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Dose-dependent disposition kinetics and tissue accumulation of boron after intravenous injections of sodium mercaptoundecahydrododecaborate in rabbits

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Summary. Kinetics of boron disposition after single intravenous injections of two different doses (25 and 50 mg/kg) of mercaptoundecahydrododecaborate sodium ($Na_2B_{12}H_{11}SH$; BSH) was studied in rabbits. Residual boron concentrations in various organs and tissues (heart, lungs, liver, spleen, kidney, adrenals, and brain) were also determined after seven daily injections of the same doses of BSH. Boron blood and tissue concentrations were measured by atomic emission spectrometry. In the majority of animals, the decline of boron blood concentrations after a single intravenous injection of either dose was biphasic, being consistent with a two-compartment model of boron disposition in the body. Although mean boron blood concentrations were roughly proportional to the BSH dose delivered, the mean total body clearance of boron from the body was 3 times lower (6.5 ± 1.9 ml min^{-1} kg^{-1}) after a dose of 50 mg/kg than after the injection of 25 mg/kg (22.4 ± 7.9 ml min^{-1} kg^{-1}), the difference between the means being statistically significant ($P < 0.05$). Moreover, the mean terminal half-life of boron in blood was prolonged after the injection of 50 mg/kg (14.5 ± 5.5 h) as compared with that found after the 25-mg/kg dose (3.5 ± 0.9 h). On the other hand, the different BSH doses did not result in marked differences in the mean values obtained for the volume parameters – the volume of the central compartment (1.3 ± 0.4 vs 1.3 ± 0.5 l kg^{-1}) and the volume of distribution at steady state (4.7 ± 1.3 vs 6.0 ± 4.0 l kg^{-1}) – both of which were high, indicating extensive binding of the compound not only in the blood but also in tissues. Residual concentrations of boron found after seven daily injections of both doses of BSH were highest in the kidneys, the difference in the mean values being relatively small (33.6 ± 6.1 vs 39.0 ± 10.7 µg/g tissue). In the majority of other organs (heart, lung, liver, spleen, brain, adrenals), the residual concentrations after a dose of 50 mg/kg were disproportionately higher than those measured after the injection of 25 mg/kg, and the mean values corresponded to the reduced total body clearance rather than to the increased BSH dose. The saturability of BSH binding to blood and tissue proteins is suggested as a possible explanation for the dose dependency of the total clearance of boron from the body and the accumulation of BSH in organs and tissues.

Introduction

There is considerable interest in boron neutron-capture therapy (BNCT) aimed at inhibiting the recurrence of malignant cerebral gliomas after their surgical treatment [1]. This therapy is based on the elimination of remaining tumor cells by alpha particles generated in situ by the following nuclear reaction:

$${}^{10}_{5}B + {}^{1}_{0}n = {}^{7}_{3}Li + {}^{4}_{2}He + 2.4\ MeV.$$

Such a reaction can be induced by a beam of low-energy neutrons that produces short-range radiation following its "capture" by a stable isotope of boron (boron 10). Sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$; BSH, Fig. 1) is the compound most frequently investigated as a potential agent for the transport of boron (${}^{10}B$) into tumor cells.

An atomic emission-spectroscopy method has recently been developed for the specific determination of small amounts of boron in biological material tested in samples of murine blood, brain, liver, and kidneys [10]. In the present study, this method was used to investigate the disposition kinetics and tissue accumulation of boron in rabbits in relation to the dose of BSH delivered by bolus intravenous injection.

