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Kidney Function Changes in Rats After Single-Dose Administration of Borocaptate Sodium

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Summary

Kidney function changes after single-dose administration of borocaptate sodium (mercaptoundecahydro-closo-dodecaborate, $B_{12}H_{11}SH$, BSH) were studied in rats. Changes of glomerular filtration rate (GFR) measured as ^{14}C-inulin clearance, renal plasma flow rate (^{3}H-p-aminohippuric acid clearance) and urine flow rate (UFR) after a slow intravenous injection of BSH (25 mg/kg b.w.) were investigated in rats under pentobarbital anaesthesia. It was found that a slow BSH injection induces a gradual decrease of renal plasma flow and glomerular filtration rate resulting in an almost constant reduction of the filtration fraction. These alterations were accompanied by a temporary increase of urine flow rate. Although a direct effect of BSH on the nephron cannot be excluded, it is suggested that the observed changes in kidney function might at least partly be mediated by disturbances in the function of the cardiovascular system following BSH injection. The role of the dianionic sulfhydryl group present in the borocaptate molecule in inducing these renal functional changes is discussed.

Key words

Borocaptate sodium – Boron neutron capture therapy – Glomerular filtration rate – Effective renal plasma flow – Urine flow – Filtration fraction

Introduction

Borocaptate sodium is the generic name for the disodium salt of mercaptoundecahydro-closo-dodecaborate ($B_{12}H_{11}SH$, BSH), one of the most frequently investigated boron delivery agents for prospective use in boron neutron capture therapy (BNCT) of intracranial tumours. In this therapeutic procedure (Dewit *et al.* 1990a) BSH enriched by the stable boron-10 isotope is aimed at the malignant tissue where a nuclear fission reaction is activated *in situ* by an incident beam of slow, i.e. low energy neutrons (thermal or epithermal). The short range cytotoxic alfa-radiation carrying 2.79 x 10^6 eV of energy released by the *in situ* occurring nuclear reaction is then responsible for the death of tumour cells.

In a previously performed preclinical study of BSH short-term toxicity in rabbits (Janků *et al.* 1993) severe nephrotoxic lesions were detected after repeated seven daily injections of BSH in doses 25 and 50 mg/kg b.w. Subsequently, measurements of inulin clearance and of the volume of urine excreted revealed that even single-dose administration of BSH, as was suggested for clinical use, might induce reversible kidney function changes in rats (Horn *et al.* 1996). In our opinion, the presence of the strongly nucleophilic dianionic sulfhydryl group in the molecule of borocaptate was responsible for the diuretic and GFR reducing effects. However, a suspicion was raised (Gabel, personal communication) that these effects might rather be due to some impurities (1.1 % of dimerized oxidation products of borocaptate) contained in the lyophilized BSH preparation (Janků *et al.* 1993) not employed by other investigators. In order to throw more light on the question whether the effects on kidney function are mediated by sulfhydryl groups of the borocaptate molecule or rather by the presence of a slightly higher percentage of oxidation products of borocaptate, we decided to repeat the rat experiments using a BSH preparation more commonly in use (Centronic Ltd., England), in which the presence of less than 0.5 % of the oxidation products is guaranted by the manufacturer. Since it was known from preclinical experiments (Slánský, unpublished results) that an

intravenous injection of BSH may provoke changes of blood pressure in anaesthetized rats, renal plasma flow (RPF) alterations were also investigated by measuring the clearance of p-aminohippuric acid (PAH) in addition to the glomerular filtration rate (GFR) and urine flow rate (UFR) measurements.

Methods

Borocaptate sodium was a product of Centronic Ltd., England. ^{14}C-inulin (4 MBq/ml) was purchased from UVVR (Prague, CR), unlabelled inulin (Inutest ^{TM}) from Laevosan G.m.b.H. (Linz, Austria). ^{3}H-para-aminohippuric acid with a specific activity 165.39 GBq/mmol was obtained from New England Nuclear, unlabelled para-aminohippuric acid (Nephrotes ^{TM}) from BAG G.m.b.H. (Lich/Hessen, Germany). All other chemicals were of reagent grade.

