Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 395-403 (2001)
DOI: 10.1002/jlcr.467

SYNTHESIS OF COBALT-55/57-COMPLEXES FOR RADIOLABELLING OF PLATELETS AS A POTENTIAL PET IMAGING AGENT

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SUMMARY

Complexes of oxine, tropolone and mercaptopyridine-N-oxide (MPO) with ^{111}In have been widely used for platelet labelling. The medium half-life PET radioisotope cobalt-55 would combine the higher sensitivity and resolution of PET with a half-life allowing quantitative uptake and cell kinetic studies. We therefore investigated the synthesis and platelet uptake of ^{57}Co-oxine, ^{57}Co-tropolonate and ^{57}Co-MPO in vitro with cobalt-57 as model for the cobalt-55. The complexes were formed in high yields >90%, with the exception of ^{57}Co-tropolonate 38%, and were stable over several days. The platelet uptake was generally low with 5-13%. Thus, these complexes are not ideal for platelet labelling and the eventually following PET application.

Key Words: cobalt-55-complexes, platelet labelling, PET, atherosclerosis

INTRODUCTION

During the last 3 decades platelets radiolabelled with various tracers were successfully used in nuclear medicine for imaging of thrombosis and / or atherosclerosis. The scintigraphic detection of venous thrombosis,

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Received 9 August 2000
Revised 11 November 2000
Accepted 29 January 2001

atherosclerotic lesions, left ventricular thrombus, chronic arterial disease and thromboembolic complications are some of the most important clinical applications of radiolabelled platelets, based on the monitoring of their passage through the circulation and the fact that platelets accumulate only in active thrombus (for review see 1).

Since the first report of indium-111 (^{111}In) oxine labelled platelets in 1976 (2) the development and evaluation of various lipophilic complexes of ^{111}In like ^{111}In-MPO (Merc) (3) and ^{111}In-tropolone (17) have been reported as carrier mole-cules for the intracellular radiolabelling of platelets and blood cells (for review see 1). But the half-life of ^{111}In (2.8d), the other physical characteristics and the physiological pooling of the platelets in the spleen are limiting its use for imaging (14). Radiolabelling of platelets with gallium-68 (^{68}Ga) combines the advantage of using higher doses (74-222 MBq), to receive more counts and better statistics, and of the PET-technique with high sensitivity, high resolution and the possibility of quantification (4). The short half-life of ^{68}Ga (68min) limits the clinical use of ^{68}Ga-labelled platelets, because the slow kinetic of the platelet adhering to the thrombus results in a low target to background ratio and thus in a restricted visualisation of the tracer accumulation at early imaging time points (5). The ^{99m}Tc-complexes of oxine and hexamethylpropylaminooxime (HMPAO) are also suitable for labelling platelets with a high labelling efficiency of 91% and 40-50%, respectively. However, the disadvantages of a high elution rate (about 8%/h) and, like ^{68}Ga, a short physical half-life (6h) limit its use for platelet survival studies (12,13).

The labelling of platelets with cobalt-55 (^{55}Co) complexes, due to its relative long half life of 18.2h, could be an alternative to the most commonly used ^{111}In. In this paper no-carrier–added (n.c.a.) cobalt-57 (^{57}Co) (with the longer half life of 271.7d, EC-decay) has been used for the investigation of the radiotracers cobalt-oxine (8-hydroxyquinoline), cobalt-tropolonate (2-hydroxy-2,4,6-cycloheptatrien-1-one) and cobalt-MPO (2-mercaptopyridine-N-oxide), and the in vitro platelet labelling. The in vitro result should be applicable to the labelling with ^{55}Co without restriction.

RESULTS AND DISCUSSION

Synthesis of the ^{57}Co-complexes

For the in vitro evaluation of the platelet labelling with Co-complexes we used n.c.a. Co-57, because of its longer half-life and commercial availability. We investigated the formulation of the complexes with the three ligands oxine, tropolone and MPO (scheme 1).

