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SYNTHESIS AND RADIOIODINATION OF A *MESO*-TETRA (HYDROXYNAPHTHYL) PORPHYRIN AND ITS SULPHONATED DERIVATIVE AS POTENTIAL TUMOUR LOCALISERS

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SUMMARY

The synthesis and radiolabelling with iodine-125 of a sulphonated and non-sulphonated *meso*-tetra(hydroxynaphthyl) porphyrin is described. Complete demethylation of the *meso*-tetra (methoxynaphthyl) porphyrin precursor to form the corresponding hydroxynaphthyl derivative could not be achieved using HBr. Sulphonation and radioiodination reactions were thus carried out on a mixture of hydroxy/methoxynaphthyl porphyrins. The weak mass spectrum exhibited by the sulphonated porphyrin derivative may reflect the highly polar nature of the compound. Neither of the iodinated porphyrins were found to localise in tumour tissue to any appreciable extent. Spleen uptake of the iodinated hydroxynaphthyl porphyrin was found to be much greater in tumour bearing mice than in normal (non-tumour bearing) mice. This may be a result of porphyrin aggregation due to its limited water solubility.

Key Words: hydroxynaphthyl, porphyrin, radioiodination, sulphonation, tumour-localiser, ^{125}I.

INTRODUCTION

A number of *meso*-tetra (hydroxyphenyl) porphyrins have recently been

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reported as possessing favourable tissue selectivity with regard to the phototherapeutic treatment of malignant tumours (1). Previous investigations with a series of radioactively labelled porphyrin analogues (2) have led us to conclude that porphyrins containing highly aromatic groups (e.g.naphthyl) substituted in the meso positions of the tetrapyrrole ring system exhibit increased tumour localisation properties compared with the corresponding phenyl substituted compounds. Good water solubility of porphyrins also appears to be an essential requirement for tumour localisation (3) although, interestingly, the latter property may be lost following occupancy of the vacant central position in the tetrapyrrole ring by a metal ion (4). This would appear to preclude the use of porphyrins for tumour detection using radiolabelling methods involving the introduction of radioactive metals such as ^{99m}Tc or ^{111}In. Several methods are available for introducing radiohalogens into highly activated aromatic rings such as phenols and aromatic amines (5). Incorporation of ^{125}I into a porphyrin containing phenolic groups in the meso positions would thus appear to be a feasible route to the synthesis of a radiolabelled porphyrin whilst still retaining the tumour localising properties of the molecule.
In the event of a porphyrin exhibiting enhanced tumour localising properties being synthesised the ^{125}I label could be replaced by ^{123}I for gamma camera imaging.

This work reports on the synthesis of ^{125}I-labelled (4-hydroxy-1-naphthyl) porphyrin and its sulphonated derivative and, utilising a tumour-mouse model, compares and contrasts both tumour uptake and distribution characteristics of the two compounds in a variety of tissues.

MATERIALS AND METHODS

General

Pyrrole, propionic acid and 4-methoxy-1-naphthaldehyde were obtained from the Aldrich Chemical Co., Ltd., New Road, Gillingham, Kent. Concentrated sulphuric acid (s.g. 1.84) and carrier-free Na ^{125}I were purchased from FSA Laboratory Supplies, Bishop Meadow Road, Loughborough,

Leics. and Du Pont NEN Research Products, Dreieich, W. Germany respectively. Chloramine T, sodium metabisulphite and sodium iodide together with the buffer reagents sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were purchased from BDH Ltd., Reagents Division, Broom Road, Poole, Dorset.

Removal of unbound (free) Na ^{125}I from ^{125}I-labelled porphyrins and the separation of sulphonated porphyrins from residual sulphuric acid was achieved using C_{18} Sep-Pak cartridges supplied by Millipore (U.K.) Ltd., Ascot Road, Croxley Green, Watford, Herts. Before use, cartridges were pre-wetted with methanol (2ml) and then with distilled water (5ml). An aqueous solution of the porphyrin was then applied to the cartridge and unwanted materials removed by elution with water and the porphyrin obtained following elution with methanol.

UV/visible spectra were obtained using a Pye-Unicam Model SP 1800 spectrophotometer. Porphyrins were examined dissolved in either methanol or chloroform (10-15 µg/ml). Mass spectral data were obtained using a Jeol JMS DX 303 mass spectrometer using either thioglycerol or 3-nitrobenzyl alcohol matrices.

