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Short communication

Metabolism of borono-phenylalanine–fructose complex (BPA–fr) and borocaptate sodium (BSH) in cancer patients—Results from EORTC trial 11001

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ABSTRACT

Within the clinical trial EORTC 11001, patients were infused with ^{10}B-enriched borono-phenylalanine–fructose complex (BPA–fr), or borocaptate sodium (BSH) solutions, which are used as boron carriers for boron neutron capture therapy. Urine samples were periodically collected and analyzed by ^{10}B NMR spectroscopy. The results revealed time-dependent metabolic changes of the administered compounds. BPA–fr dissociated to the constituents BPA and fructose, and the borate group was partly cleaved from BPA. BSH was partly aggregated to a dimer form, BSSB. These observations were previously reported for cultured cells and animal models, and are confirmed here in human cancer patients.

1. Introduction

Boronated phenylalanine (BPA), and sodium mercaptoundecahydro-closo-dodecaborane (BSH) are the two compounds which are used clinically for boron neutron capture therapy (BNCT). In this binary radiation treatment, boron-containing molecules, enriched in the ^{10}B isotope, are targeted to the tumor, and irradiated with thermal or epithermal neutrons. Capture of these neutrons by ^{10}B nuclei generates cell-damaging radiation, confined to single cell dimensions [1,2].

The development, improvement and optimization of BNCT depend on understanding the pharmacological and metabolic properties of the administered compounds. Many of the investigations in this field rely on monitoring the temporal and spatial concentration changes of ^{10}B, the active nucleus for BNCT [3]. However, using techniques such as inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), or prompt gamma-ray analysis (PGRA), the molecular environment of the detected ^{10}B nuclei cannot be established. On the other hand, NMR spectroscopy has the potential for quantitative on-line monitoring of molecules *in vivo*, as well as in urine, blood, and tissue samples [4]. Several studies that employed NMR spectroscopy, revealed that both BPA and BSH underwent certain changes from the molecular form in which they were administered. These changes were observed in mouse kidney, rat blood, implanted mouse tumor, cultured malignant cells, as well as in the culture medium itself [5–8]. Other studies reported previously on metabolic changes of BPA in a patient's blood sample, using HPLC [9], as well as on evidence for the presence of urinary metabolites and oxidation products of BSH (including BSSB), using electrospray ionization mass spectrometry [10]. In this report, we submit further evidence for *in vivo* metabolic changes, of the BPA–fructose complex (the preferred administered form of BPA), and of BSH, following i.v. infusion of both compounds to tumor patients, and provide some approximate time scales for these changes, based on the analysis of urine samples using ^{10}B NMR spectroscopy.

2. Materials and methods

2.1. The clinical trial EORTC 11001

The samples where collected from patients who participated in the clinical trial EORTC 11001, "^{10}B-uptake in different tumors using the boron compounds BSH and BPA" [11,12]. The Protocol Review Committee of the EORTC and the Ethics Committee of the Medical Faculty of the University Duisburg-Essen approved the trial. All patients gave written informed consent prior inclusion and agreed that the samples taken might be investigated by innovative procedures that were not available at the time of inclusion, which made this study possible.

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2.2. Boron compounds

BSH and BPA were purchased from KATCHEM Ltd. (Praha, Czech Republic). The quality of the study medication was strictly controlled, including examination of the identity of compounds by infra-red spectroscopy, monitoring of purity by high pressure liquid chromatography and test for pyrogenicity. The enrichment of ^{10}B was ≥99.6% in both compounds and was tested with PGRA and ICP-AES. Injection-solutions were prepared according to standard operating procedures established for the EORTC trials 11961, 11001 and 11011 [12].

2.3. Patient 1 infused with BPA

This patient suffered from squamous cell carcinoma of head and neck. To improve solubility, BPA (100 mg/kg) was infused as BPA–fructose complex [13], within 1 h. Urine samples were taken before and 1.5 h, 3.0 h and 7.5 h after start of infusion. Samples were frozen on the day of collection at −20 °C until analysis. After defrosting the samples were centrifuged, evaporated to dryness, and re-dissolved for the NMR experiments.

2.4. Patient 2 infused with BSH

This patient suffered from squamous cell carcinoma of head and neck. BSH (50 mg/kg) was dissolved in saline and infused within 1 h. Urine samples were taken before start of infusion and at 2.0 h, 10.0 h, 11.2 h, 12.1 h, 13.5 h, 15.1 h, 23.0 h, 38 h, 41.5 h, 53.0 h, 58 h, 64 h, and 71 h after start of the BSH infusion. Samples were frozen on the day of collection at −20 °C until analysis, and re-thawed prior to the NMR experiments

