

Enhancement of the radiation response of EMT-6 tumours by a copper octabromotetracarboranylphenylporphyrin

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Objective: The carborane-containing porphyrin, copper (II) 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(3-[1,2-dicarba-closo-dodecaboranyl]methoxyphenyl)-porphyrin (CuTCPBr), was investigated as a potential radiation enhancing agent for X-ray radiotherapy (XRT) in a subcutaneously implanted EMT-6 murine carcinoma.

Method: The biodistribution and toxicological profile of this porphyrin has been shown to be favourable for another bimodal radiotherapy technique, boron neutron-capture therapy. For the XRT studies, CuTCPBr was formulated in either 9% Cremophor® (BASF Corporation, Ludwigschafen, Germany) EL and 18% propylene glycol (9% CRM) or a revised formulation comprising 1% Cremophor ELP, 2% Tween 80® (JT Baker, Mansfield, MA), 5% ethanol and 2.2% PEG 400 (CTEP formulation), which would be more clinically acceptable than the original 9% CRM formulation. Using the 9% CRM formulation of CuTCPBr, doses of 100, 210 or 400 mg kg⁻¹ of body weight were used in combination with single doses of 25–35 Gy 100 kVp X-rays.

Results: While doses of 100 mg kg⁻¹ and 210 mg kg⁻¹ did not result in any significant enhancement of tumour response, the 400 mg kg⁻¹ dose did. A dose modification factor of 1.20 ± 0.10 was obtained based on the comparison of doses that produced a 50% local tumour control probability. With the CTEP formulation of CuTCPBr, doses of 83 and 170 mg kg⁻¹ produced significant radiation enhancement, with dose modification factors based on the TCP₅₀ of 1.29 ± 0.15 and 1.84 ± 0.24 , respectively.

Conclusion: CuTCPBr significantly enhanced the efficacy of XRT in the treatment of EMT-6 carcinomas in mice. The CTEP formulation showed a marked improvement, with over 9% CRM being associated with higher dose modification factors. Moreover, the radiation response in the skin was not enhanced.

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Radiology

Porphyrins are used in the treatment of cancer, as photosensitisers in clinical photodynamic therapy (PDT) and as boron carriers in preclinical studies of boron neutron-capture therapy (BNCT) [1–4]. Both approaches use the porphyrin as part of bimodal therapy, in which the cell-killing chemical species are only formed when both the porphyrin and radiation are present in the form of light or thermal neutrons, respectively. Selectivity is attained because the porphyrins predominantly localise in tumour tissue within the irradiated volume. Such a strategy can also be used with conventional X-ray therapy (XRT), by using a porphyrin with a different set of physicochemical requirements, while maintaining similar biological requirements, such as tumour selectivity and low toxicity. For example, haematoporphyrin, used for PDT, has been reported to have some activity in enhancing the effect of X-rays [5, 6]. The porphyrin, verteporfin, has also been used in combination therapy involving both PDT and XRT [7]. Results from animal studies, based on the endpoint of tumour growth delay, suggested a synergistic rather than just an additive effect

[7]. The manganese tetrapyridylporphyrins (known as mimetics of superoxide dismutase) have also been shown to increase tumour response to XRT [8, 9]. They are believed to act by inhibiting tumour angiogenesis, which is activated by oxidative stress, a well-recognised occurrence after exposure to ionising radiation.

The only porphyrin-like compound currently being clinically investigated as a radiation enhancement agent is an expanded porphyrin with gadolinium at the centre, known as gadolinium texaphyrin (Gd-Tex) or motexafin [10–12]. It has a high electron affinity with a relatively positive reduction potential, which is believed to be at least partially responsible for its effectiveness. It has been shown that electron-affinic aromatic compounds can act as oxygen mimetic sensitisers in hypoxic radioresistant cells, which are frequently found in malignant tissue [13, 14]. Reactive oxygen species (ROS), comprising free radicals, peroxides and superoxides, are believed to be the active species created primarily from the radiolysis of water during exposure to ionising radiation. Although many potential XRT sensitisers that maximise the concentration of ROS have been studied in pre-clinical and clinical trials, none are used routinely in the clinic. Temozolomide and cetuximab have been used with radiation, but their function is primarily chemotherapeutic

