Physiol. Res. 52: 629-635, 2003

The Subcellular Targets of Mercaptoborate (BSH), a Carrier of ^{10}B for Neutron Capture Therapy (BNCT) of Brain Tumors

V. MAREŠ[1,2], D. KRAJČÍ[3], V. LISÁ[1]

[1]Joint Laboratory of Cancer Cell Biology of the Institute of Physiology, Academy of Sciences and the First Faculty of Medicine, Charles University, Prague, [2]Chair of Biology, Purkinje University, Ústí nad Labem, Czech Republic, [3]Faculty of Medicine, Kuwait University, Department of Anatomy, Safat, Kuwait City, State of Kuwait

Received August 22, 2002
Accepted October 5, 2002

Summary

The transformed C6 glial cells in cultures were treated with sodium mercaptoborate ($Na_2B_{12}H_{11}SH$, BSH), a carrier of atomic targets (^{10}B) of thermal neutrons for the neutron capture therapy of brain tumors. As shown by light microscopy, the therapeutic dose of BSH (100 μg/ml) did not alter the gross morphology and growth of the population of cells within a 72 h treatment interval. Electron microscopic analysis of these cells revealed activation of nucleoli and, occasionally, enlarged and bifurcated mitochondria. After 200 μg BSH/ml and 72 h treatment, growth of the cell population was inhibited and ultrastructural changes became more profound. They included condensation of chromatin and its allocation to the nuclear envelope which formed deeper invaginations. Mitochondria further increased in size and were characterized by slim or angular cristae. Moreover, in circumscribed segments of some of the slightly swollen mitochondria their cristae disappeared or were reduced to fine pouch-like structures localized near the continuous outer membrane, suggestive for a non-destructive restructuring of the inner mitochondrial membrane. The smooth pinocytotic vesicles near the plasma membrane, lysosomes and heterogeneous dense bodies were more frequent. The revealed subcellular targets of BSH may initiate the development of pharmacological protocols aimed to further improve the tolerance to BSH by the healthy tissues of patients undergoing BNCT of brain tumors, e.g. by intervention into the oxidative stress triggered likely by the altered mitochondria.

Key words
Borocaptate (BSH) • Brain tumors • Mitochondria • Nucleus • Neutron Capture Therapy (NCT)

Introduction

Irradiation of the stable isotope ^{10}B with biologically low effective thermal neutrons is followed by transmutation of ^{10}B to ^{7}Li and emission of high energy alpha particles which can lethally damage cell organelles and their molecules. Furthermore, the energy of alpha particles is absorbed within the cell in which interception of ^{10}B and thermal neutrons had occurred (Linear energy transfer <10 μm). This principle of cell damaging was applied for the development of a new approach to selective destruction of tumor cells, referred to as the neutron capture therapy (NCT). For NCT of brain tumors, sodium mercaptoborate (synonyms: sodium borocaptate,

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

$Na_2B_{12}H_{11}SH$, BSH), is the most often used carrier of ^{10}B (Barth *et al.* 1999). At therapeutic doses (≤ 100 mg/kg), BSH is reported to be well tolerated by experimental animals (Kliegel 1980, Haselsberger *et al.*, 1994a, Stragliotto and Fankhauser 1995). Nevertheless, infusion of 50 mg BSH/kg b.w. in dogs led to vomiting and 100 mg/kg b.w. even the death of some animals (5 %) during or soon after its administration (Gavin *et al.*1994). In man, a transient facial flush, mild nausea and chest pains occasionally also occur after sub-therapeutic or therapeutic doses of BSH. The patients with renal, hepatic, cardiopulmonary or cognitive abnormalities are not eligible for BSH-NCT (Haselsberger *et al.* 1994a, Stragliotto and Fankhauser 1995). Administration of supra-therapeutic or repeated doses of BSH to small laboratory animals can lead to increased salivation, transient alterations of cardiovascular, respiratory and renal functions, motor restlessness, changes in blood cell counts and body growth retardation (Janků *et al.* 1993, LaHann *et al.* 1997a, Horn *et al.* 1997).

