RADIOBROMINE LABELED CHOLESTEROL ANALOGS. SYNTHESIS AND TISSUE DISTRIBUTION OF BROMINE-82 LABELED 6-BROMOCHOLESTEROL

Liu Bo-Li*, Jin Yu-Tai*, Pan Zhong-Yun†, Minoru Maeda#, and Masaharu Kojima#
*Chemistry Department, Division of Radiochemistry, Beijing Normal University, Beijing, China; †Isotope Laboratory, The First Teaching Hospital of Beijing Medical College, Beijing, China; and #Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan

SUMMARY

Bromine-82 labeled 6-bromocholesterol (CL-6-Br-82) was prepared by the reaction of 6-chloromercurycholesterol with bromine-82 labeled bromine in chloroform. Tissue distribution of CL-6-Br-82 in rats was determined. The adrenal uptake of CL-6-Br-82 reached a maximum of 136 %dose/g at 5 days after injection. The adrenal-to-liver ratio increased from 57 at 3 days to 141 at 5 days. The substitution of radiobromine for radioiodine in 6-iodocholesterol (CL-6-I) resulted in an agent which demonstrates less affinity for the adrenal gland than CL-6-I itself.

Key Words: adrenal imaging agent, ^{82}Br-6-bromocholesterol, tissue distribution

INTRODUCTION

The demand for a scintigraphic agent for adrenal visualization has led to the development of various radiolabeled analogs of cholesterol (1,2). The first successful images of human adrenals were obtained with ^{131}I-19-iodocholesterol (CL-19-^{131}I) synthesized by Counsell et al. (3). Thereafter, a homoallylic isomer of this compound, ^{131}I-6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-^{131}I) has shown to provide better human adrenal images than CL-19-^{131}I because of its more rapid uptake and greater target-to-background ratio (4,5), and is the current agent of choice for adrenal imaging. On the other hand, ^{131}I-6-iodocholesterol

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(CL-6-^{131}I), synthesized by Wang and Liu et al. (6,7), has been used clinically as a new diagnostic agent for adrenal gland in China due to its notable stability in-vivo and in-vitro, though demonstrating lower adrenal-liver and adrenal-kidney radioactivity ratios than NCL-6-^{131}I (8). However, the limitations of diagnostic imaging of the adrenal gland with these radioiodinated cholesterol analogs are the time required and the high radiation dose to patients.

The potential use of radioactive isotope of bromine as an alternative to radioiodine in nuclear medicine has been pointed out (9). ^{75}Br ($T_{1/2}$=101 min), ^{76}Br ($T_{1/2}$=15.9 hr) and ^{77}Br ($T_{1/2}$=56 hr) are the most suitable nuclides in-vivo as radiopharmaceutical labels. It is of interest to use a bromine labeled cholesterol analog which would give a radiopharmaceutical with more favorable characteristics. As a part of study of analog synthesis of cholesterol and subsequent structure-activity relationship evaluation, we planned to synthesize ^{82}Br labeled 6-bromocholesterol (CL-6-^{82}Br) and to evaluate its ability to selectively localize in adrenals. The rationale for selecting ^{82}Br ($T_{1/2}$=35.4 hr) in the present research was that ^{82}Br is readily available and a suitable model for ^{75}Br, ^{76}Br or ^{77}Br.

RESULTS AND DISCUSSION

The nonlabeled compound, 6-bromocholesterol, was prepared by the reaction of 6-chloromercurycholesterol with Br_2. Initial attempts to obtain ^{82}Br-6-bromocholesterol (CL-6-^{82}Br) by the Br-for-Br exchange using Na^{82}Br in various solvents or in a melt resulted in markedly resistant incorporation of the label. The preparation of CL-6-^{82}Br by the direct bromination of 6-chloromercurycholesterol with $^{82}Br_2$ in chloroform was attempted. The results of the time dependence of labeling yield is shown in Fig. 2, indicating that

