RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

[^{125}I]Iodobenzoyl Derivatives of Acebutolol as Potential Myocardial Imaging Agents

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Three new iodobenzoyl derivatives of acebutolol, a cardioselective beta antagonist, were synthesized and labeled with iodine-125. The biodistributions of these labeled compounds were determined in normal rats and compared with that of thallium-201. Fifteen minutes following i.v. administration, the iodine-125-labeled meta- and para-iodobenzoyl acebutolols possessed the greatest ventricular uptake and the highest ventricle-to-blood and ventricle-to-lung ratios of the new agents. The corresponding values for thallium-201 were 2.5 to 3.0 times as high. The data in this study suggest that more lipophilic derivatives of the cardioselective beta antagonists will possess increased uptake and cardioselectivity, and thereby will compare more favorably with thallium-201 as myocardial imaging agents.

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The development of gamma-emitting radiopharmaceuticals that could provide more accurate imaging of regional myocardial perfusion than thallium-201 would be useful in the early detection of coronary disease and assessment of therapy. Our approach to the development of such agents utilizes radiolabeled derivatives of drugs, such as the beta-adrenergic antagonists, that exert a selective pharmacologic effect upon the heart.

The beta antagonists have the ability to decrease heart rate, myocardial contractility, and myocardial metabolism. As defined by Lands et al. (*1*), these are beta-1 responses, and structure-activity relationship studies (*2,3*) have demonstrated that selective inhibition of these beta-1 responses can be achieved by the selective introduction of functional groups onto the parent 3-phenoxy-1-isopropylaminopropan-2-ol structure. For example, the placement of hydrogen-bonding moieties in the para position of the phenoxy group, such as the acetamido and butyramido groups present in practolol (*4*) and acebutolol (*5*), respectively, tends to enhance the cardioselectivity of the drug.

Since benzoylated analogs of practolol and acebutolol are cardioselective (*4,5*), it seemed reasonable to expect that the iodobenzoyl derivatives would also show this pharmacologic selectivity. Derivatives of the beta antagonists were synthesized in which the iodine-containing moiety is distal to the oxyisopropylaminopropanol pharmacophore. These compounds should be more resistant to deiodination in vivo than the *o*-iodophenolic beta antagonists previously prepared (*6–8*). To test the theory that derivatives of cardioselective antagonists would have better myocardial uptake and selectivity than derivatives of noncardioselective antagonists, a series of three I-125-labeled beta antagonists was made (*9*). Meta-[^{125}I]-iodobenzoyl practolol (*m*-IBP) and -acebutolol (*m*-IBA) were prepared as the cardioselective derivatives, and 4′-[^{125}I]iodopropranolol (IP) was prepared as a nonselective beta antagonist. The biodistribution data indicated that *m*-IBP and *m*-IBA had significantly better myocardial uptake and target-to-nontarget ratios (T/NT) than the noncardioselective IP. These data supported the decision to examine derivatives of the cardioselective beta-adrenergic antagonists as possible cardiovascular imaging agents.

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Pharmacokinetic studies with radiolabeled β-adrenergic antagonists have demonstrated that i.v. administration of the drug results in a rapid distribution phase followed by a slower elimination process that is consistent with a two-compartment body model. At doses less than 2 mg/kg there is little distribution to a tissue compartment with a slower equilibration constant (*10,11*). This is reflected in the biodistributional studies conducted by other investigators (*8,12-15*), and by ourselves (*9*), where the target- (heart) to-nontarget ratios for the radiolabeled β-adrenergic antagonists remain relatively constant between 5 min and 2 hr after administration. Therefore, for the purpose of comparing the uptake and selectivity of radiopharmaceuticals, we chose a single time interval that falls in the early elimination phase for the rat.

