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Drug Monitoring and Toxicology

Atomic Emission Method for Total Boron in Blood during Neutron-Capture Therapy

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Background: **Boron neutron-capture therapy (BNCT) is a drug-targeted binary radiotherapy for cancer. The ^{10}B capture of thermal neutrons induces secondary radiation within cells during irradiation. The most widely used boron carrier is 4-dihydroxyborylphenylalanine (BPA). The duration and timing of the irradiation is adjusted by monitoring ^{10}B concentrations in whole blood.**

Methods: **We developed a new method for boron determination that uses inductively coupled plasma atomic emission spectrometry (ICP-AES) and protein removal with trichloroacetic acid before analysis. This method was compared with the established but tedious inductively coupled plasma mass spectrometry (ICP-MS), which uses wet ashing as sample pretreatment. Erythrocyte boron concentrations were determined indirectly on the basis of plasma and whole blood boron concentrations and the hematocrit. The hematocrit was determined indirectly by measuring calcium concentrations in plasma and whole blood.**

Results: **Within- and between-day CVs were <5%. The recoveries for boron in whole blood were 95.6–96.2%. A strong correlation was found between results of the ICP-AES and ICP-MS ($r = 0.994$). Marked differences in plasma and erythrocyte boron concentrations were observed during and after infusion of BPA fructose complex.**

Conclusions: **The present method is feasible, accurate, and one of the fastest for boron determination during BNCT. Our results indicate that it is preferable to determine boron in plasma and in whole blood. Indirect erythrocyte-boron determination thus becomes possible and avoids the impact of preanalytical confounding factors, such as the influence of the hematocrit of the patient. Such an approach enables a more reliable estimation of the irradiation dose.**

Boron neutron-capture therapy (BNCT)[6] is an experimental binary radiotherapy in which a tumor-localizing, boronated carrier drug is allowed to accumulate in cancerous tissue before neutron irradiation. The neutron irradiation produces a $^{10}B(n,\alpha a)^{7}Li^{*}$ nuclear reaction in boron-containing cells, yielding high linear-energy-transfer particles capable of destroying structures within the radius of a single cell *(1)*. Two malignant tumors, glioblastoma multiforme and malignant melanoma, have been treated with BNCT to this date. Because BNCT is the only radiation treatment in which short-range, high linear-energy-transfer radiation is generated within cells, it promises to be more effective than other types of radiation [for a review, see Ref. *(2)*]. Consequently, BNCT has attracted worldwide interest. Treatments are being given and planned in the near future at seven centers around the world: one in the US, four in Europe, and two in Japan. In addition, several research groups are tackling the pharmacologic, oncologic, physical, and radiobiologic aspects of BNCT.

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[6] Nonstandard abbreviations: BNCT, boron neutron-capture therapy; BPA, 4-dihydroxyborylphenylalanine; BPA-F, fructose complex of BPA; ICP-AES, inductively coupled plasma atomic emission spectrometry; and ICP-MS, ICP mass spectrometry.

Two boron carriers are currently being used in clinical BNCT trials. The most widely used boron carrier, 4-dihydroxyborylphenylalanine (BPA), given as water-soluble fructose complex (BPA-F), is being used in the Finnish BNCT project. At the time of writing, 16 patients with malignant brain tumors have been treated since 1999 in the Finnish BNCT study.

Fast and accurate blood boron monitoring is required for BNCT for the following reasons.

(*a*) Clinically, the most important part of BNCT, the high linear-energy-transfer radiation dose in the tumor and in the surrounding healthy tissue, is dependent solely on the ^{10}B concentration in the respective cells and tissues. With the current amount of BPA-F given and with the neutron beams used, up to 50% of the tumor irradiation dose is estimated to originate from the neutron-capture reaction of boron.

(*b*) At present, there is no way to routinely determine tissue boron concentrations during BNCT. As a surrogate for determining the in vivo tissue boron content, whole blood boron concentrations are used instead. The irradiation time for BNCT is then adjusted on the basis of the preirradiation whole blood boron concentration, assuming an mean boron concentration ratio of 1:1 for blood to healthy brain tissue and 1:3.5 for blood to tumor tissue *(3)*.

(*c*) There are interindividual variations in the blood boron concentration–time profiles, and the pharmacokinetic characteristics of BPA-F during and after the BPA-F infusion are not fully understood. The boron concentrations decline after the end of the infusion; therefore, the irradiation has to be carried out within a few hours. Irradiations take from 15 to 40 min, during which time the preirradiation blood boron concentrations are needed to adjust the irradiation time.