Materials and methods

Sodium mercaptoundecahydrododecaborate was synthesized and characterized by thin-layer (TLC) and high-performance liquid chromatogra-

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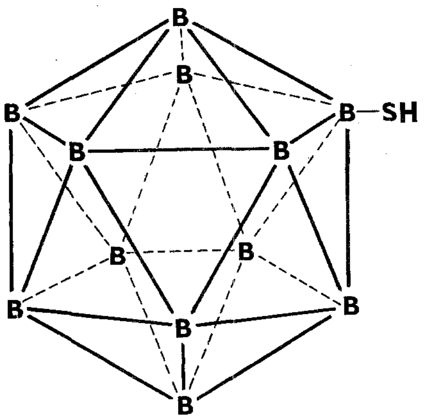

Fig. 1. Polyhedral structure of the BSH anion. A hydrogen atom is linked to each atom of boron except that bearing the -SH group

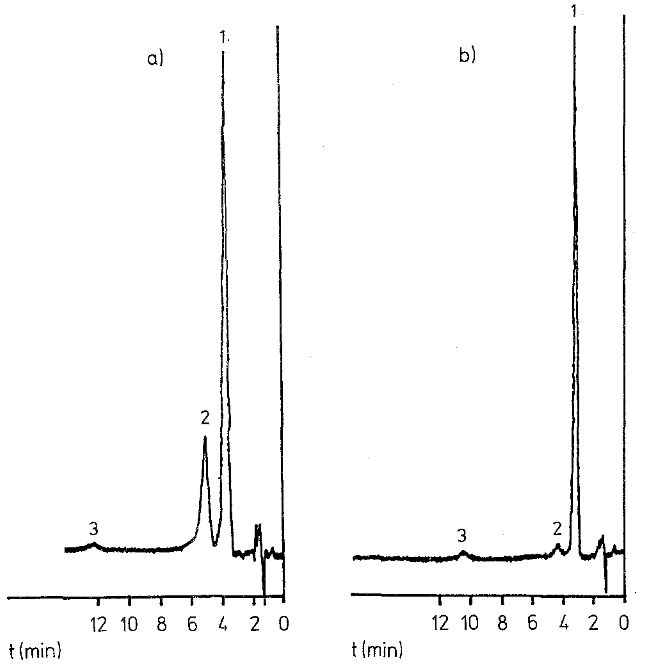

Fig. 2 a, b. Separation of borate anions by HPLC with UV detection. Elution conditions are described in Materials and methods. *1*, $(B_{12}H_{11}SH)^{2-}$; *2*, $(B_{24}H_{22}S_2)^{4-}$; *3*, $(B_{24}H_{22}S_2O)^{4-}$. **a** Standard BSH sample (0.25 mg/ml) containing 11% $(B_{24}H_{22}S_{22})^{4-}$ anions and <0.5% $(B_{24}H_{22}S_2O)^{4-}$ anions. The magnitude of UV absorption at 204 nm is approximately 2.5 times higher for oxidation products than for intact BSH. **b** Separation of the lyophilized BSH substance (0.3 mg/ml) used in the experimental study. The content of anions in the sample was 98.5% for $(B_{12}H_{11}SH)^{2-}$, 0.6% for $(B_{24}H_{22}S_2)^{4-}$, and 0.5% for $(B_{24}H_{22}S_2O)^{4-}$

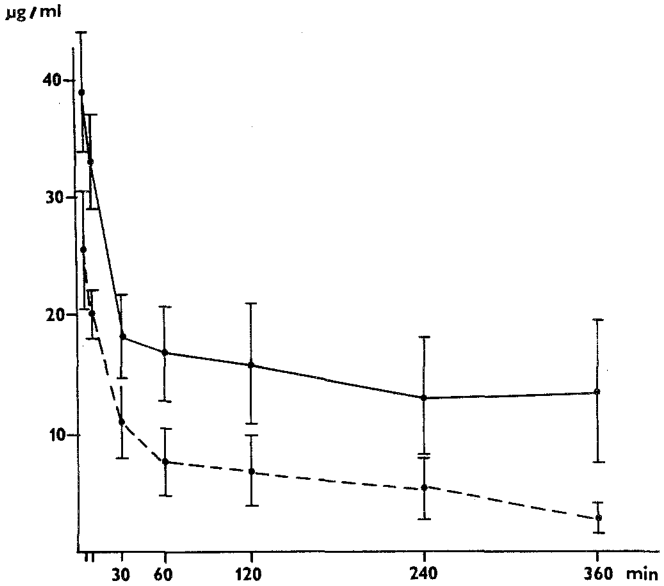

Fig. 3. Decline of boron concentration in the blood of rabbits after a single intravenous injection of 25 (*dashed line*) and 50 mg/kg (*solid line*) BSH. Mean values ± SEM are shown

