^{123}I-IODOBENZOYLGLUCOSAMINES: GLUCOSE ANALOGUES FOR HEART IMAGING.

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SUMMARY

The ortho-, meta- and para-[^{123}I]-2-deoxy-2-N-(iodobenzoyl)-D-glucosamine (BGA) derivatives were investigated to determine the effect of iodine position and lipophilicity on tissue distribution. There was no correlation between tissue uptake and lipophilicity. Maximum uptake was observed for *o*-BGA displaying a heart:blood ratio of 36.0 at 18 hours post injection. Yeast hexokinase phosphorylation studies *in vitro* and an *in vivo* insulin experiment were carried out on *o*-BGA. No phosphorylation was detected, but the insulin study indicated that *o*-BGA uses the glucose transporter. *o*-BGA showed maximum tissue uptake in mice at an optimal specific activity of 0.004mg/μCi. Mouse biodistribution studies of *o*-[^{123}I]-iodobenzamide(*o*-^{123}IBA) indicated that the glucose moiety of *o*-BGA may be involved in the heart accumulation process in mice. Heart tissue extraction studies showed unmetabolized *o*-[^{123}I]BGA was the predominant species. A rabbit image of *o*-[^{123}I]BGA, recorded at 14 hours post injection, showed significant heart uptake.

Key words: iodobenzoylglucosamine, heart imaging and biodistribution studies.

INTRODUCTION

It has been well established in the literature that a radiolabelled glucose analogue is of great benefit in the diagnosis of various brain and heart disorders(1-3). The majority of this work has been accomplished using ^{18}F as the radionuclide and therefore the imaging procedure can only be carried out if a PET camera is available. Unfortunately, one must also be in close proximity to a cyclotron. The above drawbacks as well as the significant cost have motivated numerous researchers to investigate various glucose analogues that can be labelled with radioiodine, a radionuclide which can be readily utilized by all nuclear medicine departments.

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Work in this area includes the investigation of 3-deoxy-3-[^{123}I]-D-glucose(4), (E)-3-[^{125}I]iodovinyl-D-allose(5), methyl-2-deoxy-2-iodo-ß-D-glucopyranoside and 3,4,6-tri-O-acetyl-2-deoxy-2-iodo-ß-D-glucopyranoside(6), 2-deoxy-2-iodo-D-glucose(7), 2-deoxy-2-O-(*p*-iodobenzyl)-D-glucose(8,9), 2-deoxy-2-N-(*m*-iodobenzoyl)glucosamine(*m*-BGA)(10,11), 1,3,4,6-tetra-O-acetyl-N-(*m*-iodobenzoyl)glucosamine and 1,3,4,6-tetra-O-pivaloyl-N-(*m*-iodobenzoyl)glucosamine(12). None of these have shown any significant heart or brain uptake. We have investigated the use of some iodophenyl ether derivatives of glucose(9) and found that uptake in the brain and heart correlated with lipophilicity. Magata et al.(10) found *m*-BGA had good *in vivo* stability and good blood clearance properties. We felt that this analogue may therefore be worth persuing.

As noted by other authors(13-16) iodine position has an effect on tissue uptake. Some authors designing drugs that bind to receptor sites have correlated octanol:water partition coefficients with potential agents, enabling beneficial modifications to be made in the design of future agents. Correlations between iodine position and octanol:water partition coefficients for the *o*-, *m*-, *p*-BGA series were therefore investigated to determine if lipophilicity had an effect on biodistribution as seen with the iodophenyl ether derivatives(9).

D-glucose is transported across cell membranes by a carrier-mediated facilitated diffusion process using a glucose specific transporter(17-19). Skeletal muscle, adipocytes and heart tissues all contain a glucose transporter that is activated by insulin resulting in a marked increase in glucose transport(19). The effect of insulin in mice was examined as a means of elucidating the transport properties of *o*-[^{123}I]BGA.

Once transported, D-glucose is phosphorylated by hexokinase to form D-glucose-6-phosphate. D-glucose-6-phosphate is then a substrate for the next of a series of steps of the glycolytic pathway. However, phosphorylation of D-glucose is a very substrate specific process, such that any alteration to the D-glucose structure either decreases the rate of phosphorylation or inhibits phosphorylation completely(20). D-glucose analogues that are phosphorylated by hexokinase but not further metabolized (such as 2-deoxy-2-fluoro-D-glucose ^{18}FDG) provide an effective mode of "trapping" the glucose analogue in the tissue, resulting in a useful imaging agent. Phosphorylation of glucose analogues by yeast hexokinase *in vitro* was therefore investigated and compared to the work done by Maley and Lardy(21).

In summary, this study concentrates on the affects that various factors, such as iodine position and lipophilicity have on the biodistribution of a series of iodobenzoylglucosamine

analogues in mice, as well as studying their phosphorylation properties *in vitro*. In order to study the accumulation mechanism of *o*-[^{123}I]BGA in the heart, the effects of insulin, metabolism, and specific activity were more extensively studied.

MATERIALS AND METHODS

Ammonium Sulphate (Mallinckrodt), *o*- and *p*-iodobenzoyl chloride (Aldrich), *m*-iodobenzoic acid (Aldrich), *o*-bromobenzoyl chloride (Aldrich), glucosamine hydrochloride (Aldrich), thionyl chloride (Aldrich), ATP-disodium (Sigma), glyclylglycine (Aldrich), cresol red (Aldrich), C18 Sepac(Waters) and yeast hexokinase (Sigma H5500) were used as supplied. Glucosamine hydrochloride was converted to glucosamine by the method of Westphal and Hozmann(22).

