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Uptake and metabolism of boronophenylalanine in human uveal melanoma cells in culture
Relevance to boron neutron capture therapy of cancer cells

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Summary. The transport of boronophenylalanine (BPA) and its metabolic fate have been studied in a human uveal melanoma cell line isolated from a primary enucleated tumor. The boronated compound was rapidly incorporated into the cells reaching a peak of incorporation in two hours. This was followed by a trough between 10 and 24 hours and by an increase thereafter. The analogy with the amino acids phenylalanine (Phe) and tyrosine (Tyr) was studied in competition experiments incubating cultures of cell line MK-T, isolated in this laboratory, with [^{3}H]-Phe and [^{125}I]-Tyr, in the presence or absence of various concentrations of BPA, between 0 and 5 min. The presence of BPA severely reduced the uptake of both amino acids. The kinetics of the transport of [^{3}H]-Phe and [^{3}H]-Tyr in the presence of BPA, measured after 10 sec of incubation, showed that the boronated compound exerted a competitive inhibition on both transport systems. The intracellular metabolism of BPA was followed by measuring boron concentration (measured with Ionization Coupled Mass Spectrometry) in subcellular fractions and after membrane extraction by the detergent Triton X-100. The results showed that BPA remained in the supernatant and was not metabolized into macromolecules. These results and the relative absence of melanine in these cells, as observed by electron microscopy, suggest that BPA may be actively transported into melanoma cells but not metabolized. The results may have a relevance in studies on Boron Neutron Capture Therapy.

Keywords: Amino acids – Human – Uveal – Melanoma cultures – Borophenylalanine – Transport kinetics

Introduction

Several reports have shown that Boron Neutron Capture Therapy (BNCT) may be used with a certain efficacy in the treatment of tumors readily attainable by a beam of thermal neutrons (Barth et al., 1992). BNCT relies upon the intense ionising effect of the nuclear reaction: ^{10}B (n, α) ^{7}Li (Taylor and Goldhaber, 1935; Locher, 1936) to deliver localized damage to cancer cells. The main issue of BNCT is to bring specifically and, when possible, selectively boronated molecules into tumor cells while keeping the concentration of these molecules in blood and normal tissues as low as possible. Several boron containing compounds have been developed and tested in animals and, in some cases, in humans. Among these, sodium borocaptate (a sulphydryl-containing polyhedral borane, BSH) (Hatanaka, 1975), carborane containing amino acids (Leukart et al., 1976; Spielvogel et al., 1989), porphyrins (Kahl et al., 1990) and nucleosides (Schinazi and Prusoff, 1978; Anisuzzam et al., 1990; Tjarks and Gabel, 1991).

Clinical trials have been performed with BSH in the treatment of gliomas (Hatanaka et al., 1986) and with boronophenylalanine (BPA) for the treatment of skin melanomas (Mishima et al., 1989). Animal studies have recently been published showing the treatment of Greene's melanomas with BNCT after single intragastric injections of BPA in rabbits (Coderre et al., 1990). Ratios of boron concentrations in the tumor to boron concentration in the surrounding normal tissues were in the range of 3–4: 1 indicating a sufficient delivery of ^{10}B to the tumors for an efficient action of BNCT. The irradiation of this tumor with thermal neutrons has led to encouraging results in 8 of 11 animals thus treated (Packer et al., 1992).

BPA may be considered as an analog either of tyrosine (Tyr) or of phenylalanine (Phe) or of both. To date, and to our knowledge, only one study has been performed on the mechanisms of uptake of BPA across the cell plasma membrane of skin B-16 melanoma cells (Ichihashi et al., 1986) and none on its metabolism into proteins. The present experiments have been undertaken in an attempt to show the analogy of BPA with the two amino acids with regards to transport mechanisms and to protein metabolism in a newly isolated human uveal melanoma cell line. The results show that BPA should be considered as a competitive analog for the transport of both Phe and Tyr but that it is not metabolized into macromolecules after its entry in the cells. The relative absence of pigmentation in the melanosomes of the tested cell line further suggests that the observed cellular accumulation of BPA may not represent its metabolism into melanin.