Because the myocardial uptake and heart-to-lung ratio for meta-[^{125}I]iodobenzoyl acebutolol were comparable to those of the corresponding practolol derivative, and because of the side effects reported in the clinical use of practolol (*16*), the acebutolol derivatives were selected for primary consideration. A comparison of the organ distribution with that of Tl-201 and the elucidation of the effect of positional isomerism in the iodobenzoyl prosthetic group upon iodine exchange, biodistribution in normal rats at 15 min, and myocardial selectivity were thought to be reasonable objectives. These data would provide a basis for determining the relative merits of the labeled beta antagonists vis-a-vis a currently used myocardial perfusion imaging agent.

MATERIALS AND METHODS

Chemical reagents. Ortho- and para-iodobenzoyl chloride and meta-iodobenzoic acid were obtained commercially.* Sodium [^{125}I]iodide in 0.08 *N* sodium hydroxide, and [^{201}Tl]thallous chloride, were carrier free. The acebutolol hydrochloride was used without purification.†

Chemical and radiochemical purity analyses. The chemical and radiochemical purity of the products were determined by thin layer chromatography using the following analytical systems: (a) Silica gel plates‡ (250 μ with fluorescent indicator) and chloroform:methanol (85:15); (b) Silica gel plates‡ (250 μ with fluorescent indicator) and butanol:acetic acid:water (4:1:2); and (c) KC_{18} plates (200 μ with fluorescent indicator) and methanol:water:acetic acid (60:40:1). The unlabeled starting materials were applied adjacent to the radiolabeled products, the chromatograms were developed, and 0.5-cm segments of the adsorbent were removed and counted in a NaI(Tl) well counter. In all three systems, 95-99% of the radioactivity was associated with a single component having an R_f value identical to that of the unlabeled material. No other organic products could be visualized by ultraviolet light. The nonlabeled compounds were characterized by their infrared, nuclear magnetic resonance, and mass spectra. Elemental analyses (C, H, N, I) were performed commercially and agreed to within ±0.4% of the theoretical values.

Chemical synthesis and exchange. The ortho-, meta-, and para-iodobenzoyl derivatives of acebutolol (IBA) were prepared using the previously published procedure (Fig. 1) (*9*). The radioisotopic exchange with the ortho-iodo isomer was achieved by autoclaving an ampoule containing a buffered solution of the compound and sodium [^{125}I]iodide (5-9 mCi) at 130°C for 90 min (*17*). Adjustment of the cooled solution to pH 10, fol-

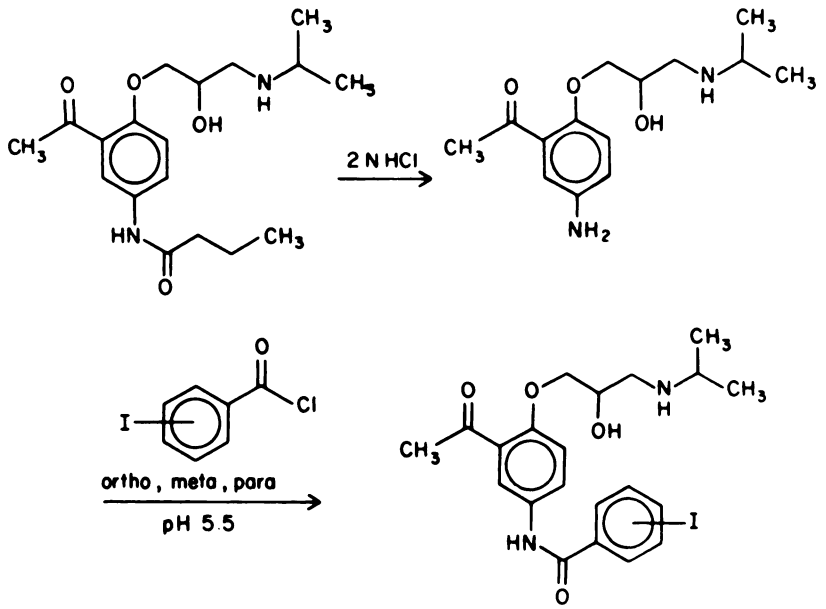

FIG. 1. Preparation of isomeric iodobenzoyl derivatives of acebutolol.