Preparation of 2-deoxy-2-N-(*o*-iodobenzoyl)-D-glucosamine (*o*-BGA)(21)

To a 125 mL erlenmeyer flask was added glucosamine·HCl (4.0g, 18.5 mmol), water (20mL), and solid Na_2CO_3 (1.97g, 18.5mmol). The flask was placed in an ice bath and *o*-iodobenzoyl chloride (3.1g, 11.6mmol) was added. The chilled mixture was stirred for 2 days. During this time, portions of 1M Na_2CO_3 were added to maintain pH 9. A bulky white precipitate appeared. The reaction mixture was brought to pH 6 by addition of concentrated HCl (1.5mL). The precipitate was harvested by filtration followed by washes of water, methanol, and vacuum drying to yield 2.50g of *o*-BGA (52.6%). Subsequent purification of *o*-BGA (150mg) was carried out using SiO_2 column chromatography (2x10cm), eluting with N,N dimethylformamide/acetonitrile (1/2) to give 94mg of *o*-BGA (62.7%). ^{1}H NMR (400MHz, d^6-DMSO, ppm); 3.129-3.207(m, 1H), 3.456-3.550(m, 1H), 3.607-3.653(m,3H), 3.684-3.720(m, 1H), 4.444(t,1H), 4.643(d, 1H), 4.927(d, 1H), 5.106(t, 1H), 6.462(d, 1H), 7.146(td, 1H), 7.375-7.426(m, 2H), 7.863(d, 1H), 8.079(d, 1H). Other signals were observed due to the presence of approximately 20% ß anomer, causing considerable overlap with α anomer signals in the 3.45-3.78 ppm region. Elemental analysis for $C_{13}H_{16}O_6NI$ Calc.(found): C; 38.16(37.91), H; 3.94(3.96), N; 3.42(3.57), I; 31.02(30.86). Mp:decomposes at 210°C. Purity of *o*-BGA was assayed by HPLC(50:50:0.1 EtOH:H_2O:conc.H_2SO_4, 1.3 mL/min, C18μBondapak, anomers were resolved at R_f=6.2 min and R_f=5.5 minutes by UV detection at 254nm) and SiO_2 TLC(30% MeOH in $CHCl_3$ R_t=0.62 and 0.67 for the α and ß anomers of *o*-BGA).

Preparation of 2-deoxy-2-N-(*o*-bromobenzoyl)-D-glucosamine (*o*-BrBGA)(21)

To a 50 mL erlenmeyer flask was added glucosamine·HCl (0.62g, 3.46 mmol), water (3.5mL). The flask was placed in an ice bath and *o*-bromobenzoyl chloride (0.77g, 3.5 mmol) was added in small amounts. After the last of the *o*-bromobenzoyl chloride was added, the reaction was left to stir for 2 hours while maintaining the pH between 8-9 by adding portions of 1M Na_2CO_3. A bulky white precipitate appeared. After 2 hours, the reaction mixture was brought to pH 6 by addition of 1 M HCl. The precipitate was harvested by cold filtration followed by washes of water, methanol, and vacuum drying to yield 0.54g of *o*-BrBGA (43%). *o*-BrBGA was recrystallized from ethanol giving an analytically pure product. ^{1}H NMR (300MHz, d^6-DMSO, ppm); 3.059-3.295(m, 1H), 3.456-3.560(m, 1H), 3.607-3.683(m,3H), 3.706-3.740(m, 1H), 4.487(t,1H), 4.688(d, 1H), 4.936(d, 1H), 5.126(t, 1H), 6.491(d, 1H), 7.447(td, 1H), 7.356-7.577(m, 2H), 7.648(d, 1H), 8.182(d, 1H). Other signals were observed due to the presence of approximately 20% ß anomer, causing considerable overlap with α anomer signals in the 3.45-3.78 ppm region. Elemental analysis for $C_{13}H_{16}O_6NBr$ Calc.(found): C; 43.11(43.38), H; 4.45(4.52), N; 3.88(3.86). Purity of *o*-BrBGA was assayed by HPLC(50:50:0.1 EtOH:H_2O:conc.H_2SO_4, 1.3 mL/min, C18μBondapak, anomers were resolved at R_f=6.2 min and R_f=5.5 minutes by UV detection at 254nm) and SiO_2 TLC(30% MeOH in $CHCl_3$ R_t=0.62 and 0.67 for the α and ß anomers of *o*-BrBGA).

Preparation of 2-deoxy-2-N-(*m*-iodobenzoyl)-D-glucosamine (*m*-BGA)

m-BGA was synthesized by a method similar to that used for *o*-BGA. *m*-Iodobenzoyl chloride was obtained by the reaction of *m*-iodobenzoic acid with thionyl chloride(23). Glucosamine (4.0g, 22.3mmol) was placed in a 250mL erlenmeyer flask with water (20mL) and *m*-iodobenzoyl chloride (3.0g, 11.2mmol). The reaction mixture was chilled with ice and agitated for 1 day. A total of 8 mL of 1M Na_2CO_3 was added to maintain pH 9. Finally the mixture was neutralized with HCl; the solids were filtered, washed with water, methanol, and dried *in vacuo* to give 1.633g of *m*-BGA (35.7%). Purification of 200mg of crude *m*-BGA was carried out using SiO_2 column chromatography (DMF/CH_3CN, 1/2), giving 137mg of *m*-BGA (68.5%). ^{1}H NMR (400MHz, d^6-DMSO, ppm); 3.130-3.208(m, 1H), 3.480(ddd,1H), 3.589-3.670(m, 2H), 4.442(t, 1H), 4.687(d, 1H), 4.954(d,1H), 5.041(t, 1H), 6.455(d, 1H), 7.258(t, 1H), 7.867(dd, 1H), 7.898(dd, 1H), 8.263(s, 1H), 8.273(s, 1H). Elemental analysis for $C_{13}H_{16}O_6NI$ Calc.(found): C; 38.16(38.12), H; 3.94(4.02), N; 3.42(3.62), I; 31.02(30.97).