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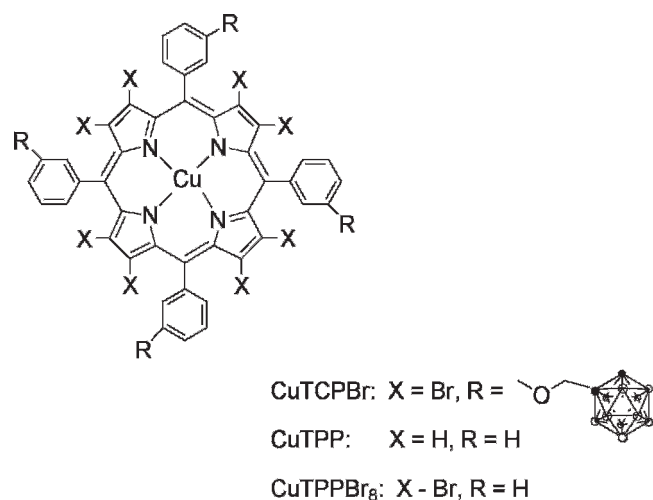


Figure 1. Porphyrin structures of CuTCPBr, CuTPP and CuTPPBr₈. Closed circles represent C or CH and open circles represent BH in CuTCPBr.

[15, 16]. However, there is evidence that temozolomide will inhibit the repair of radiation-induced damage to DNA in the presence of the methylated version of the *MGMT* (*O*⁶-methylguanine-DNA methyltransferase) DNA-repair gene, which explains the greater efficacy of this drug/radiation combination in the treatment of glioblastoma multiforme [17].

Copper (II) 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(3-[1,2dicarba-closo-dodecarboranyl]methoxyphenyl)-porphyrin (CuTCPBr) (Figure 1) was initially synthesised as a boron carrier for use in BNCT. It has been shown to possess the biological properties needed for this treatment modality, *i.e.* high tumour boron localisation and low toxicity [18]. Moreover, besides having the necessary high boron content, it should also have a significantly higher electron affinity owing to the electron-withdrawing bromo groups on the macrocycle. To determine its electron affinity, redox potentials were measured and compared with similar copper brominated porphyrins.

The purpose of this article is to study the biodistribution of CuTCPBr and to evaluate its therapeutic efficacy in combination with single doses of X-rays using the murine EMT-6 tumour model. Data for this porphyrin were acquired using direct-current plasma atomic emission spectroscopy (DCP-AES) to assay boron concentrations, since the boron and porphyrin are covalently linked. CuTCPBr is not water soluble and, therefore, requires formulation for *in vivo* studies. The first approach was to use the standard preclinical formulation used for lipophilic porphyrins, which has been adopted in the Medical Department, Brookhaven National Laboratory, and comprises 9% Cremophor® (BASF Corp., Ludwigschafen, Germany) EL and 18% propylene glycol (9% CRM formulation) with a CuTCPBr concentration of approximately 3.5 mg ml⁻¹ [4, 19]. However, for clinical use, more concentrated solutions with significantly lower amounts of Cremophor are required. To this end, a formulation comprising 2% Cremophor ELP, 1% Tween 80® (JT Baker, Mansfield, MA), 5% ethanol and 2.2% PEG 400 (CTEP formulation), developed by Applied Analysis Ltd (Beverly, East Yorkshire, UK) was also used.

Methods and materials

Chemicals

CuTCPBr was synthesised by brominating CuTCPh, as previously reported [18]. The resulting porphyrin was solubilised using the two formulations described in more detail below. Each formulation was made prior to the start of the experimental animal study and then stored in the dark at 4 °C.

Electrochemistry/optical spectroscopy

Electrochemistry measurements to determine oxidation and reduction potentials were performed with a Voltalab 50 (Radiometer Analytical SAS, Lyon, France) electrochemical analyser. A three-electrode system was used, which consisted of a platinum-disc working electrode, a platinum-wire counter electrode and a saturated calomel reference electrode. The solvent was dichloromethane with 0.1 M tetrabutylammonium perchlorate as the supporting electrolyte. Optical absorption spectra were measured in dichloromethane using a Cary 500 spectrophotometer (Agilent Technologies, Lexington, MA).

Animal tumour model

All animal studies were approved by the Brookhaven National Laboratory Institutional Animal Care and Use Committee. Female BALB/c mice (18–20 g) were used in this study (Taconic Farms, Germantown, NY) and admitted to the animal facility approximately 1 week in advance of any procedures being undertaken. Food (Purina mouse chow #5015; Purina Mills LLC, Gray Summit, MO) and water were provided *ad libitum*.