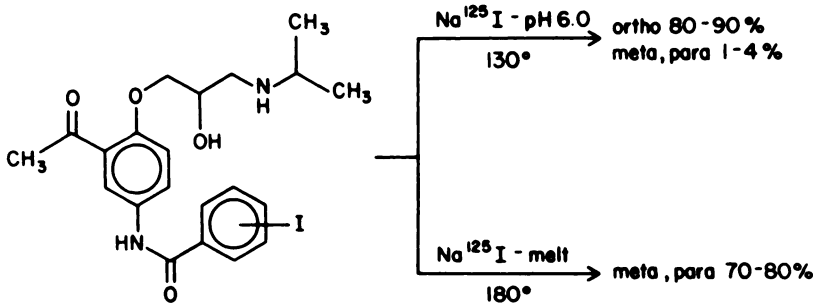

FIG. 2. Radioisotopic labeling of isomeric iodobenzoyl derivatives of acebutolol.

lowed by extraction with chloroform, gave the product, which was virtually free of inorganic [^{125}I]iodide. Incorporation proceeded with an 89% yield, 99% purity, and specific activity of 3.2 Ci/millimole. The same procedure with the meta and para isomers gave only a 1-4% incorporation, and therefore exchange was achieved by using the melt method (*18*) to give a 75-80% incorporation and specific activities of 0.38 and 1.9 Ci/millimole, respectively (Fig. 2).

Biodistribution. A solution of iodine-125-labeled product or [^{201}Tl]thallous chloride (0.1 ml, 10 μCi per rat) was injected intravenously into 150-250 g Wistar or Sprague-Dawley outbred rats. Fifteen minutes after administration the animals were killed by ether asphyxiation and the organs of interest excised. Blood samples were obtained from a vein in the thoracic cavity. The tissue samples were weighed, counted in a NaI(Tl) well scintillation counter, and the percent of the dose per gram of tissue (% ID/g) and/or per organ (% ID/organ) determined.

RESULTS AND DISCUSSION

The chemical syntheses for the three iodobenzoyl acebutolol isomers resulted in yields adequate to provide

TABLE 1. TISSUE CONCENTRATIONS OF RADIOPHARMACEUTICALS 15 MIN AFTER INTRAVENOUS ADMINISTRATION TO RATS*

	Tl (n = 6)	*o*-IBA† (n = 3)	*m*-IBA (n = 6)	*p*-IBA (n = 5)
Liver	0.72‡	1.57	1.84	1.70
	(0.49-0.93)	(1.39-1.88)	(1.54-2.04)	(1.40-2.11)
Spleen	0.86	0.60	0.65	0.91
	(0.67-1.12)	(0.49-0.70)	(0.57-0.82)	(0.67-1.16)
Lungs	2.03	1.07	1.76	1.34
	(1.79-2.70)	(0.96-1.16)	(1.45-2.63)	(1.08-2.16)
Kidneys	5.83	0.95	3.42	3.30
	(4.23-7.49)	(0.92-0.99)	(2.57-4.03)	(2.63-3.96)
Pelt	0.19	0.19	0.15	0.19
	(0.16-0.22)	(0.06-0.26)	(0.11-0.22)	(0.16-0.23)
Muscle	0.28	0.21	0.15	0.17
	(0.24-0.33)	(0.18-0.24)	(0.12-0.17)	(0.15-0.22)
Bones	0.49	0.19	0.17	0.19
	(0.36-0.71)	(0.12-0.24)	(0.14-0.21)	(0.15-0.29)
Blood	0.07	0.24	0.07	0.17
	(0.05-0.08)	(0.20-0.28)	(0.05-0.09)	(0.09-0.23)
Thyroid	10.91	5.65	4.25	5.88
	(7.83-16.32)	(5.13-6.20)	(2.39-6.29)	(5.58-6.44)
Atria	2.16	0.42	0.77	0.80
	(1.45-3.46)	(0.36-0.51)	(0.59-1.02)	(0.67-0.87)
Ventricles (normal)	3.09	0.43	1.06	0.93
	(1.90-4.11)	(0.36-0.55)	(0.74-1.19)	(0.75-1.07)

* Dose = 0.01 mCi in 0.1 ml saline.