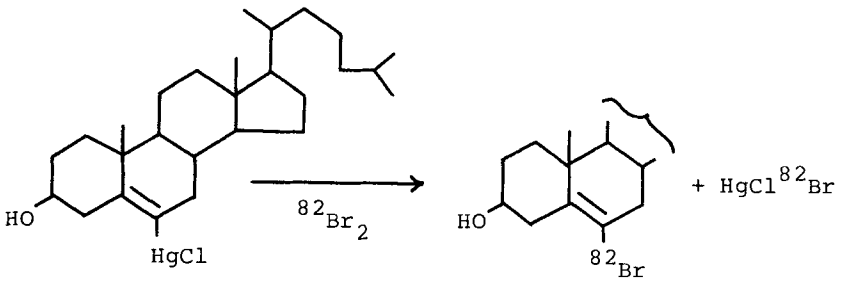

Fig. 1. Reaction scheme for the synthesis of ^{82}Br-6-bromo-cholesterol (CL-6-^{82}Br)

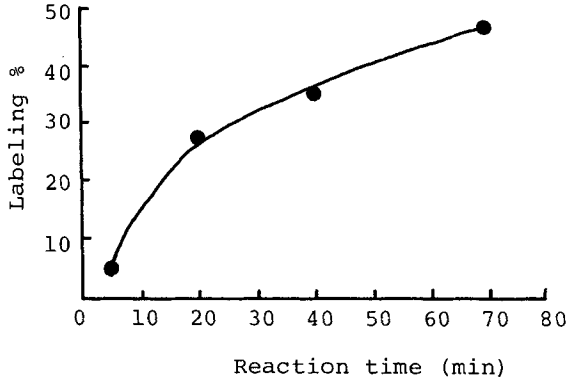

Fig. 2. Time dependence of the reaction of 6-chloromercury-cholesterol with $^{82}Br_2$

the yield of CL-6-^{82}Br reaches a theoretical maximum value of about 46% in 70 min. Using 18 mCi of $^{82}Br_2$ we have synthesized 8.1 mCi of CL-6-^{82}Br with more than 95% radiochemical purity and a specific activity of 0.87 mCi/mg.

The distribution of radioactivity in tissues of male rats was determined at time intervals from 1 day to 5 days following the administration of CL-6-^{82}Br and the results are summarized in Table 1. The distribution profile of radioactivity from CL-6-^{82}Br showed many similarities with the data previously reported for the

Table 1. Rat tissue distribution of ^{82}Br-6-bromocholesterol (CL-6-^{82}Br) at various time intervals*

Tissue	Days after administration 1 day	3 days	5 days
Adrenal	115.74±22.02	130.78±72.14	136.28±41.10
Liver	21.44±9.26	2.26±1.02	0.96±0.18
Kidney	10.20±6.12	3.08±0.94	2.24±0.62
Lung	21.30±9.66	5.34±1.86	3.28±0.86
Spleen	55.72±21.74	4.84±1.98	1.72±0.32
Testicle	2.38±0.38	1.44±0.32	1.42±0.32
Blood	6.84±1.44	1.64±1.16	0.46±0.08
Thyroid	4.20±1.06	0.92±0.12	0.86±0.68

*Values represent mean % administered dose per gram of tissue for 3 rats at 1 day and 3 days, for 5 rats at 5 days, with SD of mean.