Materials and methods

Cell cultures

Human uveal melanoma cell line MK-T was isolated in this laboratory as a primary culture of a dissociated tumor obtained after surgical enucleation (Massarelli et al., 1994). Pieces of the tumor collected under sterile conditions, were chopped with fine scissors and incubated with 0.125% trypsin (Gibco) in Krebs-Ringer medium without Ca^{++} and Mg^{++} at room temperature for 5 min. The reaction was stopped by adding RPMI 1640 growth medium (Gibco) containing 50 μg/ml of Gentamycin (Gibco), 2.5% Donor calf serum (Gibco), 2.5% calf newborn serum (Gibco) and 2% Ultroser G (IBF). After centrifugation (90 × g for 10 min) the pellet was suspended in the same growth medium and seeded onto plastic Petri dishes. The medium was renewed every 3 days. In the present experiments cells were tested between the 30th and 40th passage and were used in the log phase of their growth cycle as established in pilot experiments. The cell line has presently reached over 70 passages. Cell integrity was regularly controlled by the incubation of the cultures with dimethyl-thiazolyl-diphenyl-tetrazolium which reacts with viable cells forming, after incorporation into the living cells, Formazan blue which may be quantified by spectroscopy (Manthorpe et al., 1986). Cell viability was also checked after incubation with BPA.

The cell cycle parameters of the MK-T cell line and of its morphological characteristics have been presented elsewhere (Massarelli et al., 1994). Here we show the aspect of MK-T cells under phase contrast microscopy (Fig. 1a) and electron microscopy (Fig. 1b). The presence of melanosomes, often devoid of melanin, indicates the melanotic nature of the cells, but a poor capacity to synthesise the pigment once the cells are grown in vitro.

Incubation conditions

Three series of incubations were performed: *incorporation* of amino acids and BPA intended as the relative long term intracellular accumulation of the labelled molecules (hours), *uptake* intended as short term accumulation (minutes) and *transport* intended as the movement of labelled molecules across the plasma membrane in seconds.

Incorporation

L-BPA hydrochloride (final concentration: 0.45 mM, Boron Biologicals, Raleigh, NC, USA) was added to the growth medium of cultured cells and the incubation performed under normal growth conditions. At different time points the medium was discarded and the cultures washed 3 times with 5 ml of Krebs-Ringer phosphate solution, pH 7.4 (137 mM NaCl, 2.7 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 3.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 10 mM Glucose). Cells were homogenized in 2 ml of distilled water. An aliquot was tested for protein content and another for boron content measured by Ionization Coupled Plasma Mass Spectrometry (performed at the Service Central d'Analyse of the CNRS, Lyon, France). Cultures were incubated (up to 48 hours) in their growth medium with 0.3 μCi/ml [^{3}H]-Phe (final specific activity: 3.7 Ci/mole, Amersham) or with 0.5 μCi/ml of [^{3}H]-Tyr (final specific activity: 3.9 Ci/mole, Amersham). At the end of each incubation time the radioactivity was measured (with 0.6 ml of water and 10 ml of Biofluor, DuPont) in an aliquot of the growth medium (20 μl) to confirm that the radioactivity present in the medium remained constant during the time of the experiment. The remaining medium was discarded and the cell layer washed with Krebs-Ringer phosphate solution as above and dried at room temperature. Two ml of 2 N NaOH were added to dissolve the dry material and aliquots (100 μl) were used to measure protein content and radioactivity (600 μl) as above.