Scheme 1:

$$^{57}CoCl_2 \xrightarrow[\text{0.5M NaOAc pH5 / EtOH : 5/1 or saline}]{\text{L = oxine, tropolone or MPO}} {}^{57}CoL_{2,3}$$

^{57}Co-oxine can be prepared in high radiochemical yields (95±2%) with 0.4µmol oxine in 0.05M sodium acetate pH 5 buffer in 15 minutes (fig. 1,2). In neutral or alkaline solutions the reaction to the Co-oxine-complex is much slower due to the reversible formulation of Co-hydroxide at pH >5 (tab. 1). The necessity of adding 17% ethanol to the aqueous phase in order to achieve complete solubility shows the high lipophilicity of the complex.

Table 1: RCY of ^{57}Co-oxine at different pH (0.8µmol oxine, pH 5: 0.05M sodium acetate buffer ; pH 7: saline solution ; pH 8: phosphate buffer ; $\bar{x}$±SD ; n≥3)

pH 5	pH 7	pH 8
92±3%, 15min	88±3%, 70min	80±5%, 3h

The Co-tropolonate-complex required the oxidation of the Co^{2+} to Co^{3+} . The radiochemical yield is 38±1% at 1h with 0.8µmol tropolone and 10-20µl H_2O_2 (30%) at pH5 (fig. 1,2). Higher amounts of H_2O_2 should be avoided in order to prevent further oxidation of the cobalt (tab. 2). For platelet labelling the ^{57}Co-tropolonate was purified by extraction with chloroform. Higher radiochemical yield (70-97%) were reported by Ellis and Sharma (18), but these results were obtained at pH8 and with an excess of tropolone.

*Table 2: RCY of ^{57}Co-tropolonate vs. H_2O_2 amount (pH5, 0.8µmol tropolone, 20min , $\bar{x}\pm SD$; *n≥3)*

µl H_2O_2 (30%)	1	5	10	20	30	50	100	200
RCY %	16	15	25*±9	27*±7	22	24	8	16

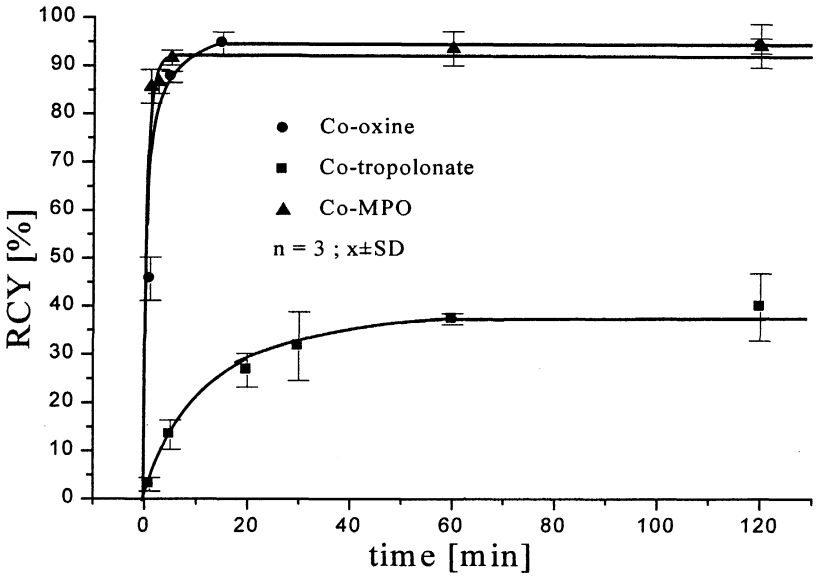

Figure 1: Radiochemical yield (RCY) vs. time (oxine: pH 5, 4.0µmol ; tropolone: pH5, 0.8µmol, 10/20µl 30% H_2O_2 ; MPO: saline solution, 0.2µmol)

^{57}Co-MPO can be easily prepared in high radiochemical yields (92±2% in 5 minutes) in physiological sodium chloride solution (fig. 1). Acid reaction conditions are not necessary (data not shown). There is no dependence of the ligand amount in the range of 0.04-2.4µmol MPO (fig. 2). The stability of the complex is unchanged for 4 days. The lipophilicity of ^{57}Co-MPO is lower in comparison to ^{57}Co-oxine, due to its good solubility in water not requiring ethanol addition and to its lower chloroform extraction rate.

