose phosphorylation, its inhibitory action on ATP effect was competitive (Fig. 2B). As such, binding competitively to the allosteric ATP binding site of

hexokinase rendered BGA to elicit certain stereostructural changes in the hexokinase molecule - a non-competitively inhibitory effect of BGA on the binding of glucose to the hexokinase was thus postulated (12, 13). Not only was IBG not phosphorylated by hexokinase (Table 1), this compound per se did not elicit any influences on the hexokinase-regulated phosphorylation as well (4). The structural difference between BGA and IBG is probably attributed to the presence of an amide bond in the former. The spatial disposition of the amide bond of BGA appeared to relate closely to the reaction associated with hexokinase. Moreover, changes attributed to the iodination site were prompted; introduction of ^{125}I at the o-position elicited negligible effects on the hexokinase reaction, whereas introduction at the m- and p-positions indicated increasing effects on the hexokinase reaction in that order, implicating that the planar structure of the benzene ring was closely associated to the influence exerted on hexokinase. Further, as indicated in Table 1, lipophilicity increased in the following rank order: m- < p- < pm-, rendering effects to be exerted on the interaction degree of hexokinase.

In a manner similar to the labelling process for IBG, an exchange reaction using Cu^{2+} as the catalyst was performed. Except for o-BGA, yields for the other iodinated compounds were excellent. The low yield for o-BGA was probably attributed to a steric hindrance imposed on its structure, impairing formation of the intermediate product of the reaction with Cu^{2+} serving as the nucleus (14). The three radioiodinated derivatives remained chemically stable for a 3-month period when stored at 4 °C, advocating that introducing the benzene ring enabled the radioiodinated compounds to achieve enhanced stability in the living system.

Except for o-BGA, which indicated a non-reactive labelling reaction, biodistribution of the other 3 labeled derivatives were investigated in mice. The results reveal a low accumulation of radioactivity in the stomach when the accumulation was expressed by a deiodination index in the body system(15). Their high chemical stability was indicated *in vivo* as well as *in vitro*. On investigating the biodistribution in mice, all 3 labeled derivatives indicated clearance from the brain with a similar elimination rate from the blood, implicating that it was difficult for ^{125}I-BGA to transport into the brain via the BBB. However, unlike radioiodinated BGA, IBG is able to permeate across the BBB and transport into the brain (4). As such, the structure of amide bond in BGA is closely related to permeability across the BBB.

Further, beside the radioactivity accumulation that lasted for 3 hr, p- and pm-BGA manifested much higher uptakes into the liver compared to that registered for m-BGA. In addition to these unfavorable data, the former 2 derivatives were showing a very slow clearance from the kidneys. These could have been due to the higher lipophilicity indicated by p- and pm-BGA as

compared to that of m-BGA. Taking imagings of the myocardia and pancreas into consideration, the background radioactivity of a useful radiopharmaceutical, representing the basal uptake into the liver, has to be low in general. As regard to the requirement of a clinical radioligand with rapid radioactivity clearance from the living system, the data in our present study advocate that m-BGA fulfills the purpose much more appropriately than the other 2 radioiodinated derivatives.

From our above findings, BGA was related closely to the hexokinase-regulated glucose phosphorylation. As such, it is highly possible that we could develop an ^{123}I-labeled agent for measurement of the pharmacokinetic profile and quantitative changes related with hexokinase reaction in animals and human. However, we have hitherto yet to clarify and enhance the specificity of hexokinase and BBB permeability to achieve the best clinical radioligand for the study of pharmacokinetics and other biochemical aspects of glucose in the living system.

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