*Anti-Cancer Drugs 1994*, **5**, pp. 463–466

# Enhanced boron uptake in RG 2 rat gliomas by electropermeabilization *in vivo* — a new possibility in boron neutron capture therapy

**Crister P Ceberg,[1] Arne Brun,[2] Lluis M Mir,[3] Bertil RR Persson[1] and Leif G Salford[4]**

Departments of [1]Radiation Physics, [2]Neuropathology and [4]Neurosurgery, Lund University Hospital, S-221 85 Lund, Sweden. Fax: (+46) 31 419481. [3]URA 147 CNRS and U.140 INSERM, Institut Gustave-Roussy, Villejuif, France.

**Accumulation of boron in tumor tissue is an indispensable requirement for boron neutron capture therapy and it is important that the uptake is as high as possible. In this work we have studied the influence of electropermeabilization *in vivo* on the uptake of boron in normal and RG 2 glioma bearing Fischer 344 rats. Two different boron compounds, a sulfhydryl boron hydride (BSH) and a boronated porphyrin (BOPP), have been investigated. The rats were infused intravenously during 5 min with 175 μg BSH/g body weight or 12 μg BOPP/g body weight. Two electrodes were placed 5 mm apart in the brain and electropermeabilization was performed with eight square 400 V pulses at 4 and 7 min after the end of the infusion. After 6 h the animals were killed, and the boron content in the tumors and the surrounding brain was measured with neutron-activated autoradiography. In electropermeabilized healthy animals the BOPP uptake was low and limited to the electrode lesions, whereas BSH was spread extensively throughout the hemisphere. Rats with gliomas showed doubled (BOPP) to 10-fold (BSH) uptake of boron in the tumor when electropermeabilization was performed as compared with untreated animals. We conclude that electropermeabilization in the future may provide an interesting possibility to increase the uptake of certain boron compounds before neutron capture therapy.**

*Key words:* Boron, electropermiabilization, neutron capture therapy.

## Introduction

In boron neutron capture therapy (BNCT), the goal is to selectively accumulate boron in tumor cells. The rationale for this is that stable $^{10}B$ atoms can capture thermal neutrons, whereas the short-ranged, high-LET reaction products (an α particle and a recoiling $^{7}Li$ fragment) may kill the cell. In this way the advantageous local effect of internal radiotherapy is combined with a minimal unwanted exposure of distant organs, outside the primary neutron beam. These principles will be tested in upcoming clinical trials with astrocytoma grades III–IV within the European Collaboration on BNCT.[1]

The neutron radiation field cannot be made completely free from other contaminating radiation, such as fast neutrons and photons, and these, together with hydrogen recoils and protons from neutron capture reactions in nitrogen, will render also the boron free tissue an unavoidable background absorbed dose. In order to minimize this undesired absorbed dose to the healthy brain, it is therefore important to select a boron carrier with high and specific uptake in the tumour tissue. Within the European Collaboration it is planned to use BSH, a sulfhydryl borane. We have earlier shown that both BSH, and a boronated porphyrin, BOPP, are accumulated *in vivo* in the RG 2 rat glioma.[2]

Electropermeabilization has been used for almost a decade by molecular and cellular biologists to transiently open cell membranes *in vitro*. Although the exact molecular membrane processes of electropermeabilization are not yet fully understood, there is an increasing number of practical applications of the technique.[3] It has been shown recently that electropermeabilization *in vivo* opens cell membranes and allows hydrophilic substances to pass into the cell, and we have previously proposed that electropermeabilization could be used together with bleomycin as a new therapy of gliomas.[4]

In this work, the aim was to study whether electropermeabilization can be used to enhance the boron uptake in rat gliomas after intravenous injections of the boron carrying substances BSH and BOPP.

This study was supported by the Crafoord Foundation, Lund, Ms. Bertha Kamprads Foundation, Lund, The Medical Faculty, Lund University, Gunnar Nilssons Foundation and the Swedish Cancer Society.

Correspondence to LG Salford

## Materials and methods

### The animal model

In this study 24 normal male and female Fischer 344 rats were used. During surgical procedures, the animals were under chloral hydrate anaesthesia. By a stereotactic technique, 16 of these rats were injected with 5000 RG 2 glioma cells in 5 μl nutrient solution into the head of the right caudate nucleus of the brain using a Hamilton syringe. After 17 days tumors had developed. The animals were then divided into six groups with four rats in each. The rats in these groups were treated with different combinations of boron and electropermeabilization according to Table 1.

### Boron compound

The sulfhydryl borane (BSH, $Na_2{}^{10}B_{12}H_{11}SH$) (Centronic, UK) was injected at a dose equivalent to 175 μg B/g body weight. BSH, which is a highly soluble, white microcrystalline powder, has a boron weight percentage of about 53%, enriched to <95% $_{10}$B.