Prior to implantation into the mice, aliquots of EMT-6 tumour cells frozen in 10% dimethylsulfoxide in liquid nitrogen were thawed and grown in culture for several passages in Dulbecco's Modified Eagle Medium with 10% foetal bovine serum [20]. Initially, EMT-6 tumours were grown subcutaneously on the dorsal thorax after injection of single-cell suspensions of 2.5 × 10⁵ cells in 0.05–0.10 ml using a 27-gauge needle. Subsequently, for the XRT study, EMT-6 tumours were implanted in the thighs of mice using tumour tissue from the dorsal thorax. Fragments ≤ 0.5 mm were implanted subcutaneously into the thigh using an 18-gauge trocar. Tumour fragments were used in this site because tumours produced by the injection of a single-cell suspension tend to spread out as a thin subcutaneous layer and invade the subjacent muscle, making tumour response difficult to assess. Using tumour fragments, the tumours were palpable but thinner in the thigh compared with the dorsal thorax. Tumours produced using fragments can invade muscle tissue, but not as frequently as tumours produced by the injection of a cell suspension. Local irradiation of tumours in the thigh was carried out 10–11 days after tumour implantation.

Porphyrin formulations

9% CRM formulation

The 3.5 mg ml⁻¹ concentration of CuTCPBr in the 9% CRM was prepared by first dissolving the porphyrin in

tetrahydrofuran (THF) (1.5% final volume) in a round-bottomed flask equipped with a stir bar. Cremophor EL was added and the mixture stirred at 70 °C for 2 h, so that most of the THF had evaporated. The mixture was allowed to cool to approximately 20 °C and then propylene glycol (18% final volume) was added as a single aliquot. After stirring for 30 min, sterile water was added dropwise with vigorous stirring. The solution was then filtered through a 0.45 µm and then a 0.2 µm nylon filter (Nalgene, Fischer Scientific Pittsburgh, PA) into a sterile injection vial.

CTEP formulation

A CuTCPBr concentration of 8–10 mg ml⁻¹ was achievable using the CTEP formulation. This formulation was developed by Applied Analysis Ltd. The procedure was more labour intensive: it entailed stirring Cremophor ELP (1% final weight) and Tween 80 (2% final weight) in a small round-bottomed flask, equipped with a stir bar and closed with a ground-glass stopper, heated in an oil bath at 70 °C for 15 min. Powdered solid CuTCPBr was then added to the flask, followed by absolute ethanol (5% final volume). The mixture was stirred for a further 1 h at 70 °C and then cooled to 20 °C. After this, PEG 400 (2.2% final volume) was added and the mixture was stirred at 70 °C for 16 h. After cooling to 20 °C, sterile water was added dropwise with vigorous stirring. At this point the black translucent solution was homogenised (IDA Ultra-Turrax T25, Staufen, Germany) for 3 min and then filtered through a 0.45 µm followed by a 0.2 µm nylon filter (Nalgene) into a sterile injection vial.

CuTCPBr administration

The lower doses of CuTCPBr (100 mg kg⁻¹ or 210 mg kg⁻¹ in 9% CRM) required administration as three equally spaced intraperitoneal (IP) injections over 8 h, since the porphyrin concentration was only approximately 3.5 mg ml⁻¹. This was administered approximately 8 days after the subcutaneous implantation of tumour fragments into the thigh. A total dose of 400 mg kg⁻¹ of CuTCPBr required six IP injections to be given over a total period of 32 h (three equally spaced IP injections over 8 h on two consecutive days). 2 days were allowed after the end of porphyrin administration (9% CRM formulation) for CuTCPBr clearance from blood prior to irradiation. Groups of five mice, used for biodistribution studies, were humanely killed at the time irradiation would have occurred and samples of tumour, liver, skin (pinna), muscle (thigh) and blood were removed for boron analysis. CuTCPBr in CTEP (approximately 10.5 mg ml⁻¹) was given as a single IP injection of approximately 0.01 ml g⁻¹ or 0.02 ml g⁻¹ of body

weight per injection for the 83 mg kg⁻¹ or 170 mg kg⁻¹ dose, respectively. Groups of 12 mice were irradiated 24 h after CuTCPBr administration to allow for clearance of the porphyrin from the blood.

CuTCPBr analyses

CuTCPBr concentrations in tissues were determined from boron concentration, which were assayed using DCP-AES [21]. The boron concentration in the solutions injected were assayed using prompt-gamma spectroscopy at the Massachusetts Institute of Technology Reactor Prompt-Gamma Neutron Activation Facility [22]. CuTCPBr concentrations were calculated from the boron measurements by multiplying by 4.61. This was validated by extracting porphyrin from liver tissue and assaying by high performance liquid chromatography to ensure that CuTCPBr remained intact *in vivo*.