Male Wistar rats, weighing 240–400 g, were anaesthetized with sodium pentobarbital (60 mg/kg b.w., i.p.). The left carotid artery was cannulated for arterial blood sampling, the right jugular vein was cannulated for simultaneous intravenous infusion of ^{14}C-inulin and of ^{3}H-para-aminohippuric acid. Sodium heparin (500 U) was administered *via* the tail vein to prevent blood coagulation in the catheter. In order to facilitate the collection of urine samples, the left ureter was cannulated after performing a midline abdominal incision. To maintain a sufficient and constant urine flow rate, the rats received 5 % mannitol in saline infusion containing 10 % inulin. After the priming dose of inulin given during 10 min at a rate of 100 μl/min, a maintenance infusion at a constant rate of 20 μl/min continued throughout the experiment. An equilibration period of 60 min was allowed for reaching the plateau level. Both the blood and urine samples were collected in two 20-min lasting sampling periods to estimate the basal GFR, RPF and UFR values. Then a slow (5 min) injection of BSH (25 mg/kg b.w.) was delivered into the same catheter as the permanent infusion of inulin, p-aminohippuric acid and mannitol was given. Samples of blood and urine were collected in 5 additional sampling periods in order to estimate the possible changes of GFR, RPF and UFR due to BSH infusion. The urine volume was calculated from the gain in weight of the tube (Eppendorf, Germany) into which urine samples were collected. The control group of animals received saline instead of BSH. The body temperature of the rats was maintained by radiant heat and a heat-controlled table.

Radioactivities of ^{14}C-inulin as well as of ^{3}H-para-aminohippuric acid in the blood and urine were measured by a liquid scintillation counter. The counts were corrected using appropriate standards.

The clearances of both compounds were calculated according to the usual formula: $CL = U.V/P$ where U represents the urine concentration of the compound, P its plasma concentration and V is the volume of urine excreted per unit of time. Inulin clearance and PAH clearance were taken as measures of the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. Simultaneous measurement of GFR and ERPF enabled us to evaluate the changes of the filtration fraction (FF) using the formula: $FF = GFR/ERPF$.

For all the data, geometric means with 95 % confidence limits were calculated. Intra-group comparisons were performed using the paired t-test. Intergroup comparisons were carried out using two-way analysis of variance. Differences were considered significant when $p < 0.05$.

Table 1. Inulin clearance after BSH (Centronic) injection in rats (changes in % of pretreatment values)

Minutes after injection	10	30	50	70	90
Control (saline)	83.32	84.51	90.19	94.34	72.67
n = 4	66.80–103.80	41.00–174.21	41.31–196.90	39.58–224.84	23.94–220.74
BSH 25 mg/kg	90.47	67.45	57.09*	46.30*	35.57 *
n = 5	74.29–110.18	35.32–128.81	28.40–114.78	21.29–100.68	13.17–96.09

* *statistically significant reduction versus mean pretreatment value*

Results

The initial average pretreatment values of ^{14}C-inulin and of ^{3}H-PAH clearances were 1.7±0.11 ml/min/kg b.w. and 8.6±1.2 ml/min/kg b.w., respectively, whereas the average pretreatment filtration fraction was 0.25±0.03. The average pretreatment value of urine flow amounted to

9.86±0.72 μl/min. The percentual changes of all these parameters following a slow injection of BSH are summarized in Tables 1–4. It is evident from Table 1 that in all post-infusion sampling periods the injection of BSH reduced GFR. It can also be seen that the reduction of GFR progressively increased in time, becoming statistically significant 50 min after slow injection of BSH. A progressive reduction in time can also be observed for PAH clearance (Table 2) which, however, is already statistically significant in the second, and later on in the fourth and fifth post-infusion sampling periods. As is shown by the data in Table 3, the filtration fraction is reduced and nearly constant after BSH infusion. The reduction is, however, statistically significant only during the first post-infusion sampling interval. The data of Table 4 indicate that urine flow rate was elevated after a slow BSH injection, the increase being statistically significant only in the first post-infusion sampling period.

