[Chem. Pharm. Bull. 24(10)2322—2326(1976)]

UDC 615.357.033.076.9 : 546.15.02.131.09

A Comparison of 19-Iodocholesterol-^{131}I with 6β-Iodomethyl-19-norcholest-5(10)-en-3β-ol-^{131}I as an Adrenal-scanning Agent

Kazuo Nitta, Hiroshi Ogawa, Takayuki Ito,[1a] Masaharu Kojima, and Minoru Maeda[1b]

Daiichi Radioisotope Labs., Ltd.[1a] *and Faculty of Pharmaceutical Sciences, Kyushu University*[1b]

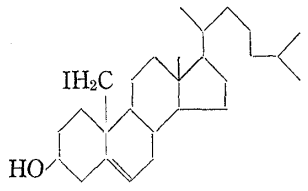
(Received December 22, 1975)

Distribution studies in male Wistar rats of pure 19-iodocholesterol-^{131}I (CL-19-^{131}I) and 6β-iodomethyl-19-norcholest-5-(10)-en-3β-ol-^{131}I (NCL-6-^{131}I) were performed. The uptake of NCL-6-^{131}I in the rat adrenal gland was ten times greater than that of CL-19-^{131}I. The ratios for adrenal-to-liver in the concentration of NCL-6-^{131}I were always higher than those of CL-19-^{131}I. Therefore, NCL-6-^{131}I seems to be a better adrenal-scanning agent than CL-19-^{131}I.

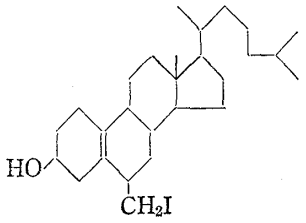
Introduction

Since 19-radioiodinated cholesterol was reported as an effective adrenal-scanning agent by Counsell, *et al.*,[2,3] this agent has been recognized as a useful diagnostic agent for unilateral adrenocortical carcinoma,[4] primary aldosterone adenoma,[5] and Cushing's syndrome.[6,7]

However, when 19-iodocholesterol-^{131}I(CL-19-^{131}I) was synthesized by isotope exchange of CL-19-I with radioactive sodium iodide according to the method of Counsell, *et al.*,[2] a considerable amount of 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol(NCL-6-I) or its radioactive iodine (NCL-6-^{131}I) analog was contained as a by-product in the preparation of CL-19-I or its radioactive iodine-labeled analog.[8,9]



CL-19-I



NCL-6-I

Chart 1

1) Location: *a) Tokuriki Build. 10–5, Nihonbashi 3-chome, Chuo-ku, Tokyo, 103, Japan; b) Maidashi 3–1–1, Higashi-ku, Fukuoka, 812, Japan.*
2) R.E. Counsell, V.V. Ranade, R.J. Blair, W.H. Beierwaltes, and P.A. Weinhold, *Steroids*, **16**, 317 (1970).
3) R.J. Blair, W.H. Beierwaltes, L.M. Lieberman, C.N. Boyd, R.E. Counsell, P.A. Weinhold, and V.M. Varma, *J. Nucl. Med.*, **12**, 176 (1971).
4) L.M. Lieberman, W.H. Beierwaltes, J.W. Conn, A.N. Ansari, and H. Nishiyama, *N. Engl. J. Med.*, **285**, 1387 (1971).
5) J.W. Conn, R. Morita, E.L. Cohen, W.H. Beierwaltes, W.J. McDonald, and K. Herwig, *Arch. Int. Med.*, **129**, 417 (1972).
6) W.H. Beierwaltes, L.M. Lieberman, A.N. Ansari, and H. Nishiyama, *J. Am. Med. Assoc.*, **216**, 275 (1971).
7) R. Morita, L.M. Lieberman, W.H. Beierwaltes, J.W. Conn, A.N. Ansari, and H. Nishiyama, *J. Clin. Endocrinol. Metab.*, **34**, 36 (1972); D.E. Schteingart, J.W. Conn, L.M. Lieberman, and W.H. Beierwaltes, *Arch. Intern. Med.*, **130**, 384 (1972).
8) *a)* M. Kojima, M. Maeda, H. Ogawa, K. Nitta, and T. Ito, *J. Chem. Soc. Chem. Commun.*, **1975**, 47; *b)* M. Maeda, M. Kojima, H. Ogawa, K. Nitta, and T. Ito, *Steroids*, **26**, 241 (1975).
9) M. Kojima, M. Maeda, H. Ogawa, K. Nitta, and T. Ito, *J. Nucl. Med.*, **16**, 666 (1975).