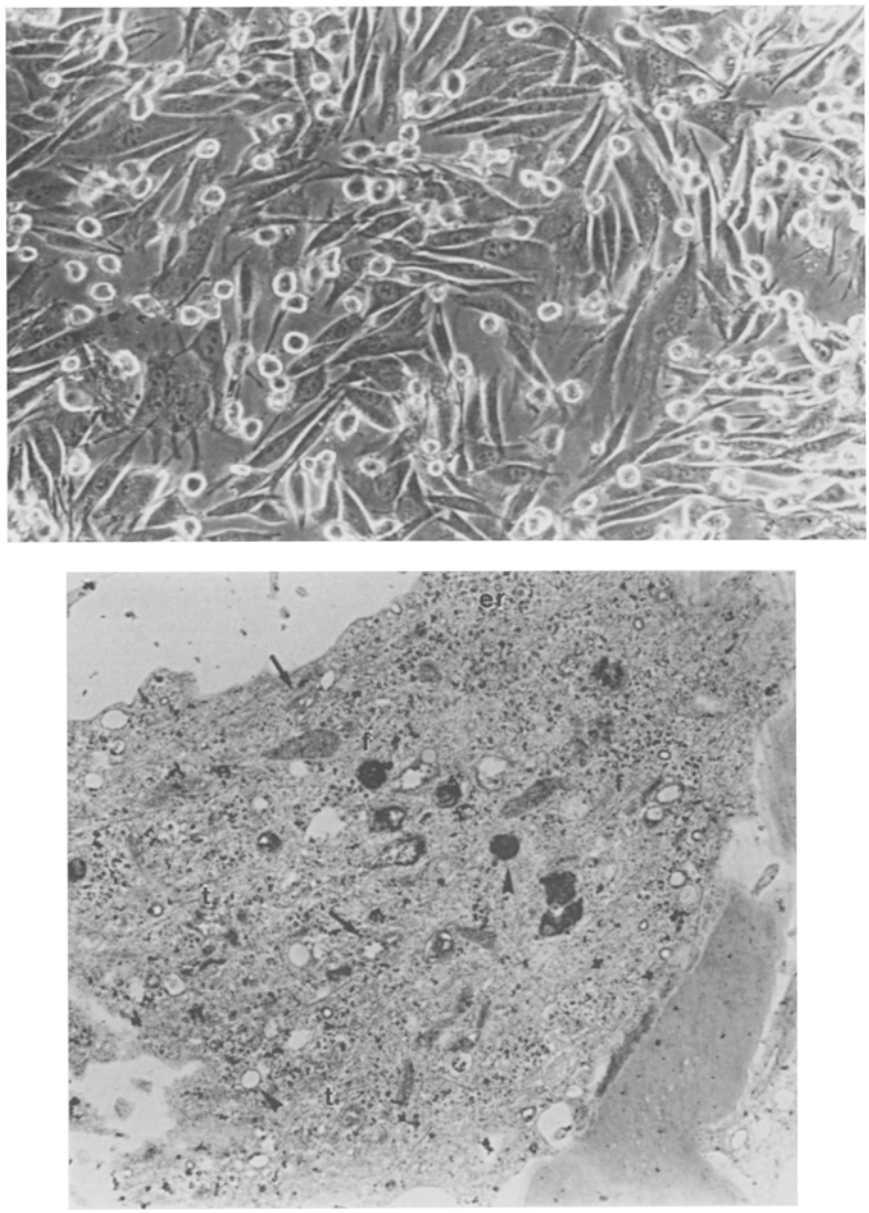


Fig. 1. Morphology of human uveal melanoma MK-T cell cultures. (**a**) Phase contrast optical microscopy. The field shows spindle-like MK-T cells with polynucleolated nuclei and round shaped cells (Magnification ×200). (**b**) Electron micrograph of a melanoma MK-T cell with endoplasmic reticulum (*er*), filaments (*f*), tubules (*t*), premelanosomes (arrows) and empty melanosomes or containing traces of pigment (arrowhead) (Magnification ×20,800)

Uptake

In experiments devised to study the competition of BPA with the uptake of [^{3}H]-Phe and [^{125}I]-Tyr (up to 5 min of incubation) the growth medium was discarded and the cultures washed 3 times with 5 ml of Krebs-Ringer solution and incubated with 5 ml of the same solution containing various concentrations of BPA and labelled amino acids: 0.29 μCi/ml