Platelet labelling

The platelet uptake of ^{57}Co-oxine, ^{57}Co-tropolonate and ^{57}Co-MPO was investigated for different platelet densities (10^7-10^9 platelets/ml), incubation temperatures (4°C-37°C), amounts of radioactivity (0.37-37kBq) and incubation

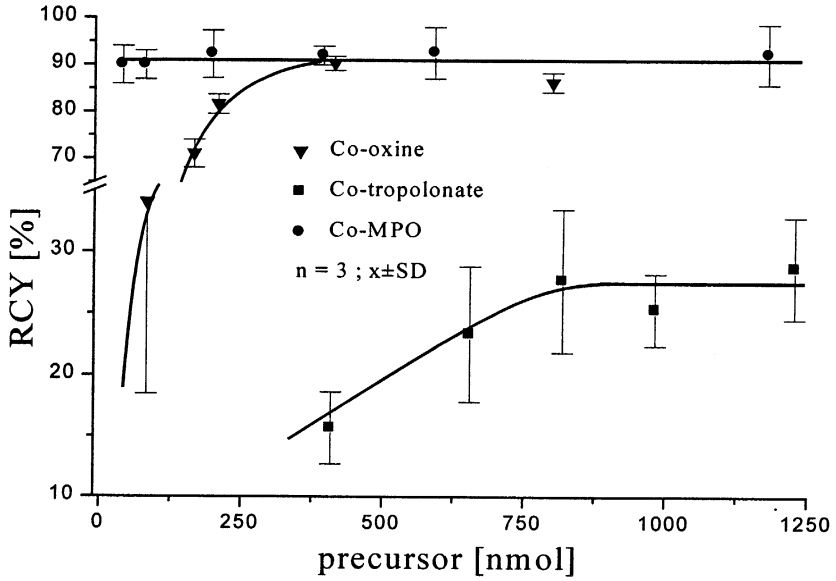

Figure 2: RCY vs. precursor amount (oxine: pH 5, 10min ; tropolone: pH 5, 20min, 20µl 30% H_2O_2 ; MPO: saline solution, 5min)

times from 1 to 30 minutes. As expected, the highest platelet uptake of the ^{57}Co-complexes was obtained at a platelet-density of 10^9 platelets/ml and an incubation temperature of 37°C (summarised in table 3, for details see (8)). The platelet uptake was independent on the amount of radioactivity in the range of 0.37-37kBq (tab. 4). Hereby, the concentration of the ligand were simultaneously reduced to a hundredth, thus the labelling efficiency was also independent on the chosen amount of ligand and a separation of the ligand was not necessary.

Table 3: Platelet uptake of the ^{57}Co-complexes at (8) (10^9 platelets/ml, 37kBq ^{57}Co-complex, 20min, 37°C ; n=6, $\bar{x}$±SD ; p<0.01)

^{57}Co-oxine	^{57}Co-tropolonate	^{57}Co-MPO
5.9±0.48%	10.4±0.70%	12.4±0.62%

Table 4: Platelet uptake of the ^{57}Co-complexes with different amounts of radioactivity in kBq (8) (10^9 platelets/ml, 37°C ; n=6, $\bar{x}\pm SD$; p<0.01)

t [min]		10	20	30
oxine	37	5.9±0.60	5.9±0.48	5.9±0.52
	3.7	4.9±.061	5.1±0.56	5.2±0.76
	0.37	5.3±0.34	5.3±0.67	5.4±0.48
tropolonate	37	9.2±0.56	10.4±0.70	11.0±0.67
	3.7	8.9±0.65	10.7±0.49	11.1±0.60
	0.37	9.0±0.57	9.9±0.71	10.9±0.73
MPO	37	9.0±0.58	12.4±0.62	13.0±0.69
	3.7	8.8±0.71	11.8±0.67	13.0±0.65
	0.37	8.7±0.46	11.5±0.66	12.4±0.61

For ^{67,68}Ga-MPO a platelet uptake of 18% and 36% has been reported (4,6,7,8). For all these tracers the same platelet labelling technique was used. ^{57}Co-oxine shows only a very low affinity for platelet labelling. The uptake of ^{57}Co-tropolonate and ^{57}Co-MPO is in the same magnitude as ^{67,68}Ga-MPO, but substantially lower as the one achieved with the respective ^{111}In-complexes (2,3,17). The reason could be a higher stability of the Co-complexes (^{57}Co-oxine > ^{57}Co-tropolonate, ^{57}Co-MPO, ^{67,68}Ga-MPO » ^{111}In-complexes), similar as postulated for ^{68}Ga-MPO (9,10) and ^{111}In-complexes (15,16). Thus, after diffusion into the platelets the strongly bound Co-complexes can more easily diffuse back across the cell membrane prior to dissociation and trapping of the ^{57}Co within the platelet (4).

