# POTENTIAL ORGAN OR TUMOR-IMAGING AGENTS. 12. ESTERS OF 19-RADIOIODINATED CHOLESTEROL

Raymond E. Counsell, Vasant V. Ranade, Prabhakar G. Kulkarni, and Parviz Afiatpour

*The University of Michigan, Ann Arbor, Michigan*

***The acetate and palmitate esters of $^{125}$I-19-iodocholesterol were synthesized for tissue distribution studies in rats. Tissues were analyzed at 48 and 96 hr after intravenous, subcutaneous, and oral administration. In contrast to $^{125}$I-19-iodocholesterol, the esters failed to show appreciable adrenal uptake following intravenous or subcutaneous administration. All forms, however, produced significant radioactivity in the adrenals when given orally. In fact, the best adrenal-to-liver ratio was achieved by this route. These results imply that esters of 19-radioiodinated cholesterol can serve as substrates for pancreatic cholesterol esterase and suggests their possible use in the diagnosis of pancreatic insufficiency.***

Recent studies have demonstrated that 19-radioiodinated cholesterol behaves similarly to cholesterol in its capacity to concentrate selectively in the adrenal cortex of laboratory animals (*1,2*). Accordingly, $^{131}$I-19-iodocholesterol has proven to be a useful radiopharmaceutical for the diagnosis of unilateral adrenocortical carcinoma (*3*), primary aldosterone adenoma (*4*), and Cushing's syndrome (*5*).

In man 60–80% of the plasma cholesterol is present as cholesterol esters (*6*). Following intravenous administration, cholesterol rapidly becomes esterified in the plasma (*7*). In contrast to its esterifying capacity, the plasma does not appear to contain enzymes which hydrolyze cholesterol esters to free cholesterol. This transformation is presumed to occur predominantly in the liver (*7*).

One of the important functions of the esters is their involvement in the transport of storage of cholesterol (*6*). In the GI tract, dietary cholesterol esters are hydrolyzed in the intestinal lumen by pancreatic cholesterol esterase, and only free cholesterol appears to be absorbed by the intestine (*6*). During the absorption process, however, a portion of the cholesterol becomes re-esterified in the mucosa and passes into the intestinal lymph in the esterified form (*6*).

A distinct difference between free and esterified forms has also been demonstrated for the transport of cholesterol to the adrenals. Dexter (*8*) noted that nonesterified plasma cholesterol is more efficiently incorporated into adrenal tissue than plasma cholesterol palmitate. Once in the adrenals, however, cholesterol is rapidly esterified by polyunsaturated fatty acids. This represents a storage form for cholesterol and in most species cholesterol ester represents 80–90% of the total adrenal cholesterol (*6*).

The question arises, does iodinated cholesterol and its esters have the same metabolic fate as cholesterol and its esters? This question prompted us to synthesize some 19-radioiodinated cholesterol esters and compare their tissue distribution properties with those found for 19-radioiodinated cholesterol. The acetate and palmitate esters were selected for this preliminary study and the distribution of radioactivity following subcutaneous, intravenous, and oral administration was examined.

## MATERIALS AND METHODS

Iodine-125-19-iodocholesterol and its corresponding acetate were prepared according to previously published procedures (*1*). The scheme for the synthesis of $^{125}$I-19-iodocholesterol palmitate (III) is illustrated in Fig. 1 and involved the following set of chemical experiments:

**1. Preparation of cholest-5-ene-3$\beta$,19-diol 3-palmitate 19-p-toluenesulfonate (II).** A mixture of cholest-5-ene-3$\beta$,19-diol 19-p-toluenesulfonate (I,19-

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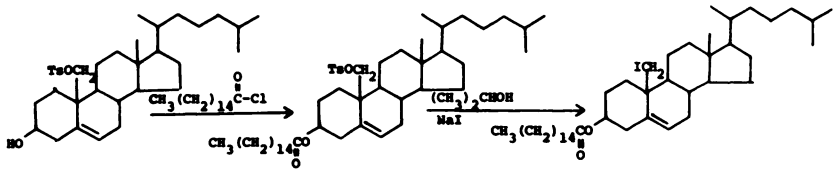
For reprints contact: R. E. Counsell, Laboratory of Medicinal Chemistry, College of Pharmacy, The University of Michigan, Ann Arbor, Mich. 48104.



