Synthesis and in Vivo Behavior of a Copper-64-labeled Dithiosemicarbazone Derivative Coupled to a Dihydropyridine Carrier

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Summary

The dihyropyridine⇄pyridinium salt redox delivery system was used to develop a perfusion agent that could be labeled with a generator-produced positron emitter ^{62}Cu. A ^{64}Cu-labeled dithiosemicarbazone (DTS) molecule attached to a dihyropyridine carrier (PDTS-DHC) was synthesized by the simple mixing of ^{64}Cu acetate and Zn-PDTS-DHC. The latter was prepared by dithionite reduction of the Zn complex of a corresponding pyridinum precursor (PDTS-QC^+). When injected into mice, ^{64}Cu-PDTS-DHC showed a high myocardial uptake and high heart-to-blood and heart-to-lung ratios. These results demonstrate that it may be possible to develop ^{62}Cu-PDTS-DHC compounds as radiopharmaceuticals for myocardial perfusion studies using positron emission tomography.

Key words: copper-62; bifunctional radiopharmaceutical; redox chemical delivery system; myocardium; positron emission tomography.

Introduction

Since the study of regional blood flow provides fundamental information that assists our understanding of the biochemical and physiological processes occurring in an organ, nuclear medical perfusion imaging is useful for the diagnosis of various diseases, especially those affecting the heart and brain (1-4). To improve the quality of imaging, radiopharmaceuticals that exhibit a high level of extraction and retention in the target organ are needed (5,6).

The brain and myocardium utilize the oxidative metabolism of substrates such as glucose and fatty acids to provide energy (7,8). Accordingly, it seems worthwhile to design a tracer for cerebral and myocardial perfusion studies that is prevented from diffusing out of the target organ by oxidative transformation after its uptake.

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Recently, Bodor et al. developed a redox system for the specific delivery of drugs based on an interconvertible dihyropyridine ⇄ pyridinium salt carrier (9-11). When combined with a lipid-soluble dihydropyridine (DHC) carrier, an agent can readily cross cell membranes, after which the carrier is oxidized to a membrane-impermeable quaternary pyridinium ion (QC^+). This results in the effective oxidative metabolic trapping of the desired agent.

Such a redox chemical delivery system appears to be applicable to the development of useful radiopharmaceuticals for cerebral and myocardial perfusion studies. In fact, the potential application of radioiodinated phenylamines coupled to DHC for the evaluation of regional cerebral blood flow has recently been reported (12).

On the other hand, our laboratory recently developed a new $^{62}Zn/^{62}Cu$ generator system for positron emitting copper-62 (^{62}Cu) (T1/2 = 9.8 min) (13). We have subsequently designed various target-specific ^{62}Cu-labeled radiopharmaceuticals (14-17), since such tracers allow positron emission tomography (PET) to be performed without the need for an in-house cyclotron. Our studies on these ^{62}Cu-labeled compounds have indicated that the dithiosemicarbazone (DTS) structure was easily labeled with generator-produced ^{62}Cu, and formed a compact and uncharged unit with a high stability both in vitro and in vivo.

Accordingly, we designed a bifunctional radiopharmaceutical (Cu-PDTS-DHC) containing a DTS structure, ρ-aminoethyl-phenylpropane-2,3-dione bis(N-methylthiosemicarbazone) (PDTS) as the Cu chelating site and a DHC structure as the site related to delivery to the target organ. A spectrophotometric study was carried out first using nonradioactive copper to obtain a basic understanding of the process of complex formation. Then radiolabeling was performed using copper-64 (^{64}Cu), since it is more suitable for basic studies than ^{62}Cu because of its ready availability and long half-life (T1/2 = 12.8 h), and the chemical and biological behaviour of the obtained compound (^{64}Cu-PDTS-DHC) were examined. Figure 1 shows the chemical structure of the Cu-PDTS-DHC complex.