phy (HPLC) and spectra ([^{11}B]-nuclear magnetic resonance (NMR) and UV) at the Institute of Inorganic Chemistry, Czechoslovak Academy of Sciences, Prague. The isotopic abundance of boron in the product was natural.

An injectable pharmaceutical formulation containing lyophilized BSH (Léčiva Pharmaceuticals, Prague) was used in the study. Quantitation of BSH in its ionic form [$(B_{12}H_{11}SH)^{2-}$] as well as of its oxidation products $(B_{24}H_{22}S_2)^{4-}$ and $(B_{24}H_{22}S_2O)^{4-}$ in the lyophilized material was carried out by HPLC using a column (CGC 150 × 3.3 mm) packed with the hydroxymethylmethacrylate sorbent Separon HEMA Bio 300 (12 µm; Tessek Ltd., Prague). The mobile phase consisted of 100 mM $NaClO_4$ in 0.01 M phosphate buffer. The flow rate of the eluent was set at 0.5 ml/min and the temperature, at 40° C. Borate anions were monitored by direct spectrophotometric UV detection at 204 nm (sensitivity, 0.16 AU; Fig. 2). The minimal concentrations detectable by this method were 2×10^{-8} mol/ml for $(B_{12}H_{11}SH)^{2-}$, 7×10^{-9} mol/ml for $(B_{24}H_{22}S_2)^{4-}$, and 1×10^{-9} mol/ml for (($B_{24}H_{22}S_2O)^{4-}$ [2]. In all, 100 mg BSH in the form of the lyophilized compound was dissolved in 0.4 ml saline immediately prior to its injection.

A total of 11 male Chinchilla rabbits weighing 1.5–1.8 kg were used; 7 received 50 mg/kg and 4 were given 25 mg/kg BSH injected as a bolus into the ear vein of each animal for 7 days. On the 1st day, blood samples (1.2 ml) were drawn from the contralateral ear vein before treatment and at 5, 10, 30, 60, 120, 240, and 360 min postinjection for pharmacokinetic analysis. Blood was heparinized (200 IU/ml) immediately after the samples had been taken. At 17 h after the last dose of BSH, all animals were killed and samples of brain, lungs, heart, liver, spleen, kidneys, and adrenals were excised for the determination of residual boron in these tissues.

The emmision spectrograph used for boron determination was a Zeiss Q-24 model. The spectra were recorded by the photographic method using ORWO-WU2 UV-sensitive plates and germanium in the form of GeO_2 (Fluka, 99.99% purity) as an internal standard. The respective regions of the spectra recorded in the vicinity of the boron and germanium lines were transformed into spectra using a Zeiss Model II photometer connected with a TZ 4200 line recorder (Laboratory Instruments, Prague). The graphite electrodes (type SU 302; Electrocarbon, Topolčany, Czechoslovakia) used in the analyses gave zero blank values for boron.

A sample of $2-4 \times 10^{-2}$ g tissue was placed on the graphite electrode and weighed quickly at an accuracy of within 10^{-5} g. After drying and ashing of the sample, an aqueous solution of the internal standard (1×10^{-6} g Ge) was added and the mixture was analyzed in an electric ac arc for 164 s. The duration of the analyses proved to be sufficient for atomization of the entire sample. After standard photographic processing and recording of the spectra, the heights of the corresponding peaks were measured at 249.678 nm for boron and 265.118 nm for germanium. The calibration curves were linear within the range of 0.05–7 µg boron, within which the error of boron estimation amounted to about 15%.