X-irradiation

Groups of 12 mice, anaesthetised with sodium pentobarbital (approximately 60 µg g⁻¹ IP), were positioned with the tumour-bearing leg extended across a flat horizontal surface for irradiation using a 25 mm diameter collimator. Tumours were irradiated with single doses of 25, 35, 42 and 50 Gy at 2.1 Gy min⁻¹ using a Philips RT-100 source (100 kVp and 8 mA) (Philips & Co. Eindhoven, Netherlands).

Results

Electrochemistry/optical spectroscopy

The electronic properties of CuTCPBr were investigated using electrochemistry and optical spectroscopy (Table 1). For comparison, boron-free copper tetraphenylporphyrin (CuTPP) and its octa-brominated analogue, CuTPPBr₈, (structures shown in Figure 1) results are also shown. Other investigators [23, 24] have shown that the addition of bromine groups to the pyrrolic positions of CuTPP to form CuTPPBr₈ caused the reduction potential ($E_{1/2}^{\text{red}}$) to become more positive, *i.e.* more easily reducible or electron affinic. Our results show that the first reduction potential has become more positive relative to the parent CuTPP by 0.56 V increasing from -1.33 V to -0.77 V ($E_{1/2}^{\text{red}}$). By contrast, the first oxidation potential ($E_{1/2}^{\text{oxid}}$) has remained fairly constant, only increasing by 0.07 V. Further information concerning the electronic energy levels was obtained from the

Table 1. Electrochemical and optical properties of copper porphyrins

Compound	$E_{1/2}^{\text{ox}}$ (V) ^a	$E_{1/2}^{\text{red}}$ (V) ^a	Absorption bands (nm) ^b
CuTPP [23]	1.25, 0.98	-1.33, -1.80	414, 538, 572
CuTPPBr ₈ [23]	1.41, 0.96	-0.87, -1.12	365, 451 (sh), 467, 582, 623
CuTCPBr	1.39, 0.92	-0.77, -1.04	366, 450 (sh), 465, 580, 625

sh, shoulder.

^aIn dichloromethane, 1M tetrabutylammonium perchlorate vs saturated calomel reference electrode, platinum working and auxiliary electrodes, 20 °C.

^bIn dichloromethane.

Table 2. Average boron or porphyrin concentrations ($\pm$ standard deviation in micrograms per gram) in various mouse tissues 2 days after the administration of three different total doses of CuTCPBr in the 9% CRM formulation by serial intraperitoneal injections (see text for details). Five mice were used for each dose group

Porphyrin dose	100 mg kg ⁻¹	210 mg kg ⁻¹	400 mg kg ⁻¹	
Tissue	($\mu\text{g g}^{-1}$ boron)	($\mu\text{g g}^{-1}$ boron)	($\mu\text{g g}^{-1}$ boron)	($\mu\text{g g}^{-1}$ porphyrin)
Tumour	49 $\pm$ 13	117 $\pm$ 34	277 $\pm$ 45	1280 $\pm$ 92.2
Blood	2.4 $\pm$ 1.2	40 $\pm$ 26	68.5 $\pm$ 19	316 $\pm$ 39.2
Leg muscle	4.2 $\pm$ 1.4	8.9 $\pm$ 1.5	12.0 $\pm$ 3.3	55.3 $\pm$ 6.8
Skin	5.7 $\pm$ 1.7	11 $\pm$ 2	19.7 $\pm$ 4.3	90.8 $\pm$ 8.8
Liver	261 $\pm$ 46	318 $\pm$ 21	725 $\pm$ 72	3340 $\pm$ 9
Tumour:blood	20:1	2.9:1	4.0:1	4.0:1

CuTCPBr, copper (II) 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(3-[1,2-dicarba-closo-dodecaboranyl]methoxyphenyl)-porphyrin.

optical data, which showed that the first absorption bands of both CuTPPBr₈ and CuTCPBr were red-shifted by 42–50 nm relative to CuTPP and also to CuTCPh, which has absorbances similar to that of CuTPP (415 nm, 538 nm) [4].