Mp:decomposes 205°C. Purity was assayed by HPLC(30:70 MeOH:H_2O, C18 μBondapak, 1.0 mL/min, the α and ß anomers are resolved at R_t=11.0 and 15.0 minutes by UV detection at 254nm) and SiO_2 TLC(85:15 $CHCl_3$:MeOH R_f=0.44).

Preparation of 2-deoxy-2-N-(*p*-iodobenzoyl)-D-glucosamine (*p*-BGA)

p-BGA was synthesized by a method similar to the procedure used for *o*-BGA. Glucosamine·HCl (4.0g, 18.5mmol) was suspended in water (20mL) with solid Na_2CO_3 (1.97g, 18.5mmol) and *p*-iodobenzoyl chloride (2.5g, 9.38mmol). The chilled mixture was stirred for three days, maintained at pH 9 by the addition of portions of 1M Na_2CO_3. The mixture was neutralized with HCl, filtered, washed with water, methanol, and dried *in vacuo* to yield 2.19g of crude *p*-BGA (55.2%). Subsequent purification of *p*-BGA (400mg) was carried out using SiO_2 column chromatography (DMF/CH_3CN, 1/1), giving 254mg of *p*-BGA (63.5%). ^1H NMR (400MHz, d^6-DMSO, ppm); 3.130-3.203(m, 1H), 3.450-3.530(m, 1H), 3.599-3.660(m, 2H), 3.701-3.765(m, 2H), 4.444(t, 1H), 4.684(d, 1H), 4.952(d, 1H), 5.049(t, 1H), 6.450(d, 1H), 7.678(d, 2H), 7.832(d, 2H), 8.153(d, 1H). Elemental analysis for $C_{13}H_{16}O_6NI$ Calc.(found): C; 38.16(38.00), H; 3.94(3.87), N; 3.42(3.38). Mp:decomposes 208°C. Purity was assayed by HPLC(30:70 MeOH:H_2O, C18 μBondapak, 1.0 mL/min, the α and ß anomers are resolved at R_t=10.2 and 11.7 minutes by UV detection at 254nm) and SiO_2 TLC(60:40 $CHCl_3$:MeOH R_f=0.35).

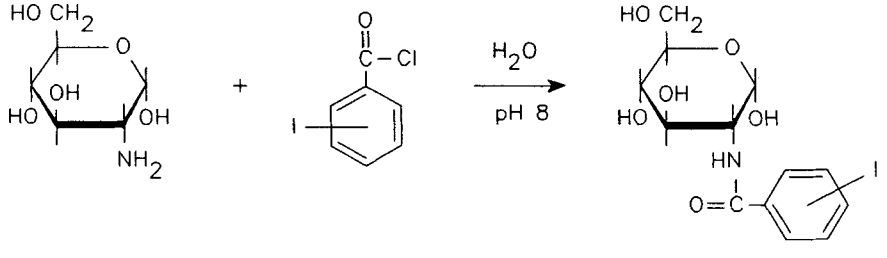

Figure 1. Synthesis of *o*-, *m*-, or *p*-BGA.

Preparation of *o*-iodobenzamide (*o*-IBA)

In a 50 mL RB flask, 0.43g (1.6mmol) of *o*-iodobenzoyl chloride was dissolved in 20 mL of acetone. To this, 0.42g (3.2mmol) of ammonium sulphate was added. To this mixture, 3 mL of 0.6M sodium carbonate was slowly added, and the solution was left to stir overnight.

Reaction completion was shown by SiO_2 TLC(70:30 hexane:EtOAc R_f=0.35). The mixture was filtered and the acetone removed. Recrystallization from ethanol gave 0.29g of *o*-IBA (74%). ^1H NMR, 200MHz ($CDCl_3$, ppm); 5.846(s,2H), 7.152(td,1H), 7.478(m,2H), 7.903(d,1H). Elemental analysis for C_7H_6NOI Calc.(found): C; 34.02(33.91), H; 2.45(2.40), N; 5.69(5.54). Purity was assayed by SiO_2 TLC(70:30 hexane:EtOAc R_f=0.35).

Radiolabelling Techniques

Labelling of all compounds with ^{123}I was carried out according to the literature(24). Copper salts were omitted in the cases of *o*-BGA and *o*-IBA due to greater labelling yields obtained without the use of copper salts. Radiochemical yield for all BGA isomers were >97% without further purification, as determined by SiO_2 TLC(30:70 MeOH:$CHCl_3$). All compounds maintained their radiochemical purity at >97% after 48 hours in 0.01 M H_2SO_4. High specific activity *o*-BGA was prepared by ^{123}I exchange label of *o*-BrBGA. The preparation of *o*-[^{123}I]BGA from *o*-BrBGA had a radiochemical yield of 61%. This crude labelled product was initially purified by a C18 Sepac, then further purified by HPLC (using the same conditions as described previously for *o*-BGA) to give *o*-[^{123}I]BGA having 98% radiochemical purity.