Table 2. Sodium para-aminohippurate clearance after BSH (Centronic) injection in rats (changes in % of pretreatment values)

Minutes after injection	10	30	50	70	90
Control (saline)	83.97	82.94	82.42	91.26	65.62
n=4	66.31–106.35	50.60–135.95	41.80–162.52	42.36–196.97	26.54–162.27
BSH 25 mg/kg	105.46	63.16 *	59.21	46.91 *	37.32 *
n=5	85.38–130.28	40.60–98.27	32.26–108.67	23.61–93.18	16.60–83.88

* *statistically significant reduction versus mean pretreatment value*

Table 3. Filtration fraction after BSH (Centronic) injection in rats (changes in % of pretreatment values)

Minutes after injection	10	30	50	70	90
Control (saline)	104.8	105.0	107.0	110.0	116.0
n=4	79.3–138.3	64.1–172.3	67.8–167.5	68.8–176.6	69.3–194.1
BSH 25 mg/kg	76.5 *	83.6	79.8	78.6	77.0
n=5	59.6– 98.1	53.7–130.1	53.0–120.2	51.5–119.8	48.8–121.9

* *statistically significant increase versus mean pretreatment value*

Table 4. Urine flow rate after BSH (Centronic) injection in rats (changes in % of pretreatment values)

Minutes after injection	10	30	50	70	90
Control (saline)	94.3	104.4	109.6	97.2	112.2
n=4	67.8–131.1	69.4–159.2	72.9–164.7	63.8–148.0	57.9–212.4
BSH 25 mg/kg	147.6 *	118.2	98.1	78.1	67.7
n=5	109.9–198.2	81.0–172.5	66.1–136.6	53.6–113.9	37.5–122.4

* *statistically significant increase versus mean pretreatment value*

Discussion

The results of the present animal experiments as well as of monitoring of kidney functions in patients with brain tumours (Horn *et al.* 1996) clearly indicate that even a single infusion of borocaptate, as is proposed for BNCT, can induce some alterations in the function of a normal kidney. This was expressed more clearly in experiments on rats. There may be two reasons for this species difference. First, the rate of BSH infusion in rats was greater as compared to that which was used in the clinical study, secondly, the sensitivity is different in the two species. La Hann *et al.* (1996a) have shown that the human 89 mg/kg BSH dose is best approximated by 125 mg/kg in rats. The present findings demonstrate that the alterations of kidney function are independent of the origin of borocaptate as well as of the content of its oxidized products in the preparation. The results of the short-term toxicity study in rabbits (Janků *et al.* 1993) also showed that monitoring of kidney functions should become more urgent if, as was proposed by Dewit *et al.* (1990b) and Gabel (1992), BSH infusions were to be repeated with the aim of increasing the efficacy of BNCT.

It should be pointed out, however, that the mechanism of the observed alterations of kidney function after BSH administration is not fully clear at present. At the organ level, the prevailing rise in post-infusion renal plasma flow of patients with malignant brain tumours (Horn *et al.* 1996) might contribute to the increasing excretory activity of the kidney. Although one cannot exclude a direct effect of BSH on the nephron, it seems that the kidney function changes in the rat are at least partly mediated by disturbances in the function of the cardiovascular system as described by La Hann *et al.* (1996b). According to these authors, the initial blood pressure elevation due to a positive inotropic effect on the heart and a massive increase of peripheral resistance resulting in a significant redistribution of peripheral blood flow is followed by hypotension.

On the other hand, it would be difficult to connect the observed kidney function changes at the molecular level with the structure of the "borohydride cage" itself. It is, however, more probable that the presence of the highly nucleophilic dianionic -SH group might be responsible for the effects described by the reaction with electron-deficient sites of biological macromolecules, namely proteins. Such a view may be supported by the several fold higher toxicity of borocaptate in comparison with the toxicity of the simple dodecaborohydride $B_{12}H_{12}^{2-}$-anion (Soloway *et al.* 1967). It is known that BSH binds to albumin either by forming covalent (Nakagawa and Nagai 1976) or electrostatic bonds (Zhu-Tang *et al.* 1995). According to the experience of our laboratory, borocaptate exerts some effects on structural protein sulfhydryl groups of several rat organs reducing the fraction of free sulfhydryl groups especially in the kidneys and their subcellular fractions (Buchar *et al.* 1994). It should also not be overlooked that the kidney represents the main excretory organ for borocaptate from the body (Sweet *et al.* 1986) and that the greatest accumulation of boron can be found in this organ when borocaptate is administered repeatedly (Buchar *et al.* 1992).

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