The pharmacokinetic analysis of BSH blood-concentration data was performed under the assumption of the two- or one-compartment pharmacokinetic model of BSH disposition in the body. The parameters of both models were estimated by nonlinear regression analysis programmed for an HP 86B desk computer.

Table 1. Pharmacokinetic parameters of boron disposition in rabbits after the intravenous injection of 25 mg/kg BSH

Rabbit number	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	V_1 (l kg^{-1})	V_{dss} l kg^{-1})	C (ml min^{-1} kg^{-1})	AUC (mg h l^{-1})
1	9.2	3.4	0.9	4.2	19.1	21.8
2	7.6	1.0	0.6	1.3	26.1	16.0
3	185.4	5.1	2.7	17.7	41.3	100.8
4	109.8	4.4	0.1	1.1	3.1	132.4
Mean	78.0	3.5	1.3	6.0	22.4	67.8
SEM	43.0	0.9	0.5	4.0	7.9	29.0
CV (%)	110.3	51.5	75.3	130.0	70.8	85.5

Table 2. Pharmacokinetic parameters of boron disposition in rabbits after the intravenous injection of 50 mg/kg BSH

Rabbit number	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	V_1 (l kg^{-1})	V_{dss} (l kg^{-1})	C (ml min^{-1} kg^{-1})	AUC (mg h l^{-1})
1	15.4	46.1	3.3	11.6	3.0	281.2
2	0.5	6.7	0.8	3.0	5.2	160.7
3	5.0	15.8	0.8	2.1	1.5	548.3
4	16.6	2.0	0.9	2.0	15.4	54.2
5	18.0	5.6	1.0	3.9	10.2	81.7
6	6.8	13.3	0.8	3.1	2.8	300.0
7	–	11.8	–	7.4	7.3	114.3
Mean	10.4	14.5	1.3	4.7	6.5	220.0
SEM	3.0	5.5	0.4	1.3	1.9	65.3
CV (%)	69.6	102.0	78.9	74.8	76.1	78.5

Table 3. Residual boron concentrations found in rabbit tissues at 17 h after the termination of 7 days' administration of 25 mg/kg BSH

	Boron concentration (µg/g tissue)						
Rabbit number	Brain	Heart	Lungs	Liver	Spleen	Kidney	Adrenals
1	<0.3	0.9	1.1	0.6	1.7	36.9	3.1
2	<0.3	1.1	1.3	1.6	1.4	22.2	3.7
3	<0.3	2.9	3.9	2.1	3.9	49.4	6.2
4	<0.3	2.5	8.4	2.1	4.2	26.0	9.8
Mean		1.9	3.7	1.6	2.8	33.6	5.7
SEM		0.5	1.7	0.4	0.7	6.1	1.5
CV (%)		53.0	92.5	44.1	51.9	36.3	53.3

Table 4. Residual boron concentrations found in rabbit tissues at 17 h after the termination of 7 days' administration of 50 mg/kg BSH

	Boron concentration (µg/g tissue)						
Rabbit number	Brain	Heart	Lungs	Liver	Spleen	Kidney	Adrenals
1	0.6	16.7	17.5	13.8	24.9	31.7	6.5
2	–	14.1	7.3	7.7	4.6	18.2	4.3
3	0.8	22.8	18.6	35.0	21.9	59.8	24.3
4	0.3	3.5	7.9	14.4	2.7	39.2	3.2
5	0.3	2.8	6.3	7.0	2.4	19.5	2.8
6	0.6	26.5	40.6	43.0	17.2	92.2	48.0
7	–	2.0	5.3	10.5	1.7	12.9	8.3
Mean	0.5	12.6	14.8	18.8	10.8	39.0	13.9
SEM	0.1	3.8	4.8	5.4	3.8	10.7	6.3
CV (%)	41.7	79.7	85.3	76.0	94.4	72.5	120.5

Results

A comparison of the mean boron concentrations in rabbit blood after a single intravenous bolus injection of two different doses (25 and 50 mg/kg) of BSH is illustrated in Fig. 3. It is evident that doubling of the BSH dose led on average to a 2-fold increase in the boron concentration in the blood; on correction of the estimated concentrations for the dose of BSH injected, no statistically significant difference was detected between the corresponding mean concentrations.