Porphyrin synthesis

The presursor *meso*-tetra (4-methoxy-1-naphthyl) porphyrin is a new compound and was prepared using minor modifications (6) of the standard method of porphyrin synthesis (7) as described below.

5,10,15,20-Tetra (4-methoxy-1-naphthyl) porphyrin (1) was prepared by adding a solution of 4-methoxy-1-naphthaldehyde (9.89g, 0.053 mole) in propionic acid (10ml) to a refluxing mixture of propionic acid (100ml) and acetic anhydride (8ml). Freshly distilled pyrrole (3.6g, 0.053 mole) dissolved in propionic acid (20ml) was added over a period of 5 min and the mixture was then refluxed for a further 30 min. The reaction mixture was allowed to cool and stand at room temperature for 24 hr. The resultant precipitate, containing polymeric material, was removed by filtration. Treatment with 15ml of dimethylformamide (DMF) dissolved the polymeric products and the remaining precipitate was removed by filtration, washed

successively with small amounts (10ml) of DMF, methanol and hot water, and oven dried (105°C, 1 hr). Product weight: 414 mg (3.3% yield). Mass spectrum (+ve FAB): M_+ +H, 935. UV/visible spectrum ($CHCl_3$), λ_{max} ($\log_{10} \varepsilon$) 656 (3.73), 593 (3.83), 554 (3.80), 519 (4.35), 423 nm (5.12).

5,10,15,20-Tetra (4-hydroxy-1-naphthyl) porphyrin (*2*) was obtained from (*1*) by demethylation using 60% HBr (8).

Tetra (4-methoxy-1-naphthyl) porphyrin (0.20g, 0.21 mmole) was refluxed in 60% HBr (13ml) for 8 hr, after which time an additional amount (13ml) of HBr was added and the reflux continued for a further 16 hr. The mixture was then cooled, filtered and the black residue dissolved in 1M NaOH. The solution was filtered and the product precipitated by addition of concentrated HCl. The precipitate was removed by filtration, washed with water, centrifuged (MSE Super Minor, 3000 rpm, 1100g, 10 min) and the resultant pellet oven dried (105°C, 1 hr). Product weight: 94.2 mg. Mass spectrum (+ve FAB): M_++H, 879 (53%), 893 (25%), 907 (14%), 921 (6%), 935 (2%). UV/visible spectrum (MeOH), λ_{max} ($\log_{10} \varepsilon$) 652 (3.50), 593 (3.71), 553 (3.73), 518 (4.19), 427 nm (5.02).

Sulphonation

Sulphonation of compound (*2*) above was carried out under very mild conditions. 5,10,15,20-tetra (4-hydroxy-1-naphthyl) porphyrin (10mg) was mixed with concentrated H_2SO_4 (0.4ml, s.g. 1.84) and allowed to react at room temperature (19°) for 1 hr. Crushed ice (≈5g) was then added to the reaction mixture and the sulphonated product separated by centrifugation (3000 rpm, 1100g, 10 min). The supernatant was removed, the dark brown pellet was dissolved in a small amount of water and the pH of the solution adjusted to ≈7 by addition of 5M NaOH. The solution was then passed through a pre-conditioned C_{18} Sep-Pak cartridge (see earlier) to adsorb the porphyrin. Water-soluble impurities were removed from the cartridge by elution with water (5 x 2 ml). The adsorbed porphyrin was eluted from the Sep-Pak cartridge using MeOH (3 x 2ml). The solvent was removed by rotary evaporation under vacuum to leave the sulphonated porphyrin (*3*) as a solid residue. Product weight: 4.5 mg. Mass spectrum (+ve FAB): M_+ +H, v. weak

(<0.05% rel. abundance) 1199. UV/visible spectrum (MeOH), λ_{max} ($\log_{10} \varepsilon$) 652 (3.44), 591 (3.67), 550 (3.65), 516 (4.12), 422 nm (4.93).