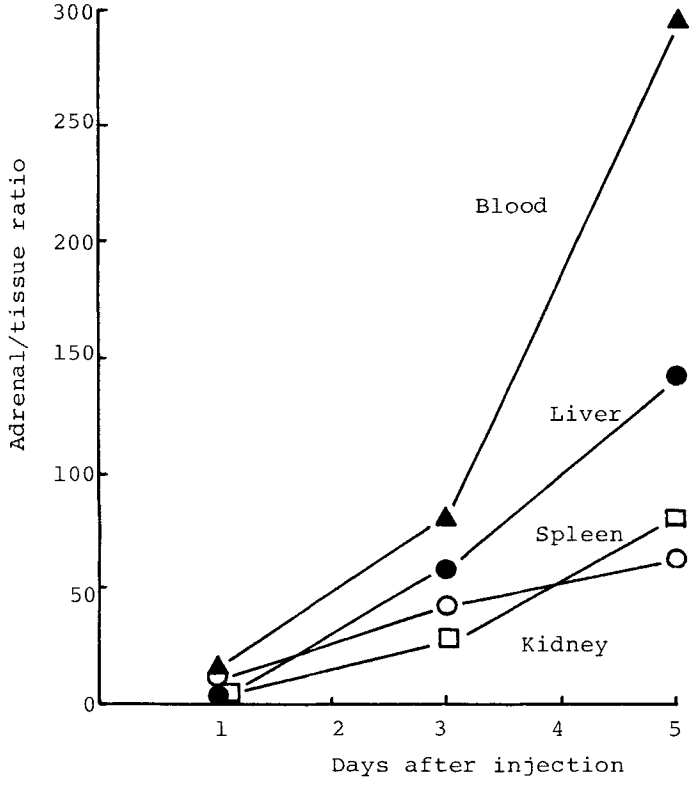

Fig. 3. The adrenal/tissue ratios of selected tissues following intravenous injection of ^{82}Br-6-bromocholesterol

other radiolabeled cholesterol analogs. This result clearly indicates a considerable selective localization of radioactivity in the adrenals. Apart from the adrenals, the other tissue showing high level of radioactivity was the spleen. However, the high splenic uptake considerably decreased at 3 days after injection. The adrenal uptake of CL-6-^{82}Br reached a maximum of 136 %dose/g at 5 days after injection. It must be mentioned that the adrenal concentration of radioactivity is less than that achieved with CL-6-^{131}I (8) or NCL-6-^{131}I (4) over the 5-day period, although it is at higher level than that observed with CL-19-^{131}I (4). The relative concentration of radioactivity in adrenals, compared with nearby organs, is very important for adrenal imaging. The adrenal to non-target ratios for several selected tissues are presented in Fig. 3. The adrenal-to-liver ratio increased from 57 at 3 days to 141 at 5 days, which are comparable to those of CL-6-^{131}I, but these values are considerably lower than those observed with CL-19-^{131}I and NCL-6-^{131}I. The value is probably not high enough to allow clean imaging of adrenals by conventional techniques. Measurements of the whole-body activity following the injection of CL-6-^{82}Br shows that 50% of the radioactivity is eliminated in 2.5 days. This means that CL-6-^{82}Br has a shorter biological half-time, compared with that of CL-6-^{131}I, CL-19-^{131}I or NCL-6-^{131}I.

The present results indicate that the substitution of radiobromine for radioiodine in the CL-6-I results in an agent which demonstrates less affinity for the adrenal gland than CL-6-I itself. By the use of CL-6-Br labeled with the positron emitter such as ^{76}Br and positron emission tomography, however, it may be possible to visualize tomographically adrenal masses.

EXPERIMENTAL

6-Bromocholesterol (CL-6-Br)———— 6-Chloromercurycholesterol (CL-6-HgCl) prepared according to the method described by Mertz (10) was recrystallized from acetic acid. To a solution of CL-6-HgCl (300 mg) was added dropwise a solution of Br_2 (200 mg) in chloroform (5 ml). The mixture was stirred at 25°C for 2 hr. After filtration, the filtrate was washed with 0.5% Na_2SO_3, extracted with ether, and dried (Na_2SO_4). The residue, after removal of the solvent, was chromatographed on silica gel (Silica ARCC-4, 100 mesh, Mallinckrodt) eluting with chloroform to give 6-bromocholesterol (CL-6-Br) (199 mg) as colorless needles, m.p. 141-142°C, after recrystallization from ethanol; IR ν_{max} (Nujol) 3400, 3240 and 1650 cm^{-1}. Anal. Calcd. for $C_{27}H_{45}OBr$: C, 69.66; H, 9.74. Found: C, 69.58; H, 9.73.