Therefore, the selective high affinity for the adrenal of radioiodinated CL-19-I reported in the literature[2,3] is questionable because of probable contamination by its radiopharmaceutical impurity. This prompted us to attempt to synthesize pure CL-19-^{131}I and NCL-6-^{131}I and to make a comparison of their tissue distribution in rats. The advantage of NCL-6-^{131}I as a possible adrenal-scanning agent is discussed.

Materials and Methods

Synthesis of 19-Iodocholesterol-^{131}I (CL-19-^{131}I)——A solution of Na^{131}I (30 mCi) was placed in a round bottom flask and the water was removed by azeotropic distillation with benzene. A solution of pure CL-19-I[8b] (25 mg) in acetone (5 ml) was added and the mixture was refluxed under nitrogen for 3 hr. The solution was allowed to cool and poured into ice-water. The resulting mixture was extracted with ether. The ether was washed with 1% $Na_2S_2O_3$ and water, and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was checked by thin-layer chromatography (TLC) (Silica gel 60 F_{254}, E. Merck) developing with $CHCl_3$–acetone (95: 5). Two spots were detected by scanning the radiochromatogram at the positions of *Rf* values of 0.30 and 0.35 (the ratio was about 9: 1). Accordingly, the product was purified by TLC on a silica gel plate (Silica gel 60 F_{254}, E. Merck, 20×20 cm) developing with $CHCl_3$–acetone (95: 5) and stripping the band of 0.30 *Rf* value. The scraped powder was extracted quickly with ether and the extract was filtered. After removal of the ether *in vacuo*, CL-19-^{131}I was obtained as a crystal (15 mg) with a specific activity of about 1 mCi/mg (radiochemical yield; 50%). The sample thus obtained was dissolved in ethanol. Polysorbate 80 (1.6%) and sufficient 0.9% NaCl were added to give a 6.4% ethanol solution having a radioactivity concentration of 1 mCi/ml. The ethanol solution was filtered by millipore filter GS (pore size 0.22 μ) to sterilize it. TLC using $CHCl_3$–acetone (95: 5) showed a single spot (*Rf* 0.30) coincident with the radioactive peak shown by radiochromatogram scanning. However, as low activity of free iodine was noticed at the origin, the radiochemical purity of the CL-19-^{131}I preparation was 95% (Fig. 1).

Synthesis of 6β-Iodomethyl-19-norcholest-5 (10)-en-3β-ol-^{131}I (NCL-6-^{131}I)——A solution of Na^{131}I (35 mCi) was placed in a round bottom flask and the water was removed by azeotropic distillation with benzene. A solution of NCL-6-I[8b] (30mg) in acetone (5 ml) was added and the mixture was refluxed for 4 hr. After the solvent was removed *in vacuo*, the residue was extracted with ether. The ether was washed with 1% $Na_2S_2O_3$ and water, and dried over Na_2SO_4. The ether was evaporated to give NCL-6-^{131}I (25 mg) with a specific activity of about 1 mCi/mg (radiochemical yield; 71.4%). The sample obtained was dissolved in ethanol, and polysorbate 80 (1.6%) and sufficient 0.9% NaCl were added to give a 6.4% ethanol solution having a radioactivity concentration of 1 mCi/ml. The ethanol solution was filtered by millipore filter GS (pore size 0.22 μ) to sterilize it. TLC using $CHCl_3$–acetone (95: 5) showed a single spot (*Rf* 0.35) coincident with the radioactive peak shown by radiochromatogram scanning. The radiochemical purity of the NCL-6-^{131}I preparation was 97% (Fig. 1).