† *o*-IBA = ortho-iodobenzoylacebutolol, *m*-IBA = meta-iodobenzoylacebutolol, *p*-IBA = para-iodobenzoylacebutolol.

‡ Percent injected dose per gram of tissue, mean and (range) for n animals.

TABLE 2. ORGAN CONCENTRATIONS OF RADIOPHARMACEUTICALS 15 MIN AFTER INTRAVENOUS ADMINISTRATION TO RATS*

	Tl (n = 6)	*o*-IBA† (n = 3)	*m*-IBA† (n = 6)	*p*-IBA† (n = 5)
Liver	7.86‡	14.4	19.8	21.4
	(6.55–11.0)	(13.0–15.8)	(18.4–21.4)	(15.6–25.3)
Spleen	0.57	0.26	0.43	0.58
	(0.48–0.83)	(0.21–0.34)	(0.35–0.51)	(0.51–0.70)
Lungs	2.26	1.56	1.96	2.63
	(2.08–2.75)	(1.46–1.70)	(1.59–2.69)	(2.12–3.25)
Stomach	1.19	1.57	1.46	2.58
	(0.46–1.96)	(0.15–2.45)	(0.63–2.59)	(0.57–3.21)
Small intestine	7.27	2.54	9.63	4.96
	(3.80–11.2)	(0.55–3.68)	(7.59–14.4)	(3.53–6.41)
Large intestine	3.32	—	1.76	0.87
	(1.60–4.72)		(0.54–5.28)	(0.60–1.42)
Kidneys	11.6	1.76	6.77	7.28
	(8.80–15.0)	(1.65–1.84)	(6.39–7.66)	(6.80–7.83)
Pelt	8.90	9.80	6.94	10.2
	(7.77–10.6)	(9.57–10.0)	(5.77–9.87)	(8.43–12.1)
Muscle	26.4	16.4	13.8	21.6
	(22.2–29.3)	(15.4–18.0)	(12.2–15.2)	(15.4–24.2)
Bones	7.07	3.70	2.25	5.85
	(5.73–10.6)	(2.20–4.74)	(2.12–2.42)	(4.13–7.13)
Blood	0.99	2.86	1.41	3.33
	(0.74–1.18)	(2.52–3.12)	(0.86–3.10)	(2.91–3.51)
Thyroid	0.22	0.11	0.09	0.12
	(0.15–0.33)	(0.10–0.13)	(0.05–0.12)	(0.11–0.13)
Heart	2.17	0.22	0.70	0.76
	(1.56–2.83)	(0.13–0.30)	(0.63–0.77)	(0.54–0.94)
Total	79.8	55.2	67.0	82.2

* Dose = 0.01 mCi in 0.1 ml saline.
† *o*-IBA = ortho-iodobenzoylacebutolol, *m*-IBA = meta-iodobenzoylacebutolol, *p*-IBA = para-iodobenzoylacebutolol.
‡ Percent injected dose per organ, mean and (range) for n animals.

well-characterized products. Either method of exchange produced good incorporation of the label. The specific activities were sufficient to permit the collection of biodistribution data at subpharmacologic (nmol) doses. The conditions for exchange have not been optimized and significant enhancement of the specific activity could be achieved by increasing the quantity of sodium [^{125}I]-iodide and/or decreasing the amount of iodobenzoyl precursor. The position of the iodine on the benzoyl moiety has a dramatic but predictable effect upon the ease of exchange. Whereas the ortho-iodobenzoyl isomer underwent facile exchange both by autoclaving in a buffer and by melting, autoclaving the meta- and para-iodobenzoyl acebutolol derivatives in pH 6.0 to 6.5 buffer in the presence of sodium [^{125}I]iodide produced only 1–4% radioisotopic exchange. This experience is analogous to that encountered in the preparation of the ortho-, meta-, and para-iodobenzoic acids (*17*).