The cellular effects of BSH, in both normal and tumor-affected organs, are supposed to be negligible, although they have only been rarely studied. After the administration of repeated or supra-therapeutic doses degenerative changes were observed histologically in the kidneys (Janků *et al.* 1993, LaHann *et al.* 1997b). Our earlier study showed that prolonged exposure of C6 glioma cells in culture to higher doses of BSH depressed proliferation, adhesiveness and viability of cells terminated by apoptosis (Mareš *et al.* 1992, 1997). In the present study we demonstrate that the main subcellular target of this drug, namely when applied at higher doses and for longer exposure times, are the nuclei and mitochondria. Our study suggests that pharmacological protection of these organelles, or interference with the subsequently triggered processes, especially the mitochondria, could further increase the tolerance of BSH by healthy tissues and organs of patients undergoing BNCT for brain gliomas.

Methods

Cell cultures and BSH treatment

Transformed C6 glial cells (CCL 107, ATCC Rockville, MD, USA) were cultured in Petri dishes (diameter 5 cm, 5 ml media, 10^5 cells seeded per dish) on strips of plastic (Thermanox, Lux Sci. Corp., USA) using Eagle's MEM with 10 % bovine serum and 5 % CO_2 atmosphere. Sodium mercaptoborate (BSH, Katchem Co., Řež near Prague, BSSB content < 0.1 %) was stored in a dry nitrogen enriched atmosphere at + 6 ºC. The drug was dissolved in PBS immediately before use and added to 3-day-old cultures in the dose of 100 or 200 μg/ml of culture media.

Gross morphology and viability of cells

The morphology of cells was monitored intravitally by phase contrast microscopy up to 72 h after BSH treatment (Diavert, Leitz, obj. 20x or Axiophot Nomarski contrast, obj. 40x). Some cultures were sampled for testing the viability of cells by staining with propidium iodide (PI) (Sigma, St. Louis, USA, 5 mg/ml in calcium ion-free phosphate-buffered saline or culture medium, 5-10 min at room temperature) viewed simultaneously in the phase contrast and epifluorescence (Axioplan, Opton, Germany, 557 nm excitation and 576 nm emission filters). The population density of cultures was expressed as the number of cells per an eyepiece grid (625 μm^2) evaluated intravitally under phase contrast (Diavert, Leitz, obj 20x).

Electron microscopy

The cells cultured on plastic strips, sampled at 48 and 72 h after BSH administration, were fixed in 2.5 % phosphate buffered glutaraldehyde (pH 7.2, 30 min), washed in phosphate-buffered saline and postfixed in 2 % buffered OsO_4 (2 h). After routine dehydration and clearing, the cells were embedded in Araldite CY212. Semithin 1 μm thick sections stained with 1 % toluidine blue were used for more detailed examination of gross morphology and targeting of cells for ultrathin sectioning. The thin sections were double stained with uranyl acetate and lead citrate and examined by transmission electron microscope JEM-1200EXII (JEOL, Japan) at 80 kV. In total, 687 cells were screened.

Statistics

Data are given as arithmetic means ± S.E.M. The differences were tested by Student's t-test.

Results

Gross morphology, growth and viability of cells

As shown by light microscopy, the cells in control cultures formed a loose network interconnected by short processes with a finely ruffled surface (Fig.1A). With the *in vitro* age, the population density of cultures increased and attained a subconfluent state by day 6. The number of dead cells revealed by propidium iodide red fluorescence in control cultures was ≤0.5 %. After

treatment with 100 μg BSH/ml, no significant changes in gross morphology, population density and the number of supravitally PI-stained cells occurred until the 72 h after treatment. Morphology and viability of cells were almost unchanged also after 200 μg/ml until 48 h. At 72 h, the cell processes became longer and thicker and of a more smooth and "stringy" appearance (Fig. 1B). The cell population density was lower than in control cultures (–35.7±1.85 %, $p<0.01$, Fig. 2). Some cells started to lose their adherence to the growth support and appeared floating in the culture medium (1.7±0.52 %). The dead PI-stained cells were rare and only insignificantly more frequent (≤1 %, $p>0.05$).