CONCLUSION

The half-life of the PET-radioisotope ^{55}Co (18.2h) would enable the determination of quantitative uptake and even cell kinetics with ^{55}Co-labelled platelets. From the 3 investigated complexes, ^{57}Co-MPO shows the best results of complex formulation (92±2% in 5min) and platelet labelling (12-13%). In comparison to the affinity of ^{111}In-complexes to platelets, however, the labelling efficiencies of platelets with ^{57}Co-oxine, ^{57}Co-tropolonate or ^{57}Co-MPO are low. Therefore, only minor advantage for in vivo PET-imaging can be expected.

However, we could show, that ^{57}Co builds stable complexes with oxine, tropolonate and MPO. They may be useful as precursor for other complexes or the labelling of other cells.

EXPERIMENTAL

Materials

Thin-layer chromatography was carried out using Si-60-TLC-strips (Merck, Darmstadt, Germany). 8-Hydroxyquinoline (oxine) and 2-hydroxy-2,4,6-cycloheptatrien-1-one (tropolone) were purchased from Aldrich, 2-mercaptopyridine-N-oxide (MPO) from Sigma, respectively. No carrier added cobalt-57 (37MBq , specific activity of 16.5-18.0 TBq/mmol, as cobalt (II) chloride in 100µl 0.1N hydrochloric acid) was obtained from Amersham (Buckinghamshire, Kent, UK). Other chemicals were obtained from commercial sources and were all analytical grade.

Blood from 96 healthy volunteers (32 women and 64 men), age range from 18 to 36 years, mean age 27 years, without any risk factor for development of atherosclerosis (smoking, hyperlipoproteinemia, hypertension, no drugs) was drawn for the isolation of platelets.

Preparation of the tracers

A cobalt-57-stock-solution 70-80kBq/µl was prepared through dilution of the 100µl 0.1N hydrochloric acid solution with 400µl distilled water. For each synthesis 5µl of the respective stock solution (250-400kBq) were mixed with 0.5ml 0.05M sodium acetate buffer pH5 (or saline solution) and the precursor (MPO in 100µl water, tropolone and oxine in 100µl ethanol) were added. In the case of tropolone H_2O_2 was added additionally.

50µl of the Co-stock solutions and 1.1µmol oxine, 0.8µmol tropolone (+ 10µl 30% H_2O_2) or 0.2µmol MPO were used for the labelling of the platelets. In the case of ^{57}Co-tropolonate the product was purified through extraction with chloroform, evaporation of the solvent and dissolving of the product in saline solution/20% ethanol. The complexes were stable for more than 1 week.

The radiochemical purity was determined using silica gel TLC and methanol/chloroform 15/85, 0,1% 0,5M acetate buffer as mobile phase (chloroform/acetone 10/90 for ^{57}Co-tropolonate) and analysed with an radioactivity imager (instant imager, Canberra-Packard, Meriden, USA).

Platelet separation and labelling:

Separation and labelling of the platelets were carried out by the technique described by Sinzinger et al (11). Shortly, in a Monovette (Sarstedt, Feldkirch, Austria) 2ml acid citrate dextrose were added to 7ml blood. After sedimentation of the red blood cells (10min) the vial was centrifuged (150g, 5min) and the platelet rich plasma removed. After another centrifugation (500g, 10min), the obtained platelet pellet was gently resuspended in 1ml Tyrode buffer (pH 6.2), the tracer added and incubated in a water bath at the respective temperature under constant stirring. After radiolabelling and centrifugation the platelet pellet and the supernatant platelet poor plasma were counted in a gamma counter. The labelling efficiency was expressed as percentage of radioacticity uptake by the platelets. The in vitro platelet function was shown by ADP-induced platelet aggregation (for details see 8).

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