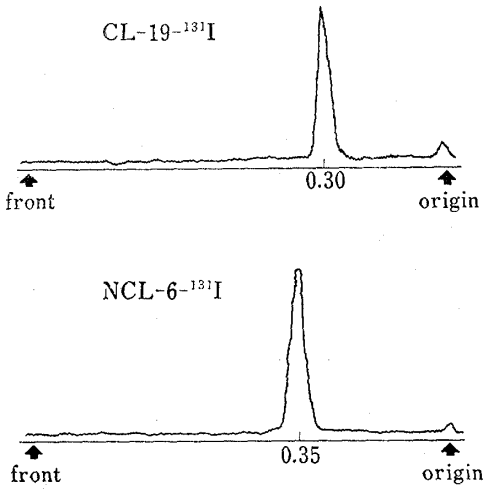


Fig. 1. ^{131}I-Activity on Thin-Layer Radiochromatograms

Stability of CL-19-^{131}I and NCL-6-^{131}I——The radiochemical purity of CL-19-^{131}I and NCL-6-^{131}I solutions (1 mCi/ml) through the course of storage at 5°, 20° and 37° was checked by TLC using a radiochromatogram scanner with a $CHCl_3$–acetone (95: 5) solvent system. The radioactive spots were scraped off to count their activity by a well-type scintillation counter.

Acute Toxicity of NCL-6-I——Male mice weighing 20—28 g were used. Because of the insolubility of NCL-6-I in water, NCL-6-I suspensions in olive oil in the final concentration of 80 mg/ml, 12 mg/ml and 3 mg/ml respectively were used for toxicity measurement. Each preparation of each 0.4 ml was given by intraperitoneal injection to 3 mice and olive oil was injected at 0.4 ml/mouse in the control group. The survival of the mice was determined at 72 hr and 7 days after the administration of NCL-6-I and olive oil.

Tissue Distribution of CL-19-^{131}I and NCL-6-^{131}I——The ethanol solutions of CL-19-^{131}I and NCL-6-^{131}I were further diluted by 0.9% NaCl to have a radioactive concentration of 250 μCi/ml, respectively. The dilute preparations were given intravenously to male Wistar rats weighing 140—150 g *via* tail vein. The dose administered was 50 μCi/rat. Rats from both groups were sacrificed at various time intervals, respectively, after the administration of the dose. After the rats were deeply anesthetized with ether, hepaline was administered and carotid was cut to remove blood. The major organs such as liver, thyroid, kidney, lung, spleen, testicle and adrenal were excised, weighed and placed in small counting vials. As thyroid was hardly taken out by itself,

it was taken out with trachea. The weight of thyroid was estimated as 30 mg.[10] Each voiding of urine and feces was collected separately and assayed for radioactivity. The radioiodine-containing tissues were directly counted in glass tubes in a well-type scintillation counter and correction was made for decay and counting efficiency. Results were expressed in dpm/mg of tissues and converted into percent administered dose per gram of tissues.

Results and Discussion

Stability During Storage

Fig. 2 and 3 show the change of purity of CL-19-^{131}I and NCL-6-^{131}I during the storage at 5°, 20° and 37° respectively. Although CL-19-^{131}I is relatively stable in a refrigerator at 5°, a marked loss of activity of CL-19-^{131}I was observed with the elevation of temperature and at 20° about 60% of the activity of CL-19-^{131}I was lost in 5 days. On the other hand, NCL-6-^{131}I is fairly stable and maintains its radiochemical purity at 80% even after 7 days at 37°.

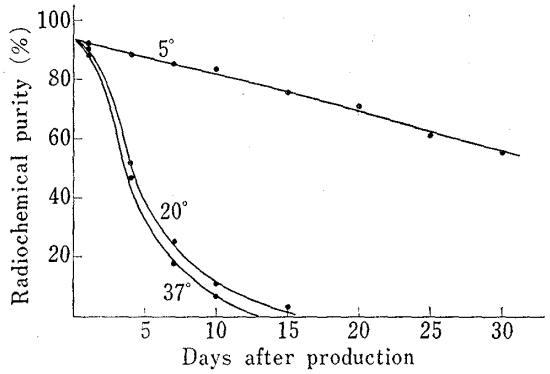


Fig. 2. Stability of 19-Iodocholesterol-^{131}I(CL-19-^{131}I)