Radioiodination

This was achieved using the chloramine-T method described by Bolton (9). Sulphonated and unsulphonated porphyrins (1mg) were dissolved in either 0.5ml of sodium phosphate buffer (0.25M, pH 8.0) or a mixture of 0.4ml dimethylsulphoxide (DMSO) and 0.1ml sodium phosphate buffer (0.25 M, pH 8.0) respectively. This solution was added to 5µl of Na ^{125}I (≈ 18.5MBq) in 10µl of sodium phosphate buffer (0.25M, pH 8.0) followed by 5µl of a solution of chloramine-T (5mg/ml in 0.05M sodium phosphate buffer, pH 8.0). After a 15 minute incubation period 50µl of a solution of sodium metabisulphite (1.2mg/ml in 0.05M sodium phosphate buffer, pH 8.0) was added to the reaction mixture which was then made up to 1.0ml with sodium iodide solution (2.0 mg/ml in 0.05M sodium phosphate buffer, pH 8.0). The free Na ^{125}I was removed from the porphyrin using a pre-wetted C_{18} Sep-Pak cartridge by first adsorbing the porphyrin onto the cartridge then eluting the free iodine with distilled water (5 x 2ml). The iodinated porphyrin was eluted from the Sep-Pak cartridge using methanol (3 x 2ml) which was subsequently removed by rotary evaporation under vacuum to leave the iodinated porphyrin as a solid residue.

Solutions

Solubility characteristics of the sulphonated and non-sulphonated 4-hydroxy-1-naphthyl porphyrins were such that whilst the former compound was easily soluble in 0.9% w/v NaCl the unsulphonated derivative was found to be more soluble in aqueous alkali, methanol and ethanol, DMSO being found a particularly good solvent (1). Administration of the porphyrins to mice by both intraperitoneal (ip) and intravenous (iv) routes was therefore based on their dissolution in either 0.9% w/v NaCl (sulphonated derivative) or a solvent system consisting of 9% v/v DMSO, 19% v/v ethanol and 72% v/v pyrogen-free water (non-sulphonated derivative). Each of the iodinated porphyrins was dissolved in the appropriate solvent system such that 1ml of solvent contained ≈35µCi of radioactivity and 35-100µg of porphyrin.

Mouse-tumour model

The tumour used in this study was a carcinoma designated as "CBA Carcinoma NT" (10). A tumour fragment (<1mm^3) was implanted subcutaneously over the rib cage and the experiments were carried out when the tumours had reached a mean diameter of approximately 7mm.

The porphyrins under study were injected iv into one of the lateral tail veins in a volume of 0.1ml using a 100µl fixed-volume "Hamilton" syringe (Scientific Glass Engineering, Melbourne, Australia). Each mouse received 3.5µCi of ^{125}I (equivalent to 3.5 to 10.0µg of porphyrin, depending on the specific activity). At predetermined times after injection the mice were killed by cervical dislocation and the tissues of interest removed, weighed to ±1mg and placed in polystyrene tubes for counting in an LKB Wallac Compugamma CS Model 1282 counter (Wallac OY, P.O. Box 10, 20101, Turku, Finland) together with standards (1% injected dose).

RESULTS

Although *meso*-tetra (4-methoxy-1-naphthyl) porphyrin was easily obtained, albeit in low yield (≃3%), complete demethylation of this compound to the corresponding tetra (hydroxynaphthyl) derivative using HBr proved to be extremely difficult to achieve. Increasing the reflux time from 3hr to 24hr including further addition of HBr after 8hr produced only a moderate increase in the amount of the tetrahydroxy compound formed (Table 1). Similar difficulties were encountered by us during earlier attempts to fully demethylate *meso*-tetra (3-methoxyphenyl) porphyrin using HBr (11). The alternative method of demethylation using boron tribromide, although reported to be superior to that using 48% HBr (12) also appears to produce a mixture of compounds rather than a single substance (13). Sulphonation of the "tetra(hydroxynaphthyl) porphyrin" was thus carried out on the mixture of hydroxy/methoxynaphthyl porphyrins as presented in Table 1.

Table 1. HBr demethylation of *meso*-tetra(4-methoxy-1-naphthyl) porphyrin

Demthylation product	Percent product formed	
	3 hr reflux	24 hr reflux
5,10,15,20-Tetra(4-hydroxy-1-naphthyl) porphyrin	32	53
5,10,15-Tri(4-hydroxy-1-naphthyl)-20-mono (4-methoxy-1-naphthyl) porphyrin	31	25
5,10,-Di(4-hydroxy-1-naphthyl)-15,20-di(4-methoxy-1-naphthyl) porphyrin 5,15-Di(4-hydroxy-1-naphthyl)-10,20-di(4-methoxy-1-naphthyl) porphyrin	23	14
5-Mono(4-hydroxy-1-naphthyl)-10,15,20-tri(4-methoxy-1-naphthyl)porphyrin	11	6
5,10,15,20-Tetra(4-methoxy-1-naphthyl) porphyrin	3	2

UV/visible spectra of all the porphyrins presented in this study were unremarkable, the spectral profiles being characteristic of compounds containing the porphyrin ring system. Mass spectra of both the tetra(methoxynaphthyl) porphyrin and its demethylation product(s) were distinct and confirmatory of the porphyrin species present. The mass spectrum for the sulphonated tetra(hydroxynaphthyl) porphyrin was very weak but indicated the presence of an M_+ +H ion (M/Z = 1199) corresponding to a tetrasulphonated derivative of *meso*-tetra (4-hydroxy-1-naphthyl) porphyrin.