Several analytical approaches may be applied for total boron determination in biologic samples. These include prompt-γ, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), spectrofluorometric methods, and direct current AES *(4–7)*. Usually, wet ashing or related procedures are used for the degradation of the sample matrix *(8)*. Such pretreatment methods are too time-consuming for clinical BNCT. Many BNCT facilities use the prompt-γ method alone *(9, 10)* or in conjunction with direct current AES *(11)* for boron determination. Lengthy counting times and low sample throughput *(10, 12)* may limit the usefulness of prompt-γ. Because prompt-γ was not technically feasible at the Finnish BNCT facility, other solutions for boron determination had to be found.

The aim of the present study was to develop a new ICP-AES-based method for fast and accurate boron determination during clinical BNCT. The purpose was also to study the distribution of boron between plasma and erythrocytes during and after BPA-F infusion. The knowledge of this distribution is important for two reasons: (*a*) it enables the estimation of the impact of the hematocrit on whole blood boron concentrations, and (*b*) it enables determination of the uptake of boron in the erythrocytes. Erythrocytes may serve as a model for the estimation of dynamic boron concentrations in other kinds of cells and tissues.

Materials and Methods

PATIENTS AND SPECIMENS

The present ICP-AES method was used to determine the blood boron concentration during and after infusion of BPA-F in 12 patients (6 women and 6 men; 44–67 years) who received BNCT treatment for glioblastoma multiforme. The patients were healthy in other respects. Each patient was on dexamethasone treatment at the time of this study and was also receiving antiepileptic medication. Two catheters were inserted, one in the antecubital vein of one arm for infusion of BPA-F and one in the other arm for drawing blood samples. The samples were taken in tubes containing lithium heparin (VT100; Terumo). Samples were taken before, during, and 3 h after the end of BPA-F infusion at intervals of ~20 min. Before initiation of the clinical study with BPA-F, appropriate notification (form 723) with appendices was presented to the Finnish National Agency for Medicines. The respective ethical committees of the clinics concerned accepted the BNCT protocol, and the patients voluntarily participated in the protocol after signing an informed consent form. The samples used for estimation of the accuracy of the present method were derived from canine experiments approved by the Animal Experimentation Committee of the Helsinki University Central Hospital.

DRUG PREPARATION AND DOSAGE

The BPA (Katchem, Ltd.) had a chemical purity of >99% and an enrichment in the ^{10}B isotope of ≥99%. The BPA-F solution for each patient was prepared on the day before the BNCT treatment in the Helsinki University Central Hospital Pharmacy. The pharmaceutical quality was verified for each individual BPA-F batch. The BPA-F solution contained 30 g/L BPA and was administered as a 2-h intravenous infusion. The dosage was 290 mg of BPA per kg of body weight.

ICP-AES METHOD

Calibrators, plasma, or whole blood samples (400 μL) were added and mixed into polypropylene tubes containing 200 μL of internal standard solution (1 mg/L beryllium). The samples were then deproteinized by adding 2 mL of 0.4 mol/L trichloroacetic acid solution under continuous mixing. After short centrifugation (2900*g*; 3 min), the clear supernatant was manually aspirated into a Perkin-Elmer 3200 DV ICP-AES instrument. To avoid potential contamination from borosilicate glass, glassware was not used at any stage of the procedure. Deionized water (18.2 megaohms of resistivity) was obtained by MilliQ and Elix systems (Millipore Inc). The most sensi-

tive boron and beryllium emission lines (249.772 and 234.861 nm, respectively) were used. Beryllium was chosen as an internal standard for the ICP-AES procedure because the first-order lines used have excitation potentials close to each other (5.28 eV for beryllium and 4.96 eV for boron). The lower end of the beryllium emission peak at 234.861 nm was manually adjusted to avoid potential interference of an adjacent peak at 234.831 nm present only in the whole blood samples. Boron and beryllium standard solutions (1000 mg/L) were certified products of E. Merck. Aqueous calibrators for boron were diluted to 2.5–100 mg/L and treated as samples. Positive displacement pipettes (Gilson Medical Electronics) were used for the plasma and whole blood samples. ICP-AES operating conditions are described in detail in Table 1. Whole blood and plasma calcium concentrations were monitored simultaneously with the boron measurements and used for determination of the hematocrit. Boron determinations for BNCT were carried out from samples processed in duplicate.

ICP-MS method

The dissolution of the whole blood samples was performed by microwave-assisted wet ashing. Whole blood (100 mg) was treated with 2.5 mL of HNO_3 and 0.5 mL of H_2O_2 in a sealed Teflon vessel in a microwave oven (Milestone 1200; Mega). All solutions were diluted up to a 30-mL volume, and then 10 μg/L beryllium was added to the samples as an internal standard.