Ultrastructure of cells

In control cultures (Fig. 3A), the nuclei contained finely distributed chromatin of low density. The lamina-surrounded nuclear envelope was smooth or formed only a few and wide-open shallow invaginations. There were 2 to 3 reticular nucleoli composed of all three characteristic components in most cells. In the cytoplasm, short cisterns of granular endoplasmic reticulum and small clusters of free ribosomes were randomly dispersed. Adjacent to the nucleus, there were several stacks of Golgi complexes surrounded with vesicles and small dense bodies, which occasionally appeared to be lamellated. Mitochondria were round or oval-shaped with transversally oriented lamellar cristae spanning the whole profile of the organelles; their matrix was moderately dense and homogeneous. Pinocytotic vesicles, either isolated or in clusters, often occurred close to the plasma membrane, namely near to the cell-to-cell contacts. They were mainly small and smooth-surfaced, or occasionally larger and of the coated type. Small dense-cored bodies resembling secretory granules were observed in the perinuclear cytoplasm.

In cultures treated with 100 μg BSH/ml, no significant changes of ultrastructure were found by 48 h. At 72 h, the nucleoli acquired a more activated appearance and cisterns of the rough endoplasmic reticulum were slightly dilated and contained fine, moderately dense material indicating enhanced protein synthesis. In some cells (≤ 20 %), mitochondria were slightly enlarged and often bifurcated with slim elongated cristae. In some cases, the cristae were angular while spanning the whole cross-section of the organelles (see also below and Fig. 3C). The mitochondrial matrix was moderately dense and contained some calcium granules.

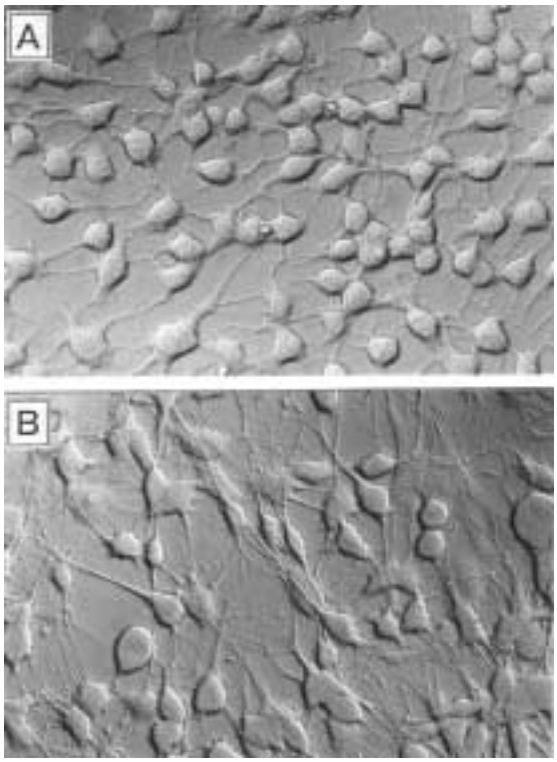

Fig. 1. *Gross morphology of fresh C6 glial cells in cultures under control conditions (A) and after treatment with 200 μg BSH/ml for 72 h (B). Intravital Nomarski-phase-contrast microscopy, Axiophot, Opton, obj. 40x.*

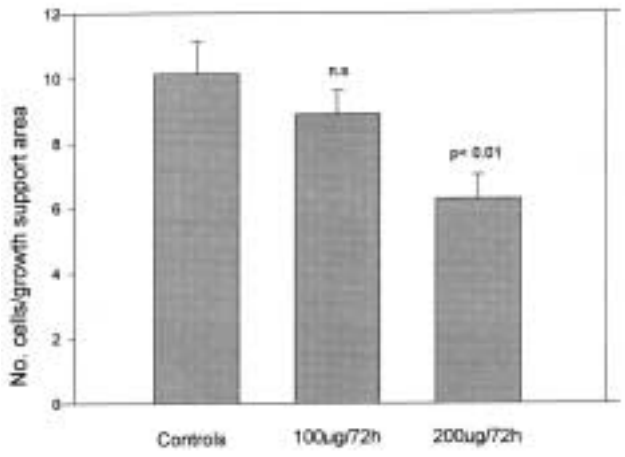

Fig. 2. *Cell population density of C6 glial cells in cultures under control conditions and after treatment with BSH.Expressed as the number of cells per a growth support unit area (625 μm^2).*