CuTCPBr biodistribution

Data for the biodistribution of CuTCPBr, as assessed by the boron measurements, in tissue and blood are shown in Tables 2 and 3. Both boron and porphyrin concentrations in tissues from mice given 400 mg kg⁻¹ of CuTCPBr in 9% CRM are shown in these tables. These data indicate that the mean boron or porphyrin concentrations in the different tissues, based on the administered dose, did not vary greatly from each other with the two different formulations used, except perhaps for the leg muscle and skin, where mean values for the CTEP formulation were higher than expected owing to the use of the 9% CRM formulation. Blood boron levels using the CTEP formulation were very low 24 h after the single-dose administration of CuTCPBr. This difference was probably due to the considerably reduced volume administered using the CTEP formulation, which was approximately three times lower than the 9% CRM formulation for similar doses of CuTCPBr administered. A consequence of this was that the 9% CRM formulation had to be given by serial IP injections over 8 h or 32 h, depending on the total dose administered. In contrast, the CTEP formulation was given as a single IP injection.

The tumour boron levels from CuTCPBr in both the 9% CRM and the CTEP formulation were dose dependent, and increased with increasing dose administered.

However, the lower blood levels found after injection of the CTEP formulation resulted in much higher tumour: blood-boron concentration ratios—75–80 compared with 20 for the lowest dose of the 9% CRM formulation and only 2.9–4.0 for the two higher doses.

Animal-irradiation studies

9% CRM formulation

Three different doses of CuTCPBr (100, 210 and 400 mg kg⁻¹) in the 9% CRM formulation were evaluated as potential radiation enhancing doses in combination with single doses of 25 or 35 Gy of X-rays. Tumours were irradiated 2 days after the completion of administration of CuTCPBr when the tumour:blood-boron concentration ratio was at least 2.9 (Table 2). Only after the highest dose of 400 mg kg⁻¹ of CuTCPBr did Kaplan–Meier plots of animal survival against time after irradiation appear to show dose enhancement when compared with X-rays alone (Figure 2a). Animals in which the tumour was uncontrolled and grew to 500 mm³ were humanely killed. Irradiation with 25 Gy alone resulted in local tumour control in 3 out of 12 animals, while with 35 Gy it was 4 out of 12 controlled at 120 days after irradiation. When 400 mg kg⁻¹ of CuTCPBr was given 2 days prior to irradiation, local tumour control was seen in 5 out of 12 animals after 25 Gy, and 7 out of 11 after 35 Gy. Only one mouse in the group that was locally irradiated with 35 Gy alone developed severe damage to the skin and subcutaneous tissue of the leg 89 days after irradiation, when the tumour was locally controlled and the mouse was humanely killed. No animals irradiated to this, or

Table 3. Average boron or porphyrin concentrations ($\pm$ standard deviation in micrograms per gram) in various tissues 1 day after a single intraperitoneal administration of two different doses of CuTCPBr in the CTEP formulation. Five mice were used for each dose group

Porphyrin dose	83 mg kg ⁻¹		170 mg kg ⁻¹	
Tissue	($\mu\text{g g}^{-1}$ boron)	($\mu\text{g g}^{-1}$ porphyrin)	($\mu\text{g g}^{-1}$ boron)	($\mu\text{g g}^{-1}$ porphyrin)
Tumour	30 $\pm$ 18	140 $\pm$ 80	96 $\pm$ 30	440 $\pm$ 140
Blood	0.4 $\pm$ 0	1.8 $\pm$ 0	1.2 $\pm$ 1.2	5.5 $\pm$ 5.5
Leg muscle	7.5 $\pm$ 5.8	36 $\pm$ 27	12 $\pm$ 2	55 $\pm$ 9.2
Skin	2.0 $\pm$ 0.9	9.2 $\pm$ 4.1	5.7 $\pm$ 2.2	26 $\pm$ 10
Liver	212 $\pm$ 28	980 $\pm$ 130	340 $\pm$ 65	1570 $\pm$ 300
Tumour:blood	75:1	75:1	80:1	80:1

CTEP, Cremophor ELP, Tween 80, ethanol, PEG 400; CuTCPBr, copper (II) 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(3-[1,2-dicarba-closo-dodecaboranyl]methoxyphenyl)-porphyrin.

