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Tri-*n*-octyl[^{32}P]phosphine Oxide. Synthesis and Biodistribution *via* the Hepatic Artery of Rats

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SUMMARY

The purpose of this study was to analyse the biodistribution of tri-*n*-octyl[^{32}P]phosphine oxide ([^{32}P]TOPO) in rats following intrahepatic arterial injection so as to assess its potential as a radiopharmaceutical for the treatment of hepatic tumours in humans. The retention of [^{32}P]TOPO remained high in liver and decreased rapidly in kidney, bone marrow and blood. The synthesis of [^{32}P]TOPO was performed in a one step procedure from [^{32}P]$POCl_3$ and *n*-octyl magnesium chloride in diethyl ether at 0°C.

KEY WORDS

Tri-n-octyl[^{32}P]phosphine oxide, Biodistribution, Autoradiography, Hepatic cancer, Metabolic Radiotherapy

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INTRODUCTION

The prognosis for patients with advanced hepatocellular carcinoma remains poor (1). Partial hepatectomy or liver transplantation have limited effects on overall survival (2). Our study was carried out to assess a novel therapeutic approach using a Lipiodol analogue substance as a pure β^- isotope carrier.

Ultra-Fluid Lipiodol (LUF), an oily lymphographic agent, has been used in hepatography since 1962 (3). It can be combined with anticancer drugs (4-5) or labelled with 131-Iodine (6), 90-Yttrium (7-8) and 32-Phosphorus combined with 131-Iodine (9-11). Radiolabelled Lipiodol has been developed for the treatment of non-resectable primary hepatoma both in animals (12-13) and humans (14-15) following hepatic arterial injection (16-18). Bretagne *et al* (1, 19) studied the biodistribution of [^{131}I]LUF in twenty-three patients with hepatocellular carcinoma. Their results suggested the usefulness of LUF as a carrier of therapeutic agents in the treatment of liver tumours. Lipiodol showed characteristic tumour tropism when injected into the hepatic artery although the mechanism of this local accumulation remained unknown.

131-Iodine is not, however, the best radiotherapeutic agent. The purpose of our study was to use a more promising candidate, 32-Phosphorus, for experimental radiotherapy. 32-P has several advantages, including a shorter half-life more suitable for therapy, lack of *gamma*-ray emissions dangerous for surrounding normal tissues, and a higher *beta* energy range sufficient to kill cells. In light of these studies it seemed appropriate to consider ^{32}P-labelled fatty derivatives, where the acid ethyl esters of Lipiodol would be replaced by long chain-hydrocarbons.

Here we report the synthesis of tri-*n*-octyl[^{32}P]phosphine oxide ([^{32}P]TOPO) performed in a one pot procedure from [^{32}P]$POCl_3$ and *n*-octyl magnesium chloride in diethyl ether at 0°C. Data on the biodistribution in rats following hepatic arterial injection is also presented.

MATERIALS AND METHODS

General

Carrier-free [^{32}P]H_3PO_4 was obtained in aqueous solutions from Amersham. *n*-Octyl magnesium chloride and phosphorus oxychloride were purchased from Aldrich

Chemical Co. Phosphorus oxychloride was distilled in the absence of moisture shortly before use. All other solvents and chemicals used were of the highest available purity. ^{31}P-NMR spectra were obtained with an AC 300P Brucker Spectrometer, operating at a frequency of 121 MHz, using conventional FT-NMR techniques. Mass spectrometry measurements were performed on a MS/MS ZABSpec TOF Micromass instrument (Centre Régional de Mesures Physiques de l'Ouest, Rennes, France). Radioactivity was determined by liquid scintillation counting using a LKB Wallac β counter. Samples were counted in scintillation fluid (Aquassure, Packard).