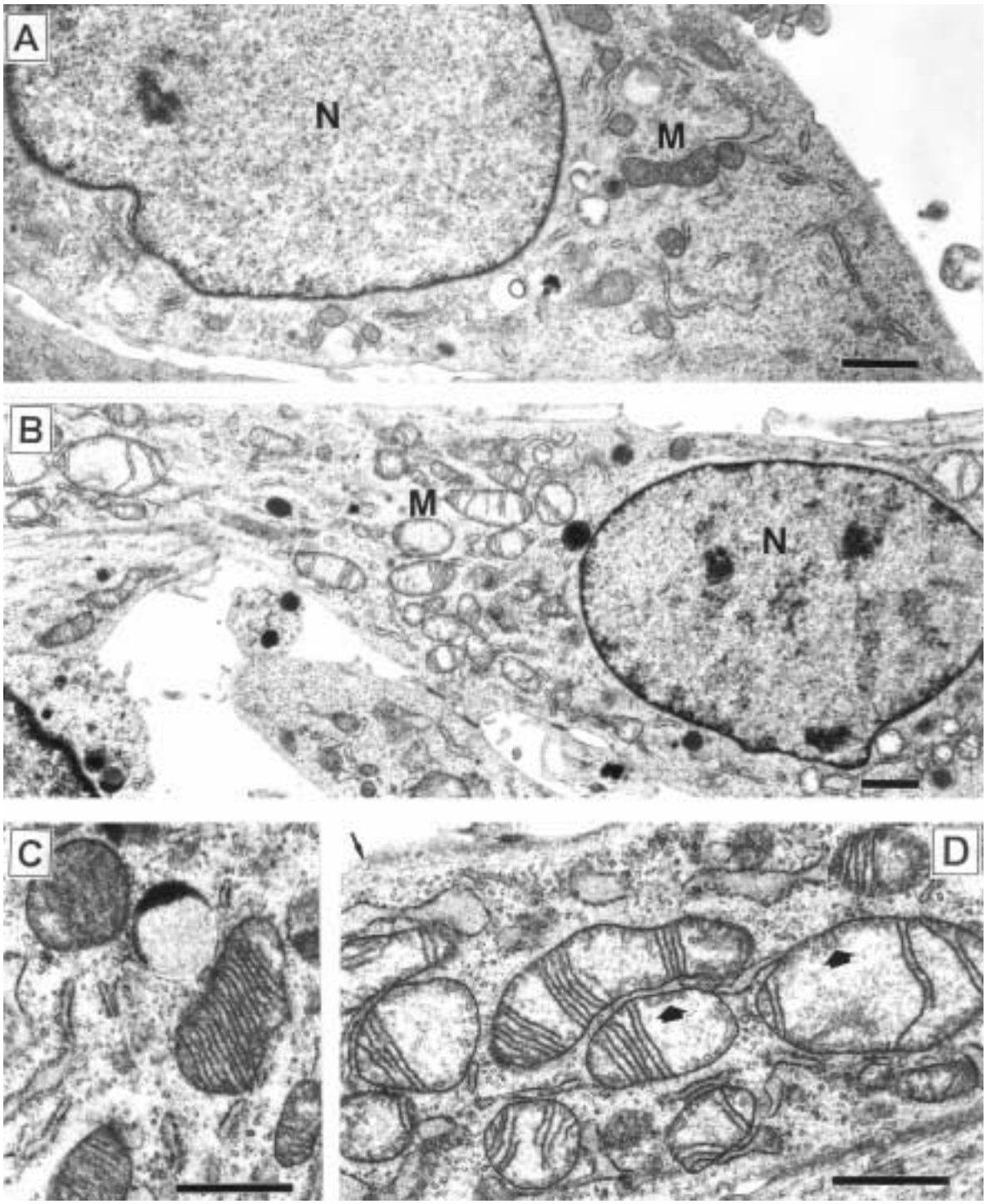

Fig. 3. *Ultrastructure of C6 glial cells under control conditions and after treatment with BSH (200 µg/ml, 72 h).*
(A) Control cells; N - an ovoid nucleus with slightly indented nuclear envelope M - slim intact mitochondria. Magnif. bar = 1 µm. (B) BSH treated cells; N − nucleus with mild peripheral accumulation of heterochromatin. M - hypertrophic mitochondria with segmental reduction of cristae. Magnif. bar = 1.0 µm. (C) A detail of the initial mitochondrial hypertrophy in BSH treated cells represented by the perpendicular or oblique cristae crossing the entire profile of the organelle. Magnif. bar = 0.5 um. (D) A detail of the mitochondrial alterations in treated cells represented by circumscribed reduction, and/or retraction of the cristae toward the periphery of inner membrane (wide arrows). Clusters of smooth-surfaced pinocytotic vesicles labeled by long arrow. Magnif. bar = 0.5 µm.

In cultures treated with 200 μg BSH/ml, the fine structure of cells was similar to that after the lower dose up to 48 h. At 72 h, the nuclear chromatin of most cells started to condense and accumulate near the nuclear envelope (Fig. 3B) which formed deeper invaginations.Mitochodria had further increased in size and their cristae were long and slim or, occasionally, angular while spanning the whole profile of the organelle (Fig. 3C). In about one half of the cell population, mitochondria were slightly swollen but without ruptures of the peripheral membrane (Figs 3B and 3D). In circumscribed segments of these organelles, the cristae were often absent or, occasionally, reduced to fine vesicle-like pouches located close to the continuous