A VG Plasma Quad 2+ instrument (VG Elemental) equipped with a Gilson autosampler and a V-groove nebulizer was used to determine the ^{10}B concentration in the sample solutions. The calibration solutions were prepared by diluting a certified boron standard (Reagecon Diagnostics). Three integrations, lasting 1 min each, were used to quantify the ^{10}B signal. Control samples for the ICP-MS measurements were prepared from the BPA-F solution.

Table 1. Operating conditions of ICP-AES.

Plasma view	15-mm radial
Nebulizer type	Concentric glass
Sample uptake rate	1 mL/min
Washing time	30 s
Plasma power	1300 W
Argon flow rate	
Plasma	15 L/min
Auxiliary	0.5 L/min
Nebulizer	0.8 L/min
Replicates	5
Read time/replicate	1 s
Emission wavelengths	
B	249.772 nm
Be	234.861 nm
Ca	317.933 nm

Method comparison

Post- and preinfusion whole blood samples (n = 24) derived from canine experiments (1-h infusions of 700 mg of BPA per kg) were used for comparison of the ICP-AES and the ICP-MS procedures. A pooled sample was prepared by taking an equal aliquot from each of the samples. This pool and two other samples derived from the same canine experiment were analyzed on different days to assess total and between-day imprecision. Because mg/L results were obtained by the ICP-AES method, the mg/kg results produced by the ICP-MS procedure were converted to mg/L by use of the specific gravity of the pool.

Indirect determination of erythrocyte boron concentration and hematocrit

The erythrocyte boron concentrations were calculated as follows: $B_{erythrocytes} = [B_{whole\ blood} - (1 - Hct) \times B_{plasma}]/Hct$, where Hct represents hematocrit, $B_{whole\ blood}$ represents whole blood boron concentration, B_{plasma} represents plasma boron concentration, and $B_{erythrocytes}$ represents erythrocyte boron concentration.

To assess hematocrit values in the whole blood samples taken during and after the infusion of BPA-F, calcium concentrations were monitored simultaneously with boron determinations. The hematocrit was then calculated as follows: $Hct = (Ca_{plasma} - Ca_{whole\ blood})/Ca_{plasma}$, where $Ca_{whole\ blood}$ represents whole blood calcium concentration, and Ca_{plasma} represents plasma calcium concentration.

The erythrocyte calcium concentration was ignored in the equation because it was below the detection limit in saline-washed erythrocytes. Because the hematocrit is calculated from a ratio, determined calcium concentrations may be replaced by calcium net intensities in the equation obtained without standardization. To evaluate the linearity of indirect hematocrit determination, a series of whole blood samples was prepared by mixing plasma and isolated erythrocytes to different hematocrit values. The erythrocyte fraction was obtained from the Helsinki University Hospital Blood Centre and contained trapped plasma. Three of these samples were measured on different days to estimate the imprecision of the method. In one patient the hematocrit values from all samples taken during and after the BPA infusion were determined in addition to the present indirect method with the Coulter Counter T850, which is in accredited clinical use in the Helsinki University Central Hospital laboratories.

Statistical analyses

One-way ANOVA was used to segregate the total variance into the within- and between-day components. For estimation of the between-day imprecision, the date of analysis was used as a grouping variable. For estimation of between-replicate imprecision the data were grouped by sample identification number. The SDs and subsequently CVs were calculated from the variances listed in