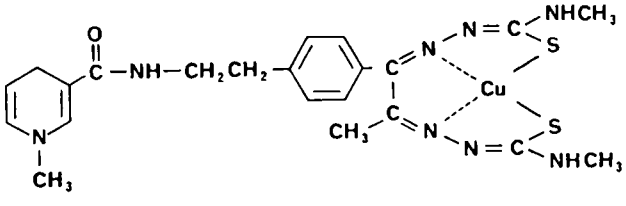

Fig. 1. Chemical Structure of 1-Methyl-3-[ρ-aminoethyl-phenylpropane-2,3-dione bis(N-methylthiosemicarbazone)]-1,4-dihydropyridine Copper(II) Complex (Cu-PDTS-DHC).

Materials and Methods

Reactor-produced ^{64}Cu was supplied as copper acetate (18.5 MBq/ml, 1.04 X 10^9 Bq/mg Cu) from the Japan Atomic Energy Research Institute. All other chemicals used were of reagent grade. Absorption spectra were recorded on a Model 330-S Hitachi spectrophotometer. Male ddY mice were supplied by Japan SLC Co., Ltd..

Synthesis of 1-Methyl-3-[p-aminoethylphenylpropane-2,3-dione bis(N-methylthiosemicarbazone)]pyridinium Iodide (PDTS-QC$^+$) (3) (Fig. 2)

PDTS hydrochloride (1) was synthesized by the previously reported method (18). N-Succinimidyl (1-methyl-3-pyridino)formate iodide (2) was synthesized by the method of Tedjamulia et al. (12). Triethylamine (0.238 ml) was added to a solution of PDTS hydrochloride (686 mg) (1) in N,N-dimethylformamide (DMF) (15 ml). This mixture was then added slowly to a solution of compound 2 (3.0 g) in DMF (10 ml) and the reaction mixture was stirred at room temperature for 12 hr. The DMF was evaporated in vacuo, and the residue was triturated with water. Then the resultant yellow solid was collected by filtration and dried. Recrystallization from methanol gave pale yellow crystals. Anal. Calc'd for $C_{22}H_{29}N_8OS_2I$: C, 43.14; H, 4.77; N, 18.30. Found: C, 43.08; H, 4.85; N, 18.00. mp 218-220°C. MS m/e:485. NMR (DMSO-d_6, ppm); 2.38 (s, 3H), 2.82 (d, 3H), 2.94 - 3.05 (m, 5H), 3.65 (q, 2H), 4.39 (S, 3H), 7.33 (q, 4H), 8.22 (t, 1H), 8.64 - 9.36 (m, 6H).

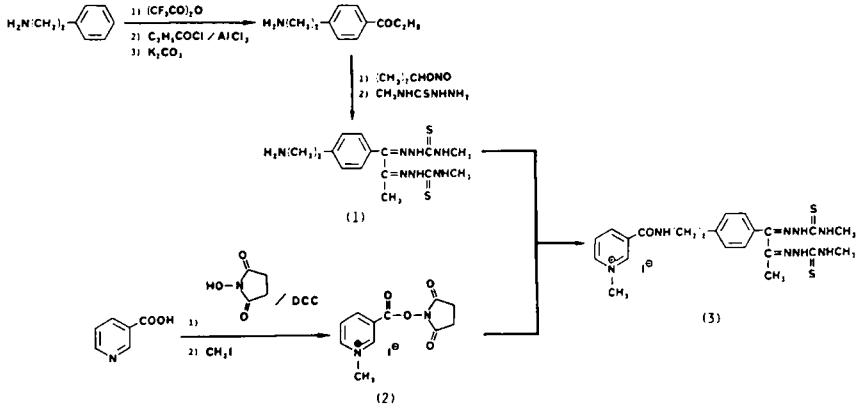

Fig. 2. Synthesis of 1-Methyl-3-[p-aminoethylphenylpropane-2,3-dione bis(N-methylthiosemicarbazone)]pyridinium Iodide (PDTS-QC$^+$).