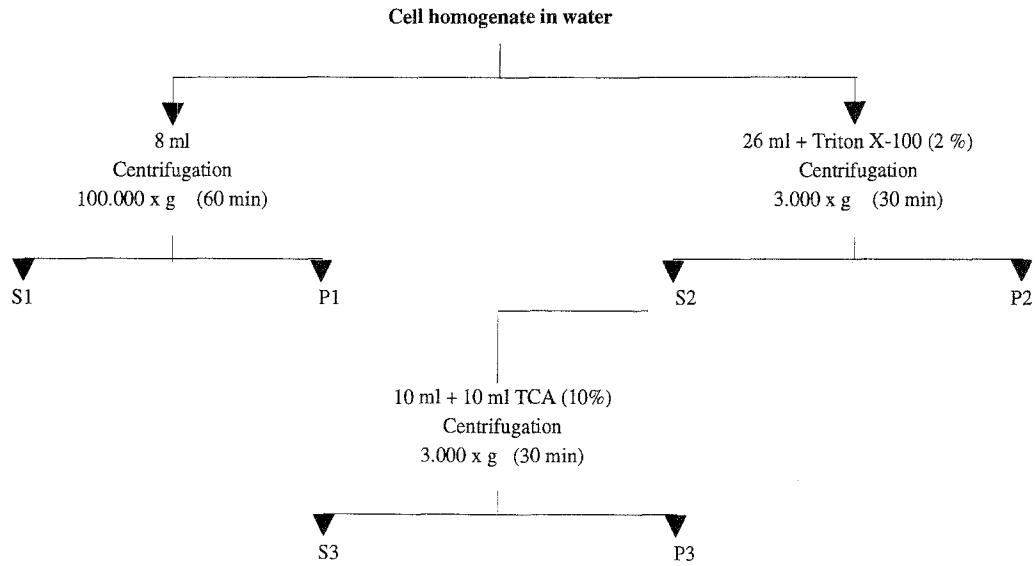
of [^{125}I]-Tyr or 0.92 μCi/ml of [^{3}H]-Phe (final specific activities: 2.62 μCi/μmol – labelled at the CEA – and 10.2 μCi/μmol – Amersham –, respectively). Control experiments have shown that [^{3}H] and [^{125}I] isotopes might be used indistinctly to label tyrosine. At each time point the radioactive medium was discarded and the cultures washed as above. The cell layer was dried at room temperature, dissolved in 2 N NaOH and aliquots used for protein and radioactive measurements as above.

Transport

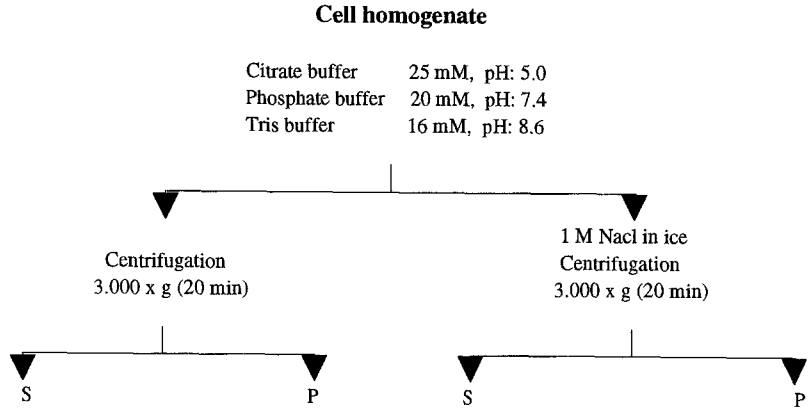
Cell cultures were washed as above and incubated with 1 μCi/ml of [^{3}H]-Tyr or [^{3}H]-Phe (specific activities: 56 Ci/mmol and 55 Ci/mmol, respectively, Amersham), but the incubation lasted 10 seconds and was rapidly followed by 3 washes with Krebs-Ringer solution. Cells were thereafter digested with NaOH as in the above mentioned procedure.