The boronated porphyrin (BOPP) was injected at a dose corresponding to 12 μg B/g body weight. BOPP, which is the tetrakiscarborane carboxylate ester of 2,4-bis-(α,β-dihydroxyethyl) deuteroporphyrin IX, was synthesized using naturally abundant boron.[5] Four closo-carborane cages are appended to the porphyrin, giving a water soluble and physiologically stable compound with a boron weight percentage of nearly 30%. Since porphyrins are known to be sensitizers to visible light, these experiments were carried out under low intensity light.

### Electropermeabilization

The rats in groups 3–6 were electropermeabilized at 4 and 7 min after boron administration using a standard electropermeabilization unit (Bioblock Scientific, France). At each time point, a series of eight square pulses with an electrical field strength of 800 V/cm and a duration of 100 μs were given. As electrodes, two acupuncture needles were used; the anterior needle was the anode. The electrodes were placed 5 mm apart and inserted 4 mm deep intracerebrally with the aid of a stereotactic instrument through two burr holes in the skull.

**Table 1.** The six different series

| Group | Tumor | Treatment |
|---|---|---|
| 1 | yes | BSH only |
| 2 | yes | BOPP only |
| 3 | yes | BSH + electropermeabilization |
| 4 | yes | BOPP + electropermeabilization |
| 5 | no | BSH + electropermeabilization |
| 6 | no | BOPP + electropermeabilization |

### Boron analysis

At 6 h after the boron infusion, all rats were sacrificed and the brains were excised. A 5 mm thick slice from the brain including the tumor was mounted in tissue glue on cork and was immediately frozen in isopentane containing dry ice (−70°C). The boron analysis was carried out with neutron capture imaging as described in an earlier publication.[6] Briefly, the specimens were freeze-sectioned at −20°C and freeze-dried for 24 h in a vacuum desiccator before they were mounted on cellulose nitrate films (LR115 type 1; Kodak Pathé, France) and irradiated with thermal neutrons ($10^{12}$–$10^{13}cm^{-2}$) at the R2-0 research reactor facility in Studsvik, Sweden. After the films had been irradiated and etched, digitized grey-scale transmission images were obtained using a light box (Novalux; ITAB, Sweden) and a video camera (CCD-72E; MTI, USA) connected to a PC486 computer running an imaging software package (ImagePro$^+$; Matrox, Canada). Using a set of 11 standard blood samples on each film, the grey-scale values were related directly to the boron concentrations in the samples. After the neutron irradiation, the slides carrying the tissue slices were removed from the films and stained with Cresyl-violet for histological examination.

## Results

No animal showed any adverse clinical side effects due to the electro permeabilization *per se* during the course of the experiment.

The rats in groups 1 and 2 exhibited a boron uptake well confined to the tumor, with only a slight tendency to spread into the surrounding brain tissue along the white matter tracts. The boron concentration in the tumor was 19 ± 10 p.p.m. (SEM) with BSH and 74 ± 5 p.p.m. (SEM) with

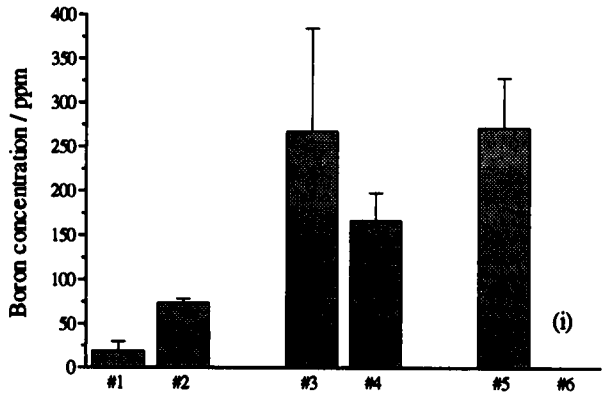


**Figure 1.** Mean boron concentration values with standard deviations for the animals in groups 1–6. (i) In the healthy rats in group 6, no BOPP uptake was seen outside the lesions.

BOPP. In the healthy brain tissue the boron uptake was negligible.

For the electropermeabilized animals in groups 3 and 4, the tumor boron concentrations had increased dramatically. The boron concentration values in the tumor were 268±117 p.p.m. (SEM) with BSH and 167±31 p.p.m. (SEM) with BOPP. For the animals receiving BOPP, the boron uptake was still well confined to the tumor and the boron concentrations in the surrounding brain tissue was negligible. For BSH, on the other hand, the boron was spread diffusely far away from the bulk of the tumor and even entered the contra lateral hemisphere.