^{82}Br-6-Bromocholesterol (CL-6-^{82}Br)————An aqueous solution of ^{82}Br-NaBr (20 mCi, 5 mCi/mg) was added to a separatory funnel with chloroform (4 ml) and an aqueous solution of $KBrO_3$ (0.8 ml) (3.45 mg/ml) was then added to the mixture. Ten drops of conc. H_2SO_4 was further added to the mixture. The $^{82}Br_2$ (18 mCi) in chloroform solution was thus obtained. A solution of $^{82}Br_2$ in chloroform was added dropwise to a solution of CL-6-HgCl (13 mg) in chloroform (1 ml) with stirring at room temperature. The mixture was allowed to stand for 2 hr at room temperature. The filtered solution was streaked on silica gel glass plates (Silica gel 60F 254, 0.5 mm layer, E. Merck) and developed in benzene-ethyl acetate (9:1). The separated CL-6-^{82}Br was scraped off and eluted with chloroform to give CL-6-^{82}Br (8.1 mCi) with a specific activity of 0.87 mCi/mg. It showed a single radioactive peak coincident with unlabeled CL-6-Br on thin-layer chromatography.

Tissue distribution———— CL-6-^{82}Br was dissolved in ethanol (0.4 ml), and Tween 80 (0.5 ml) and sufficient saline

were added to give a 10% ethanol solution having a radioactive concentration of 100 μCi/ml. Eleven male Wistar rats weighing 120-150 g received through the tail vein a dose of 20 μCi/animal of CL-6-^{82}Br. Three or five rats were killed at 1 day, 3 and 5 days after the injection. Major organs were excised, weighed and placed in small counting vials. Samples of tissues were counted in a gamma well counter, after which corrections were made for radioactive decay and counting efficiency. The concentration in each tissue was expressed as percentage of injected dose per gram.

REFERENCES

1. Beierwaltes W.H., Wieland D.M., and Swanson D.- Radiopharmaceuticals: Structure-Activity Relationships, ed., by Spencer R.P., Grune and Stratton, Inc., New York, 1981, p395.
2. Counsell R.E. and Ice R.D.- Drug Design, vol. 6, ed., by Ariëns E.J., Chap. 4, Academic Press, New York, 1975.
3. Counsell R.E., Ranade V.V., Blair R.J., Beierwaltes W.H., and Weinhold P.A.- Steroids 16: 317 (1970).
4. Kojima M., Maeda M., Ogawa H., Nitta K., and Ito T.- J. Nucl. Med. 16: 666 (1975); idem., Chem. Pharm. Bull. (Tokyo) 24: 2322 (1976).
5. Sarkar S.D., Beierwaltes W.H., Ice R.D., Basmadjian G.P., Hetzel K.R., Kennedy W.P., and Mason M.M.- J. Nucl. Med. 16: 1038 (1975).
6. Wang Shizen, Liu Cheng-Bin, and Chen Zhong- Radioisotope and Radiation Application Exhibition Information Pamphlet (1972).
7. Liu Bo-Li, Jin Yu-Tai, Liu Zhenghao, Sun Zhaoxiang, Lu Gongxu, and Li Tai-Hau - J. Nucl. and Radiochem. (China) 2: 107 (1980).
8. Jixiao M. and Ruisen Z.- Chinese Med. J. 92: 237 (1979); Pan Zhong-Yun, Li Guan-Zhong, Liang Shi-Tian, Liu Zhong-Min, Wang Yong-Chao, Xia Zhen-Min, Zhou Qian, and Liu Xiu-Jie -

J. Chinese Nucl. Med. 1: 85 (1981).

9. Stöcklin G.- Int. J. appl. Radiat. Isotopes 28: 131 (1977).

10. Merz W.- Z. Physiol. Chem. 154: 225 (1926).