POTENTIAL TUMOR OR ORGAN-IMAGING AGENTS, 24. RADIOIODINATED PREGNENOLONE ESTERS[1]

*M. Van Dort, *S.W. Schwendner, #R.W.S. Skinner, #M.D. Gross, and *R.E. Counsell, *Department of Pharmacology, The University of Michigan Medical School and the #Nuclear Medicine Service, Veterans Administration Hospital, Ann Arbor, Michigan 48109

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ABSTRACT

A series of radioiodinated pregnenolone esters was prepared in an effort to find an agent that would be rapidly and selectively taken up by adrenal cortical tissue. Achievement of such a goal would provide the basis for the development of an adrenal imaging agent having several advantages over those agents currently available for clinical use. The radioiodinated esters for this study were readily prepared by treating pregnenolone with the appropriate iodobenzoic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylamino-pyridine (DMAP). The resulting esters were readily labeled with radioiodine by isotope exchange with sodium iodide-125 in pivalic acid. Subsequent tissue distribution studies in rats revealed that those esters displaying the most stability towards hydrolysis achieved the highest concentration in adrenal cortical tissue. For example, the 2,3,5-triiodobenzoate (6) showed an adrenal uptake of 23% of administered dose per gram of tissue at 0.5 hours. The achievement of high levels of radioactivity in the adrenal with this agent at early time periods warrants further evaluation of this agent in other animals.

INTRODUCTION

Radioiodinated derivatives of cholesterol have been widely used for the diagnosis of a variety of human adrenal disorders (2,3,4). The synthesis of these agents was based upon the known capacity of cholesterol to be readily taken up by adrenocortical cells for either conversion to steroid hormones or storage as cholesteryl esters (5).

Currently, ^{131}I-labeled 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol is the most widely used radiodiagnostic for the noninvasive visualization of the adrenal gland (6,7). The clinical utility of this agent, however, is limited because of the need to wait 4 to 5 days after i.v. administration to obtain images. Moreover, the long biological half-life of this agent in the adrenal poses a significant radiation

dose to this organ. There is, therefore, a need for an agent that would not only allow for earlier visualization of the adrenal, but also display rapid clearance once adequate images are obtained. Moreover, radioiodinated agents possessing such properties would permit the use of iodine-123 that has a short life ($T_{\frac{1}{2}}$ = 13hr) which would further reduce the radiation hazard to the patient.

Attempts to achieve such a goal prompted us to evaluate a number of esters of cholesterol wherein the radioiodine was linked to the acyl rather than the sterol moiety (8,9,10). These studies clearly demonstrated that it was unnecessary for the radioiodine to be affixed to the sterol in order to achieve selective localization in adrenocortical cells. Moreover, cholesterol itself was not required since similar radioiodinated esters of other sterols such as pregnenolone (3β-hydroxy-5-pregnen-20-one) and dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one) were readily taken up by adrenocortical tissue (10). For example, radioiodinated pregnenolone iopanoate (3β-hydroxy-5-pregnen-20-one[w(3-amino-2,4,6-triiodophenyl)-α-ethyl] propionate, 1) was found to give rise to unusually high levels of radioactivity in the adrenal cortex of rat within 0.5 hr of I.V. administration (23% of administered dose/gm of tissue).

Although the radioactivity in the adrenal following administration of 1 was over 13 times blood at 0.5 hr, the adrenal to liver ratio was less than 5 at this time. Such a low adrenal/liver ratio prevents adequate resolution of the adrenal unless liver subtraction techniques are employed. The present study was undertaken in an attempt to find radioiodinated esters of pregnenolone that would localize in the adrenal similar to 1, but would be more rapidly cleared from other tissues such

as the liver. Accordingly, a series of radioiodinated benzoate esters of pregnenolone was synthesized and their tissue distribution properties analyzed.

EXPERIMENTAL SECTION

Melting points were obtained in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. NMR spectra were obtained on a Varian EM360 A spectrometer with $CDCl_3$ as solvent. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane, which was used as the internal standard. IR spectra were obtained in the form of thin KBr wafers and recorded on a Perkin-Elmer 281 spectrophotometer. Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, IN. All analyses (C,H,I) are within ± 0.4% of the calculated values. Thin-layer chromatography (TLC) was done with Analtech preadsorbent glass backed silica gel plates with fluorescent indicator. TLC's of radiolabeled compounds were scanned with a Vanguard 930 autoscanner. Column chromatography was done on silica gel from Grace Davison Chemical, Baltimore, Maryland. CH_2Cl_2 was freshly distilled over phosphorous pentoxide and dried with molecular sieves (4A) prior to use Pregnenolone was kindly supplied by G.D. Searle and Co., Skokie, IL. Dicylohexylcarbodiimide, 4-(dimethylamino)-pyridine and other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. Sodium iodine-125 was obtained from New England Nuclear, Boston, MA. Tween 20 was obtained from Sigma Chemcial Co., St. Louis, MO. Rats were obtained from Harlan Sprague Dawley, Inc., Hasslett, MI. TLC's of tissue extracts were performed on plastic-backed silica gel plates with fluorescent indicator from Eastman Kodak Co.