Octanol:water Partition Coefficients

Octanol:water partition coefficients were performed by the shake-flask method(25) and their UV spectrum was recorded between 190 and 260 nm before and after octanol extraction. The samples were extracted using 1:1 volumes of octanol:water (where the water contains 0.05M Trizma buffer pH 7.4 and 0.15M NaCl) at approximately 0.1 mM sample concentrations.

Hexokinase Phosphorylation Assay

Phosphorylation was investigated according to the modified method of Lazarus et al.(26). The assay solution was prepared by mixing 1.8% $MgCl_2 \cdot 6H_2O$, 10 mL of 0.006% cresol red solution, 2 mL of 0.1 M ATP-disodium salt solution, and 5 mL of 0.1 M glycylglycine solution. The pH was adjusted to 8.5 using 0.1N NaOH, then the volume was adjusted to 50 mL. Glucose was used initially as the phosphorylation substrate (0.40 mL of 0.2 M) in order to validate the assay technique prior to the testing of the BGA series. Phosphorylation was determined by the addition of 2.5 mL of assay solution, 0.9 mL of substrate, 4.0μL of hexokinase (15 units), and recording any change in absorbance at 574 nm between 10 and 120 seconds after mixing. The

change in absorbance is due to cresol red pH indicator absorbing at 574 nm when exposed to acid in a quantitative manner, which is released in the phosphorylation of glucose. Due to the low solubility of the BGA series, 0.9 mL of 14 mM BGA was used. These maximum volumes and concentrations of the BGA series were used in order to detect the smallest amount of phosphorylation. Inhibition studies of glucose phosphorylation were also carried out on the BGA series using the same method as described above except 0.40 mL of $1x10^{-4}$M of the desired BGA isomer and 0.40 mL of varying concentrations of glucose ($6.1x10^{-4}$M to $2.4x10^{-3}$M) were used. These results were compared to a control experiment using 0.40 mL water and 0.40 mL of varying concentrations of glucose($6.1x10^{-4}$M to $2.4x10^{-3}$M)(27).

Biodistribution Studies

Biodistribution studies were carried out using male CDI/UBC mice (20-25g). Animals were injected in the tail vein with 37 KBq (1 μCi) of [^{123}I]BGA in saline with a range of specific activities from 0.001- 0.88mg/mouse as specified in the biodistribution tables 1-8. The mice were sacrificed at 0.25, 0.5, 1, 3, or 18 hours post injection. The activity in the blood, liver, kidneys, spleen, pancreas, stomach, lung, heart, muscle and brain were determined and results expressed as % injected dose per gram tissue. For the insulin study, the same procedure was used as described above with the exception that the solution injected into each mouse contained 0.2 units of insulin as well as 37 KBq (1μCi) of *o*-[^{123}I]BGA as described by Cooney et al.(28). Five animals were used at each time period.

Heart Tissue Extractions

Heart tissue extraction studies were carried out for *o*-[^{123}I]BGA. Mice were injected in the tail vein with 3.7 MBq (100μCi) of *o*-[^{123}I]BGA and sacrificed at 18 hours post injection. Heart tissue was cut up finely and a 5% trichloroacetic acid(TCA) solution was added and stirred vigorously. The supernant was removed resulting in 70% of total activity as a suspension. The fine suspension was then centrifuged for 10 minutes at 4000 rpm. The supernant of the centrifuged mixture was then loaded onto a C18 Sepac and eluted with methanol:water:conc. sulphuric acid (50:50:0.2). The solutions pre and post C18 Sepac treatment were then assayed by RP HPLC and SiO_2 TLC(using the same condition as described in the preparation of *o*-BGA).

Rabbit Image

A male New Zealand White rabbit (2Kg) was injected with 185 MBq (5.0 mCi) of *o*-[^{123}I]BGA containing 0.9mg of cold *o*-BGA and fasted overnight. According to approximate blood volume differences in mice and rabbits, this is equivalent to a mouse receiving 0.016mg cold *o*-BGA. At 14 hours post injection, the rabbit was anesthetized using 0.5ml/kg of ketamine:acepromazine:rompun (1000mg:20mg:100mg), and an image was recorded using a Siemans Basic camera.

DISCUSSION

The BGA series were prepared by the addition of an unprotected glucosamine with the appropriate iodobenzoyl chloride according to the modified method of Maley and Lardy(21) averaging 48% yield. The preparation of *m*-BGA by Magata et al. involves a five step synthesis(10). The modified method of Maley and Lardy(21) used in this work is a one step synthesis, eliminating several unnecessary synthetic steps involving protection and deprotection of glucosamine.

Upon examination of this series of glucosamines, it became evident that each compound had its own unique biodistribution pattern even though they only differ by the iodine position on the phenyl ring. Both the most lipophilic glucosamine, *p*-BGA (log P=0.28±0.01), and the most hydrophilic glucosamine, *o*-BGA (log P=-0.83±0.02), exhibited similar trends in tissue uptake with the major exception being their blood clearance capabilities. *m*-BGA (log P=0.18±0.01) however, displayed a very different biodistribution pattern than either the *o*- or *p*-BGA isomers (Tables 1-3). An 18 hour biodistribution study was carried out due to other receptor binding agents showing improved target:ratios at these longer time periods(29). The use of radionuclides like I-123($T_{1/2}$=13 hours) enables images to be recorded at longer time periods.

m-BGA exhibited good blood clearance, in agreement with the work done by Magata et al.(10), but it showed a continual decrease in tissue uptake after 0.25 hour post injection in all tissues except the muscle. The muscle tissue had an increase in uptake from 0.30±0.06 to 0.92±0.07 %dose/g between 3 to 18 hours post injection. See Table 2.