outer membrane (Fig. 3D). The mitochondrial matrix was less dense in these segments while the remaining cristae in these organelles were of normal appearance. The slightly swollen cisterns of the rough endoplasmic reticulum held a fine granular material. Clusters of smooth pinocytotic vesicles near the plasma membrane, especially at cell-to-cell contacts, as well as lysosomes and heterogeneous dense bodies were more frequent than in the controls (Fig. 3B).

Discussion

As shown by light microscopy, BSH affected gross morphology of cells only at the supra-therapeutic dose (200 μg/ml) and after 72 h treatment. The changes included reshaping of cell processes and a deficit in the cell population density attributable to inhibition of DNA synthesis (55 % reduction of BrdU incorporation, Vlach and Mareš, unpublished data). Furthermore, the adhesiveness of some cells to the growth support decreased. At the subcellular level, the most auspicious changes occurred in the nuclei and mitochondria. In the latter, mitochondrial cristae underwent an unusual non-destructive reduction of cristae limited to circumscribed segments of the organelles which, otherwise, were morphologically relatively well preserved, i.e. without signs of a lethal damage. The residual fine pouches observed occasionally close to the outer mitochondrial membrane in the lamellar cristae-free segments resembled *pediculi cristae* (initial segments, bridge contact sites) (Deams and Wisse 1996, Perkins *et al.* 1998, Frey and Mannella 2000), the fine tubular connections of the lamellar cristae to the inner boundary membrane, a new structural paradigm of the inner mitochondrial membrane (Perkins *et al.* 1998).