Studies on Cu Complex Formation Using Nonradioactive Cu

To obtain a basic understanding of Cu complex formation with PDTS-QC$^+$ and 1-methyl-3-[p-aminoethyl-phenylpropane-2,3-dione bis(N-methylthiosemicarbazone)]-1,4-dihydropyridine (PDTS-DHC), spectrophotometric studies were carried out using nonradioactive copper (Cu acetate, 1 X 10^{-5} - 5 X 10^{-4} M) and the ligand (1 X 10^{-4} - 5 X 10^{-4} M).

Preparation of ^{64}Cu-PDTS-QC^{+}

PDTS-QC^{+} (3) (1.9 mg) was first dissolved in 1 ml of 1 N NaOH and adjusted to pH 8.0 with 1 N HCl, and then the total volume of the solution was adjusted to 10 ml with 0.1 M Tris-HCl buffer (pH 8.0). The ^{64}Cu acetate was diluted with 0.1 M Tris-HCl buffer (pH 8.0) to give a working solution of 3.7 MBq/ml (207 MBq/mg Cu). Then, 1 ml of this ^{64}Cu acetate solution was added to 1 ml of PDTS-QC^{+} (3) solution and stirred gently for 5 min. The radiolabelled ^{64}Cu-PDTS-QC^{+} was analyzed by thin-layer chromatography (TLC) which was developed with a mixture of chloroform and methanol (7:3) and the radiochemical purity was determined.

Preparation of ^{64}Cu-PDTS-DHC

Zinc acetate (1.3 mg) was dissolved in 10 ml of 0.1 M Tris-HCl buffer (pH 8.0) (6 X 10^{-4} M). Then 1 ml of this solution was added to 1 ml of PDTS-QC^{+} (3) solution (6 X 10^{-4} M in methanol) and stirred gently for 30 min. After the mixture was deaerated by bubbling with argon gas, 1 ml of 2.4 X 10^{-3} M sodium dithionite solution in deaerated 0.1 M Tris-HCl buffer (pH 8.0) was added gradually. Argon was bubbled through the mixture during the entire subsequent course of the reaction. Stirring was continued for 30 min, and 2 ml of chloroform was added. After stirring for an additional hour, the chloroform layer was separated. Then 1 ml of ^{64}Cu acetate (3.7 MBq, 0.018mg Cu) solution was evaporated and dissolved in 1 ml of deaerated dimethyl sulfoxide (DMSO). This ^{64}Cu acetate solution was added to the chloroform layer, the mixture was stirred for 5 min, and then the reaction mixture was evaporated to remove the chloroform. The radiochemical purity of the product was determined by TLC (chloroform : methanol = 7 : 3).

Measurement of Octanol/water Partition Coefficient

The partition coefficients for the various ^{64}Cu complexes were determined according to the procedure described previously (19). A 20-μl aliquot of each ^{64}Cu complex was mixed with 3 ml each of 1-octanol and 0.06 M phosphate buffer (pH 7.4) in a test tube. This tube was stirred with a vortex mixer (3 X 1 min), incubated for 1 hr at room temperature, and then centrifuged for 5 min. Then, 0.5-ml aliquots of each phase were removed and counted for ^{64}Cu activity in a well-type NaI scintillation counter. The partition coefficient was determined by calculating the ratio of the cpm/ml for the octanol to that for the buffer.

Biodistribution in Mice

A dose of 61.5 kBq of ^{64}Cu-PDTS-QC^{+}, ^{64}Cu-PDTS-DHC, or ^{64}Cu-acetate was injected intravenously into male ddY mice weighing about 30 g. At appropriate time intervals, the mice were killed by decapitation, the organs of interest were excised, and blood samples were collected by cardiac puncture. All samples were weighed and the radioactivity was counted in a well-type NaI scintillation counter.

Results and Discussion

Chemistry

The process of complex formation of Cu-PDTS-DHC was first studied spectrophotometrically using nonradioactive copper. In this study, sodium dithionite was used as a reducing agent for formation of the dihydropyridine moiety from the pyridinum moiety, since it was reported that the reduction of 3-substituted pyridinum salts with sodium dithionite exclusively affords the corresponding 1,4-dihydropyridine (9-11, 20-22).