Subcellular boron distribution

Cells were preincubated with 0.45 mM BPA for 48 hrs. Two sets of experiments were performed to follow boron distribution and metabolism in proteins. In the first set, after discarding the growth medium containing BPA and washing the cell layer with 154 mM NaCl solution (kept at 37 °C), the cells were scraped and homogenized (by sonication) in distilled water. The homogenate was divided in 2 aliquots one of which was centrifuged at 100,000 × g, 60 min, and gave rise to a first supernatant (S1), containing essentially soluble proteins, and pellet (P1) (Scheme 1). The second aliquot was extracted with Triton X-100 (2% final concentration) to solubilize membrane bound proteins and centrifuged 3,000 × g, 30 min. The supernatant thus obtained (S2) contained soluble and solubilized membrane bound proteins. An aliquot of S2 was precipitated with 10% TCA in ice, followed by centrifugation 3,000 × g, 30 min. Proteins, under these conditions, would precipitate in the P3 fraction (Scheme 1). The second set of experiments (Scheme 2) took advantage of the known behaviour of proteins at different pH and ionic strength. Cells were homogenized either with 25 mM citrate buffer, pH 5.0, or with 20 mM phosphate buffer, pH 7.4 or with 16 mM Tris buffer, pH 8.6. An aliquot of the homogenate was centrifuged at 3,000 × g, 20 min and an equal aliquot was extracted with 1 M NaCl (final concentration) prior centrifugation. The solubilization of proteins increases with



Scheme 1. Flow sheet of subcellular fractionation of melanoma MK-T cells. The explanation are in the text. *S* Supernatant; *P* Pellet



Scheme 2. Extraction of MK-T cells at different pH. *S* Supernatant; *P* Pellet

increasing pH. The high ionic strength in the presence of NaCl favors furthermore such a solubility.

Kinetic analysis and statistics

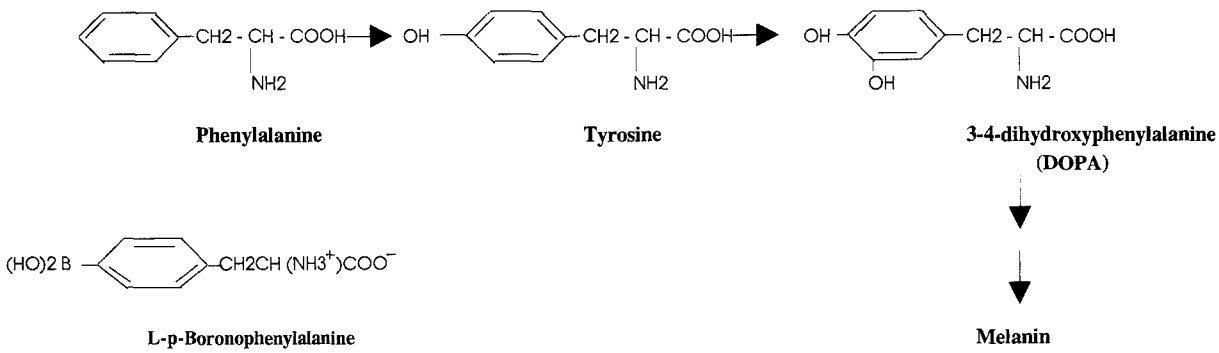
Transport kinetic parameters (Vmax and Km) were measured by regression analysis. Statistical significance was assessed by Peritz' test (Harper, 1984).

Proteins were measured according to the procedure of Lowry et al. (1951).

Results

The incorporation of BPA into MK-T cells reached a maximum in 2 hours, decreased between 10 and 24 hours and increased thereafter (Fig. 2, left). The incorporation did not follow a linear increase as those of [^{3}H]-Phe and [^{3}H]-Tyr which were continuous for at least 24–30 hours (Fig. 2, right).

