Comparison of 19-Iodocholesterol and 6-Iodomethylnorcholesterol as Adrenal-Scanning Agents

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The two adrenal-specific compounds, 19-iodocholest-5-en-3β-ol (19-iodocholesterol) and 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (6-iodomethylnorcholesterol), synthesized pure in gram amounts have been labeled with iodine-125 and used for tissue-distribution studies in the rat. Radioactivity from 6-iodomethylnorcholesterol is accumulated in the rat adrenal gland at least 18 times more than is radioactivity from 19-iodocholesterol at 24 hr, and 50 times more at 72 hr. The use of radioactive 6-iodomethylnorcholesterol gave superior adrenal images in rats at earlier times than did radioactive 19-iodocholesterol, and the former should be equally superior when used in humans.

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In 1975 Kojima et al. (*1,2*) showed that the adrenal-scanning agent 19-iodocholest-5-en-3β-ol (19-iodocholesterol), as synthesized by the method of Counsell et al. (*3*), contained an impurity that they identified as its homoallylic isomer 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (6-iodomethylnorcholesterol). This observation was later confirmed by Basmadjian et al. (*4*). Kojima et al. (*5,6*) then showed 6-iodomethylnorcholesterol to be 10 times as active than was 19-iodocholesterol in concentrating in the rat adrenal, whereas Sarkar et al. (*7*) reported it to be five times as active in the rat and dog. Both groups concluded that the isomeric "impurity" was far superior to 19-iodocholesterol as an adrenal-scanning agent. Because of the obvious importance of 6-iodomethylnorcholesterol as a potential radiopharmaceutical for human use, we undertook to synthesize gram amounts of both compounds and have since reported the synthesis of 19-iodocholesterol (*8*) and 6-iodomethylnorcholesterol (*9*) in greater than 98% chemical and radiochemical purity. We now report the distribution of the two pure isomers in the rat.

MATERIALS AND METHODS

Radiopharmaceuticals. *[^{125}I]-19-iodocholesterol.* 19-iodocholest-5-en-3β-ol acetate (10 mg) was refluxed (N_2) for 4 hr in 15 ml acetone with 10 mCi carrier-free $Na^{125}I$, in 0.05 *N* NaOH and was neutralized with ascorbic acid before use. After evaporation of the acetone (N_2), 2 ml H_2O were added and the mix extracted with three 2-ml portions of ether. The combined ether washes were dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in dioxane, added dropwise to methanolic NaOH, and stirred for 2 hr at room temperature. Cold water (1 ml) was then added, and the resulting mixture extracted with ether (×3). The combined extracts were dried over Na_2SO_4 and evaporated. The resulting white residue was placed on a preparative thin layer chromatography (TLC) plate* and developed with $CHCl_3$. Three bands were observed under uv light. The R_f 0.13–0.20 band was scraped off, extracted with $CHCl_3$, filtered, and blown to dryness (N_2). Yield: 4.8 mg [^{125}I] 19-iodocholesterol (= 46% yield); total radioactivity, 1.69 mCi (34% exchange); specific activity, 0.33 mCi/mg. Thin layer chromatography ($CHCl_3$) showed a single spot at the R_f of pure nonradioactive 19-iodo-

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cholesterol (*8*) that was identical to the single radioactive spot detected by autoradiography.

[*^{125}I*] *6-iodomethylnorcholesterol.* Pure 6-iodomethylnorcholesterol (*9*) (10 mg) was refluxed (N_2) for 4 hr in 15 ml acetone with 10 mCi of carrier-free $Na^{125}I$, in 0.05 N NaOH and neutralized with ascorbic acid before use. The solvent was removed (N_2), 2 ml H_2O added, and the mix then extracted 3 times with 2-ml portions of ether. The combined extracts were dried (Na_2SO_4) and the solvent removed (N_2). The residue was dissolved in $CHCl_3$, placed on a preparative TLC plate, and developed with $CHCl_3$ to give three bands, which were observed under uv light. The R_f 0.14–0.25 band was scraped off, extracted with $CHCl_3$, filtered, and blown to dryness (N_2). Yield: 7.2 mg [^{125}I] 6-iodomethylnorcholesterol; (= 70% yield); total radioactivity, 4.7 mCi (64% exchange); specific activity, 0.66 mCi/mg. Nonradioactive 6-iodomethylnorcholesterol was added to reduce the specific activity to 0.33 mCi/mg. With $CHCl_3$, TLC showed a single spot at the R_f of pure nonradioactive 6-iodomethylnorcholesterol that was identical to the radioactive spot detected by autoradiography.

Formulation. Both isomers were formulated to give a concentration of 1 mg/ml. This formulation contained 8% ethanol, 1.6% Tween 80, and 90.4% normal saline.