At first, the formation of a Cu-PDTS-QC$^+$ complex and its subsequent reduction was attempted. When Cu-acetate was mixed with PDTS-QC$^+$, the reaction was completed rapidly, and the solution had an absorption maximum of 485 nm, indicating complex formation with Cu at the DTS moiety (Fig. 3) (13,14). However, when sodium dithionite was added to the resulting solution, the absorption peak at 485 nm disappeared, although there was a slight increase in the peak at 340 nm indicating formation of the dihydropyridine moiety (20-22). Since the oxidation-reduction potential of quaternary pyridinium analogs such as NAD$^+$ and NADP$^+$ (- 0.32 V) (23) is more negative than that of Cu^{2+}-complexed DTS derivatives (0.18-0.28 V) (24), the results obtained suggest that Cu^{2+} was reduced in preference to the pyridinum moiety.

Thus, since Zn^{2+} has a much higher resistance to reduction (25) and a lower chelation stability than Cu^{2+} (26), we planned to transform PDTS-QC$^+$ into a Zn chelate complex followed by its reduction, and then carry out a metal exchange reaction between Zn^{2+} and Cu^{2+}. When Zn-acetate was added to PDTS-QC$^+$, PDTS-QC$^+$ readily formed a complex with Zn that gave an absorption peak at 425-430 nm, which characterizes Zn complex formation with the DTS moiety (14). When the Zn-PDTS-QC$^+$ complex was reduced by sodium dithionite, the absorption maximum at 340 nm appeared and the peak at 425-430 nm did not change, indicating that the pyridinum moiety had been reduced with Zn still complexed at the DTS moiety. Subsequent addition of Cu acetate to a solution of the Zn-PDTS-DHC complex increased the optical density at 485 nm, indicating Cu complex formation at the DTS moiety (Fig. 3). Thus, the desired Cu-PDTS-DHC complex was formed by reduction of the Zn-PDTS-QC$^+$ complex, followed by metal exchange between Zn and Cu.

Based on the results obtained using nonradioactive Cu, preparation of a ^{64}Cu-PTDS-DHC complex was carried out as described in Materials and Methods. Labeling was completed through a simple and rapid procedure, which could readily be applied to a short-lived positron emitter ^{62}Cu. The TLC profile of the solution obtained was compared with that of the ^{64}Cu-PTDS-QC$^+$ complex. As shown in Fig. 4 (a), the ^{64}Cu-PDTS-QC$^+$ had a single peak at an Rf of 0.18. On the other hand, in the ^{64}Cu-PDTS-DHC solution, most of the ^{64}Cu radioactivity (85-90%) was detected near the solvent front, although a small portion (10-15%) was detected at the origin (Fig. 4 (b)). The presence of some radioactivity at the origin may have been the result of oxidative decomposition of the product during development of the chromatogram, since spectrophotometry showed that Cu-PDTS-DHC was decomposed rapidly by contact with air.

In the n-octanol extraction study, ^{64}Cu-PDTS-DHC (30.8 ± 1.6) showed a more than 100-fold greater partition coefficient than that for ^{64}Cu-PDTS-QC^{+} (0.288 ± 0.006). The high lipophilicity of ^{64}Cu-PDTS-DHC was also supported by the high Rf value shown by TLC.

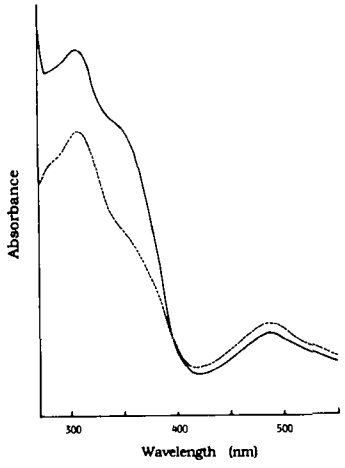

Fig. 3. Absorption Spectra of Cu-PDTS-QC^{+} (---) and Cu-PDTS-DHC (—).