I II III

**FIG. 1.** Scheme for synthesis of 19-iodocholesterol palmitate (III).

tosyloxycholesterol, 278 mg, 5 m*M*) and palmitoyl chloride (150 mg, 6 m*M*) in dry benzene (1.1 ml) was stirred at room temperature for 3 hr. The benzene was removed in vacuo at room temperature and the residue treated with $H_2O$. The mixture was extracted with ether and the ether extract washed with $H_2O$ and dried over anhyd $Na_2SO_4$. Evaporation of the ether afforded a solid which was purified by recrystallization from 95% EtOH to give II (220 mg, 55%) as a colorless solid, mp 70–71°; and nmr as expected.

Anal: Calcd: $C_{50}H_{82}SO_5$: C, 75.53: H, 10.40; Found: C, 75.56; H, 10.56.

**2. Preparation of 19-iodocholest-5-en-3β-ol palmitate (III).** A solution of II (795 mg, 1 m*M*) and NaI (223 mg, 1.5 m*M*) in isopropyl alcohol (30 ml) was refluxed gently under an atmosphere of $N_2$ for 4 hr. The solution was concentrated to one tenth the original volume and poured slowly into ice water. The mixture was extracted with ether and the ether extract washed with $H_2O$ and dried over anhyd $Na_2SO_4$. Evaporation of the ether afforded a solid which was purified by recrystallization from acetone to give III (450 mg, 60%), mp 40–41°; ir and nmr as expected.

Anal. Calcd. $C_{43}H_{75}O_2I$: C, 68.74; H, 10.07. Found: C, 68.92; H, 10.14.

**3. Preparation of radioiodinated palmitate ester.** An aqueous solution of $Na^{125}I$ (5 mCi) was placed in a 50-ml round bottom flask and the $H_2O$ removed by distillation with benzene. A solution of III (100 mg) in acetone (10 ml) was added and the solution refluxed under an atmosphere of $N_2$ for 4 hr. The flask was allowed to cool and the contents poured into ice-$H_2O$. Extraction with ether and workup as above gave a residue which upon crystallization from acetone afforded radioiodinated III with a specific activity of 10 μCi/mg (33% exchange). Thin-layer chromatography using $CHCl_3$:EtOH (1:1) showed a single spot ($R_f$ 0.60) coincident with the radioactive area shown by a radiochromatogram scan.

**Tissue distribution studies.** Radioiodinated sterols were given subcutaneously, intravenously (tail vein) or orally to albino male Sprague-Dawley rats weighing 175–200 gm. The dose administered was approximately 50 μCi/rat. The vehicle used for $^{125}I$-19-

**TABLE 1. TISSUE DISTRIBUTION OF $^{125}I$-19-IODOCHOLESTEROL AND**

| Tissue | Compound | 48 hr | | |
|---|---|---|---|---|
| | | Subcutaneous | Intravenous | Oral |
| Adrenal | Free alcohol | 7,862 ± 1,644† | 24,928 ± 3,873 | 12,465 ± 1,111† |
| | Acetate | 292 ± 53 | 81 ± 5 | 1,218 ± 279 |
| | Palmitate | 86 ± 17† | 112 ± 16 | 969 ± 275† |
| Blood | Free alcohol | 264 ± 15† | 1,050 ± 113 | 346 ±48† |
| | Acetate | 28 ± 9 | 19 ± 3 | 48 ± 9 |
| | Palmitate | 34 ± 2† | 44 ± 3 | 252 ± 99† |
| Kidney | Free alcohol | 92 ± 14† | 179 ± 14 | 120 ± 9† |
| | Acetate | ‡ | ‡ | 28 ± 3 |
| | Palmitate | ‡ | ‡ | 28 ± 1† |
| Liver | Free alcohol | 223 ± 45† | 434 ± 21 | 282 ± 50† |
| | Acetate | 20± 0 | 30 ± 4 | 22 ± 6 |
| | Palmitate | ‡ | 25 ± 5 | 43 ± 3† |
| Lung | Free alcohol | 266 ± 36† | 784 ± 86† | 384 ± 37† |
| | Acetate | 13 ± 1 | 2,207 ± 553 | 81 ± 38 |
| | Palmitate | ‡ | 779 ± 365 | 64 ± 9† |
| Thyroid | Free alcohol | 186,512 ± 42,009† | 114,255 ± 15,780 | 89,074 ± 18,882† |
| | Acetate | 14,344 ± 2,901 | 7,210 ± 99 | 73,192 ± 20,075 |
| | Palmitate | 58,820 ± 12,780† | 15,081 ± 5,450 | 36,999 ± 5,825† |