p-BGA showed an increase in uptake with time in the spleen, lung, heart, muscle, and brain, but *p*-BGA also showed an increase in blood uptake. The poor blood clearance capabilities of *p*-BGA and the lower accumulation resulted in a heart:blood ratio of 1.67±0.29 at 18 hours post injection. *p*-BGA also showed a great increase in uptake in the liver between 0.5 and 1 hour post injection, having a liver:blood ratio of 17.6±0.66 at 1 hour post injection. See Table 3.

o-BGA showed the greatest uptake in the heart and pancreas tissues and good blood clearance after 0.5 hour post injection. At 18 hours post injection *o*-BGA displayed a heart:blood ratio of 36.0±0.96. This high ratio was due both to efficient blood clearance and to an increase in uptake from 1.61 to 6.48 %dose/g between 3 and 18 hours. See Table 1.

Table 1. Biodistribution Data for 0.004mg *o*-[^{123}I]BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	1.17±0.05$^{\sigma}$	0.96±0.15	0.65±0.06	0.27±0.04	0.18±0.01
Liver	11.30±1.05	10.74±1.57	7.17±0.65	1.92±0.22	0.84±0.06
Kidney	24.79±2.43	20.95±3.58	12.34±2.19	3.40±0.69	1.11±0.12
Spleen	0.76±0.04	0.80±0.15	0.81±0.18	0.53±0.05	2.28±0.19
Pancreas	3.01±0.31	2.98±0.85	3.17±0.71	2.07±0.28	1.51±0.41
Stomach	2.17±0.22	2.01±0.22	2.41±0.38	1.74±0.28	2.18±0.36
Lung	1.23±0.09	1.18±0.09	1.04±0.08	0.82±0.14	3.81±0.42
Heart	1.13±0.18	0.91±0.28	0.97±0.19	1.61±0.65	6.48±0.97
Muscle	0.46±0.16	0.37±0.11	0.26±0.08	0.25±0.01	2.11±0.29
Brain	0.07±0.01	0.06±0.01	0.05±0.01	0.04±0.01	0.26±0.04

* values represent %dose/g. σ mean of five animals.

Table 2. Biodistribution Data for 0.004mg *m*-[^{123}I]BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	3.66±0.24$^{\sigma}$	1.87±0.28	1.15±0.22	0.44±0.07	0.08±0.02
Liver	10.75±0.99	4.99±0.94	3.42±1.10	0.50±0.06	0.21±0.02
Kidney	15.36±2.21	5.63±1.51	4.08±0.84	0.49±0.07	0.16±0.02
Spleen	1.66±0.38	1.04±0.16	0.68±0.05	0.29±0.06	0.10±0.06
Pancreas	9.99±2.07	6.72±1.52	5.14±0.70	0.69±0.14	0.39±0.23
Stomach	6.36±1.38	5.35±0.48	2.36±0.09	2.26±0.48	0.25±0.02
Lung	3.65±0.58	1.80±0.46	1.39±0.72	1.15±1.06	0.12±0.07
Heart	1.84±0.14	1.28±0.18	0.96±0.09	0.39±0.11	0.17±0.02
Muscle	0.84±0.18	0.60±0.08	0.46±0.06	0.30±0.06	0.92±0.07
Brain	0.15±0.03	0.10±0.03	0.08±0.03	0.02±0.01	0.02±0.01

* values represent %dose/g. σ mean of five animals.

Magata et al. prepared the *o*-, *m*- and *p*-BGA, but concentrated mainly on *m*- and *p*-BGA due to labelling difficulties with the *o*-BGA isomer(11). However, from our above results, *o*-BGA showed the most promising biodistribution properties. Further experiments were therefore carried out to elucidate the mode of accumulation of *o*-[^{123}I]BGA in the heart. To determine if

Table 3. Biodistribution Data for 0.004mg *p*-[^{123}I]BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	1.55±0.12$^{\sigma}$	1.33±0.37	0.95±0.10	0.69±0.09	1.32±0.12
Liver	4.65±1.20	6.28±1.82	16.73±1.97	10.48±1.61	2.97±0.24
Kidney	17.76±1.86	11.96±3.49	9.16±1.00	4.19±0.23	1.52±0.19
Spleen	1.40±0.20	1.40±0.29	1.32±0.17	1.29±0.10	1.95±0.23
Pancreas	4.63±0.36	3.83±1.11	4.17±0.53	3.32±0.45	3.66±0.61
Stomach	2.67±0.32	2.44±0.57	2.56±0.24	2.11±0.24	1.22±0.14
Lung	2.14±0.19	2.01±0.49	1.90±0.12	1.60±0.24	1.98±0.23
Heart	1.37±0.23	1.06±0.24	1.11±0.10	1.07±0.10	2.21±0.45
Muscle	0.51±0.06	0.50±0.07	0.47±0.06	0.56±0.16	1.84±0.19
Brain	0.01±0.03	0.08±0.03	0.06±0.01	0.05±0.01	0.15±0.03

* values represent %dose/g. σ mean of five animals.

