A new ^{99m}Tc labeled porphyrin for specific imaging of Sarcoma 120 : synthesis and biological study in a Swiss mouse model

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SUMMARY

A new porphyrin *meso*-5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxyphenyl]porphyrin (T3,4BCPP) was synthesised and efficiently labeled with ^{99m}Tc. On injecting this ^{99m}Tc labeled porphyrin to abdominal Sarcoma 120 bearing Swiss mice it accumulated in the abdominal tumour. This radiolabeled complex can thus be used for tumour detection and diagnosis.

Key Words: ^{99m}Tc, porphyrin, tumour, radioscintigraphy, cancer detection.

INTRODUCTION

In recent years, there has been an increasing importance of ^{99m}Tc labeled radiopharmaceuticals in the field of diagnostic nuclear medicine (1-3). The extensive use of ^{99m}Tc emerges from its favourable nuclear properties ($t_{1/2}$ = 6.02 h, E_γ = 140 KeV), its ready

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availability and its relatively low cost (2). In the development of ^{99m}Tc radiopharmaceuticals for assessing the status of organ function and morphology, ^{99m}Tc complexation with suitable chelating and nonchelating agents is necessary. In nonchelating agents this may be achieved by a suitable modification such as conjugation with a bifunctional chelator and if disulfide bonds are present by their reduction (2,3). A large number of these radiopharmaceuticals show organ selectivity with little or no specificity for neoplastic tissue and are therefore unsuitable for detection of tumours. It has been shown that some porphyrins accumulate in tumour tissues (4,5) and are acceptable to metabolic processes (6). Some attempts have been made to label hematoporphyrin derivative (HpD) with ^{99m}Tc (7) and subsequent study has shown high uptake of the radiopharmaceutical in mammary adenocarcinomas of mice (8). However, as the labeled product remained stable at room temperature up to 3 hours only, its clinical utility seems rather limited.

Following a similar approach to obtain tumour specific imaging agents, we report the results of the synthesis of a new water soluble porphyrin, its ^{99m}Tc labeling and the tumour uptake of this labeled species in abdominal Sarcoma 120 bearing Swiss mice.

EXPERIMENTAL:

Chemicals: Sodium [^{99m}Tc]pertechnetate was eluted from a $^{99}Mo/^{99}Tc$ generator (BRIT at B.A.R.C., Bombay, India). All other chemicals and reagents were obtained commercially and were of the highest available grade of purity.

Porphyrins: *meso*-tetrakis[3,4-bis(carboxymethyleneoxy)phenyl]porphyrin 4 (T3,4BCPP) was prepared as shown in Fig. 2 by refluxing 3,4-dicarboethoxymethyleneoxybenzaldehyde 2 and pyrrole (molar ratio 1:1) in a mixture of propionic acid and nitrobenzene (v/v 3:1) (9). The diester of the above porphyrin 3 was purified by silica gel column chromatography using a mixture of $CHCl_3$:MeOH (9.5:0.5, v/v) and this purified diester was hydrolysed with KOH (2N) in tetrahydrofuran to

get T3,4BCPP **4**. The T3,4BCPP was purified on a Dowex 500X8 ion exchange column using water as an eluant. Yield: 70% ; Elemental Analysis; Found: C, 59.96; H, 3.64; N,4.52. Calc. for $C_{60}H_{46}O_{24}N_4$: C, 59.71; H, 3.84; N, 4.14. UV-Vis (0.01 M phosphate buffer of pH 7.4): 422.1 nm (ε $102X10^3$), 519.8 nm ($32.2X10^3$), 554.8 nm ($20X10^3$), 593 nm ($11.5X10^3$) and 650 nm ($11.8X10^3$); IR (KBr):2500-3000 cm^{-1} (OH); 1660 cm^{-1} (CO); ^1H NMR (300 MHz) DMSO-d_6:δ (in ppm), 5.02 (s,8H,-OCH_2-) 7.24 -7.28 (m,12H,phenyl) and 8.83 (s,8H,pyrrole); FAB mass spectra of the ester of T3,4BCPP :molecular ion peak;m/z obsd. 1432, calcd. 1432.2.