Radioiodination of the porphyrins tested in this study was initially carried out using iodine monochloride. However, the chloramine-T method was subsequently found to give better labelling yields and specific activities: 12% and 3.6 GBq/mmol and 60% and 11.5 GBq/mmol for sulphonated and non-sulphonated hydroxynaphthyl compounds respectively.

The tissue distribution of the sulphonated and non-sulphonated porphyrins are shown in Figure 1(a) and (b) respectively. Certain tissue distribution characteristics of the two porphyrins are very similar, e.g. the liver and spleen take up appreciably more of the injected activity than almost any of the other tissues. Relatively small amounts of the injected activity are taken up by the blood and kidneys (≃ 2-3%) whilst tumour

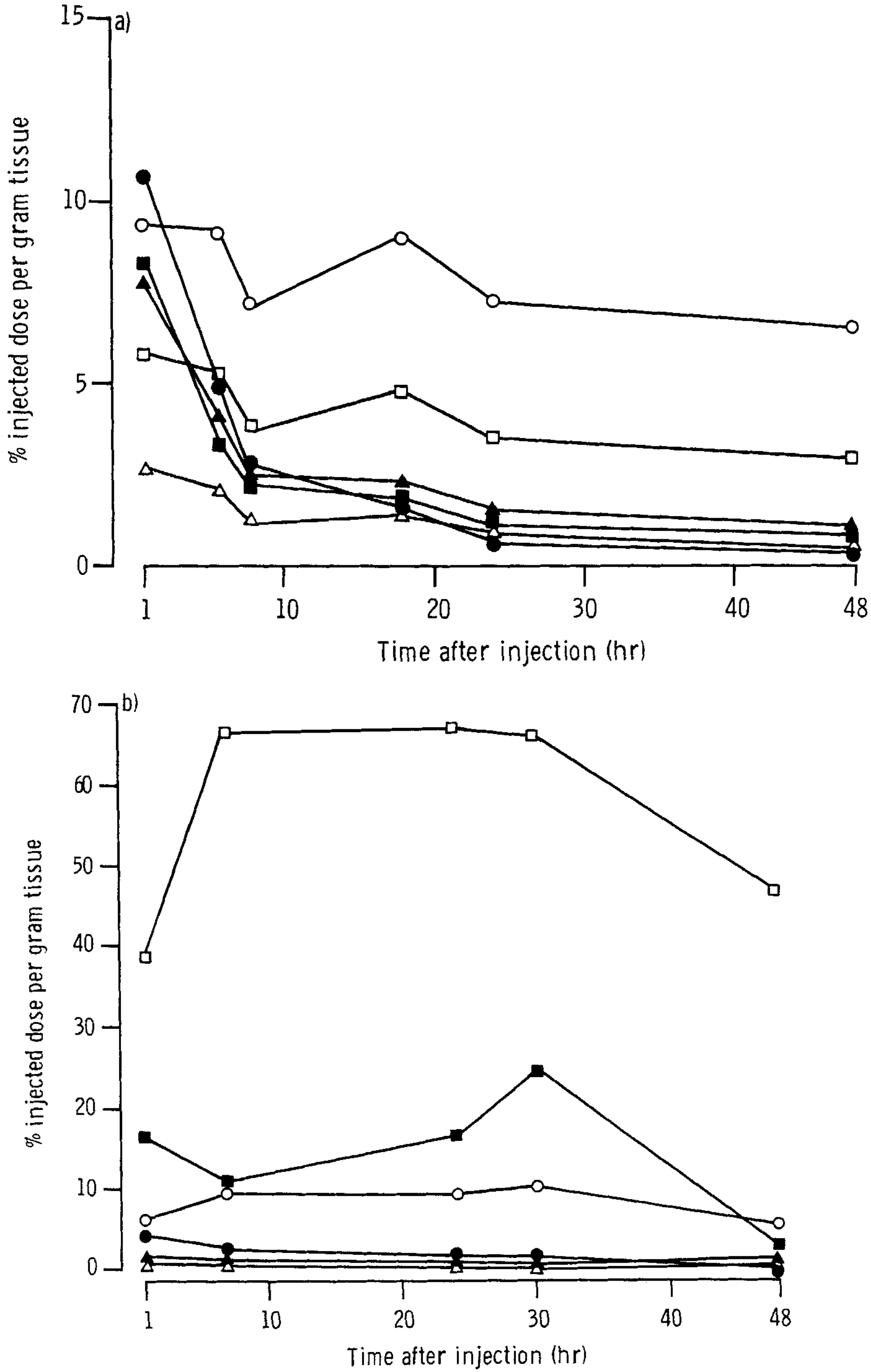

Figure 1: Tissue distribution of: a) sulphonated and (b) non-sulphonated ^{125}I labelled *meso*- tetra (4-hydroxy-1-naphthyl) porphyrin in mice. Tissue