Table 4. Biodistribution Data for 0.004mg *o*-^{123}IBA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	4.14±0.34$^{\sigma}$	3.73±0.28	3.68±0.72	3.32±0.58	0.56±0.10
Liver	13.44±0.85	12.43±1.48	12.14±1.70	9.99±1.11	1.55±0.10
Kidney	6.96±0.50	6.12±1.43	5.87±0.76	4.55±0.48	0.70±0.07
Spleen	3.97±0.21	3.50±0.23	3.12±0.37	2.58±0.33	0.29±0.05
Pancreas	4.98±1.19	5.15±0.53	4.50±0.22	3.49±0.44	0.37±0.12
Stomach	5.06±0.45	5.32±0.38	5.64±0.77	6.57±1.19	0.72±0.12
Lung	5.38±0.78	4.80±0.29	4.07±0.36	3.45±0.40	0.49±0.06
Heart	4.41±0.41	3.93±0.68	3.20±0.37	2.36±0.29	0.26±0.03
Muscle	3.39±0.39	3.12±0.19	2.40±0.19	1.83±0.25	0.22±0.01
Brain	3.75±0.26	3.33±0.20	2.70±0.29	1.90±0.24	0.20±0.01

* values represent %dose/g. σ mean of five animals.

the glucose moiety was involved in the accumulation of *o*-[^{123}I]BGA, *o*-iodobenzamide (*o*-IBA) was prepared and its biodistribution studied (see Table 4). *o*-IBA is much more lipophilic (log P=0.88) than the BGA series(log P=-0.83 to 0.28) such that the amount of tissue uptake was

Table 5. Biodistribution data for <0.001mg *o*-BrBGA and *o*-[^{123}I]BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	5.33±0.20$^{\sigma}$	1.12±0.11	0.55±0.06	0.30±0.06	0.02±0.01
Liver	41.44±2.55	10.71±0.46	5.67±0.50	1.69±0.27	0.08±0.01
Kidney	83.72±8.42	16.18±1.55	8.11±0.69	2.42±0.48	0.11±0.02
Spleen	3.76±0.40	0.77±0.05	0.67±0.06	0.63±0.12	0.22±0.04
Pancreas	11.25±2.25	3.72±0.13	3.09±0.89	2.46±0.58	0.04±0.04
Stomach	9.75±1.62	2.67±0.30	2.30±0.26	2.16±0.38	0.26±0.05
Lung	5.35±0.21	1.21±0.04	0.88±0.05	0.84±0.13	0.39±0.10
Heart	4.53±0.82	1.09±0.24	1.26±0.26	1.41±0.40	0.39±0.13
Muscle	1.85±0.19	0.43±0.11	0.29±0.03	0.32±0.09	0.21±0.04
Brain	0.34±0.02	0.06±0.01	0.05±0.01	0.04±0.006	0.02±0.005

* values represent %dose/g. σ mean of five animals.

Table 6. Biodistribution Data for 0.02mg *o*-BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	2.30±0.20$^{\sigma}$	1.38±0.21	0.73±0.39	0.50±0.05	0.05±0.02
Liver	11.56±3.27	12.99±1.01	6.97±3.63	3.82±0.55	0.15±0.01
Kidney	20.69±4.02	20.14±2.84	10.31±5.74	5.91±0.80	0.21±0.02
Spleen	1.09±0.11	0.97±0.09	0.83±0.37	1.89±0.28	0.44±0.11
Pancreas	2.85±0.47	1.87±1.31	2.46±1.25	4.12±1.75	1.06±0.94
Stomach	2.29±0.51	2.43±0.26	2.32±1.19	5.07±0.47	0.44±0.09
Lung	1.98±0.21	1.36±0.15	0.94±0.48	2.23±0.53	0.49±0.14
Heart	1.22±0.12	1.10±0.20	0.78±0.39	2.49±0.35	0.74±0.14
Muscle	0.76±0.10	0.78±0.55	0.31±0.15	0.95±0.10	0.36±0.12
Brain	0.12±0.02	0.10±0.01	0.07±0.04	0.14±0.02	0.06±0.03

* values represent %dose/g. σ mean of five animals.

reflected by the efficiency of diffusion across the cell membrane. The biodistribution of *o*-^{123}IBA showed much higher initial uptake in all tissues compared to *o*-[^{123}I]BGA, but unlike *o*-[^{123}I]BGA, all tissues exhibited a decrease in uptake at a slow rate with no indication of accumulation. These results suggest that the glucose moiety imparts some tissue specificity and

Table 7. Biodistribution Data for 0.88mg *o*-BGA.*

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	2.28±0.27$^{\sigma}$	2.12±0.58	1.23±0.42	0.44±0.07	0.03±0.008
Liver	14.96±1.88	15.96±2.08	11.47±5.17	1.46±0.38	0.13±0.02
Kidney	25.92±1.84	34.94±12.55	17.49±9.42	2.20±0.38	0.18±0.02
Spleen	1.15±0.15	1.43±0.43	1.29±0.49	0.66±0.18	0.36±0.08
Pancreas	3.33±0.35	3.84±0.73	3.39±1.10	1.43±0.49	0.27±0.10
Stomach	2.84±0.59	3.40±0.29	4.26±1.45	1.67±0.31	0.32±0.09
Lung	2.09±0.20	2.06±0.25	1.56±0.47	0.66±0.06	0.43±0.09
Heart	1.33±0.24	1.45±0.27	1.19±0.17	0.80±0.13	0.44±0.09
Muscle	0.66±0.09	0.67±0.22	0.52±0.12	0.29±0.02	0.34±0.03
Brain	0.12±0.02	0.12±0.01	0.13±0.05	0.07±0.03	0.05±0.01

* values represent %dose/g. σ mean of five animals.

Table 8. Biodistribution Data for 0.004mg *o*-BGA per 1μCi of *o*-[^{123}I]BGA and 0.2 Units insulin.