Rats. Ten mature male Sprague–Dawley rats, weighing 400–450g and being fed a regular diet, were given 72 μCi (0.2 mg in 0.2 ml) of [^{125}I] 6-iodomethylnorcholesterol through a tail vein after they had been anesthetized with intraperitoneal sodium pentobarbital (30 mg/kg). Ten similar rats were injected with 68 μCi (0.2 mg in 0.2 ml) of [^{125}I] 19-iodocholesterol. None of these animals received Lugol's iodine.

Adrenal scans. Adrenal I-125 scintiscans were performed at 9 days on two rats in the preceding series, one having received 19-iodocholesterol and the other 6-iodomethylnorcholesterol. Two additional rats each received one of the tracers similarly except for the administration of Lugol's iodine—3 drops daily in the drinking water, starting one day before the injection and continuing until autopsy. These rats were anesthetized with intraperitoneal sodium pentobarbital and scanned on days 1, 2, 3, 4, and 7 after injection. Posterior scans were obtained with each rat lying supine on the face of a scintillation camera† with a high-resolution collimator.

Tissue samples. Six rats (three injected with 19-iodocholesterol and three injected with the 6-isomer) were killed by a lethal dose of intraperitoneal sodium pentobarbital at 1, 3, or 7 days after injection. Ten tissues—adrenal, thyroid, lung, liver, kidney, testes, heart, spleen, brain, and blood—were obtained, cleaned of adipose and connective tissue, weighed, placed in thin-walled plastic test tubes, and counted in an automatic gamma well counter for at least 10^4 counts, after which corrections were made for radioactive decay. The concentration in each tissue was expressed as percentage of injected dose per gram.

RESULTS

Radiopharmaceutical purity. *6-iodomethylnorcholesterol.* Sarkar et al. (*7*) gave no details of the method of purification of [^{131}I] 6-iodomethylnorcholesterol whereas Kojima et al. (*6*) merely extracted the compound with ether and then formulated it. Neither group gave any spectroscopic evidence for its purity. When we prepared [^{131}I] 6-iodomethylnorcholesterol from 6-iodomethylnorcholesterol by isotope exchange with $Na^{131}I$ in either refluxing acetone (*5*) or ethanol (*4*), we obtained 6-iodomethylnorcholesterol that consistently contained 10–25 mole% impurity as proved by C-13 nuclear magnetic resonance (CMR) spectroscopy. From CMR it was evident that the impurity consisted of two or more compounds, neither of which was 19-iodocholesterol. It is clear that isotope exchange with $Na^{131}I$ by refluxing will yield a product that is not more than 75% to 90% 6-iodomethylnorcholesterol. For this reason we purified this crude material by preparative TLC and proved it to be 97% chemically pure 6-iodomethylnorcholesterol by CMR. We conclude that the radioactive 6-isomer prepared as described by Kojima et al. and Sarkar et al., if not purified further, could not have been better than 75%–90% pure.

19-iodocholesterol. Sarkar et al. (*7*) do not specify the degree of purity of labeled 19-iodocholesterol used for comparison with 6-iodomethylnorcholesterol. Kojima et al. obtained "pure" radioactive 19-iodocholesterol by preparative TLC of a 9:1 mixture of 19-iodocholesterol and 6-iodomethylnorcholesterol (*6*). The R_fs of these two compounds are within 0.1 unit of each other; therefore, contamination of the 19-isomer with the 6-isomer is possible. Since all three groups of workers report that 19-iodocholesterol, prepared by the method of Counsell et al. (*3*) does contain the 6-isomer as an impurity (Kojima et al. 30%; Sarkar et al. 10%–25%; Couch et al. 10%–60%), it is possible that the radioactive 19-iodocholesterol used in the experiments of Sarkar et al. contained some of the 6-isomer.

Animal studies. Table 1 presents the relative distribution of I-125 from 6-iodomethylnorcholesterol and 19-iodocholesterol in rats as expressed in percentage of injected dose per gram of tissue.