* Values represent mean dpm/mg for 3 rats ± s.e.m.
† Values represent mean dpm/mg for 2 rats ± s.e.m.
‡ Below minimum detectable level; 10 dpm/mg.

iodocholesterol was 90% ethanol (0.2–0.3 ml) and, for the esters, the vehicle was isopropyl myristate: ethanol 2:1 (0.2–0.3 ml). Groups of two or three animals were killed by exsanguination through the ventricle at 48 and 96 hr postinjection. The liver, kidney, adrenal, thyroid, and lung were excised, weighed, and homogenized. The organs were washed thoroughly with isotonic saline and blotted dry. The liver, kidney, and lung were minced with scissors and homogenized. Two samples each of liver, kidney, and lung homogenates, entire adrenal, thyroid, and heparinized blood were placed in liquid scintillation vials. To each vial was added 0.5 ml of 10% NaOH and the samples left overnight at room temperature. The vials were then heated for 10 min at 60° in a waterbath to complete digestion, allowed to cool, and five drops each of glacial acetic acid and 30% hydrogen peroxide added. Ten milliliters of thixotropic liquid counting system (*9*) were then added and each vial shaken using a vortex mixer. The vials were kept in a cool, dark place for at least 4 hr before counting. Radioactivity was assayed in a Beckman LS-200 liquid scintillation spectrometer. Sufficient counts were accumulated to reduce the probable error of counting to less than 5%. All counts were corrected for quench by using $^{125}$I-quench standards curves.

## RESULTS AND DISCUSSION

Preliminary tissue distribution results for 19-iodocholesterol and its acetate and palmitate esters following subcutaneous, intravenous, and oral administration to rats are recorded in Table 1. Since the liver and kidney are the most prominent interfering organs for adrenal photoscanning, a high adrenal-to-liver or adrenal-to-kidney ratio is a prerequisite for any radiopharmaceutical intended to be used for imaging the adrenals. Accordingly, Fig. 2 compares the adrenal-to-liver ratios of radioactivity observed 48 hr after various routes of administration. The adrenal-to-kidney ratios exhibited a similar profile of distribution. Although less of the administered dose was taken up by the adrenals after oral administration, the highest target-to-nontarget ratios were observed following this mode of administration. Moreover, the achievement of an adrenal-to-liver ratio approaching 165 at 96 hr following the oral administration of $^{125}$I-19-iodocholesterol suggests that this route of administration should be further evaluated. An oral preparation of 19-radioiodinated cholesterol would have many pharmaceutical advantages over the small volume parenteral product preparation procedure currently used.

Irrespective of the route of administration, $^{125}$I-19-iodocholesterol always produced a significant concentration of radioactivity in the adrenals, and, except for thyroid, this amount was considerably greater than all other tissues examined at 48 and 96 hr. The high levels of radioactivity in the thyroid presumably reflect in vivo liberation of free iodide either chemically or enzymatically. Studies in dog (*1*) and man (*10*), however, have shown that most of the thyroid uptake can be blocked by predosing with Lugol's solution.

In contrast to the free sterol, much less radioactivity was apparent in the adrenals following subcutaneous or intravenous administration of the acetate or palmitate esters. This observation is consistent with the previously noted inefficient incorporation of cholesterol palmitate into adrenal tissue (*8*). As in the case of cholesterol, free 19-iodocholesterol is necessary before appreciable adrenal uptake occurs.

The ability of the iodinated steroids to behave similarly to their noniodinated counterparts is further supported by the results obtained after oral administration of the 19-radioiodinated cholesterol esters. In contrast with the parenteral results, radioactivity concentrated in the adrenal much more efficiently when the sterol esters were given orally. Moreover, there was little difference in the adrenal-to-liver ratios when all preparations were given by the oral route. On the basis of current knowledge concerning cholesterol ester metabolism, it seems reasonable to speculate that 19-iodocholesterol acetate and palmitate are hydrolyzed in the gastrointestinal tract by pancreatic cholesterol esterase to liberate free 19-