	0.25 hour	0.5 hour	1 hour	3 hour	18 hour
Blood	1.07±0.12$^{\sigma}$	0.99±0.20	0.65±0.03	0.39±0.03	0.05±0.01
Liver	9.07±0.65	9.15±0.78	8.12±0.80	3.11±0.46	0.21±0.04
Kidney	12.55±0.69	10.49±1.61	7.00±0.83	3.10±0.31	0.28±0.07
Spleen	0.66±0.16	-	0.75±0.08	0.71±0.05	0.38±0.10
Pancreas	1.89±0.28	2.18±0.40	2.58±0.21	2.60±0.35	0.37±0.10
Stomach	1.85±0.25	2.21±0.16	2.38±0.17	2.17±0.18	0.54±0.10
Lung	1.04±0.08	1.11±0.14	0.98±0.09	0.97±0.12	0.66±0.10
Heart	2.22±0.20	2.55±0.38	2.51±0.63	2.83±0.66	1.78±0.34
Muscle	0.90±0.36	1.07±0.55	0.65±0.07	0.62±0.10	0.60±0.11
Brain	0.07±0.006	0.08±0.02	0.07±0.008	0.06±0.009	0.03±0.002

* values represent %dose/g. σ mean of five animals.

the glucose moiety rather than the phenyl substituent may be involved in the "trapping" of *o*-[^{123}I]BGA in the heart.

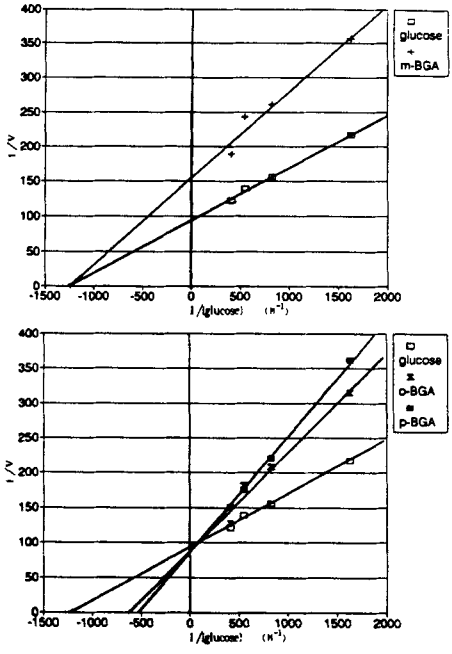

Figure 2. Inhibition of glucose phosphorylation by *o*-BGA ($1.3x10^{-4}$M), *m*-BGA ($1.2x10^{-4}$M) and *p*-BGA ($1.2x10^{-4}$M) using yeast hexokinase. The velocity is expressed as change in absorbance (574nm) per second.

Hexokinase phosphorylation studies *in vitro* were carried out to elucidate the mode of trapping of *o*-[^{123}I]BGA in the heart as determined by UV spectroscopy according to the procedure described in the method section. *o*-, *m*-, and *p*-BGA showed no change in absorbance at 574 nm between 10 and 120 seconds after mixing. By this assay method, phosphorylation of *o*-, *m*-, and *p*-BGA by hexokinase was not observed. Glucose was used as a control, without inhibitor, resulting in a $K_m = 8.07x10^{-4}$M. Crystallographic work done by Shoham and Steitz(30) showed *o*-BGA to be in the active site of yeast hexokinase. In agreement with this work, our inhibition studies showed both *o*- and *p*-BGA were competitive inhibitors of glucose phosphorylation, *p*-BGA being a stronger inhibitor than *o*-BGA, having K_i's of $1.48x10^{-4}$M and $9.09x10^{-5}$M respectively. *m*-BGA, on the other hand, was a non-competitive inhibitor of glucose phosphorylation having a K_i of $1.76x10^{-4}$M. See Figure 2.

To determine if *o*-[^{123}I]BGA used the glucose transporter, we stimulated glucose transport in mice by the simultaneous injection of insulin. Since glucose transport is greatly increased in heart and muscle tissue in the presence of insulin, we added insulin (approx. 10U/kg(28)) to the injection solution of *o*-[^{123}I]BGA such that each mouse received 0.004mg *o*-BGA and 0.2 units of insulin. (See Table 8). As expected for compounds which use the glucose transporter, there was greater uptake in both heart and muscle tissues compared to *o*-[^{123}I]BGA without insulin(Table 1). Constant heart activity was observed between 0.25 and 3 hours post injection followed by only a slight decrease in heart activity between 3 and 18 hours post injection. Although simultaneous injection of insulin shows a decrease in the absolute heart activity (%dose/g) at 18 hours post injection, more efficient blood clearance at this time resulted in a heart:blood ratio of 35.6.

When *o*-[^{123}I]BGA was injected into a fasted rabbit and imaged at 14 hours post injection, approximately equal heart and thyroid uptake was observed. See Figure 3.

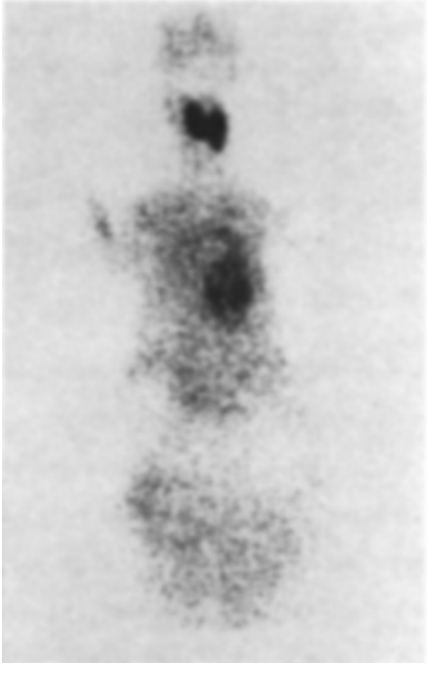

Figure 3. Significant activity is observed in the heart of a male New Zealand White rabbit that has been injected with *o*-[^{123}I]BGA and imaged at 14 hours post injection. Thyroid activity is also observed due to *in vivo* breakdown of *o*-[^{123}I]BGA.

