BNL-42522

BORONATED PORPHYRINS IN NCT: RESULTS WITH A NEW POTENT TUMOR LOCALIZER

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BNL--42522
DE89 010568

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Introduction

Porphyrins of various types have received increasing attention recently as potential carriers of boron for use in boron neutron capture therapy.[1-4] The precise biochemical mechanism for selective uptake of porphyrins in cancerous cells is not known, but the phenomenon extends across all classes of porphyrins from tetraphenylporphines to hematoporphyrin-like compounds. Indeed, the successful clinical use of photoradiation therapy (PRT) is based upon the accumulation of an oligomeric hematoporphyrin derivative (HPD) of unknown structure in superficial tumors of such diverse organs as the bladder, lung and skin. Like BNCT, photoradiation therapy is based upon the localization of a non-cytotoxic tumor-seeking agent, HPD, and its subsequent activation through external means. In PRT, activation is by visible light in the red region and the lethal product is singlet oxygen. The substantial advantage of neutron capture therapy over PRT is that epithermal

neutrons have far greater penetrability and can thus be used to treat such deep-seated malignancies as gliomas.

Structure-activity studies to date give the chemist little clue as to what factors determine the tumor localizing ability of a porphyrin. A recent study by Gabel[5] and coworkers has confirmed earlier work by Moan and Somer[6] that hydrophobicity is an important parameter in both TPP and HP classes of porphyrins. Jori has suggested that hydrophobicity may be significant in that it determines the ability of a porphyrin to be carried in the interior of low density lipoproteins.[7] These particles are the major cholesterol carriers in human plasma and enter cells through receptor-mediated endocytosis, thus providing a potential active transport pathway for the entry of porphyrins. Nevertheless porphyrins must possess at least minimal aqueous solubility in order to be administered to animals or humans and to remain solubilized in the plasma.

Several chemical methods are available for the solubilization of boronated porphyrins. We have previously reported the tumor localization of nido carboranyl porphyrins in which the icosahedral carborane cages have been opened to give B_9C_2 anions.[1,3] One of these species has shown tumor boron levels of nearly 50 μg B/g when delivered by week-long subcutaneous infusions.[1] We report here recent in vivo experiments with a new, highly water-soluble porphyrin based on the hematoporphyrin-type of compound in which aqueous solubility is achieved using the two propionic acid side chains of the "natural" porphyrin frame.

Methods

Details of the synthesis and structure of this compound, designated SBK-II, will be published as soon as the patenting process allows. The compound was administered as the

highly water-soluble di-potassium salt obtained by ion exchange of the free acid through a Dowex 50x2-400 column in the K^+ form. Solution boron levels were obtained by neutron induced prompt gamma spectrometry and converted to porphyrin dose per animal. Mice bearing a subcutaneously implanted Harding-Passey melanoma on the flank were used for i.p. administration and with an intraperitoneal solid tumor for tail vein i.v. infusions. Animals were sacrificed by ether euthanasia and organs removed for boron analysis by prompt gamma techniques.

Results and Discussion

When administered by serial intraperitoneal injection, SBK-II is found to be taken up by tumors in an approximately dose-dependent manner, as shown in Table 1. Mice were allowed only an eighteen hour clearance period at the end of the three day series of i.p. injections. Each boron concentration represents the average of four mice. Tumor boron in the 35-40 μg/g range was a consistent feature of the higher dose and represents the highest concentration of porphyrin-carried tumor boron reported to date. Whole body neutron autoradiographic thin sections of a mouse treated with this compound are presented elsewhere in this volume and demonstrate that tumor boron is distributed throughout the tumor including hypoxic areas. The tumor/blood ratios of approximately unity achieved with both doses of this compound are in line with other porphyrins when measured at short clearance times. This is particularly true with intraperitoneal administration where a significant amount of time is required for the compound to pass into the blood and then be cleared by the liver and kidneys. The lack of any statistically significant boron uptake by the brain is not surprising, since this compound would be predicted to be unable to pass the normal blood brain barrier, but it is gratifying and suggests the possible application of this compound for gliomas. It is worth noting that the compound was exceptionally well-tolerated by these mice. None showed any apparent ill effects save for some discomfort

upon injection. This may be due to the relatively high pH of the carboxylate solution which in more recent experiments is buffered with bicarbonate.