TABLE 1. RAT TISSUE DISTRIBUTION OF I-125 FROM TAGGED 6-IODOMETHYLNORCHOLESTEROL AND 19-IODOCHOLESTEROL

	% injected dose/g (mean and range)					
	6-iodomethylnorcholesterol (3 animals)			19-iodocholesterol (3 animals)		
Tissue	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Adrenal	23 (17–29)	45 (42–48)	53 (30–77)	1.3 (0.9–1.9)	0.88 (0.6–1.2)	1.9 (1.0–3.6)
Thyroid	38 (35–44)	74 (51–109)	70 (51–103)	93 (89–101)	190 (156–217)	184 (131–251)
Liver	0.78 (0.68–0.98)	0.38 (0.36–0.40)	0.11 (0.09–0.15)	0.18 (0.17–0.19)	0.04 (0.03 –0.04)	0.01 (0.01 –0.01)
Kidney	0.24 (0.17–0.30)	0.22 (0.21–0.23)	0.14 (0.11–0.17)	0.07 (0.07–0.08)	0.02 (0.02 –0.02)	0.01 (0.01 –0.01)
Testes	0.07 (0.05–0.09)	0.07 (0.06–0.08)	0.05 (0.04–0.08)	0.04 (0.04–0.04)	0.01 (0.01 –0.01)	0.003 (0.003–0.003)
Brain	0.03 (0.02–0.03)	0.03 (0.03–0.03)	0.03 (0.02–0.03)	0.01 (0.01–0.01)	0.002 (0.002–0.003)	0.001 (0.001–0.001)
Spleen	1.0 (0.75–1.28)	0.64 (0.53–0.78)	0.16 (0.13–0.20)	0.26 (0.20–0.31)	0.04 (0.04 –0.05)	0.01 (0.01 –0.01)
Lung	0.87 (0.61–1.08)	0.66 (0.64–0.72)	0.26 (0.17–0.36)	0.18 (0.16–0.21)	0.04 (0.04 –0.04)	0.01 (0.01 –0.01)
Heart	0.23 (0.17–0.29)	0.19 (0.18–0.20)	0.07 (0.06–0.09)	0.05 (0.05–0.05)	0.01 (0.01 –0.02)	0.003 (0.003–0.003)
Blood	0.41 (0.31–0.48)	0.20 (0.18–0.22)	0.06 (0.05–0.08)	0.14 (0.13–0.16)	0.03 (0.03 –0.03)	0.005 (0.005–0.006)

Adrenal uptake. The peak adrenal concentration of I-125 from 6-iodomethylnorcholesterol was 53% injected dose per gram (2.6% dose per total organ) on day 7 compared with 2% injected dose per gram (0.06% dose per total organ) on day 7 for the 19-isomer. The uptakes at 1, 3, and 7 days were 18, 51, and 27 times as great, respectively, as those for 19-iodocholesterol.

Adrenal-to-liver ratio. Adrenal-to-liver concentration ratios for 6-iodomethylnorcholesterol were 29, 118, and 482 on days 1, 3, and 7. The same ratios for 19-iodocholesterol were 7, 22, and 63 on the same days.

Adrenal-to-kidney ratio. Adrenal-to-kidney concentration ratios on days 1, 3, and 7 were 82, 204, and 379 for 6-iodomethylnorcholesterol and were 19, 44, and 190 for 19-iodocholesterol.

Thyroid uptake. The concentrations of I-125 from 6-iodomethylnorcholesterol in the thyroid were approximately half of those from 19-iodocholesterol at days 1, 3, and 7.

Scintiscans. Excellent adrenal images were obtained at 9 days with 6-iodomethylnorcholesterol in the rat without Lugol's iodine. No adrenal tissue could be detected in the comparable rat injected with 19-iodocholesterol, virtually all the radioactivity being localized in the thyroid gland (Fig. 1). In the two rats that were given Lugol's iodine, excellent adrenal images were obtained on days 2, 3, 4, and 7 after injection of labeled 6-iodomethylnorcholesterol; but in no case could the adrenals be successfully imaged after the injection of the labeled 19-iodocholesterol, even when prolonged imaging times were used (Fig. 2).

DISCUSSION

Our studies are in qualitative agreement with those of Kojima et al. (*5,6*) and Sarkar et al. (*7*), demonstrating higher adrenal uptake, higher adrenal-to-tissue ratios, and superior images with [^{125}I] 6-iodomethylnorcholesterol when compared with [^{125}I] 19-iodocholesterol. Our results, however, are not in quantitative agreement. All three groups have shown the superiority of 6-iodomethylnorcholesterol over 19-iodocholesterol as an adrenal-scanning agent. Kojima et al. (*5*), comparing disintegrations per minute per mg of tissue, reported that about 10 times as much of the 6-isomer accumulated in the adrenal as did the 19-isomer. Sarkar et al. (*7*) (using % kg dose/gram tissue values) reported that 3 to 5 times as much of the 6-isomer accumulated. Our data (% injected dose/gram tissue) indicate 6-isomer to 19-isomer uptake ratios of 18 (day 1), 51 (day 3), and 27 (day 7). These differences may be attributed in part to differences in rat weights [140–150g (*5*), 210–260g (*7*), 425–450g]; or in specific activities of the radiopharmaceutical [1 mCi/mg (*5*), 1.3 mCi/mg (*7*), and