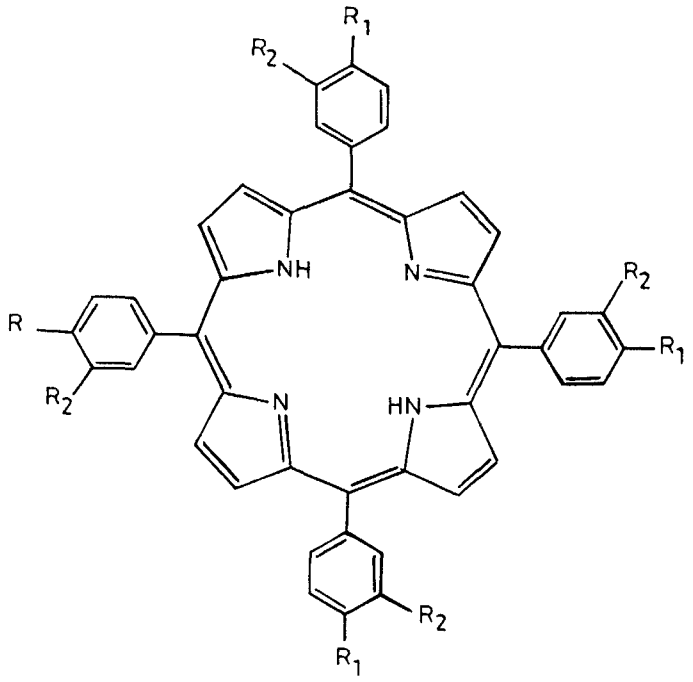
meso-tetrakis[4-(carboxymethyleneoxy)phenyl]porphyrin (T4CPP) was prepared by a modified procedure as reported earlier (10). The UV-vis and ^1H nmr spectral data are in complete agreement with the reported values.

Labeling and Scintigraphy: The porphyrins T3,4BCPP and T4CPP were labeled with ^{99m}Tc by dissolving 1 mg of the porphyrin in 1 ml of buffered saline in a sterile vial. Sn^{2+} (20 μg) as $SnCl_2$ (0.1% stock solution) was added to the above solution followed immediately by the addition of 111 MBq of $^{99m}TcO_4^-$ ions. The final pH of this mixture was observed to be 7. This mixture was incubated for 30 minutes at room temperature (7,11). The radiochemical analysis indicated that ^{99m}Tc labeled T3,4BCPP and T4CPP were 97% and 65% respectively (the radiochemical purity was checked by ascending paper chromatography using normal saline as a solvent system). The ^{99m}Tc labeled T3,4BCPP was administered by intravenous injection into the lateral tail vein of normal and Sarcoma-120 bearing Swiss mice. The accumulation of the radiolabeled porphyrin was measured by whole body scintigraphy using a gamma camera (Elscint) fitted with parallel hole collimators (3). The ^{99m}Tc labeled T3,4BCPP was stable *in-vivo* for more than 5 hours.

RESULTS AND DISCUSSION

The porphyrins used in this study are shown in Fig. 1. The reaction scheme for the preparation of the porphyrin T3,4CPP **4** is given in Fig.

2. Radioscintigraphy has been employed to depict the accumulation of the radiolabeled porphyrin in a tumour model in Fig. 3.



$R_1 = OCH_2COOH$, $R_2 = H$ (T4CPP)
$R_1 = R_2 = OCH_2COOH$ (T3,4BCPP)

Fig. 1: Structures of the porphyrins T4CPP and T3,4BCPP.

Our initial attempts in developing a tumour specific imaging agent were directed towards the labeling of T4CPP (Fig. 1) with ^{99m}Tc. This ^{99m}Tc labeled T4CPP resembles ^{99m}Tc-HpD in many ways. However, the latter has at least two major labeled species whereas the former is a single chemical species. The binding efficiency of ^{99m}Tc with T4CPP as assessed by radiochromatography was found to be about 65%. At such low binding efficiency values, the distribution of ^{99m}Tc-T4CPP cannot be monitored effectively and, therefore, the biological distribution and retention of this radiolabeled porphyrin was not further examined.

If the labeling of ^{99m}Tc takes place at the $-OCH_2COOH$ side chain of T4CPP, this results in a weak and less stable form of ^{99m}Tc-porphyrin complex as revealed above by its low binding efficiency. Therefore, we attempted to enhance the binding efficiency by incorporating tetradentate chelating groups at the phenyl side chains of the porphyrin. To achieve this another oxymethylenecarboxylic acid group was introduced adjacent to the already existing one at the phenyl groups of T4CPP.