This compound also lends itself well to intravenous administration as it is quite soluble in water. Table 2 presents the results of a 3-day i.v. tail vein infusion at a 3.1 mg/mouse dose. As with intraperitoneal injection, average tumor boron levels are consistently found in the 35-40 μg/g range following a 7-hour clearance at the end of the infusion. The advantage to this mode of administration is that liver, kidney and spleen boron levels are lower, suggesting an increased ability to metabolize and dispose of the boronated porphyrin. The consequent drop in liver/tumor and kidney/tumor ratios is from about 4-5 with i.p. injection to about 2-3 and 0.5-1 for liver and kidney, respectively. Studies are now being initiated which will determine the nature and extent of any possible changes in hepatic function with this compound. Although the blood boron determination was inadvertantly omitted from these samples, more recent data suggests that slow tail-vein infusions produce tumor/blood ratios in the 2-5 range at relatively short clearance times. One of the more interesting results is the exceptionally large amount of boron in the tail, and illustrates one of the disadvantages of administering the compound in this fashion. The tail vein of a mouse is rather small and keeping a catheter in place and open during a prolonged infusion is problematical. It was noted that some mice had tail boron levels in the ≥ 1000 ppm range. These invariably had little or no tumor boron and very little liver or kidney boron. We concluded that in these mice tail vein infusion was physically unsuccessfully and removed them from the study. It is also possible that precipitation of the porphyrin at the injection site occurred. This seems unlikely given its high solubility. Intravenous administration is likely to be used in humans and large animal studies, but for screening of potential candidates for further development, the intraperitoneal model appears to be adequate.

The effects of prolonged clearance times on tumor boron uptake are presented in Tables 3 and 4. When mice were treated with 1.5 mg/mouse and a 48-hour clearance allowed the results in Table 3 were obtained. Tumor boron levels between 18 and 48 hours have risen whereas all other organs except spleen have decreased. In particular, the tumor/blood ratio changes from unity to roughly 1.7, the liver/tumor drops from 3.5 to 2.2 and the kidney/tumor falls from 2.1 to 1.5. The boron levels in muscle and lung obtained here warrant further investigation as it had been hoped these would be smaller. The negligible brain values are again noteworthy. As before, the animals showed no apparent signs of morbidity and appeared to behave quite normally. Table 4 presents data from individual or duplicate mouse experiments in which very long clearance times were allowed. The dose in these experiments was 3.1 mg/mouse. The finding of >30 μg/g nine days after injection is truly unique. Even at 33 and 43 days the levels of tumor boron are unprecedented, especially when it is noted that at this stage the tumors have more than doubled in mass since the beginning of the experiment. Most of the tumor uptake clearly occurs in the first seven days of the experiment so the values of ~10 μg/g are certainly lower limits at this stage. This study will now be repeated with a longer number of animals in order to give better statistics. However, we conclude that this compound has an exceptionally long tumor lifetime at least insofar as the ability to fix boron within the tumor is concerned.

Conclusions

We have demonstrated that a unique new boronated porphyrin of the hematoporphyrin class provides consistent tumor boron levels in the 35-40 μg B/g tumor region with no apparent ill effects on mice bearing a Harding-Passey melanoma. These levels are obtained using both i.p. and i.v. administration of the compound. Tumor boron

levels appear to peak between 4 and 7 days and recede only very slowly. This compound clearly shows promise as a tumor-localizing agent.

Research carried out in part under the auspices of the U.S. Dept. of Energy under contract no. DE-AC02-76CH00016

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