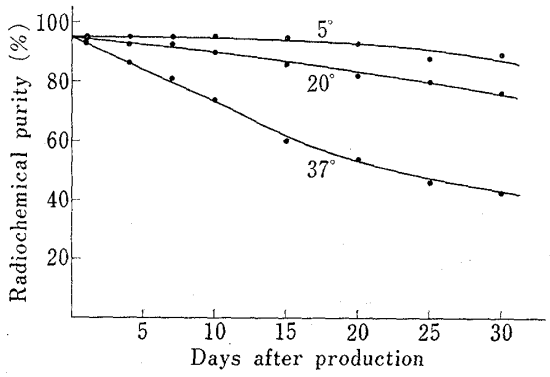


Fig. 3. Stability of 6β-Iodomethyl-19-norcholest-5(10)-en-3β-ol-^{131}I(NCL-6-^{131}I)

Acute Toxicity of NCL-6-I

All mice in every group survived at 72 hr and also 7 days after administration of NCL-6-I. No acute toxicity was observed even by the intraperitoneal administration at 1000 mg/kg. This dosage amounts to 60000 times as much as the ordinary intravenous dose in man.

Tissue Distribution Studies

Tables I and II show the mean accumulation (% dose per g) in each organ following administration of CL-19-^{131}I and NCL-6-^{131}I, respectively. The mean concentration in the adrenal gland of NCL-6-^{131}I-dosed rats was 208±80%/g at 7 days, while the CL-19-^{131}I-dosed rats averaged 18.22±8.60%/g. Thus, it is concluded that the rat adrenal accumulates ten times more NCL-6-^{131}I than CL-19-^{131}I and retains the higher radioactivity throughout the experimental period in the case of the former drug compared with the latter. CL-19-^{131}I produces a significant concentration of radioactivity in the thyroid and this amount is considerably greater than that of NCL-6-^{131}I-dosed rats. This result suggests that CL-19-^{131}I is liable to decompose to liberate free iodine either chemically or enzymatically. This result is consistent with the data of its thermal stability.

Generally, the excretion of CL-19-^{131}I from organs other than the adrenal and thyroid glands appears to be faster than the excretion of NCL-6-^{131}I. Fig. 4 illustrates excretion of ^{131}I into urine and feces after the administration of CL-19-^{131}I and NCL-6-^{131}I, respectively. NCL-6-^{131}I is excreted to feces much more than to urine, while CL-19-^{131}I is excreted to urine much more than to feces. We speculate that these differences might have been caused by the

10) Y. Koyama, and E. Kinoshita, "Dobutsu Jikken Shugi," Kyodoishoshuppansha, Tokyo, 1964, p. 312.

TABLE I. Tissue Accumulation of 19-Iodocholesterol-^{131}I(CL-19-^{131}I)[a]
(Mean Percent Administered Dose Per Gram of Tissues)

Tissue	Days after administration		
	1	3	7
Adrenal	17.30± 0.77	20.60± 7.05	18.22± 8.60
Liver	0.56± 0.01	0.15± 0.04	0.07± 0.03
Kidney	0.32± 0.01	0.08± 0	0.04± 0.01
Lung	0.87± 0.02	0.16± 0.08	0.03± 0.02
Spleen	0.96± 0.02	0.62± 0.29	0.40± 0.49
Testicle	0.13± 0.01	0.04± 0.01	0.01± 0
Blood	0.31± 0.02	0.05± 0	0.02± 0
Thyroid	377 ±99	359 ±150	294 ±165

a) Values represent mean percent administered dose per gram of tissues for 2 rats at 1 day, 3 rats at 3 days, and 4 rats at 7 days with standard deviations of mean.

TABLE II. Tissue Accumulation of 6β-Iodomethyl-19-norcholest-5(10)-en-3β-ol-^{131}I(NCL-6-^{131}I)[a]
(Mean Percent Administered Dose Per Gram of Tissues)

Tissue	Days after administration		
	1	3	7
Adrenal	141 ±17	163 ±20	208 ±80
Liver	1.65± 0.26	0.55± 0.12	0.19± 0.07
Kidney	0.92± 0	0.74± 0.09	0.42± 0.05
Lung	2.89± 0.31	1.29± 0.20	0.42± 0.04
Spleen	2.68± 0.38	0.90± 0.16	0.27± 0.08
Testicle	0.29± 0.07	0.21± 0.02	0.18± 0.13
Blood	0.85± 0.06	0.25± 0.15	0.07± 0.01
Thyroid	90 ±10	149 ±30	80 ±30

a) Values represent mean percent administered dose per gram of tissues for 2 rats at 1 day, 3 rats at 3 days, and 4 rats at 7 days with standard deviations of mean.