The typical biphasic decline of boron blood concentration, with a rapid distribution phase and a slower elimination phase being consistent with the two-compartment model of BSH disposition in the body, was observed in all rabbits after the injection of the lower dose of BSH. The same type of time-concentration curve was also detected in the majority of animals receiving the higher dose of the compound, with the exception of one rabbit exhibiting an evidently monoexponential decrease in boron concentration.

Individual as well as mean values for pharmacokinetic parameters evaluated from the levels of boron in plasma after intravenous bolus injection of 25 and 50 mg/kg BSH in rabbits are summarized in Tables 1 and 2, respectively. It is evident that a 3-fold decrease in the total clearance of boron occurred when the BSH dose was doubled, the difference between the mean values for the two groups being statistically significant ($P < 0.05$). On the other hand, similar differences in the mean values for most of the other parameters of boron disposition could not be regarded as being statistically significant because of the relatively large interindividual variation manifested by the values for the corresponding coefficients of variation.

The residual boron concentrations found in rabbit tissues at 17 h after the termination of short-term administration (7 days) of 25 and 50 mg/kg BSH are presented in Tables 3 and 4, respectively. The highest residual levels after the administration of both doses were detected in the kidneys and the lowest were measured in the brain; in the latter tissue, the values found after the injection of the lower dose lay under the detection limit of the assay. In a comparison of the residual level of boron found in the same type of tissue after the two doses, it appeared that the ratio corresponded to the dose ratio only in the adrenals. In the majority of tissues examined, the ratio of residual boron after the higher dose to that following the lower dose was at least 3, whereas the difference in residual concentrations in the kidneys was very small (only 16% on average). Nevertheless, the large interindividual variability in residual concentrations prevented the detection of statistically significant differences except in the liver, for which a ratio of about 10 was found.

Discussion

In addition to the accumulation of information on the distribution of boron in various tumors and tissues [3, 6, 7], the prospective use of BSH in neutron-capture therapy of tumors requires a detailed knowledge on the kinetics of boron disposition in the body. In the present study we extended our preliminary data on the pharmacokinetic parameters of boron after intravenous injection of BSH in rabbits [10] using an injectable lyophilized formulation of this compound. From the values obtained for the terminal half-lives in this study, we could deduce that despite considerable interindividual variation, the persistence of boron in the body of rabbits was relatively high in the majority of animals. However, even larger terminal half-life values (95–106 h) have been found in patients presenting with brain tumors who have received BSH at doses ranging up 34 mg/kg [11]. The relatively slow elimination of boron from the body of rabbits was further confirmed by the residual concentrations that were found in various organs and tissues at 17 h after the last injection of BSH.

The high values found for the volume of the central compartment as well as for the volume of distribution at steady state suggest that the relatively slow elimination of boron from the body might be attributable to extensive binding of BSH not only in the blood but also in tissues. It is known that BSH avidly binds to bovine and human serum albumin, largely by the formation of a disulfide linkage between the boron "cage" and the protein [4, 5, 8]. The presence of boronated proteins following the administration of BSH has also been demonstrated in neoplastic cells [9].

In terms of tissue accumulation, it is noteworthy that residual boron concentrations measured in the kidneys after the termination of BSH administration highly exceeded those found in other organs. Since a boron content of 60%–80% of the delivered dose of BSH was excreted in the urine within 2 days after BSH infusion in patients presenting with brain tumors [11], our finding can be regarded to reflect that the kidney is most probably the main organ responsible for the elimination of BSH from the body in the rabbit as well. In this context, it is noteworthy that definite nephrotoxic lesions, which will be described in detail elsewhere, were observed in the kidneys of the majority of rabbits independently of the dose of BSH given repeatedly for 7 days.