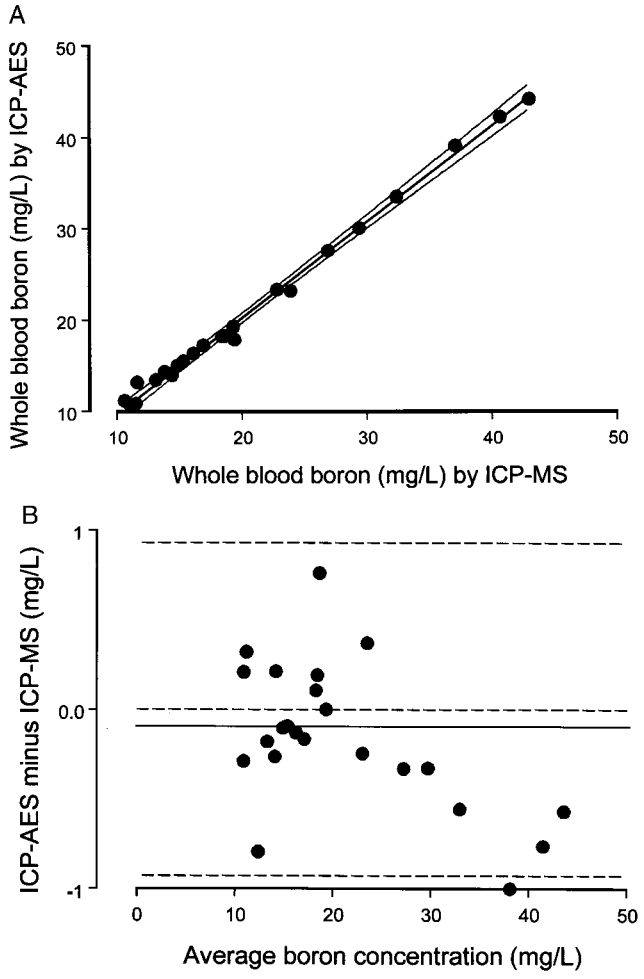

Fig. 1. Correlation of the ICP-AES and the ICP-MS methods (*A*) and Bland-Altman plot of the difference (n = 24) against mean values of results produced by the ICP-AES and the ICP-MS methods (*B*).

(*A*), regression line [(1.054 ± 0.017) − (0.895 ± 0.394); $r = 0.994$] and 95% confidence intervals are presented. (*B*), data depicted represent single measurements from the ICP-AES and the ICP-MS methods. The *upper* and *lower dashed lines* indicate 95% confidence limits (1.96 SD). The *solid line* represents the mean difference between the methods *(13)*.

the ANOVA program output. Regression analysis was used to compare the data from the ICP-AES and ICP-MS. All statistical calculations were performed with the SPSS 8.0 program (SPSS, Inc.).

Results

METHOD COMPARISON

The difference between results obtained with the present ICP-AES method and those obtained with the ICP-MS was compared by use of linear regression (Fig. 1A), as well as by use of the Bland-Altman plot (Fig. 1B) *(13)*.

IMPRECISION

Between-day and total imprecisions at different boron concentrations are presented in Table 2. Between-replicate imprecision of 0.90% was observed by one-way ANOVA from whole blood samples (n = 77), processed in duplicate, showing a concentration range of 7.0–28.9 mg/L. The limits of detection and quantification were estimated from the SD of the boron signal obtained by repeated measurements (n = 10) of a zero calibrator containing the internal standard. The limit of detection was 50 μg/L, and the limit of quantification was 160 μg/L (corresponding to the boron signals equivalent to 3 and 10 times the SD of the boron signal in the zero calibrator, respectively).

RECOVERIES

The mean boron concentrations of the individual whole blood samples determined by either method did not differ significantly (20.7 ± 10.2 mg/L for ICP-AES and 20.5 ± 9.7 mg/L for ICP-MS). Assuming that the ICP-MS wet ashing procedure yields 100% recoveries, the ICP-AES result would correspond to a mean recovery of 101% for the ICP-AES procedure (Fig. 1A). Analytical recovery in the ICP-AES procedure was further studied by supplementing whole blood with BPA-F (Table 3). The observed recoveries were 95.6–96.2% for whole blood and 102.9–104.2% for plasma.

SPECIFICITY AND LINEARITY

The ICP-AES results obtained at different wavelengths were studied by Bland-Altman comparison plots and are presented in Fig. 2. The results revealed a close agreement between different wavelengths, indicating that the analytical bias deriving from spectral interferences was negligible. Comparison of boron concentrations determined at 208.957 nm revealed a positive bias of 0.2–0.5 mg/L compared with other wavelengths. The linearity of the present ICP-AES method for boron determination was good ($r > 0.99$) for a boron concentration range of 2.5–100

Table 2. Imprecision of total boron in pooled whole blood samples by ICP-AES.

Concentration, (mg/L)	Accuracy	Between-day imprecision			Total imprecision		
		S_w[a]	CV_w (%)	Days, n	S_t	CV_t (%)	N_t
28.4[b]	ND	0.67	2.35	9	0.79	2.77	44
20.5[b]	−0.2[c]	0.45	2.19	20	0.75	3.65	87
11.8[b]	ND	0.32	2.72	11	0.49	3.83	53

[a] S_w, between-day component SD; CV_w, between-day component CV; S_t, total SD; CV_t, total CV; N_t, total number of determinations; ND, not determined.
[b] Mean concentration of a pooled whole blood sample determined with the ICP-AES method.
[c] As compared with the mean value of individual samples determined by the ICP-MS method.