concentrations of porphyrin are expressed as percent of injected dose per gram of wet weight tissue for blood (●), liver (○), kidneys (▲), tumour (△) lungs (■) and spleen (□).

uptake by both porphyrins is disappointingly low (<1%). Tissue clearance of the sulphonated porphyrin is generally more rapid than that of the non-sulphonated compound especially from the blood and kidneys.

Spleen uptake of the non-sulphonated porphyrin is very high in tumour bearing mice compared with that exhibited by the sulphonated derivative (Figure 2). Studies using the non-sulphonated porphyrin in normal

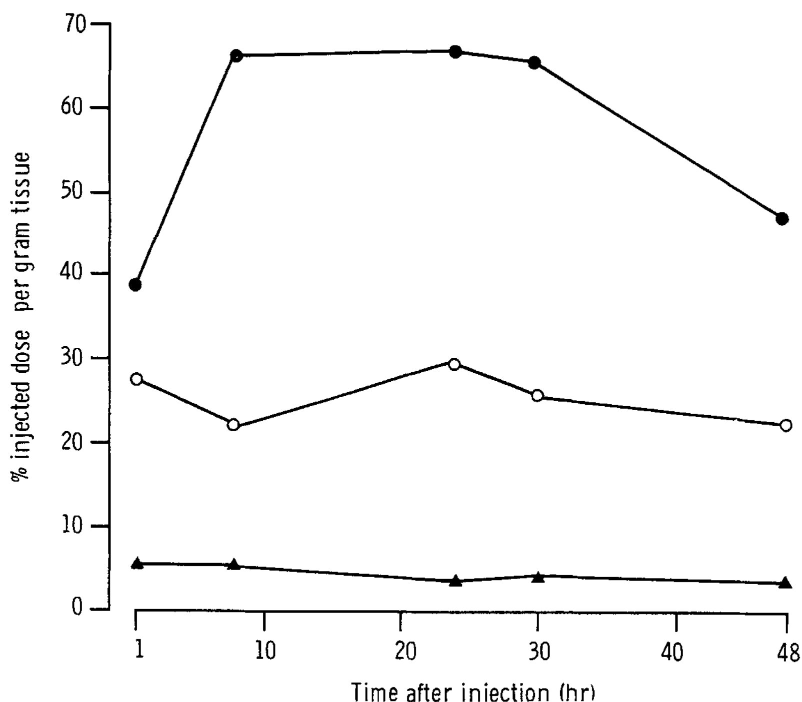

Figure 2: Mouse spleen uptake of ^{125}I labelled (hydroxynaphthyl) porphyrin. Tissue concentrations are expressed as percent of injected dose per gram of wet weight tissue for sulphonated porphyrin (tumour bearing mice), ▲ , non-sulphonated porphyrin (tumour bearing mice), ● , and non-sulphonated porphyrin (normal mice), O .