The literary data on the response of mitochondria to defined physiological or sub-lethal pathohysiological conditions are, except the terminal swelling, mainly biochemical (Drahota *et al.* 1999, Kucharská *et al.* 2000, Lotková *et al.* 2001, Kasapovic *et al.* 2001). The hitherto reported sub-lethal morphological changes include, for instance, vesiculation of cristae and their dichotomous branching under a mild hypoxia (Shepard *et al.* 1998) or HIV virus infection (Radovanovic *et al.* 1999). Reduction, curving and spiralization of the mitochondrial cristae were observed in mtDNA deprived organelles (Gilkerson *et al.* 2000). Recently, transformation of the lamellar cristae into tubules was described in the mitochondria with cytochrome P450scc- (Yoshinaga-Hirabayashi and Yoneda 2001) and ΔmtDNA4696 mutations (Nakada *et al.* 2001). The increase in relative area of cristae occurred in brown adipocytes after stimulation of thermogenesis (Cousin *et al.* 1992). Therefore, the cristae restructuring observed in the present study may represent a specific regressive morphological counterpart of the BSH-induced metabolic perturbations, for instance, inhibition of the mitochondrial ATPase, described in liver cells treated with this drug *in vitro* (Drahota *et al.* 1994). The main morphological effect of BSH on mitochondria and nuclei reported in the present study is in good agreement with its preferential subcellular accumulation (Hatanaka *et al.* 1975, Haselsberger *et al.* 1994b, Otersen *et al.* 1997).

It is noteworthy that BSH is almost not metabolized (Kliegel 1980) and is excreted mainly by the kidneys (60-80 % within 2 days) (Sweet *et al.* 1986, Haselsberger *et al.* 1994) in which drug concentration can substantially exceed the levels in other organs (Buchar *et al.* 1992). It is also important that the drug is sensitive to oxidation and that its –S–S– derivatives are more toxic than the reduced BSH (Kliegel 1980). However, in our drug experiments the BSH dimers were kept under control. Their content in the purchased substance did not exceed 0.1 % (see Material and Methods) and it was dissolved just prior to its administration to the cultures. The effect of some BSSB dimers formed intravitally in the cultured cells (Koudinova *et al.* 2000) and the organism *in situ* (Gibson *et al.* 2001) cannot, however, be excluded.

We conclude that BSH at therapeutic doses and short-time exposure is morphologically well tolerated by transformed C6 glial cells used in this study. After higher doses and longer treatment intervals, the drug induced discrete sub-lethal changes of cell organelles, namely in

the nuclei and mitochondria. These findings may initiate the development of pharmacological protocols aimed to further improve of BSH tolerance by healthy tissues and organs of patients undergoing BNCT for brain tumors by, for instance, suppression of the oxidative stress triggered probably by the altered mitochondria revealed in this study. In this way, the BNCT of brain tumors might be extended to patients with nephropathies, neuropathies, hepatopathies or cardiopulmonary abnormalities. In addition, the effectiveness of this therapy could be enhanced by a safer dose escalation of BSH.

Acknowledgements

The authors express their appreciation to the technical staff of the Electron Microscopy Unit, Faculty of Medicine, Kuwait University for the sectioning of tissue culture specimens and Mrs. Amald Wagdi for excellent photography work. Authors also extend their thanks to Dr. Zdeněk Drahota, D.Sc., Institute of Physiology, Ac. Sci, Prague for his long-term interest and encouraging discussion of our data. Supported by the Grant Agency of the Ministry of Industry & Commerce (GA MPO, Project No. FD-K/048) and the Czech Academy of Sciences, Project AVOZ 501 1922.

References

BARTH RF, SOLOWAY AH, GOODMAN JH, GAHBAUER RA, GUPTA N, BLUE TE, YANG W, JARKS W: Boron neutron capture therapy of brain tumors: an emerging therapeutic modality. *Neurosurgery* **44:** 433-451, 1999.

BUCHAR E, BEDNÁŘOVÁ S, GRUNER B, ŠTROUF O, JANKŮ I: Dose-dependent disposition kinetics and tissue accumulation of boron after intravenous injections of purified mercaptoundecahydrododecaborate in rabbits. *Cancer Chemother Pharmacol* **29:** 450-454, 1992.

COUSIN B, CINTI S, MORRONI M, RAIMBAULT S, RICQUIER D, PENICAUD L, CASTEILLA L: Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J Cell Sci* **103:** 931-942, 1992

DEAMS WT, WISSE E: Shape and attachment of the cristae mitochondriales in mouse hepatic cell mitochondria. *J Ultratstruct Res* **16:**123-140, 1966.