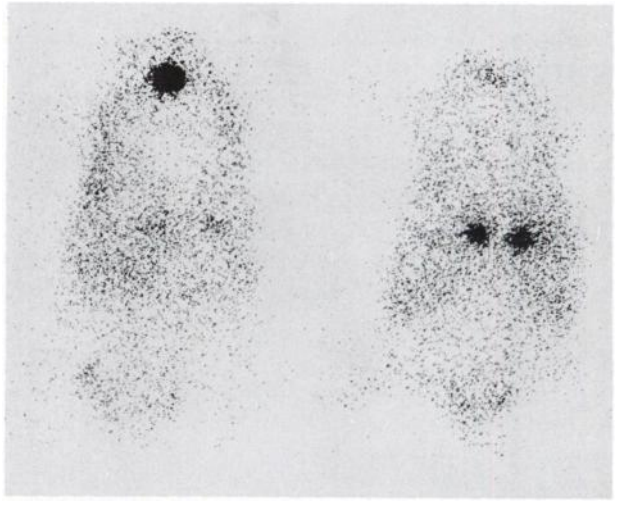

FIG. 1. Scintiscans of rats injected 9 days previously with (left) 19-iodocholesterol and (right) 6-iodomethylnorcholesterol. For both images 23,000 counts were collected. Times: 36 min and 10 min, respectively.

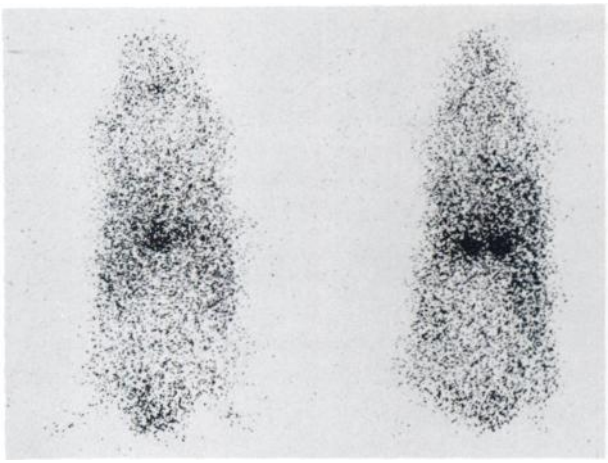

FIG. 2. Scintiscans of rats injected 2 days previously with (left) 19-iodocholesterol and (right) 6-iodomethylnorcholesterol. Both rats were receiving Lugol's iodine, 3 drops/day in the drinking water. For both images 23,000 counts were collected. Times: 27 min and 5 min, respectively.

0.33 mCi/mg]; or in purity of material prepared by the three laboratories. Although the discrepancies cannot be explained until exactly comparable experiments are carried out with radiopharmaceuticals from the three laboratories, the available data indicate that radioactive 6-iodomethylnorcholesterol from the three different laboratories is of comparable adrenal-concentrating activity but that the radioactive 19-iodocholesterol prepared by us has considerably less adrenal-concentrating activity than that reported by the other two laboratories. A 10–20% impurity in the 6-norcholesterol isomer should decrease its accumulation in the adrenal to a small extent. The presence of 10–20% 6-iodomethylnorcholesterol in 19-iodocholesterol, however, should greatly enhance the uptake of radioactivity in the adrenal. It has been shown by CMR that the preparation of radioactive 6-isomer, as outlined in the experimental section, does not give rise to any 19-isomer, and preparation of radioactive 19-isomer does not give rise to any 6-isomer (*8,9*). Consequently, to eliminate the possibility of contamination of the 19-isomer with the 6-isomer (or vice versa), radioisotopic exchange must be performed with compounds of >98% chemical purity, and the final radioactive product must then be purified by TLC to eliminate nonisomeric contaminants.

Our observations, taken in conjunction with those of Kojima et al. (*5,6*) and Sarkar et al. (*7*), establish that the apparently high selective concentration of radioactive 19-iodocholesterol prepared by the method of Counsell et al. (*3*) was due to the unrecognized presence of its homoallylic isomer, radioactive 6-iodomethylnorcholesterol. We have been entirely unable to detect rat adrenal tissue by scintillation scanning with pure [^{125}I] 19-iodocholesterol at time intervals up to 9 days. With pure [^{125}I] 6-iodomethylnorcholesterol it is possible to image the rat adrenal gland easily at 48 hr, possibly as early as 24 hr. It should be possible to label 6-iodomethylnorcholesterol with I-123, which has a principal gamma (159 keV) more favorable for imaging than that of I-131 (364 keV), and has a much shorter half-life (13.3 hr compound with 8.1 days). This should result in diagnostic images at 48 and, possibly, 24 hr accompanied by a significant reduction in radiation dose to the adrenal.

Finally, Sarkar et al. (*7*) report that 6-iodomethylnorcholesterol concentrates to about the same extent in adrenal medulla as in adrenal cortex. This raises the possibility of imaging such abnormalities of the adrenal medulla as neuroblastoma and pheochromocytoma.

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FOOTNOTES

* Silica gel with fluorescent indicator, Merck, Darmstadt, Germany.

† Ohio-Nuclear, series 100, Solon, Ohio.

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