Table 3. Analytical recoveries of boron by the ICP-AES method.[a]

Boron in whole blood			Boron in plasma		
Amount, mg/L			Amount, mg/L		
Added[b]	Recovered	Recovery, % (n = 5)	Added[b]	Recovered	Recovery, % (n = 5)
9.8	9.4 ± 0.3	96.5 ± 2.8	10.9	11.2 ± 0.4	102.9 ± 4.3
18.1	17.4 ± 0.2	96.2 ± 0.9	21.7	22.2 ± 0.3	104.4 ± 2.7
43.9	42.2 ± 0.6	96.2 ± 1.3	43.9	45.6 ± 0.3	104.2 ± 2.7

[a] Boron was added as BPA-F solution to reconstituted whole blood (hematocrit = 0.5) and to plasma.
[b] The amount of boron corresponding to the amount of BPA-F added.

mg/L. The corresponding regression equation was: $y = (1751.4 \pm 9.9)x + (1319.0 \pm 454.4)$.

DETERMINATION OF HEMATOCRIT BY ICP-AES

The hematocrit determination of reconstituted whole blood samples, based on monitoring of the calcium signal in plasma and whole blood by ICP-AES, revealed a good linearity ($y = 0.973x + 0.023$; $r = 0.9998$; Fig. 3). The erythrocyte fraction we used to construct the whole blood samples of various hematocrit values had a hematocrit of 0.92 because of trapped plasma. The calcium signal was linear ($r > 0.99$) over a concentration range of 0.5–4 mmol/L.

Comparison of hematocrit values obtained by either ICP-AES or Coulter Counter revealed a slope that significantly differed from 1.0 ($y = 0.845x + 0.046$; $r = 0.92$; see Fig. 4A). The hematocrit results obtained by ICP-AES were corrected accordingly in the calculation of erythrocyte boron concentrations. The mean difference between the results of the indirect ICP-AES method and those of the Coulter Counter T850 was 0.009 units in the hematocrit range of 0.31–0.42 (see Fig. 4B). The within-day and between-day imprecisions for hematocrit determination are presented in Table 4.

DISTRIBUTION OF BORON BETWEEN PLASMA AND ERYTHROCYTES

Substantial differences were found between the plasma and the erythrocyte boron concentrations during and several hours after the BPA-F infusion (Fig. 5). At the onset of the first irradiation, the boron concentration in plasma was ~3 times higher than in the erythrocytes. At the end of the second irradiation, the plasma concentrations were approximately the same as in the erythrocytes.

THE EFFECT OF HEMATOCRIT ON WHOLE BLOOD BORON CONCENTRATIONS

The effect of patient hematocrit on the peak whole blood boron concentration at the end of the infusion is presented in Fig. 6. The peak whole blood boron concentrations varied from 21.8 to 27.6 mg/L. The regression equation was: $y = -31.0x + 36.3$, where y represents whole blood boron concentration and x represents hematocrit ($r = -0.58$; $P < 0.05$; n = 12). A difference in hematocrit of 0.05 L/L corresponds to a difference of 2.05 mg/L in whole blood boron concentration. Maximal changes in hematocrit during infusion were within ±0.07 L/L in the 12

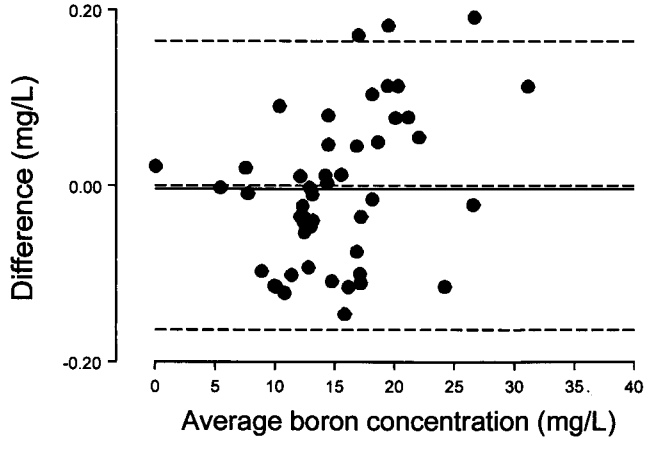

Fig. 2. Bland-Altman comparison plot of ICP-AES boron concentrations determined simultaneously at two different wavelengths (249.772 and 182.578 nm).

The *upper* and *lower dashed lines* indicate 95% confidence limits (1.96 SD) *(13)*. The *solid line* represents the mean difference between the determinations carried out at the two wavelengths.