**ITS ESTERS IN RATS* AT TWO TIME PERIODS**

| 96 hr | | |
|---|---|---|
| Subcutaneous | Intravenous | Oral |
| 21,956 ± 1,278 | 60,065 ± 18,800 | 11,933 ± 500 |
| 395 ± 68 | 177 ± 14 | 1,988 ± 355 |
| 59 ± 5 | 162 ± 14 | 1,080 ± 76† |
| 735 ± 97 | 778 ± 192 | 885 ± 410 |
| 99 ± 33 | 19 ± 2 | 38 ± 8 |
| 266 ± 17 | 82 ± 21 | 25 ± 4† |
| 166 ± 9 | 493 ± 118 | 86 ± 6 |
| 19 ± 5 | ‡ | 17 ± 0 |
| 18 ± 2 | ‡ | ‡ |
| 233 ± 18 | 736 ± 91 | 72 ± 7 |
| 28 ± 4 | 43 ± 10 | 22 ± 3 |
| 12 ± 1 | 33 ± 13 | 11 ± 2† |
| 493 ± 50 | 1,411 ± 250 | 151 ± 20 |
| 21 ± 2 | 637 ± 35 | 32 ± 4 |
| 65 ± 15 | 497 ± 175 | 20 ± 2† |
| 129,488 ± 15,920 | 145,785 ± 14,235 | 44,997 ± 2,562 |
| 19,608 ± 5,783 | 19,132 ± 1,406 | 51,516 ± 4,470 |
| 68,382 ± 12,369 | 27,704 ± 7,257 | 24,104 ± 7,102† |

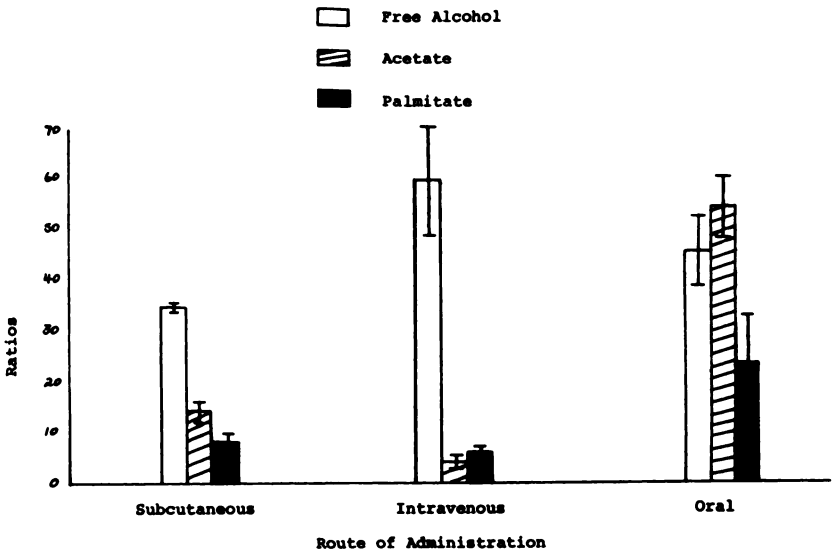



**FIG. 2.** Comparison of adrenal-to-liver radioactivity concentration ratios of 19-radioiodinated cholesterol and esters at 48 hr following different routes of administration. Values represent mean ratios ± s.e.m.

iodocholesterol which is then absorbed. Whether such radioiodinated cholesterol esters would offer any advantage over the currently used $^{131}$I-triolein or $^{131}$I-oleic acid in the diagnosis of pancreatic insufficiency is questionable, but, nontheless, seemingly worthy of investigation.

## CONCLUSIONS

The ability of 19-radioiodinated cholesterol to concentrate in the adrenals of rats following various modes of administration has been compared for the first time. In all instances, a significantly high concentration of radioactivity was observed in the adrenals following subcutaneous, intravenous, or oral administration. The highest target-to-nontarget ratios were achieved when this preparation was given orally.

In addition, administration of esters of 19-radioiodinated cholesterol produced high concentrations of radioactivity in the adrenals only after oral administration. The apparent ability of these radioiodinated sterol esters to serve as substrates for pancreatic cholesterol esterase suggests their possible usefulness in the diagnosis of pancreatic insufficiency. In all cases, the distribution pattern of radioactivity following the administration of 19-radioiodinated cholesterol or its esters was similar to cholesterol and its esters.

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