Synthesis of Tri-n-octyl[^{32}P]phosphine Oxide (n-$C_8H_{17})_3{}^{32}PO$

Labelled phosphorus oxychloride was obtained applying the method of Keenan (20). A screwed teflon-capped vial containing 840 μL (9 mmol) of freshly distilled phosphorus oxychloride and 33 MBq of carrier-free [^{32}P]phosphoric acid (900 μL, 7.4 TBq/mmol) was incubated for 65 h at 105°C to give a theoretical specific activity of 3.7 MBq/mmol (calculated for 100% conversion to $POCl_3$); 5 mL of dry ether were added and cooled at 0°C under' argon. After the dropwise addition of a 2 M ether solution of *n*-octyl magnesium chloride (14.8 mL, 29.7 mmol), a white precipitate formed. The mixture was stirred for about 10 min before the temperature was allowed to rise to ambient temperature. The mixture was maintained at this temperature for about 2 h and then quenched at 0°C with dry methanol (20 mL). The solvents were removed at atmospheric pressure and the residue, dissolved in dichloromethane (40 mL), was washed with 6 M HCl (2 x 40 mL) and shaken with an aqueous solution of potassium permanganate (6.5 mL, from 1 g $KMnO_4$ in 10 mLwater). The organic layer was then washed with 12 M H_2SO_4 (5 x 40 mL), water (3 x 40 mL), 6 M NaOH (40 mL), water (2 x 40 mL), and dried ($MgSO_4$). Evaporation to dryness (atmospheric pressure) afforded tri-*n*-octyl-[^{32}P]phosphine oxide as a clear oil which slowly solidifies on cooling (3.5 mL, 33%, 0.9 MBq/mmol).

^{31}P-NMR (121 MHz, $CDCl_3$, H_3PO_4 85% external standard), δ ppm: 49.3 (1, 96%), 58.2 (2, 4%). HRMS Calc. for $C_{24}H_{52}OP$: 387.3756. Found: 387.3758.

Animals

The animal studies were performed according to the French code of practice for the care and use of animals. Male Wistar rats weighing 250-300 g were kindly given by Dr MOULINOUX (Unité CNRS 1529). They were anaesthetized by intra peritoneal injection of 50 mg of Ketalar (0.5 mL/IP route).

Hepatic Arterial Injection of Tri-n-octyl[^{32}P]phosphine Oxide

Under anaesthesia by IP injection of Ketalar, midline laparatomy was performed. The hepatic artery was identified and 6 μCi of ^{32}P in a volume of 10 μL were injected with a 25G(5/8) needle.

Biodistribution of Tri-n-octyl[^{32}P]phosphine Oxide in Rats Following Hepatic Arterial Injection

Three rats were sacrificed at the same time in order to determine tissue biodistribution of tri-*n*-octyl-[^{32}P]phosphine oxide at 17, 24, and 48 h after injection. Samples (about 200 mg) of blood, liver (artery and lobe), spleen, lung, bone, kidney were taken and weighed. Two millilitres of tissue solubilizer (Soluen 350 Packard) were added, after which, the samples were incubated at 50°C for 4 h to achieve tissues lysis. Bone marrow was treated with 2 mL10 N HCL during 8-24 h at room temperature. A small amount of hydrogen peroxide was added to bleach the samples. 10 mLof scintillation liquid were added before counting the radioactivity.

Autoradiography by Imstar Image Analysis System

The rats were embedded after sacrifice in a hemicellulose block, 24 h after injection of the radioactive substance. The calibration range was obtained adding known increasing quantities of ^{32}P activity (0.06, 0.6, and 6 μCi) to 1 mL blood samples. The block was then frozen, and 30 μm thick sections of the rats taken. The calibration range was made using a cryomacrotome (cryomacrocut, Leica). The sections were mounted on transparent tape and placed directly on film (Kodak Biomax MR) for 5 days at –20°C. Radioactivity was calibrated from the calibration range and the half-life of the

radionuclide. Autorad software from IMSTAR image analysis system (IMSTAR, Paris, France) was used to digitalize the autoradiographies. Quantitative analysis was done with NIH Image 1.55 software.

Imaging Plate

The imaging plate consists of a flexible plastic plate coated with fine photostimulable phosphor crystals ($BaFBr:Eu^{2+}$) capable of storing a fraction of the absorbed incident energy from irradiation with electrons or photons. When later stimulated by visible or infrared radiation, these crystals emit photostimulated luminescence at an intensity proportional to the absorbed radiation energy. The imaging plate system has several advantages compared to others imaging sensors: ultra-high sensitivity, a wider dynamic range, superior linearity and better spatial resolution.