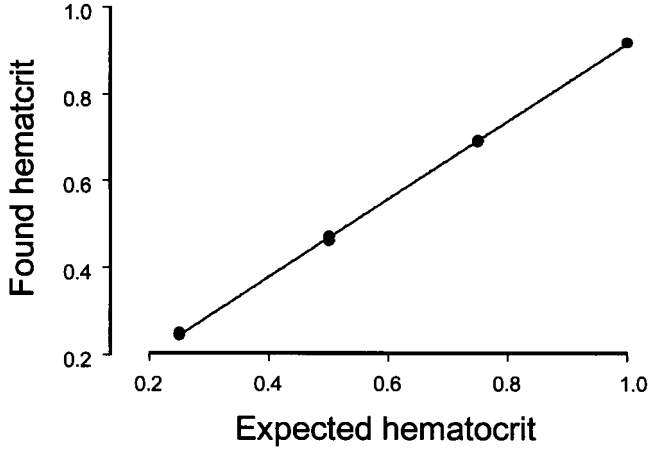

Fig. 3. Comparison of the hematocrit in whole blood samples with hematocrit values determined by measuring plasma and whole blood calcium concentrations.

Whole blood samples were prepared from erythrocyte suspension and plasma. Three samples were prepared and measured for each hematocrit value (see *Materials and Methods* for equation). The regression equation is: $y = 0.973x + 0.023$ ($r = 0.9998$).

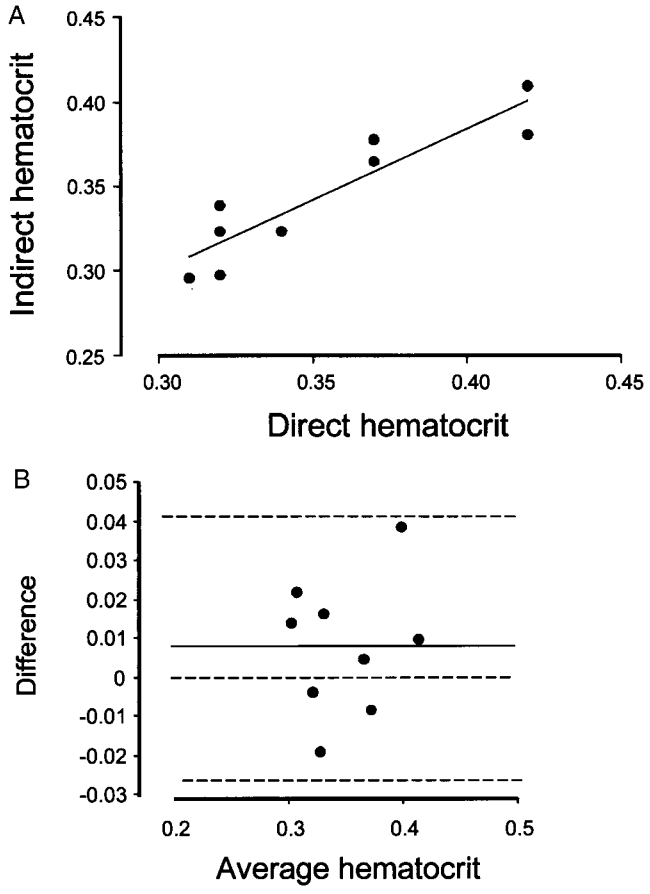

Fig. 4. Correlation of the hematocrit determined directly by Coulter Counter and indirectly by determining calcium concentrations in whole blood and plasma (*A*) and Bland-Altman comparison plot of the direct and indirect hematocrit determinations (*B*).

(*A*), regression equation was as follows: $y = 0.8452x + 0.046$ ($r = 0.92$; $P < 0.001$). (*B*), the *upper* and *lower dashed lines* indicate 95% confidence limits (1.96 SD). The *solid line* represents the mean difference between the determinations.

patients. The specific gravity of the preinfusion whole blood samples was 1.046 ± 0.004 kg/L.

Discussion

The current fast determination method of boron by ICP-AES was developed to be used in conjunction with BNCT treatments with BPA-F as the boron carrier. With the method presented here, the whole blood, erythrocyte, and plasma boron concentrations are available within minutes. The intra- and interassay imprecisions achieved were <5%. This compares favorably with the 5% intra-assay imprecision reported for prompt-γ *(14)*.

Although ICP-MS and prompt-γ can differentiate between the two isotopes of natural boron: ^{10}B (≈20%) and ^{11}B (≈80%), and ICP-AES cannot, this difference is not important for clinical BNCT. Although only ^{10}B participates substantially in the neutron-capture reaction, the direct determination of ^{10}B is not necessary because the boron carriers for BNCT are ^{10}B-enriched (≈99%). The background endogenous boron concentration has no

Table 4. Imprecision of hematocrit in whole blood samples by ICP-AES.