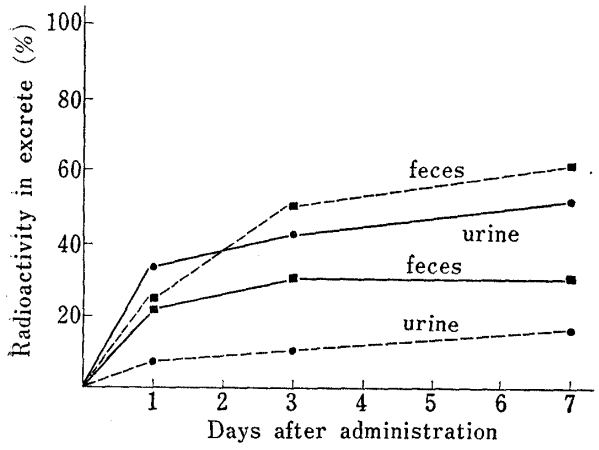


Fig. 4. Excretion of ^{131}I-Activity in Urine and Feces

—— CL-19-^{131}I dosed rats
------ NCL-6-^{131}I dosed rats

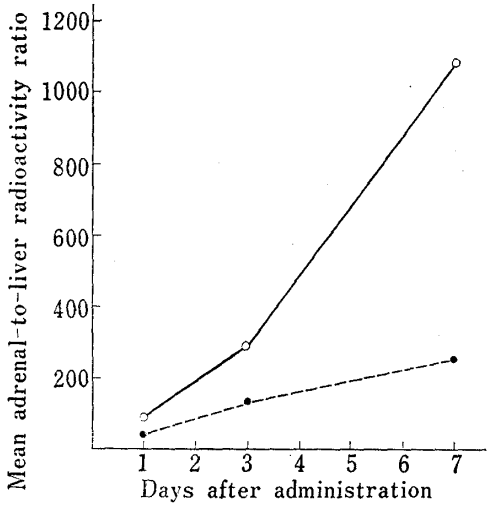


Fig. 5. Changes of Mean Adrenal-to-liver Radioactivity Ratios after Administration

CL-19-^{131}I (—●—)
NCL-6-^{131}I (—○—)

difference in the metabolic pathways. It was revealed by TLC method that the most part of the radioactive products in urine was free iodine.

As the liver is one of the main interfering organs for adrenal scintigraphy, a high adrenal-to-liver ratio is a prerequisite for any scanning agent intended to be used for imaging the adrenals. In Fig. 5 comparison of the adrenal-to-liver ratios of radioactivity between CL-19-^{131}I and NCL-6-^{131}I is demonstrated. The NCL-6-^{131}I groups attained considerably higher value than the CL-19-^{131}I groups throughout the period of the experiment. The ratio of mean adrenal-to-liver concentration at 7 days was 260 for CL-19-^{131}I, while that for NCL-6-^{131}I was 1096.

From the results described above, NCL-6-^{131}I accumulates in adrenal much more than CL-19-^{131}I and, therefore, is more suitable as an adrenal-scanning agent.

The original idea for CL-19-^{131}I as a useful adrenal-scanning agent was developed from the fact that the adrenal cortex is the richest tissue for cholesterol and concentrates it to greater extent than other steroid analogs.[11] Beierwaltes, *et al.*[3] reported that 19-iodocholesterol could not be metabolically utilized, though 19-iodocholesterol accumulated in the adrenal as well as ^{14}C-cholesterol. One of the most important problems that is confronted to us is the relationship between affinity of NCL-6-^{131}I analogues in rat adrenal and their chemical structure. Our finding is just one interesting fact about NCL-6-^{131}I, however, and it should be studied more deeply.

11) *a*) R.P. Cook, "Cholesterol," ed by C.P. Cook, Academic Press, Inc., New York, 1958, p. 173; *b*) L.E. Appelgren, *Acta. Physiol. Scand.*, 71, suppl. 301, 1 (1967).