The electropermeabilized healthy animals in groups 5 and 6 also exhibited high boron uptake. For the BOPP infused animals, the boron uptake, 212 ± 47 p.p.m., was limited to the lesions from the electrode needles and the boron concentration in the surrounding brain was unmeasurable. After the BSH infusion, however, the boron was spread extensively throughout the whole hemisphere with concentrations in the same range as for the tumor rats, 272 ± 57 p.p.m. (SEM).

The mean boron concentration values with standard deviations for groups 1–6 are shown for comparison in Figure 1.

Thus, electropermeabilization · increased the tumor uptake of BOPP with a factor of about 2 ($p = 0.03$, Mann–Whitney *U*-test). For BSH, the intracerebral boron concentration in the electropermeabilized animals was increased to 14 times the tumor values of the untreated animals ($p = 0.03$, Mann–Whitney *U*-test), even in the healthy rats without tumors.

## Discussion

In the normal brain, boron compounds, as any other substances, are prevented from reaching normal cells by the intact blood–brain barrier. This provides a therapeutically important margin between healthy and tumoral tissue, in which the blood–brain barrier is broken down. A limited leakage of boron from the tumor into the surrounding brain does not necessarily have to be undesirable, since through this route it has a chance to reach and accumulate in migrating tumor cells dwelling in the surroundings behind the intact blood–brain barrier. Thus, as long as the boron leakage is not extensive, the brain seems to be a very favorable site for electropermeabilization enhanced uptake of targeting boron compounds.

After boron administration to tumor carrying rats without electropermeabilization, the spread of boron into the brain tissue was limited and would, especially for BOPP, probably not be a restraining factor in the case of treatment with this technique. Hill *et al.* have reported tumor:brain boron concentration ratios after BOPP administration in the 400:1 range.[7] In combination with electropermeabilization, the BOPP uptake was doubled without any significant increase of boron content in the brain tissue. This strongly suggests that a continued development of this technique may be useful in BNCT.

With BSH, however, the more than 10-fold increase of the boron uptake by electropermeabilization was accompanied by an extensive leakage into large areas of the brain, which was seen also in the healthy animals. This situation would give unacceptable high radiation doses to the healthy brain if the neutron field was applied.

In a parallel study, animals treated with electropermeabilization only in our laboratory were investigated with respect to albumin leakage. This study revealed a concentration of albumin near the electrode lesions, with an initial spread in the surroundings that was washed away already during the first day after the treatment. Thus, the electrode insertion was followed by a regional opening of the blood–brain barrier, lasting over at least the 6 h duration of the experiment. Yet, it is not clear why BSH leaks so abundantly through this opening into the healthy brain, while BOPP does not.

We conclude that for some boron compounds, e.g. for BOPP as shown in this work, electropermeabilization may in the future provide an interesting instrument to increase the boron uptake in connection with BNCT.

## Acknowledgements

The authors wish to thank Catarina Blennow and Kerstin Sturesson for skilful technical assistance. The work of the European Collaboration on BNCT is supported by the commission of the European Community, which supplied the sulfhydryl boron hydride (BSH). Boronated porphyrin (BOPP) was kindly supplied by Prof SB Kahl at UCSF.

## References

1. Gabel D. 1990 *EPAC 90, 2nd European Particle Accelerator Conference,* Gif-sur-Yvette: Edition Frontiéres **1**: 1990.
2. Ceberg CP, Brun A, Kahl SB, *et al.* A comparative study on the pharmacokinetics and biodistribution of boronated porphyrin (BOPP) and sulfhydryl boron hydride (BSH) in the RG 2 rat glioma model. *J Neurosurg,* submitted.
3. Chang DC, Chassy BM, Saunders JA, *et al.* San Diego: Academic Press 1992.
4. Salford LG, Persson BRR, Brun A, *et al.* A new brain tumor therapy combining bleomycin with *in vivo* electropermeabilization. *Biochem Biophys Res Commun* 1993; **194**: 938–43.
5. Kahl SB, Koo M-S. Synthesis and properties of tetrakis-carborane-carboxylate esters of 2,4-bis($\alpha$,$\beta$-dihydroxyethyl) deuteroporphyrin IX. *J Chem Soc Chem Commun* 1990; 1769–71.
6. Ceberg CP, Salford LG, Brun A, *et al.* Neutron capture imaging of B-10 in tissue specimens. *Radiother Oncol* 1993; **265**: 139–46.
7. Hill JS, Kahl SB, Kaye AH, *et al.* Selective tumor uptake of a boronated porphyrin in an animal model of cerebral glioma. *Proc Natl Acad Sci USA* 1992; **89**: 1785–9.

*(Received 31 March 1994; accepted 12 May 1994)*