	Between-day imprecision			Total imprecision		
Hct,[a] *L/L*	S_w	CV_w, %	Days, n	S_t	CV_t, %	N_t
0.23	0.9	3.9	7	1.52	6.5	14
0.33	0.0	0.0	7	1.51	4.6	14
0.46	0.0	0.0	7	0.74	1.6	14

[a] Hct, hematocrit; S_w, between-day component SD; CV_w, between-day component CV; S_t, total SD; CV_t, total CV; N_t, total number of determinations.

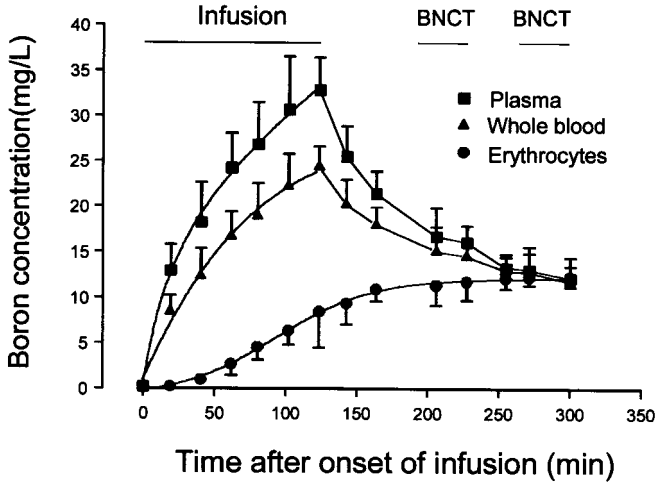

Fig. 5. Mean concentration-time profile for total boron in plasma, whole blood, and erythrocytes of 11 patients who received BPA-F (290 mg/kg) as a 2-h intravenous infusion.

Some data points represent measurements from 10 patients because samples from all data points were not available.

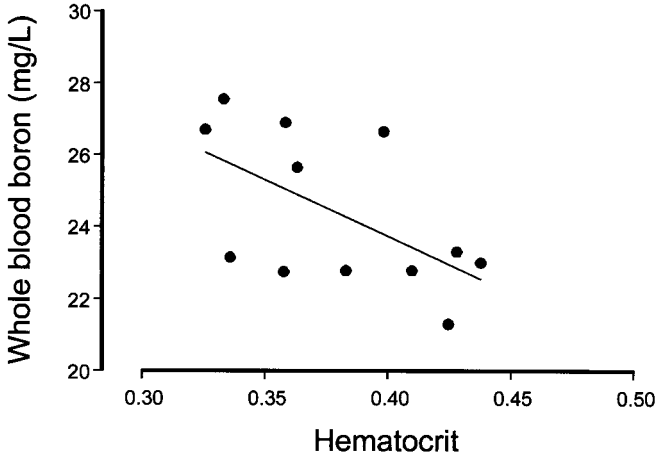

Fig. 6. Correlation ($r = -0.58$; $P < 0.05$; n = 11) of patient hematocrit with whole blood boron concentrations at the end of the 2-h infusion of BPA-F (290 mg/kg).

Regression equation: $y = -31.0x + 36.3$, where y is the whole blood boron concentration and x is the hematocrit.

practical significance because it is only 4–26 μg/L in serum *(15)* and 30–90 μg/L in whole blood *(16)*. The background boron concentration is thus ~500–1000 times lower than the total boron concentration during the BNCT irradiation.

ICP-AES is superior to ICP-MS for trace boron determinations with respect to the length of analysis, memory and matrix effects, linearity, and the cost of the instrument *(4, 5)*. Protein removal by trichloroacetic acid may also be used with ICP-MS. Because of matrix effects, ICP-MS requires an additional dilution step after protein precipitation. Potential spectral interferences are a disadvantage for boron determinations with ICP-AES. The most prominent of these is iron, the concentration of which in whole blood samples may reach 15 mmol/L. More than 99% of the iron in whole blood is tightly bound to hemoglobin. The potential spectral interference of iron is avoided in our method because hemoglobin is precipitated along with other proteins. The residual iron concentrations were undetectable in the supernatant.

We found a mean peak concentration of 24.4 ± 2.3 mg/L (n = 12) whole blood boron at the end of the infusion. This is in agreement with the 26 ± 2.3 mg/L (n = 8) concentration reported previously by Chanana et al. *(17)* in an identical experimental setting. Their value is a mean of the results of two methods: prompt-γ and direct current AES. In the present study, we report a comparison between ICP-AES and ICP-MS methods. These results revealed a close agreement between the two methods. To obtain a large range ratio of the boron concentrations, samples derived from canine experiments were used for the intercomparison study. For a more comprehensive comparison, more data would have been required. Because the radiation dose estimates in BNCT are based on blood boron concentration, systematic intercalibration studies of boron measurements between different methods and different laboratories would be important. Because of a lack of suitable reference materials, the limited scope of clinical studies, and the high cost of BPA-F, no such studies have been carried out.