The comparison of pharmacokinetic parameters derived from the analysis of individual curves constructed for boron disappearance from plasma also revealed that the clearance of boron from the body of rabbits was not constant but depended on the dose of BSH that was injected. Saturability of some processes involved in BSH elimination or distribution might offer a plausible explanation for this dose dependency. The finding that doubling of the dose of BSH resulted in only a slight increase in the residual boron concentration in the kidney relative to other organs suggests that BSH binding in this organ was previously near saturation. Therefore, it is not surprising that the incidence and extent of nephrotoxicity was almost the same after the two different doses of BSH.

It is known that the kinetics and the tissue distribution of the disulfide form of BSH ($Na_2B_{24}H_{22}S_2$; BS-SB) in glioma-bearing rats exhibit a higher degree of boron penetration and a slower rate of decrease in tumor tissue as compared with BSH [3]. It could thus be speculated that the presence of a small amount of BS-SB in the lyophilized material could to some extent contribute to a more extensive distribution of boron in and to its slower elimination from the bodies of rabbits. However, the proportion of the oxidized products in the formulation was too small (1.1%) to change the overall features of boron disposition after intravenous injection of the lyophilized BSH material as described in this report.

References

1. Dewitt L, Moss R, Gabel D (1990) New development in neutron capture therapy. Eur J Cancer 8: 912–914
2. Grüner B, Plzák J, Vinš I (1991) Purity assay of closo-$Na_2B_{12}H_{11}SH$. J Chromatogr (in press)
3. Joel D, Slatkin DM, Fairchild RG, Micca PL, Nawrocky M (1989) Pharmacokinetics and tissue distribution of the sulfhydrylboranes (monomer and dimer) in glioma bearing rats. Strahlenther Onkol 165: 167–170
4. Nakagawa T, Nagai T (1976) Interaction between serum albumin and mercaptoundecahydrododecaborate ion (an agent for boron-neutron capture therapy of brain tumor): I. Introductory remarks and preliminary experiments. Chem Pharm Bull (Tokyo) 24: 2934–2941
5. Nakagawa T, Nagai T (1976) Interaction between serum albumin and mercaptoundecahydrododecaborate ion (an agent for boron-neutron capture therapy of brain tumor): III. Results of analysis. Chem Pharm Bull (Tokyo) 24: 2949–2954
6. Slatkin DN, Micca PL, Laster BH, Fairchild RG (1986) Distribution of sulfhydryl boranes in mice and rats. In: Fairchild RG, Bond VP (eds) Proceedings, Workshop on Neutron Capture Therapy. BNL-51994, Brookhaven National Laboratory, Upton, New Zealand, pp 173–177
7. Slatkin DN, Micca PL, Forman A, Gabel DH, Wielopolski L, Fairchild RG (1986) Boron uptake in melanoma, cerebrum and blood from $Na_2B_{12}H_{11}SH$ and $Na_4B_{24}H_{22}S_2$ administered to mice. Biochem Pharmacol 35: 1771–1776
8. Soloway AH, Hatanaka H, Davis MA (1967) Penetration of brain and brain tumor: VII. Tumor-binding sulfhydryl boron compounds. J Med Chem 10: 714–717
9. Soloway AH, Alam F, Barth RF, Bapat BV (1989) Boron chemistry and target cell affinity. Strahlenther Onkol 165: 118–120
10. Štrouf O, Mertenová E, Schneiderová L, Zámečníková H, Janků I (1989) Boron determination for neutron capture therapy by colorimetry and emission spectrometry. Strahlenther Onkol 165: 174–176
11. Sweet WH, Messer JR, Hatanaka H (1986) Supplementary pharmacological study between 1972 and 1977 on purified mercaptoundecahydrododecaborate. In: Hatanaka H (ed) Boron neutron capture therapy for tumors. Nishimura Co. Ltd, Niigata, pp 59–76