2.5. NMR spectroscopy of urine samples

^{10}B NMR spectra were obtained with a 9.4 T vertical-bore spectrometer (Avance III, Bruker, Karlsruhe, Germany), using 10 mm boron-free quartz NMR tubes, and a boron-free broad-band probe, tuned to the ^{10}B resonance frequency of 41 MHz. 1 ml from the original urine samples was transferred to the NMR tube, in addition to 0.2 ml D_2O which was added to provide a field-frequency lock. The spectra were acquired by single-pulse excitations, using 90° pulses and repetition times of 0.08 s (for the samples containing BPA), and 0.1 s (for the samples containing BSH). For the samples containing BSH, proton decoupling was applied. The repetition times were sufficiently long for providing fully relaxed signal intensities. Between 2000 and 200,000 scans were averaged, depending on the ^{10}B concentration, to achieve adequate S/N (signal-to-noise) ratios. Absolute concentrations for the different metabolites were calculated by integrating the relevant peaks in the NMR spectra, and comparing the integrals to those obtained from a reference sample, containing a known amount of boric acid, normalizing for differences in the number of averages. For the BPA signals, which have a very short T_2, an additional small correction to the calculated intensities was applied by estimating the signal loss during the 0.09 ms 'dead time' from the initial decay rate of the FID. Lorentzian deconvolution was applied to resolve overlapping signal components in the spectra.

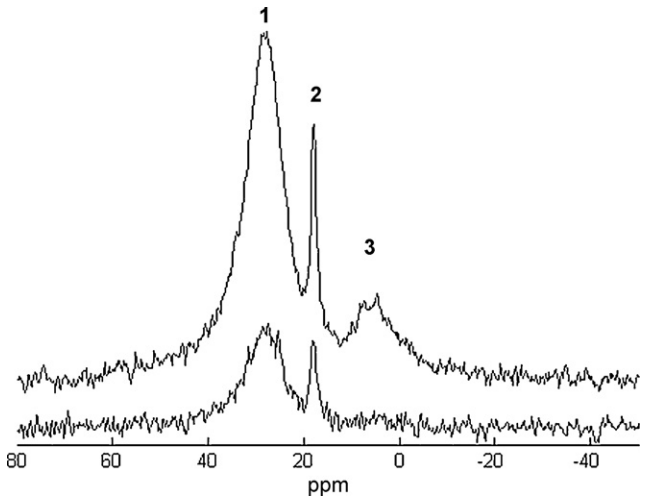

Fig. 1. ^{10}B NMR spectra of the first (upper) and last (lower) urine samples from patient 1. Each spectrum was acquired with 32,000 scans for a time of 43 min. The signals from BPA (**1**), Bi (**2**), and BPA–fr (**3**) are labeled.

3. Results

3.1. BPA–fr complex

The ^{10}B NMR spectra of the urine samples from patient 1 showed three main signal components from the BPA–fr complex, free BPA, and free borate (B_i), as previously assigned by ^{11}B NMR [6]. The spectra from the earliest and latest time points are shown in Fig. 1, and the absolute and relative concentrations of the molecular species are summarized in Table 1. It should be kept in mind, that dominant form in the injected solution (>90%) is the BPA–fr complex [13], so that all the examined urine samples show the substantial dissociation of the complex *in vivo*, as well as partial hydrolysis of the borate group, which also progresses with time during the observation window.

3.2. BSH

Representative spectra of the urine samples from patient 2, collected 10 h and 53 h after starting the administration of BSH, are shown in Fig. 2. Although the spectral resolution of the ^{10}B spectra is not as good as that of ^{11}B spectra, the partially overlapping signals of the BSH monomer and dimer could be resolved, based on the previous assignments [6,7]. BSSB was identified as the main contributor to the spectra (apart from BSH), but for late times other oxidation products could also have appeared [10]. Fig. 3 shows a graph of the total ^{10}B concentration, as well as the amount of ^{10}B nuclei in BSH. The results indicate a gradual decrease in the fraction of ^{10}B signal stemming from BSH, until there is no detectable BSH left in the urine after about 50 h.

Table 1

Time after start of infusion [h]	Total ^{10}B concentration in urine sample (μg/ml)[a]	BPA–fr fraction (%)[a]	Borate (B_i) fraction (%)[a]
1.5	110	19	11
3.0	66	15	17
7.5	28	10	20

Results of analysis of urine samples from patient 1 by ^{10}B NMR.

[a] The relative precision of the measurements is about ±10%.