Boronated phenolic acid has the ability to bind tightly to glycated proteins *(18)*. Consequently, BPA and its boron-containing metabolites may tightly bind to glycated proteins in plasma and whole blood samples in addition to nonspecific binding. Analytical recoveries obtained by supplementing samples with different concentrations of BPA-F were 96–104%. Thus, it seems that complexing BPA with fructose impedes the formation of stable BPA-protein complexes, which might be precipitated along with other proteins in the ICP-AES sample pretreatment procedure. The acidic milieu we used for protein removal may also break possible BPA-protein complexes because uncomplexed BPA is highly soluble at low pH. We compared our method involving protein precipitation with a method involving wet ashing. The latter method determines total boron concentrations regardless of the chemical speciation and protein binding. Because the differences between results obtained by either method were rather small, the results indicate that the tightly bound BPA fraction is also very small.

BPA-F enters into the plasma fraction during infusion. Our preliminary results indicate that >90% of plasma boron is unbound 1 h after the end of infusion. This free fraction is then circulated and subsequently actively and passively taken up into tissues, whereas the boron in erythrocytes is most likely not free to diffuse or be transported to other tissues. Therefore, one of the most obvious starting points to estimate boron biodistribution after BPA-F infusion would have been determination of BPA itself and possibly its metabolites in plasma. BPA may be determined by HPLC as a fluorescent derivative *(19)*, but the method is too time-consuming for BNCT.

Whole blood samples have been used for boron determination in BNCT to avoid the time requirement for plasma separation. Furthermore, the use of whole blood boron determinations for this purpose is supported by positron emission tomography studies using ^{18}F-labeled BPA. These studies have suggested that the ratio of plasma to whole blood boron concentration of BPA is constant and close to 1.3 during and after the infusion of BPA-F *(20)*. The experimental setting in these positron emission tomography studies is, however, quite different from the clinical studies. The dose in positron emission tomography studies is minute, and the whole amount is usually given as single, bolus injection. In the present study, we have shown that boron, given as BPA-F, is slowly taken up by the erythrocytes, leading to an uneven distribution between erythrocytes and plasma. Therefore, at the BPA-F doses administered in BNCT, the hematocrit of the patient is a major preanalytical confounding variable in boron determinations in whole blood. As a consequence, different irradiation doses have been given according to patient hematocrit, which is different, for example, in men and women. Our data revealed that the hematocrit may cause up to 30% variation in the peak boron concentrations in whole blood at the end of infusion. Erythrocyte phenylalanine is not available for transport through the blood-brain barrier *(21)*. Because BPA presumably behaves like phenylalanine, the same is likely true for erythrocyte BPA.

The possibility of monitoring hematocrit simultaneously with boron concentrations is one of the advantages of the present method. Because the BNCT facility is far from our hospital laboratory, like most BNCT treatment facilities are, we sought an alternative method for hematocrit determination, although we did not intend to replace conventional hematocrit determination methods. Our method permits monitoring of infusion-induced changes in the hematocrit during BNCT in the same samples used for boron determinations. It also provides hematocrit data for indirect determination of erythrocyte boron. An indirect determination of erythrocyte phenylalanine concentrations in a similar manner has been

shown to give more accurate results than direct determinations from erythrocytes *(22)*.

The widespread practice of expressing whole blood boron concentrations as mg/kg instead of mg/L or mmol/L among BNCT groups in previous studies hinders the use of the hematocrit to improve accuracy of the tissue boron estimations. Furthermore, this unit expression introduces the tedious step of weighing samples in these urgent analyses. Gray, the radiation dose unit, is defined as J/kg. This may be one of the reasons to use the mg/kg unit. The density of whole blood, however, is not necessarily the same as the density of the irradiated target tissue.

To improve boron accumulation in tumors, the use of disodium mercaptoundecahydro-*closo*-dodecaborate in conjunction with BPA has been suggested *(23)*. Disodium mercaptoundecahydro-*closo*-dodecaborate is a thiol-containing molecule and has a strong affinity for plasma proteins. The biodistribution and pharmacokinetic properties of disodium mercaptoundecahydro-*closo*-dodecaborate are different from those of BPA-F, necessitating the differentiation of the chemical species. In such a case, mere total boron determinations would not be valid for BNCT radiation estimates. More advanced methods using extraction procedures before ICP-AES would, therefore, be essential. Even so, ICP-AES will provide a useful, selective, and accurate means for future BNCT quantification of boron-containing compounds for complicated pharmacologic settings that involve several simultaneous boron-carrier drugs.

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