Before and after use, the imaging plate was exposed to visible light emitted by Molecular Dynamics Model 410 A Image Eraser in order to eliminate residual images and secondary background noise due to cosmic and environmental radiations. The 30 μm thick section of the whole animal was protected with Saran Wrap before being placed on the screen inside a Molecular Dynamics Exposure Cassette. After exposure at the room temperature for 6 h scanning was performed on a Molecular Dynamics 400APhospho Imager. Quantitative analysis was done with N I H Image 1.55 software

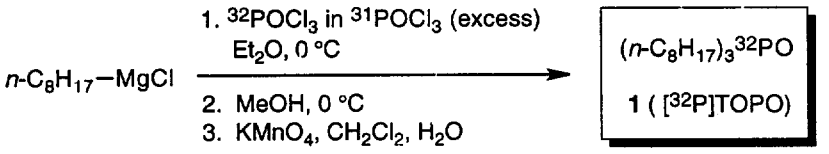
RESULTS

Synthesis of Tri-n-octyl[^{32}P]phosphine Oxide (n-$C_8H_{17})_3{}^{32}PO$

[^{32}P]$POCl_3$ was easily produced by heating [^{32}P]H_3PO_4 in the presence of a massive excess of $POCl_3$ (20). Reaction with *n*-octyl magnesium chloride in diethyl ether at 0°C gave the labelled tri-*n*-octylphosphine oxide **1** (Fig. 1) with a radioactivity of approximately 0.9 MBq/mmol and a radiochemical purity of 95-96%. Dioctylphosphine oxide **3** which was formed as a by-product resulted presumably from the reduction *via* a single electron transfer of dioctylphosphinate **2** by the Grignard reagent. After successive treatment of the crude reaction mixture with potassium permanganate and aqueous sodium hydroxide, **3** was easily removed and **1** and **2** were obtained in a 96:4 ratio

(^{31}P-NMR determination) in 33% chemical yield. The chemical yield was determined by ^{31}P-NMR (internal standard method). A known amount of trimethylphosphate was added to the sample to be analysed and the peak-area was compared to the area of the standard peak.

Fig. 1. Synthesis of Tri-*n*-octyl[^{32}P]phosphine Oxide ([^{32}P]TOPO)



(n-C_8H_{17})$_2$^{32}P(O)(OMe) (n-C_8H_{17})$_2$^{32}POH

2 **3**

The specific radioactivity of the phosphine oxide was 0.24 of the theoretical value, a result which can be explained by incomplete exchange with [^{32}P]phosphoric acid and low rate of phosphorus incorporation. The synthesis is a one-pot reaction requiring no distillation or other difficult manipulation.

Biodistribution of Tri-n-octyl[^{32}P]phosphine Oxide in Rats Following Hepatic Arterial Injection

The biodistribution of [^{32}P]TOPO in rats is shown in Table 1.

Table 1. Tissue Distribution of [^{32}P]TOPO in Healthy Rats[a,b]

Time (h)	Blood	Liver[c]	Spleen	Lung	Marrow[d]	Kidney
17	0.12	0.89	0.23	0.62	0.15	0.12
24	0.41	2.50	2.10	0.21	0.11	0.36
48	0.07	1.60	0.20	0.49	0.07	0.19

[a] Average value of three rats for each time interval. [b] Tissue concentration (% inj. dose/g). [c] Artery and lobe. [d] Bone.

The distribution of radioactivity in tissues or blood at 17 to 48 h after injection showed a high level of radioactivity in liver 24 h after injection of tri-*n*-octyl-[^{32}P]phosphine oxide compared with other sites. It remained high at 48 h in the liver lobe while it declined in other tissues and especially in blood, bone and kidney where a low radioactivity was found. Nevertheless, a moderate radioactivity was observed within the 48 h in lungs.