DRAHOTA Z, MAREŠ V, RAUCHOVÁ H, ŠAF P, KALOUS M: Inhibition of mitochondrial ATPase by dicarbopolyborate, a new enzyme inhibitor. *J Bioenerget Biomembr* **26:** 583-586, 1994.

DRAHOTA Z, RAUCHOVÁ H, SEDLÁK V, KOČÍ J, ČERVINKOVÁ Z: The effect of triiodothyronine on changes of membrane fluidity in regenerating rat liver. *Physiol Res* **48:** 167-170, 1999.

FREY TG, MANNELLA CA: The internal structure of mitochondria. *Trends in Biochem Sci* **25:** 319-324, 2000.

GAVIN PR, KRAFT SL, DEHAAN SE, SWARTZ CD, GRIEBENOW ML: Large animal normal tissue tolerance with boron neutron capture. *Int. J. Radiation Oncology Biol Phys* **28:** 1099-1106, 1994.

GIBSON CR, STAUBUS AE, BARTH RF, YANG W, KLEINHOLZ NM, JONES RB, GREE-CHURCH K, TJARKS W, SOLOWAY AH: Boron neutron capture therapy of brain tumors: Investigation of urinary metabolites and oxidation products of sodium borocaptate by electrospray ionization mass spectroscopy. *Drug Metabolism and Disposition* **29:** 1588-1598, 2001.

GILKERSON RW, MARGINEANTU DH, CAPALDI RA, SELKER JM: Mitochondrial DNA depletion causes morphological changes in the mitochondrial reticulum of cultured human cells. *FEBS Lett* **474:** 1-4, 2000.

HASELSBERGER K, RADNER H, PENDL G: Boron neutron capture therapy: boron biodistribution and pharmacokinetics of $Na_2B_{12}H_{11}SH$ in patients with glioblastoma. *Cancer Res* **54:** 6318-20, 1994a.

HASELSBERGER K, RADNER H, GOSSLER W, SCHLAGENHAUFEN C, PENDL G: Subcellular boron-10 localization in glioblastoma for boron neutron capture therapy with $Na_2B_{12}H_{11}SH$. *J Neurosurg* **81:** 741-744, 1994b.

HATANAKA H, SWEET WH: Slow neutron capture therapy for malignant tumors. Its history and recent development. In: *Advances of Biomedical Dosimetry.* International Atomic Energy Agency Press, Wien, 1975, pp 146-178.

HORN V, BUCHAR E, JANKŮ I: Kidney function changes in rats after a single-dose administration of borocaptate sodium. *Physiol Res* **46:** 279-283, 1997.

JANKŮ I, BUCHAR E, JIŘIČKA Z: Nephrotoxicity of borocaptate after short-term administration on rabbits. *Toxicology* **79:** 99-108, 1993.

KASAPOVIC J, PAJOVIC SB, PEJIC S, MARTINOVIC JV: Effects of estradiol benzoate and progesterone on superoxide dismutase activity in the thymus of rats. *Physiol Res* **50**: 97-103, 2001.

KLIEGEL W: *Bor in Biologie und Pharmazie.* Springer-Verlag, Berlin,1980.

KOUDINOVA J, ELHANATI G, SALOMON Y, BENDEL P: Uptake of BSH in M2R melanoma cells monitored by NMR spectroscopy. In: *Frontiers in Neutron Capture Therapy.* MF HAWTHORNE, K SHELLY, R WIERSMA (eds), Plenum Press, New York, 2000, p. 289.

KUCHARSKÁ J, BRAUNOVÁ Z, ULIČNÁ O, ZLATOŠ L, GVOZDJAKOVÁ A: Deficit of coenzym Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. *Physiol Res* **49:** 411-418, 2000.

LAHANN TR, HEMATILAKE G, LARSEN J, DANIEL D: Cardiovascular toxicity associated with a single dose administration of the boron delivery drug borocaptate sodium. In: *Advances in Neutron Capture Therapy, vol. II.* J. CRAWFORD, AND R. WEINRICH (eds), Elsevier, 1997a, pp.192-196.