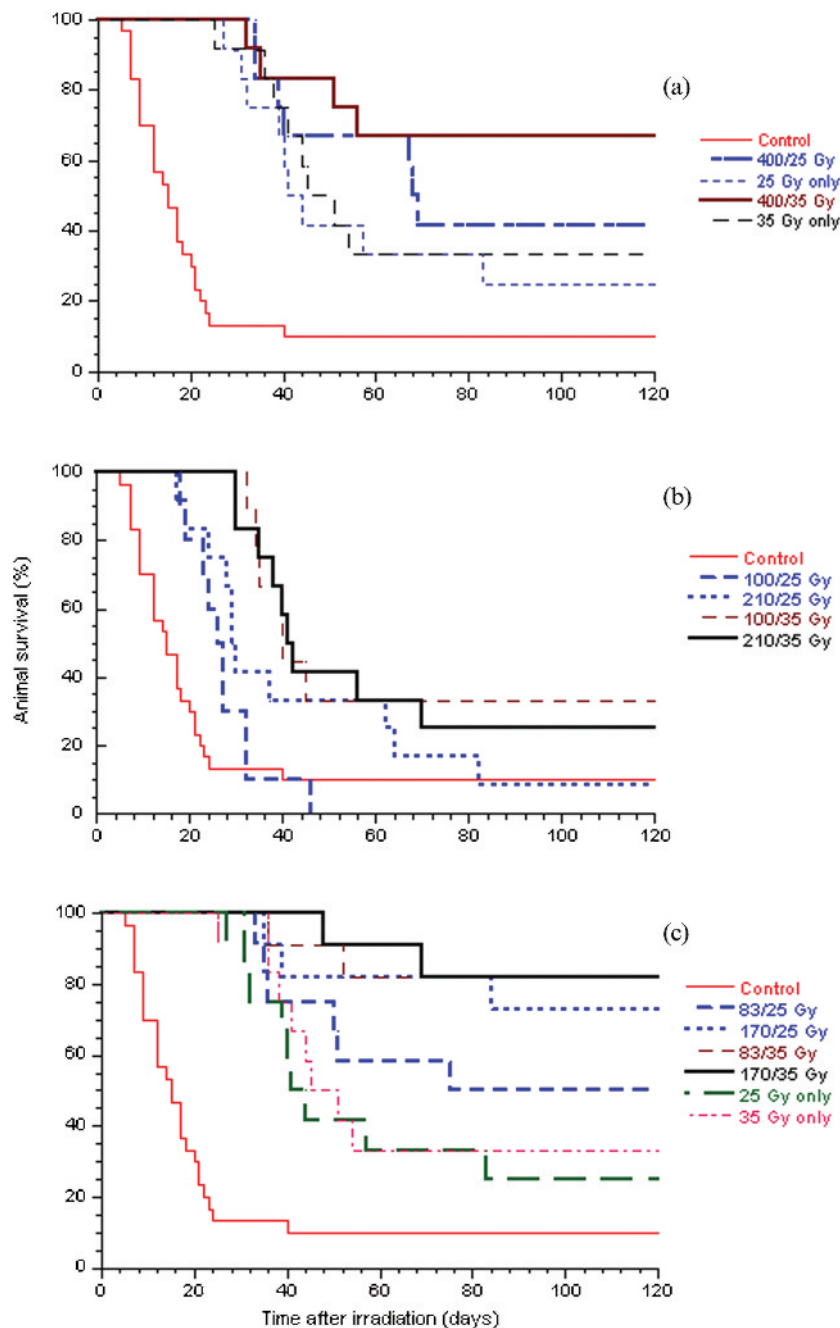


Figure 2. Time-related changes in the percent survival of animals (mice were humanely killed when the tumour volume exceeded 500 mm³) after the local irradiation of subcutaneously implanted EMT-6 tumours. (a) Irradiation was given as single doses of either 25 Gy or 35 Gy, alone or in combination with 400 mg kg⁻¹ body weight of CuTCPBr in the 9% CRM formulation. (b) Irradiation was given as single doses of either 25 or 35 Gy in combination with 100 mg kg⁻¹ or 210 mg kg⁻¹ body weight of CuTCPBr in the 9% CRM formulation. (c) Irradiation was given as single doses of either 25 Gy or 35 Gy in combination with 83 mg kg⁻¹ or 170 mg kg⁻¹ body weight of CuTCPBr in the CTEP formulation.

the lower radiation dose in combination with CuTCPBr or X-rays alone, showed adverse skin or subcutaneous tissue changes.

CuTCPBr doses of 100 mg kg⁻¹ and 210 mg kg⁻¹ resulted in time-related changes in animal survival that were not significantly different to those when tumours were locally irradiated with X-rays alone (Figure 2b).

CTEP formulation

Owing to the high concentration of CuTCPBr in the CTEP formulation, only a single IP injection was required to administer doses of either 83 mg kg⁻¹ or 170 mg kg⁻¹ of CuTCPBr. Moreover, because levels of the porphyrin declined rapidly in the blood with this formulation, tumours were irradiated 1 day after drug administration (Table 3).