Autoradiography - Results of IMSTAR Image Analysis System

Fig. 2a. Histological 30μm thick section of the whole rat made using a cryomacrotome

Fig. 2b. Autoradiography of the whole rat, 24 h after injection of Tri-*n*-octyl[^{32}P]phosphine Oxide

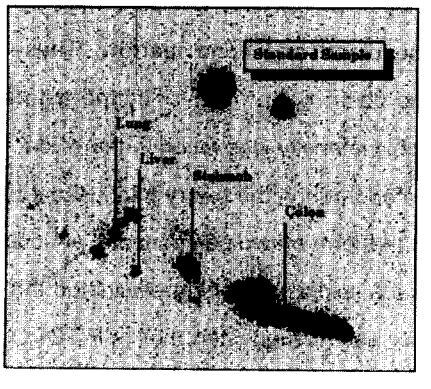
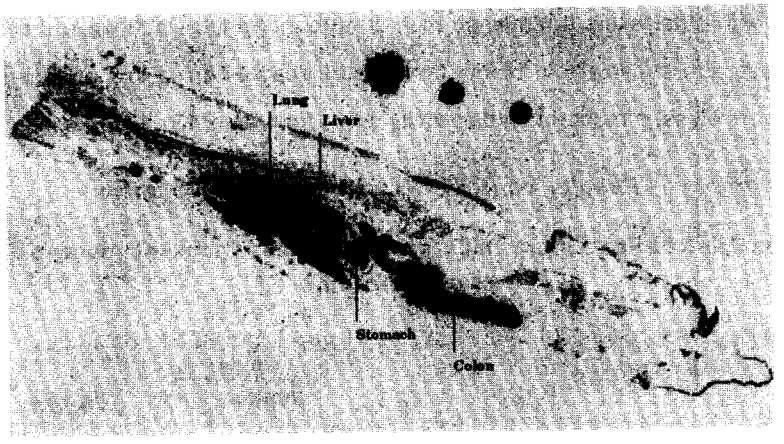


Fig. 2c. Whole Animal Autoradiography Showing Localisation of Tri-*n*-octyl[^{32}P]phosphine Oxide by superposition of Fig. 2a and Fig. 2b



The recorded image obtained after 6 h exposure (Fig. 2) showed a high relative quantitative intensity of radiation especially in the colon (39%), in the liver (27%) and in the stomach (8%). The value of each organ radioactivity was expressed by comparison with standard samples. No background was observed outside these tissues.

DISCUSSION

Our results showed a peak in the radioactivity in all organs tested one day after injection into the hepatic artery. Then the radioactivity decreased, except in the liver where it remained high. These observations suggested that tri-*n*-octyl[^{32}P]phosphine oxide is catabolized by the liver as Lipiodol (18). This was confirmed by the absence of significant radioactivity in the kidney, blood and bone marrow. These results agree with the relatively high intensity also observed in the liver by the autoradiographic method (21). The maximal autoradiographic intensity was found in the colon. Tri-*n*-octyl[^{32}P]phosphine oxide seemed to be eliminated through the biliary tract. Raoul *et al* reported (1-2, 19, 22) that LUF was eliminated in the form of two components, an iodine component eliminated through the urinary tract and a lipidic component eliminated through the biliary tract. The discrepancy between these results suggested that [^{32}P]TOPO was extremely stable *in vivo* and was eliminated without degradation, in the form of one component. The

persistent radioactivity observed in the lung may be a result of the arterial-portal-systemic shunting in the liver, or may be attributed to embolism by small oily drops leaked from the liver (23).

CONCLUSION

We synthesised and evaluated tri-*n*-octyl[^{32}P]phosphine oxide. This agent is extremely stable *in vivo*. Our results are encouraging. ^{32}P emerged as a radioisotope of choice for the metabolic radiotherapy (24). It is a pure *beta*-emitter of maximum energy 1.71 MeV, with a mean range in tissue of 3 mm and a maximum of 8 mm. It has a half-life of 14.3 days. It is known that the biological half-life of ^{32}P is higher in tumour tissue (8, 25) than in normal tissue. In order to increase the retention time of tri-*n*-octyl-[^{32}P]phosphine oxide in the body, studies on rats with hepatoma are in progress. The specific radioactivity of the product can be increased using the method of Keller (26) to synthesise labelled phosphorus oxychloride.

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