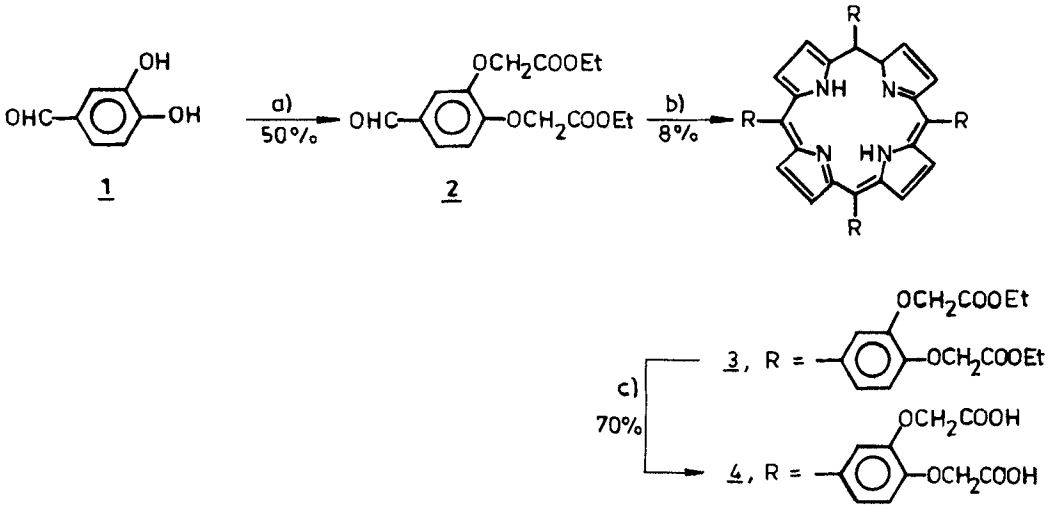


Fig. 2: Reaction Scheme for T3,4CPP synthesis.
a. $ClCH_2COOEt$, anhydrous K_2CO_3, acetone, reflux, 8h
b. Pyrrole, propionic acid, nitrobenzene, reflux, 1h, standing overnight at room temp.
c. 2(N) KOH, THF, room temp. 96h.
The percentages represent the yields of the various steps.

The ^{99m}Tc complex of this porphyrin *meso*-5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)phenyl]porphyrin (Fig. 1), showed a 97% binding efficiency and remained stable for more than 5 hours at 25^0C. Thus this complex was suitable for radioimaging.

The chemical nature of the ^{99m}Tc-T3,4BCPP complex was ascertained by comparing its UV-Vis spectrum in normal saline with the spectrum of

free T3,4BCPP. No change was observed, suggesting that the porphyrin core was unaffected (12) in the ^{99m}Tc-T3,4BCPP complex and that the reduced ^{99m}Tc binds the two oxymethylenecarboxylic acid groups as tetradentate ligands of T3,4BCPP in its complex. This mode of binding of ^{99m}Tc to the porphyrin was further supported by preparing a ^{99m}Tc-Mn^{III}T3,4BCPP complex, where Mn^{III} was strongly bonded by the four nitrogen atoms (of pyrroles) in T3,4BCPP. This ^{99m}Tc labeled Mn^{III} porphyrin like the ^{99m}Tc-T3,4BCPP complex also exhibited a high binding efficiency of 97% besides a similar accumulation pattern in tumour bearing mice.

Although ^{99m}Tc exhibited a high binding efficiency with HpD (6,7), the heterogeneous chemical composition of Hpd caused the formation of two major labeled species. Therefore, the chemical nature of these species could not be determined though the possibility of ^{99m}Tc attachment to the carboxylic side in one of the fractions was suggested. Moreover, the fraction responsible for accumulation in tumour tissue could not be identified. Most of these problems have been overcome by ^{99m}Tc complexation of the porphyrin T3,4BCPP which results in the formation of a chemically pure and stable ^{99m}Tc-T3,4BCPP species for tumour detection.

The ^{99m}Tc-T3,4BCPP complex was injected to both control and abdominal Sarcoma-120 bearing Swiss mice. Scintigraphic images of both control and tumour bearing mice were obtained 5 hours post injection (Fig. 3) to allow for reduction of blood pool activity. Fig. 3 shows the localisation of the ^{99m}Tc-T3,4BCPP complex in the abdominal tumour. Significant liver and kidney uptake was also observed in both set of animals suggesting that these organs were involved in the excretion of the radiolabeled porphyrin (1). A slight uptake of this labeled porphyrin was also observed in the brain of the tumour bearing animal and could be attributed to a metastasized cancer in the brain because no corresponding uptake was observed in the control animal.

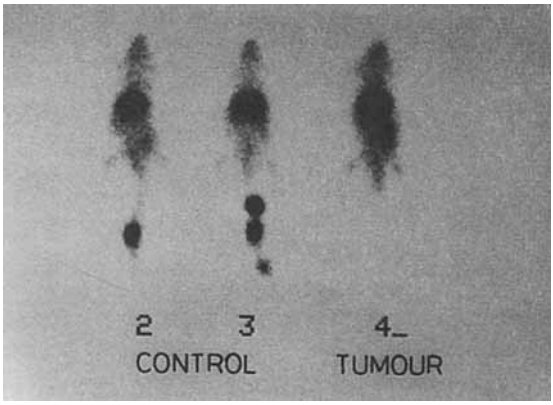


Fig. 3: Scintigrams of control and abdominal Sarcoma-120 bearing Swiss mice obtained 5 hr post injection of ^{99m}Tc-T3,4BCPP. Note the spot of injection in the tail.

The above results show the specific accumulation of ^{99m}Tc labeled T3,4BCPP complex in the tumour tissue. The uptake of this radiolabeled complex in the brain suggests that it has crossed the collapsed blood brain barrier which generally surrounds tumour tissue (13).

Our present study thus demonstrates the potentiality of the ^{99m}Tc-T3,4BCPP complex in specific detection of tumours by a non invasive method (14).

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14. Patent application is in progress.