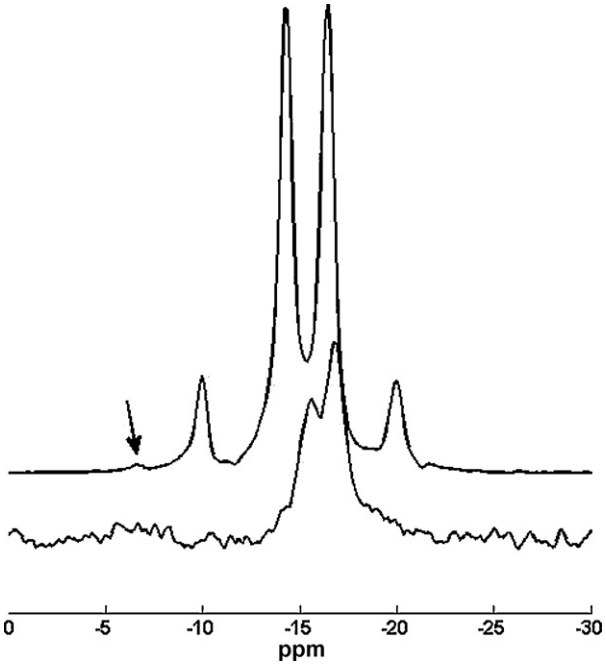

Fig. 2. ^{10}B NMR spectra of urine samples from patient 2, corresponding to collection times of 10 h (upper) and 53 h (lower). The upper spectrum was acquired with 2000 scans for 3.3 min, and the lower spectrum (plotted with 5× increased vertical scale) with 200,000 scans for 5.5 h. In the upper spectrum, most of the ^{10}B nuclei (~92%) are in the BSH monomer (a resolved peak originating only from the dimer is indicated by the arrow), while in the lower spectrum there is hardly any BSH monomer left.

4. Discussion

BNCT is a complex therapeutic process, the success of which depends upon a variety of factors, some of which are still poorly understood. The nature of the drugs used as boron carriers is almost certainly a decisive factor. The biological uptake and washout properties of BSH and BPA were extensively studied in the past [14]. However, the experimental detection techniques used for accumulating the pharmacokinetic data were mostly targeted at the ^{10}B nuclear isotope, without considering its molecular environment, with the exception of a study by Gibson et al. [10], where MS was used to demonstrate and measure the presence of a variety of BSH metabolites in urine samples of brain tumor patients. One of their findings was that 24 h after infusion about 80% of the parent compound (BSH) was still excreted unchanged. Our results agree with this finding (see Fig. 3), and also show a time-dependent decrease in the proportion of BSH, ultimately leading to a dominance of the altered metabolites.

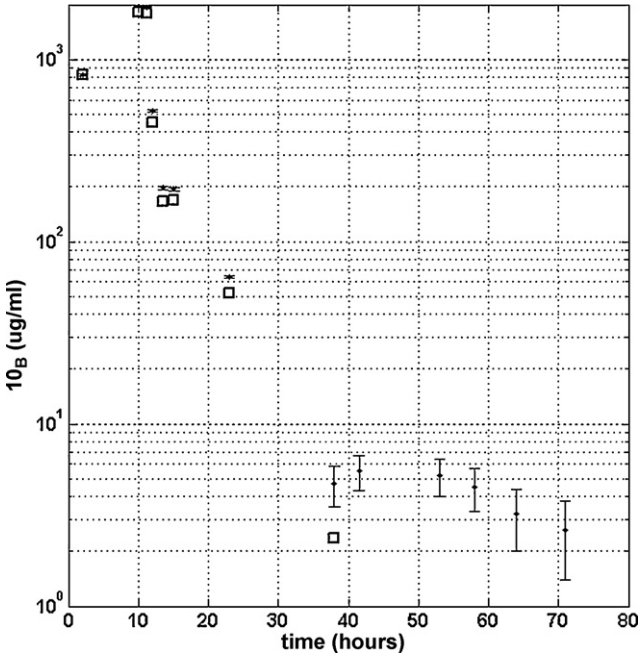

Fig. 3. The total ^{10}B concentration (in μg/ml) in the urine samples of patient 2 vs. time measured from the start of infusion (dots with error-bars). The squares represent the estimated amount of ^{10}B in BSH. For the last five measured spectra, the BSH contribution to the spectrum was too small for reliable determination (under 10% of the total signal).

The molecules administered for clinical BNCT to date are BSH and the BPA–fr complex, but as we have confirmed here, the molecules which are left in the body after a sufficiently long time, are not the same molecules which were present in the infused solution. These findings could have significant implications for the understanding and further development of BNCT. For example, the bio-distribution between extra- and intracellular compartments is most likely different for BSH and BSSB, or for BPA and BPA–fr. Nguyen et al. have shown that BSSB leads to a much higher intracellular level of boron than BSH in exponentially growing rat 9L gliosarcoma cells, if exposed to iso-effective concentrations of BSH and BSSB [15]. Moreover, the retention of BSSB in mouse M2R melanoma and rat C6 glioma cells was shown to be significantly longer than that of the monomer BSH [7]. The biological impact of the dissociation of BPA–fr to its constituents BPA and fructose has not yet been investigated.

Further studies require determining the biological significance of the detected metabolites, and whether they accumulate to significant amounts in the tumor. Other open questions are the mechanisms that led to a diminishing content of BSH and BPA–fr in the examined urine samples, in which organs these metabolic changes took place, or whether the results merely reflect differences in clearance rates, rather than progressing metabolic changes [7]. In any case, it appears that ^{10}B NMR could be a valuable tool in addressing such questions in more detail in future studies.

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