Animal survival was significantly increased to >90 days after irradiation when doses of 83 mg kg⁻¹ or 170 mg kg⁻¹ of CuTCPBr in the CTEP formulation were used in combination with 25 Gy compared with 25 Gy alone ($p < 0.02$ or $p < 0.04$, respectively). Survival was also significantly enhanced for the same CuTCPBr doses following an X-ray dose of 35 Gy ($p < 0.03$ or $p < 0.01$, respectively) (Figure 2c).

Dose-related changes in local tumour control at 120 days after irradiation with single doses of X-rays and in combination with either 400 mg kg⁻¹ (9% formulation) or 83 mg kg⁻¹ and 170 mg kg⁻¹ (CTEP formulation) of CuTCPBr are shown in Figure 3. These curves, fitted by probit analysis, were parallel to each other. The doses, associated with a 50% incidence of local tumour control probability (TCD₅₀), were obtained from the fitted curves. These TCD₅₀ values were then compared with obtain dose

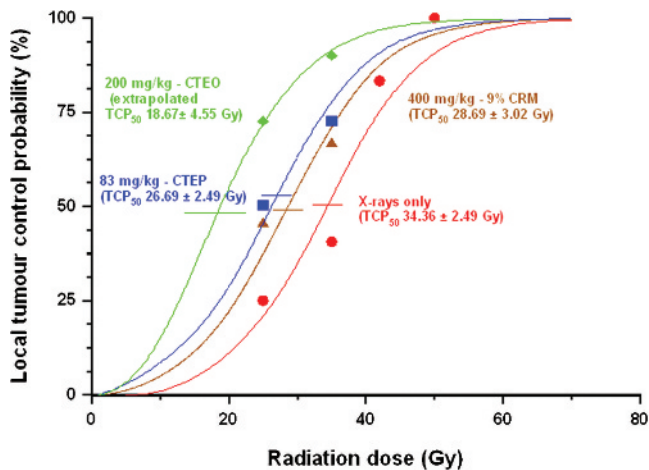


Figure 3. Dose-related changes in local tumour control after single doses of X-rays given either alone or in combination with CuTCPBr. Error bars indicate $\pm$ standard error.

modification factors (DMFs) for the combination treatment in relation to radiation alone. TCP_{50} [$\pm$ standard error (SE)] values were 34.4 ± 2.5 Gy and 28.7 ± 3.0 Gy for X-rays alone and X-rays in combination with CuTCPBr (9% CRM formulation), respectively. In the case of irradiation with X-rays in combination with CuTCPBr (CTEP formulation), the TCP_{50} ($\pm$ SE) values were 26.7 ± 2.5 Gy and 18.9 ± 4.6 Gy for the CuTCPBr doses of 83 and 170 $mg\ kg^{-1}$, respectively. The DMFs ($\pm$ SE) obtained from these values were 1.2 ± 0.1 for CuTCPBr with the 9% CRM formulation ($400\ mg\ kg^{-1}$), compared with values of 1.29 ± 0.15 and 1.84 ± 0.24 CuTCPBr using the CTEP formulation at doses of $83\ mg\ kg^{-1}$ and $170\ mg\ kg^{-1}$, respectively.

Discussion

The reduction potential of CuTCPBr was determined to establish whether or not it was sufficiently electron-affinic to be an efficient radiosensitiser (Table 1). The reduction potential of -0.77 V indicates that CuTCPBr should be capable of enhancing XRT. The approximate 45 nm red shift in absorbance peaks indicates that the electronic energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) has significantly decreased (Table 1). Using the four-orbital approach of Gouterman [25, 26], the red shift has been rationalised, owing, mainly, to the lowering of the LUMO, and also reflected in the positive shift in the reduction potential, so that the reduced porphyrin is of lower energy or is more stable than that of CuTPP [23]. This is further substantiated by the oxidation potentials ($E_{1/2}^{oxid}$), which have essentially not changed because these electrons come from the HOMO. Both are indications that the electron-withdrawing bromine groups result in a more easily reducible electron-affinic porphyrin.

It is well established that electron-affinic compounds can enhance radiation responses by acting as an oxygen substitute in hypoxic cells, which are commonly found in tumours, and the sensitiser efficiency appears proportional to reduction potential [13, 14]. Indeed, some of these compounds have an affinity for hypoxic cells and

remain a focus as agents for the imaging of hypoxia in tumours [27]. In previous studies, a large number of nitroimidazoles, with relatively high reduction potentials, such as misonidazole, have been associated with encouraging preclinical findings. However, these effects were not duplicated in clinical studies, largely owing to toxicity, in particular neurotoxicity associated with their repeated administration. For this reason, the total dose administered was reduced to levels that were not associated with significant hypoxic cell radiosensitisation. It is postulated that such compounds react with the hydroxyl adducts of DNA bases and form new radicals, which ultimately result in double strand DNA scission [13, 28].