General Procedure for Esterification. A solution of pregnenolone (1.0 mmole) and the requisite benzoic acid derivative (1.0 mmole) in 5 ml of dry CH_2Cl_2 was treated with dicyclohexylcarbodiimide (1.1 mmole) followed by 4-(dimethylamino)-pyridine (0.1 mmole). The reaction vessel was flushed with nitrogen, sealed, and stirred for 24 hr at room temperature. Dilution of the suspension to 40 ml with CH_2Cl_2, followed by filtration removed the precipitated dicyclohexylurea. The filtrate was extracted with 0.5N HCl (2x40 ml), saturated aqueous $NaHCO_3$ (1x40 ml), saturated brine (1x40 ml) and then dried ($MgSO_4$). The residue obtained following removal of the solvent under reduced pressure was chromatographed on a silica gel column with benzene/ethyl acetate (4:1) or hexane/ethyl acetate (5:2) as solvent systems. Analytically pure material was obtained by recrystalization from a suitable solvent system (Table 1).

Pregnenolone 2-iodobenzoate (2): IR (KBr) 1705 cm^{-1}; NMR ($CDCl_3$): δ 8.15-7.10 (m, 4, Ar-H), 5.56 (br m, 1, H-6), 4.99 (br m, 1, H-3), 2.17 (S, 3, 21-CH_3), 1.05 (S, 3, 19-CH_3) 0.66 (S, 3, 18-CH_3).

Pregnenolone 3-iodobenzoate (3): IR (KBr): 1715, 1695 cm^{-1}; NMR ($CDCl_3$): δ 8.43 - 7.07 (m,4, Ar-H), 5.46 (br m, 1, H-6), 4.88 (br m, 1, H-3), 2.14 (S, 3, 21 - CH_3), 1.11 (s, 3, 19-CH_3), 0.63 (S, 3, 18-CH_3).

Pregnenolone 4-iodobenzoate (4): IR (KBr) 1713, 1705 cm^{-1}; NMR ($CDCl_3$): δ 7.86 (S, 4, Ar-H), 5.47 (br m, 1, H-6), 4.87 (br m, 1, H-3), 2.14 (S, 3, 21-CH_3) 1.10 (S, 3, 19-CH_3), 0.65 (S, 3, 18-CH_3).

Pregnenolone 2,5-diiodobenzoate (5): IR (KBr) 1703 cm^{-1} NMR ($CDCl_3$): δ 8.15- 7.57 (m, 3, Ar-H), 5.49 (br m, 1, H-6) 4.83 (br m, 1, H-3), 2.17 (S, 3, 21-CH_3), 1.10 (S, 3, 19-CH_3), 0.67 (S, 3, 18-CH_3).

Pregnenolone 2,3,5-triidobenzoate (6): IR (KBr) 1728, 1703 cm^{-1}; NMR ($CDCl_3$): δ 8.36 (d, 1, Ar-H1); 7.75 (d, 1, Ar-H3), 5.47 (br m, 1, H-6), 4.89 (br m, 1, H-3) 2.13 (S, 3, 21-CH_3), 1.04 (S, 3, 19-CH_3), 0.62 (S, 3, 18-CH_3)

Pregnenolone 3,4,5-triiodobenzoate (7): IR (KBr): 1713, 1688 cm^{-1} NMR ($CDCl_3$): δ 8.47 (S, 2, Ar-H), 5.42 (br m, 1, H-6), 4.81 (br m, 1, H-3), 2.12 (S, 3, 21-CH_3), 1.02 (S, 3, 19-CH_3), 0.61 (S,3, 18-CH_3).