LAHANN TR, SPIEGEL K, SPALL R, GRIEBENOW M: Evaluation of the toxicity associated with repeat administration of the boron delivery drug borocaptate sodium. In: *Advances in Neutron Capture Therapy, vol. II,* J CRAWFORD, R WEINRICH (eds), Elsevier, 1997 b, pp. 185-191.

LOTKOVÁ H, RAUCHOVÁ H, DRAHOTA Z: Activation of mitochondrial glycerophosphate cytochrome c reductase in regenerating rat liver by triiodothyronine. *Physiol Res* **50**: 333-336, 2001.

MAREŠ V, M. BAUDYŠOVÁ, J. KVÍTEK, V. HNATOWICZ, J. ČERVENÁ, J. VACÍK, J.FOLBERGROVÁ: Accumulation of boron-10 in cell cultures exposed to mercaptododecaborate used for the neutron capture therapy. J Pharmacol Exp Therapeutics **262:** 818-822, 1992.

MAREŠ V, LISÁ V, DRAHOTA Z, BAČÁKOVÁ L, ŠPANOVÁ A, KVÍTEK J, HNATOWICZ V, BURIAN J: Toxicity, uptake and retention of sodium mercaptoborate (BSH) in glial and glioma cells in culture. In*: Advances in Neutron Capture Therapy, vol. II.* J CRAWFORD, R WEINRICH (eds), Elsevier, Amsterdam, 1997 pp. 210-215.

NAKADA K, INOUE K, CHEN CS, NONAKA I, GOTO Y, OGURA A, HAYASHI JI.: Correlation of functional and ultrastructural abnormalities of mitochondria in mouse heart carrying a pathogenic mutant mtDNA with a 4696-bp deletion. *Biochem Biophys Res Commun* **288:** 901-907, 2001.

OTERSEN B, HARITZ D, GROCHULLA F, BERGMANN M, SIERRALTA W, GABEL D: Binding and distribution of $Na_2B_{12}H_{11}SH$ on cellular and subcellular level in tumor tissue of glioma patients in boron neutron capture therapy. *J Neurooncol* **33:**131-139, 1977.

PERKINS GA, SONG JY, TARSA L, DEERINCK TJ, ELLISMAN MH, FREY TG.: Electron tomography of mitochondria from brown adipocytes reveals crista junctions. *J Bioenerg Biomembr* **30:** 431-442, 1998.

RADOVANOVIC J, TODOROVIC V, BORICIC I, JANKOVIC-HLADNI M, KORAC A: Comparative ultrastructural studies on mitochondrial pathology in the liver of AIDS patients: clusters of mitochondria, protuberances, "minimitochondria," vacuoles, and virus-like particles. *Ultrastruct Pathol* **23**: 19-24, 1999.

SHEPARD TH, MUFFLEY LA, SMITH LT: Ultrastructural study of mitochondria and their cristae in embryonic rats and primate (N. nemistrina). *Anat Rec* **252:** 383-392, 1998.

STRAGLIOTTO G, FANKHAUSER H: Biodistribution of boron sulfhydryl for boron neutron capture therapy in patients with intracranial tumors. *Neurosurgery* **36:** 285-293, 1995.

SWEET WH, MESSER JR, HATANAKA H: Supplementary pharmacological study between 1972 and 1977 on purified mercaptoundecahydrododecaborate. In: *Boron Neutron Capture Therapy of Tumors.* H. HATANAKA (ed), Nishimura Co., Ltd., Niigata, 1986, pp. 59-76.

YOSHINAGA-HIRABAYASHI T, YONEDA Y.: Expression of SCC in ovarian granulosa cells and cultured cells induced rapid structural changes in mitochondria. *Ital J Anat Embryol* **106** (Suppl 1): 51-57, 2001.

Reprint requests

V. Mareš, M.D., D.Sc., Institute of Physiology, Academy of Sciences, Vídeňská 1083, CZ-14200 Prague 4, Czech Republic. E-mail: maresv@biomed.cas.cz