It is unlikely that a large molecule, such as CuTCPBr, could ever be located in close enough proximity to the nucleus of cells to generate its effect through a similar DNA mechanism. It is far more likely that its mode of action is similar to that proposed for Gd-TeX, whereby it is proposed that it reacts with hydrated electrons to prevent quenching of cell damaging hydroxyl radicals. The reduction potential of CuTCPBr is less positive [-0.77 V *vs* saturated calomel electrode (SCE)] than that of Gd-TeX (-0.32 V *vs* SCE), although this was assessed in dimethylformamide and not dichloromethane [29]. Nevertheless, CuTCPBr is retained in tumour tissue and produces a considerable modification of the radiation response with DMFs of between 1.29 and 1.84 depending on the dose administered and the formulation used. The DMFs for Gd-TeX were in a similar range [30] based on the original *in vivo* results reported by Miller et al [11].

Unlike the previously tested electron-affinic radiosensitisers, Gd-TeX did not appear to show a selective enhancement of the radiation response of hypoxic cells *in vitro* [31]. In that particular report, the authors found no enhancement in aerated cells either, which conflicts with the original report [11]. However, it was reported that the protocol details, which were not identical, could account for the differences observed between the two reports [32]. It is not known whether CuTCPBr has any selective effect on hypoxic cells and this requires further investigation.

Gd-TeX is reported to have a lethal dose:₁₀ of 80 – $100\ \mu mol\ kg^{-1}$ [11], whereas CuTCPBr has not exhibited any toxicity at twice that dose ($200\ \mu mol\ kg^{-1}$ or approximately $400\ mg\ kg^{-1}$). The tumour uptake of CuTCPBr was also significantly higher than that of Gd-TeX. With Gd-TeX, an intramuscular implanted EMT-6 tumour showed a maximum level of $2.7\ \mu g\ g^{-1}$ at 1 h after a single intravenous injection of $9.9\ \mu mol\ kg^{-1}$ that would translate to $10.8\ \mu g\ g^{-1}$ of Gd-TeX at a therapeutic dose of $40\ \mu mol\ kg^{-1}$. In contrast, the lower dose of $42\ \mu mol\ kg^{-1}$ of CuTCPBr ($83\ mg\ kg^{-1}$) in the CTEP formulation delivered approximately $95\ \mu g\ g^{-1}$ of porphyrin. Despite these apparent limitations, it would be of interest to make a direct comparison of the effects of CuTCPBr in the CTEP formulation and Gd-TeX in the EMT-6 tumour model.

The biodistribution data show that the CTEP formulation does not produce an increase in the mean porphyrin concentration in bulk tumour tissue compared with the 9% CRM formulation (Tables 2 and 3). However, the DMF obtained in the radiation studies was markedly improved using the CTEP formulation relative to the 9% CRM formulation when comparing similar porphyrin

doses. This would suggest that the microlocalisation of CuTCPBr in tumours has probably improved using the CTEP formulation relative to the 9% CRM formulation. It has been suggested that the microlocalisation of porphyrins in 9% CRM formulation are probably heterogeneous based on BNCT studies using CuTCPh, an analogue of CuTCPBr, with similar biodistribution and solubility properties [4]. Although very high boron concentrations were achievable in tumour tissue, the local tumour control rates were lower than expected, particularly in comparison with those for the clinically used boron carrier, boronophenylalanine, a compound shown to have relatively homogeneous microdistribution [33]. With such a microdistribution, the overall concentration of a compound can be lower and yet maintain its therapeutic effectiveness. This would appear to be the case with CuTCPBr where the DMF associated with 170 mg kg^{-1} of the CTEP formulation was as effective in modifying the response of EMT6 tumours as 400 mg kg^{-1} of the original 9% CRM formulation.

Conclusion

A lipophilic boron-containing porphyrin, CuTCPBr, previously developed for use in BNCT, was demonstrated to significantly enhance therapeutic efficacy of XRT in the EMT-6 tumour model, particularly in the clinically applicable CTEP formulation. Using this formulation, the DMFs for CuTCPBr were 1.29 ± 0.15 and 1.84 ± 0.24 for single administered doses of 83 mg kg^{-1} and 170 mg kg^{-1} of CuTCPBr, respectively. Further studies of CuTCPBr are warranted to investigate its potential for clinical use.

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