Table 1
Iodobenzoate Esters of Pregnenolone

Acyl Group	No.	Recrystn. solvent	mp °C	Formula	Yield(%)
2-iodobenzoyl	2	acetone	173-174.5	$C_{28}H_{35}IO_3$	75
3-iodobenzoyl	3	$CHCl_3$/ acetone	187-188	$C_{28}H_{35}IO_3$	70
4-iodobenzoyl	4	$CHCl_3$/ acetone	203-204.5	$C_{28}H_{35}IO_3$	68
2,5-diiodobenzoyl	5	acetone	193-194.5	$C_{28}H_{34}I_2O_3$	73
2,3,5-triiodo-benzoyl	6	THF/water	199-200.5	$C_{28}H_{33}I_3O_3$	69
3,4,5-triiodo-benzoyl	7	THF/water	249-250.5	$C_{28}H_{33}I_3O_3$	79

Radioisotope Exchange in Pivalic Acid: The ester (1.0 mg) and 300 µl of THF were placed in a vial and treated with 1 mCi of $Na^{125}I$ (in 20 µl of 0.05 N NaOH). The reaction vessel was sealed and evaporated to dryness under a stream of nitrogen. Pivalic acid (25 mg) was then added, the vial sealed, and heated at 160°C for 1.0 hr in an oil bath. The vial was allowed to cool and the contents taken up in THF (300 µl) and chromatographed on silica gel with either benzene/EtOAc (4:1) or hexane/EtOAc (5:2) as eluent. Radiochemical purity was established by radiochromatography of unlabeled ester as standard.

Table 2

Distribution of Radioactivity at 0.5 hr and 24 hr after Intravenous Administration of ^{125}I-labeled Pregnenolone Esters

	Tissue	2-IODO(2)	3-IODO(3)	4-IODO(4)	2,5-DIIODO(5)	2,3,5-TRIIODO(6)	3,4,5-TRIIODO(7)
0.5hr							
	Adrenal Cortex	14.86 ± 1.06[+]	16.54 ± 1.02	13.84 ± 1.17	23.57 ± 1.43	22.90 ± 1.76	18.16 ± 1.99
	Blood	0.52 ± 0.02	0.80 ± 0.05	1.65 ± 0.08	1.50 ± 0.06	2.04 ± 0.10	2.51 ± 0.10
	Liver	5.13 ± 0.23	4.93 ± 0.17	4.40 ± 0.07	7.00 ± 0.36	7.24 ± 0.41	4.89 ± 0.16
	Thyroid	3.77 ± 0.58	0.83 ± 0.06	2.02 ± 0.34	2.78 ± 0.27	1.43 ± 0.20	3.58 ± 0.71
24hr							
	Adrenal Cortex	1.05 ± 0.10	0.17 ± 0.01	0.36 ± 0.01	3.59 ± 0.43	7.78 ± 1.02	7.72 ± 1.31
	Blood	0.04 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.15 ± 0.01	0.16 ± 0.02	0.24 ± 0.02
	Liver	0.35 ± 0.02	0.05 ± 0.01	0.51 ± 0.03	0.59 ± 0.06	1.19 ± 0.18	0.43 ± 0.09
	Thyroid	128.07 ± 17.36	23.13 ± 4.60	42.48 ± 9.15	136.66 ± 28.15	106.76 ± 10.91	194.18 ± 76.87

Corresponding values for 19-Iodocholesterol at 0.5 hr and 24 hr are 14.16 ± 2.01 and 26.49 ± 0.70, respectively.

[+]Values expressed as % administered dose per gram of tissue (n=3-4).

Tissue Distribution Studies: The radiolabeled compounds were dissolved in benzene and Tween 20 was added. The benzene was evaporated under a stream of nitrogen and physiological saline was added. Any remaining benzene was removed by a stream of nitrogen until a clear solution (2-3% in Tween 20) resulted. The radiolabeled compound, thus solubilized, was administered intravenously to adult female Sprague-Dawley rats weighing 190-300 g. Three to four rats were used for each compound at each time period, and the dose ranged between 10 and 30 µCi per animal. The rats were killed by exsanguination under ether anesthesia at 0.5 hr and 24 hr and the major organs were removed and blotted free of excess blood. Large organs were minced with scissors. Weighed samples of tissue were placed in cellulose acetate capsules and counted (81-85% efficiency) in a well scintillation counter (Searle 1185). The results are summarized in Table 2. The concentration of radioactivity in each tissue was expressed as the percentage of administered dose/gm of tissue which was calculated as follows:

$$\frac{\text{CPM - BACKGROUND/MG OF TISSUE X 1000 X 100}}{\text{EFFICIENCY X 2.2 X } 10^6 \text{ DPM/µCi X µCi DOSE}}$$

Plasma and Tissue Extraction: Radioactivity was extracted from plasma adrenal cortex and liver using the procedure described previously (9). A system of benzene:ethyl acetate (9:1) was employed for TLC analysis of the lipid extracts. The plates were then developed with the appropriate solvent system for 14.5 cm and air-dried. The plates were cut into 1 cm strips starting 0.5 cm below the origin and continuing to the solvent front. Each strip was placed in a counting tube and assayed for radioactivity. Each unlabeled ester was cochromatographed with the radioactive samples and visualized with iodine vapor to serve as a reference standard. The results for compounds 5, 6 and 7 are summarized in Table 3.