Heart tissue extraction studies were also carried out to examine possible metabolites of *o*-[^{123}I]BGA *in vivo*. Seventy percent of the radioactivity was extracted from mice hearts at 18 hours post injection by using TCA to precipitate the heart tissue. Using RP HPLC (50:50:0.1 ethanol:water:conc. sulphuric acid), only one radioactive species was seen which had the same retention time as the initially administered *o*-[^{123}I]BGA. However, using SiO_2 TLC (30% methanol in chloroform), two radioactive species were detected. One had the same R_f value as that of *o*-BGA while the other radioactive species(30% of total activity) had an R_f range of 0.0-0.2 .

Since biodistribution properties of various analogues may be effected by the specific activity at which they are used, we carried out specific activity experiments on the most promising analogue, *o*-[^{123}I]BGA, to determine the specific activity that results in maximum heart uptake. The labelling of all of the BGA analogues with ^{123}I is a very efficient exchange process, resulting in 97% labelling yields. However, the average specific activity used for the biodistribution studies was approximately 830μCi/mg of cold *o*-BGA analogue (i.e. each mouse received approximately 0.004mg of cold *o*-BGA), thus there was always a small amount of cold *o*-BGA present when the analogue was injected.

When dealing with a specific receptor site such as the glucose transporter site or the hexokinase active site, the cold analogue may saturate the binding sites resulting in a reduced binding of the labelled analogue. To determine if there was site saturation occurring, specific activity experiments were carried out on *o*-BGA using 0.004 to 0.88mg of cold *o*-BGA.

To produce an *o*-[^{123}I]BGA analogue of higher specific activity than 0.004mg per mouse, we synthesized the corresponding 2-deoxy-2-N-(*o*-bromobenzoyl) glucosamine (*o*-BrBGA) and carried out exchange labelling with ^{123}I. Reverse phase HPLC enabled isolation of *o*-[^{123}I]BGA having 98% radiochemical purity using a 50:50:0.1 ethanol:water:conc.H_2SO_4 through a C18 μBondapak column. However, this did not enable separation of the bromo and iodo isomers such that each mouse received <0.001mg of *o*-BrBGA as well as 1μCi of *o*-[^{123}I]BGA (See Table 5) compared to 0.004mg of cold *o*-BGA (See Table 1).

High specific activity *o*-[^{123}I]BGA, prepared from the bromo precursor described above, displayed an initial higher tissue uptake but decreased to 0.39 %dose/g at 18 hours post injection. Although a decrease in absolute heart uptake(%dose/g) occurred, it washed out of the blood at a faster rate than the heart resulting in a heart:blood ratio of 19.5 ± 2.65 at 18 hours post injection. The initial higher heart uptake may have been due to a greater amount of *o*-[^{123}I]BGA

transported (due to the transport sites not being saturated) or may have reflected the higher uptake observed in the blood at 0.25 hour post injection.

The three studies using 0.004mg, 0.02mg and 0.88mg of cold *o*-BGA per mouse showed similar heart uptake (approx. 1.1 %dose/g) between 0 and 1 hour post injection. At 3 hours post injection, the mice who received 0.88mg of *o*-BGA showed a gradual decrease in heart uptake. The mice who received 0.02mg of *o*-BGA, on the other hand, displayed an increase in heart uptake to 2.49 %dose/g at 3 hours post injection followed by a decrease in uptake at 18 hours post injection. Even though each specific activity experiment varied in their uptake patterns between 0 and 18 hours, they all display significant heart:blood ratios ranging from 14.7(0.88mg cold *o*-BGA) to 36.0 (0.004 mg cold *o*-BGA) at 18 hours post injection. Since differences in tissue uptake are not observed at early time periods, specific activity differences in the range between 0.004-0.88mg appear to affect the accumulation process rather than the transport process.

This study has indicated that there is definitely an effect of specific activity on uptake in mice, showing complex biodistribution kinetics. All specific activities showed high heart:blood ratio at later time periods suggesting specific activity does not effect the method of *o*-BGA accumulation in the heart. There were no trends in the observed biodistribution patterns that correlated to trends in lipophilicity. Accumulation in heart and muscle tissues were observed for both *o*- and *p*-BGA but not for *m*-BGA such that iodine position may have a steric influence on the method of accumulation. Both *o*- and *p*-BGA were also competitive inhibitors of glucose phosphorylation whereas *m*-BGA was a non-competitive inhibitor of glucose phosphorylation. The heart tissue extraction study suggests the presence of an unidentified metabolite of *o*-[^{123}I]BGA. The greater heart uptake observed in the insulin study suggests that *o*-[^{123}I]BGA uses the glucose transporter. Procurement of a rabbit image using *o*-[^{123}I]BGA showed significant heart uptake.

The mode of accumulation of *o*-[^{123}I]BGA is not clear, but trapping of this glucose analogue by hexokinase phosphorylation is not probable. Rather, it is a very complex process which is dependent on specific activity and transport rate. There may also be some binding to non-specific sites which further complicate the understanding of the specific activity biodistribution studies.

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