The biologic distribution data for the four radiopharmaceuticals are shown in Tables 1 and 2. The % ID/g and % ID/organ values for the [^{125}I]iodobenzoyl acebutolol derivatives were generally lower in most tissues at 15 min than for Tl-201. The most significant differences in organ distribution for the two classes of agents were in the liver values, where the labeled beta antagonists were two to three times as high as Tl-201, and in the muscle and bone values where Tl-201 was higher by factors of 1.3 to 2.

Greater uptake in the myocardium was also observed for Tl-201 (3.09 and 2.16 % ID/g in the ventricles and atria). Comparison of the T/NT ratios for the agents indicates that at 15 min Tl-201 possesses better cardioselectivity than the radioiodinated beta blockers (Table 3). The ratios of heart-to-lung, -blood, and -liver (1.5, 44, and 4.3, respectively) were significantly better than the corresponding values for the beta antagonists.

Positional isomerism has several effects upon the iodobenzoyl acebutolols. As described earlier, the method of radionuclide exchange is governed by whether the iodine is located in the activated ortho position or in the more resistant meta or para positions. The meta- and para-iodobenzoyl derivatives are significantly more

TABLE 3. UPTAKE AND TARGET-TO-NONTARGET VALUES FOR I-125-LABELED IODOBENZOYLACEBUTOLOLS AND Tl-201, AT 15 MIN AFTER ADMINISTRATION TO RATS*

Radiopharmaceutical	Myocardial uptake (% ID/g)	Myocardium		
		Lung	Liver	Blood
Tl-201 (n = 6)	3.09	1.58	4.85	40.8
	(1.90–4.11)	(1.05–2.06)	(3.36–6.06)	(26.1–78.8)
o-IBA† (I-125) (n = 3)	0.43	0.39	0.28	1.84
	(0.36–0.55)	(0.35–0.45)	(0.21–0.36)	(1.30–2.60)
m-IBA† (I-125) (n = 6)	1.06	0.55	0.53	12.1
	(0.74–1.19)	(0.45–0.65)	(0.48–0.58)	(10.4–14.8)
p-IBA† (I-125) (n = 5)	0.93	0.58	0.60	11.1
	(0.75–1.07)	(0.52–0.69)	(0.45–0.76)	(9.30–13.2)

* Dose = 0.01 mCi in 0.1 ml saline.

† *o*-IBA = ortho-iodobenzoylacebutolol, *m*-IBA = meta-iodobenzoylacebutolol, *p*-IBA = para-iodobenzoylacebutolol.

lipophilic than the ortho-iodobenzoyl compound (R_f = 0.46–0.47 against 0.60 on KC_{18} reversed-phase, thin-layer chromatographic plates), and this may be a contributing factor to the greater uptake and selectivity (T/NT) shown by these two radiolabeled agents compared with the ortho isomer.

On the basis of biodistribution data 15 min after i.v. administration to rats, none of the iodobenzoyl acebutolol derivatives examined was superior to Tl-201 as a selective myocardial imaging agent. However, the potential advantages of incorporating iodine-123 into these kinds of compounds make this an attractive approach to radiopharmaceutical design. In order for these derivatives to be more effective as myocardial imaging agents, it will be necessary to increase the uptake in the myocardium and decrease the concentrations in the lungs and blood. This study suggests that the meta- and para-iodobenzoyl isomers provide better uptake and selectivity than the ortho isomer. In addition, these biodistributional parameters may be further improved by adding or modifying functional groups on the molecule. Nonchemical techniques, such as exercise or positioning, may also increase the tissue uptake and T/NT values. Studies to examine these effects are in progress.

FOOTNOTES

* Eastman Kodak Company, Eastman Organic Chemicals, Rochester, NY.

† This compound was a generous gift from May and Baker, Ltd. (England).

‡ Fisher Scientific Company, Pittsburgh, PA.

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