Table 3
Analysis of Lipid-Soluble Radioactivity Extracted from Tissue Samples

	%$CHCl_3/CH_3OH$ Extractable Compound		% Parent Compound as Determined by TLC	
Tissue	0.5hr	24hr	0.5hr	24hr
2,5-DIIODO (5)				
Adrenal Cortex	91.9 ± 1.6	87.2 ± 1.3	90.7 ± 2.1	82.2 ± 3.5
Liver	88.5 ± 3.6	80.4 ± 2.6	79.1 ± 2.4	63.5 ± 3.5
Plasma	83.8 ± 2.0	60.8 ± 2.4	83.4 ± 1.0	66.1 ± 2.0
2,3,5-TRIIODO (6)				
Adrenal Cortex	93.9 ± 0.8	92.3 ± 1.3	92.5 ± 1.9	93.0 ± 0.6
Liver	93.4 ± 0.5	89.6 ± 1.1	88.8 ± 2.9	81.4 ± 3.8
Plasma	90.1 ± 0.4	39.7 ± 3.7	90.2 ± 3.9	67.7 ± 1.7
3,4,5-TRIIODO (7)				
Adrenal Cortex	88.7 ± 0.7	92.1 ± 0.8	97.2 ± 0.8	95.4 ± 0.5
Liver	88.2 ± 0.9	84.9 ± 1.4	94.1 ± 0.8	82.2 ± 1.4
Plasma	86.8 ± 2.0	16.5 ± 0.7	95.4 ± 0.7	57.8 ± 1.4

RESULTS AND DISCUSSION

As indicated in Table 1, the iodinated benzoate esters of pregnenolone were obtained in good yield by the treatment of pregnenolone with the appropriate iodinated benzoic acid in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP). The subsequent radioiodination of these esters by isotope exchange in pivalic acid afforded the desired radioiodinated esters in over 80% radiochemical yield in each case.

Adult female Sprague-Dawley rats were employed for the in vivo tissue distribution studies. These agents were formulated in a Tween/saline vehicle prior to intravenous administration. Groups of animals were sacrificed at 0.5 hr and 24.0 hr and tissues were analyzed in a γ-counter for uptake of radioactivity. The adrenal, liver and plasma were also subjected to lipid extraction by the method of Folch et al. (11). The lipid extract was subjected to TLC analysis in order to ascertain the amount of tracer present in its original form.

The distribution of radioactivity for the radiolabeled monoiodinated and polyiodinated benzoate esters is summarized in Table 2. Although up to twelve tissues were analyzed, only those that displayed a high uptake of radioactivity are shown. All of the esters showed an ability to localize in the adrenals as well as the liver. The monoiodinated analogs (2 - 4), however, were found to rapidly clear from tissues as indicated by the tissue distribution of radioactivity at 24 hr following administration. Lipid extraction of adrenal cortex, liver and plasma revealed that the monoiodinated esters underwent rapid hydrolysis following administration and the resulting radioiodinated benzoic acids were rapidly excreted. In contrast, the di- and

triiodinated benzoate esters (5 - 7) not only localized in the adrenals to a greater extent than the monoiodinated esters, but also displayed much less susceptibility to hydrolysis (Table 3). Most of the radioactivity present in the adrenals, liver and plasma for 5, 6 and 7 at 24 hr was essentially all lipid soluble and in its original esterified form. For example, over 90% of 6 was still present as the original compound even at 24 hr, although water-soluble metabolites were apparent in the plasma at this time. This stability to hydrolysis results in longer retention in the adrenals as signified by the 24 hr values. This was especially true for pregnenolone 2,3,5 triodobenzoate (6) which showed an adrenal uptake of 22.89% and 7.78% of the administered dose per gram at 0.5 hr and 24 hr, respectively.

These results suggest that the ability of these radioiodinated pregnenolone esters to produce high and sustainable levels of radioactivity in adrenal cortical tissue is related in their *in vivo* stability to hydrolysis. A similar finding was noted for radioiodinated cholesteryl esters (8,9,10). Unfortunately, those esters showing good adrenal retention were similarly retained by the liver so that the hoped for high adrenal to liver ratio was not attained in this series. Nonetheless, the high adrenal uptake shown by these esters at early time periods merits studies in other animal species to see if animal to animal variations exist for these agents. Such studies are now in progress.

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