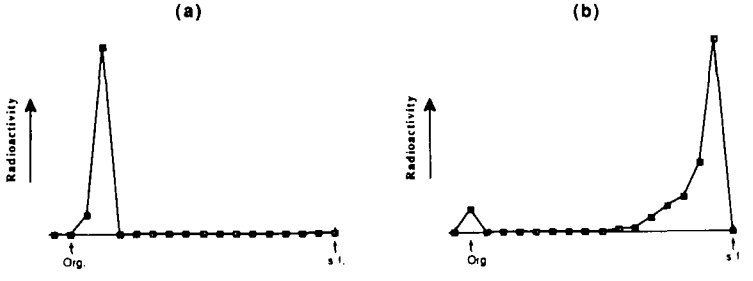

Fig. 4. TLC Profiles of ^{64}Cu-PDTS-QC^{+} (a) and ^{64}Cu-PDTS-DHC (b) (Chloroform : Methanol = 7 : 3).

Biological Studies

When assessing the potential clinical applications of ^{62}Cu-labeled radiopharmaceuticals, the biodistribution during a short time period needs to be considered because of the short half-life of the radionuclide (T1/2 = 9.8 min). Therefore, the biodistribution of ^{64}Cu-PDTS-DHC within 30 min after injection was investigated in mice. Table 1 shows the biodistribution data for ^{64}Cu-PDTS-DHC, ^{64}Cu-PDTS-QC^{+}, and ^{64}Cu acetate.

^{64}Cu-PDTS-DHC exhibited a high myocardial uptake that declined in the early period (0.5-5 min), but thereafter remained almost constant. Its uptake was respectively 1.5-2.1 and 1.8-2.4 times higher than that of ^{64}Cu-PDTS-QC^{+} and ^{64}Cu-acetate. On the

other hand, ^{64}Cu-PDTS-DHC was cleared rapidly from the blood, so a high heart-to-blood ratio (2.0-2.3) was obtained at 5-30 min after intravenous injection. In addition, the radioactivity of ^{64}Cu-PDTS-DHC taken up by the lungs, a competing organ that is generally responsible for poor resolution in myocardial imaging, was relatively low and therefore the heart-to-lung ratio was high (0.6-1.1). In fact, this ratio was higher than that obtained with ^{62}Cu-labeled pyruvaldehyde bis(N-methylthiosemicarbazonato)copper(II) (^{62}Cu-PTSM) (17), a promising radiopharmaceutical for the evaluation of myocardial perfusion (27-30). These results showed the high affinity of radiocopper-labeled PDTS-DHC for the myocardium and its potential for application to myocardial imaging studies.

On the basis of the data on some DHC derivatives reported by Bodor et al. (9-11) and Tedjiamulia et al. (12), a high brain uptake of ^{64}Cu-PDTS-DHC was expected. However, the actual uptake was low and was similar to that of ^{64}Cu-PDTS-QC$^+$ and ^{64}Cu acetate despite its high lipophilicity. Blood-brain permeability is affected by various physicochemical factors in addition to lipophilicity, such as molecular weight,

Table 1. Biodistribution of Radioactivity in Mice After the Intravenous Injection of ^{64}Cu-PDTS-DHC, ^{64}Cu-PDTS-QC$^+$, and ^{64}Cu Acetate (% dose/g)