(non-tumour bearing) mice show that the spleen uptake, although high, is still correspondingly lower than that found in tumour bearing mice. Although enlargement of mouse spleens was observed following tumour transplantation (Figure 3) no significant correlation was found either between spleen weight and tumour size or spleen weight and porphyrin uptake. During the course of these studies the level of radioactivity present in samples of mouse thyroid were also determined in order to assess the stability of the radioactive label. Thyroid uptake levels ranged

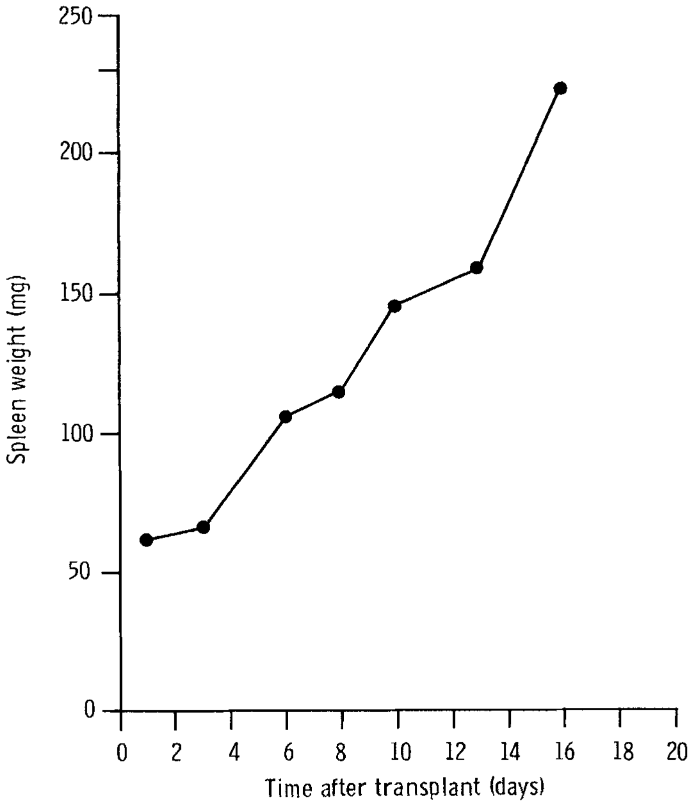

Figure 3: Effect of tumour growth upon spleen weight.

between 0.02 to 0.06 percent of the injected dose indicating a firm attachment of the iodine label to the porphyrins. Investigation of the effects of ip versus iv injection methods showed that there was no significant difference in tissue distribution between the two routes apart from the first hour after injection.

DISCUSSION

It must be stressed that, as indicated previously (see Results), it was found impossible to completely demethylate the tetra (4-methoxy-1-naphthyl) porphyrin to the corresponding tetra hydroxynaphthyl derivative. The "demethylated" derivative, therefore, consisted of a number of compounds in a variety of hydroxylated states, including a small amount of unreacted starting material (2-3%). Since the composition of this mixture is well defined (see Table 1) it can be argued that, should the demethylated

derivatives have exhibited selective tumour localisation properties, then isolation, purification and identification of the tumour-selective ingredient(s) would have been attempted. The same reasoning would also apply to the sulphonated material used in these studies.

The mass spectrum for the sulphonated porphyrin was unusually weak compared with that for the non-sulphonated hydroxynaphthyl precursor. This may be a result of the greatly increased polarity of the former compound following sulphonation since the non-sulphonated material produced distinct M_+ +H parent peaks for all components. The sulphonated preparation gave a UV/visible spectral profile consistent with that of a porphyrin ring system and its water solubility was greatly enhanced compared to the non-sulphonated porphyrin, the aqueous solution producing an intense red fluorescence when irradiated with UV light (λ=366nm). It was evident that sulphonation had taken place and that the compound(s) was a porphyrin. The non-sulphonated porphyrin was only partially soluble in aqueous media and it required a combination of DMSO, ethanol and water to achieve its effective dissolution. Although this solvent system is well tolerated by the animals there remains the possibility that the porphyrin may precipitate or aggregate following injection. Such an occurrence, coupled with an increased macrophage activity in the spleen due to the presence of a tumour, could account for the high spleen uptake seen with the non-sulphonated, less polar porphyrin.

The low tumour uptake of both porphyrins is interesting especially as the similarly related hydroxyphenyl porphyrins prepared by Berenbaum (1) as potential phototherapeutics were reported to possess favourable tissue selectivity coupled with high tumour sensitising potency. Data from the present study indicates that most of the activity detected in the tumours was in the blood vasculature. This suggests that the phototherapeutic properties of these compounds may lie in their inherently high quantum yield characteristics coupled with their ability, once sensitised by light of the appropriate wavelength, to destroy the blood supply to the tumour.

In conclusion, it must be emphasised that, since the two porphyrin

derivatives studied here seem to exhibit little preference for localisation in tumour tissue, care should be exercised if these compounds are to be used as phototherapeutic agents and unacceptable side reactions in normal tissue are to be avoided.

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