Organ	Time after injection (min)				
	0.5	2	5	15	30
	^{64}Cu-PDTS-DHC				
Blood	9.67(2.19)[a]	4.82(0.71)	2.96(0.29)	2.06(0.10)	2.03(0.16)
Intestine	2.69(0.66)	3.56(0.66)	4.66(0.49)	8.36(0.44)	9.73(1.16)
Liver	22.58(5.23)	27.73(3.26)	26.87(3.42)	24.21(2.23)	22.94(2.65)
Kidney	14.17(2.37)	17.62(1.90)	15.67(2.26)	11.36(1.03)	10.04(1.22)
Lung	11.37(1.98)	6.89(1.15)	5.48(0.65)	6.05(0.79)	7.24(0.87)
Heart	13.04(1.68)	8.64(1.89)	5.91(0.98)	4.75(0.56)	4.46(0.36)
Brain	0.26(0.06)	0.18(0.01)	0.14(0.02)	0.15(0.01)	0.19(0.02)
HE/Bl[b]	1.38(0.16)	1.85(0.65)	2.00(0.25)	2.32(0.33)	2.20(0.23)
HE/LU[c]	1.16(0.10)	1.25(0.09)	1.07(0.06)	0.79(0.05)	0.62(0.06)
	^{64}Cu-PDTS-QC$^+$				
Blood	12.13(3.88)	4.49(1.56)	2.92(0.45)	1.79(0.08)	1.63(0.19)
Intestine	2.73(0.95)	2.01(0.72)	2.80(0.47)	4.52(0.47)	5.52(0.56)
Liver	11.66(2.47)	22.35(7.98)	25.32(3.87)	21.72(0.79)	17.21(2.72)
Kidney	15.65(3.80)	15.67(4.59)	12.88(1.78)	10.02(1.07)	8.10(1.03)
Lung	8.41(1.97)	4.81(1.51)	3.87(0.91)	4.50(0.32)	5.22(1.22)
Heart	6.87(1.84)	4.19(1.22)	3.33(0.38)	3.09(1.16)	2.83(0.70)
Brain	0.37(0.06)	0.18(0.06)	0.14(0.01)	0.14(0.02)	0.18(0.03)
HE/Bl	0.58(0.11)	0.95(0.09)	1.15(0.16)	1.75(0.75)	1.77(0.49)
HE/LU	0.82(0.11)	0.88(0.10)	0.89(0.19)	0.69(0.25)	0.56(0.14)
	^{64}Cu Acetate				
Blood	22.87(1.84)	11.14(1.23)	4.74(0.27)	2.77(0.25)	2.51(0.14)
Intestine	1.65(0.08)	3.01(0.49)	3.37(0.12)	4.87(0.60)	5.91(0.64)
Liver	7.91(0.67)	16.41(2.87)	16.31(1.35)	16.78(3.48)	17.27(3.05)
Kidney	11.45(1.10)	12.65(1.94)	11.08(1.20)	10.28(1.67)	8.87(0.62)
Lung	18.73(1.63)	10.18(1.22)	6.80(0.92)	7.05(1.29)	8.07(0.80)
Heart	5.52(0.39)	3.82(0.36)	2.48(0.24)	2.29(0.58)	2.48(0.27)
Brain	0.55(0.06)	0.30(0.06)	0.21(0.04)	0.20(0.01)	0.22(0.02)
HE/Bl	0.24(0.02)	0.34(0.01)	0.52(0.03)	0.83(0.20)	1.16(0.13)
HE/LU	0.30(0.02)	0.38(0.02)	0.37(0.06)	0.33(0.05)	0.31(0.02)

a) Each value is the mean (±S.D.) of 4 animals.
b) Heart-to-blood ratio.
c) Heart-to-lung ratio.

molecular size, stereochemistry, and protein binding (31). Thus, the low brain uptake of ^{64}Cu-PDTS-DHC may have been related to any of these factors.

The uptake of ^{64}Cu-PDTS-DHC by other organs was similar to that of ^{64}Cu-PDTS-QC^{+} and ^{64}Cu acetate, and both the liver and kidneys showed a high uptake.

In conclusion, we designed a ^{64}Cu-labeled bifunctional chelate (^{64}Cu-PDTS-DHC) which coupled a DTS molecule to a DHC carrier. Labeling with ^{64}Cu was easily performed by a metal exchange reaction between Cu and Zn. Biodistribution studies in mice demonstrated that ^{64}Cu-PDTS-DHC showed a high affinity for the myocardium. Although more detailed studies of radiocopper-labeled PDTS-DHC compounds are required before its application to myocardial perfusion studies, a bifunctional radiopharmaceutical with a DTS structure and a DHC carrier seems to offer a good basis for the future design of more target-specific radiopharmaceuticals labeled with ^{62}Cu and other radiometallic nuclides such as ^{99m}Tc.

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