The structure of the BPA molecule (Scheme 3 includes as well the reactions leading to the synthesis of melanin) is that of a possible analog of both Phe and Tyr. To clarify the analogy of BPA with these amino acids the uptake of [^{3}H]-Phe and of [^{125}I]-Tyr was followed in MK-T cells in the absence and presence of varying concentrations of BPA. The results showed that increasing



Scheme 3. Simplified metabolic pathway leading to the formation of melanin. Structural analogy of BPA with Phe and Tyr

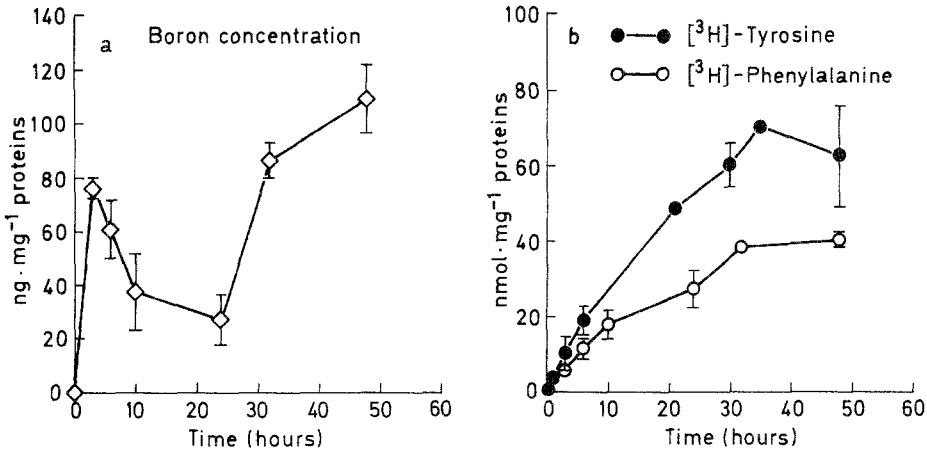


Fig. 2. Incorporation of BPA (left), [^{3}H]-Phe and [^{3}H]-Tyr (right) as a function of time in MK-T cells. Boron content was measured by Ionisation Coupled Plasma Mass Spectrometry and the radioactivity by scintillation spectrometry after digestion of the cells as explained in the Materials and methods section. The radioactive measurements (dpm's) were transformed in moles in relation to the specific activity present in the medium. Radioactivity was measured in the medium at each time point. Each point represents the average of 3–4 determinations ± the standard deviation

concentrations of BPA inhibited the uptake of both amino acids (Fig. 3a,b). To define more clearly the nature of this inibition, MK-T cells have been incubated with different concentrations of [^{3}H]-Phe and of [^{3}H]-Tyr in the presence or absence of 1.2 mM BPA. The kinetics of the transport were analysed after 10 sec of incubation and the data show (Fig. 4a,b) that BPA may act as a competitive inhibitor of the transport of both amino acids as judged in comparing the kinetic parameters (Table 1).

Further experiments were devised to determine the fate of the incorporated BPA in various subcellular fractions. In the first series of experiments (Scheme 1), in the absence of ionic strength, most soluble proteins of the homogenate remained probably attached with pelleted membranes (P1). The data show (Fig. 5) that the boron content was 24 fold higher in the supernatant S1 than in the pellet P1. After Triton X-100 extraction, 61 fold more boron was found in S2; the increased ratio was essentially due to a much higher amount of boron found in S2 which was extracted from a larger volume than S1. This suggests that the incorporated boron appeared to be present soluble in the supernatant and not in the proteins solubilized by the Triton extraction. 97 fold more boron was found in the S3 after TCA precipitation, hence under conditions which exclude the presence of proteins in S3 (the results are not expressed per mg proteins because of the known effect of Triton X-100 on Lowry's protein determination).

In a further experiment (Table 2) the cell homogenization was performed at pH 5.0, 7.4 and 8.6, hence at increasing protein solubility. The results confirm that more boron is present in the supernatants than in the pellets.

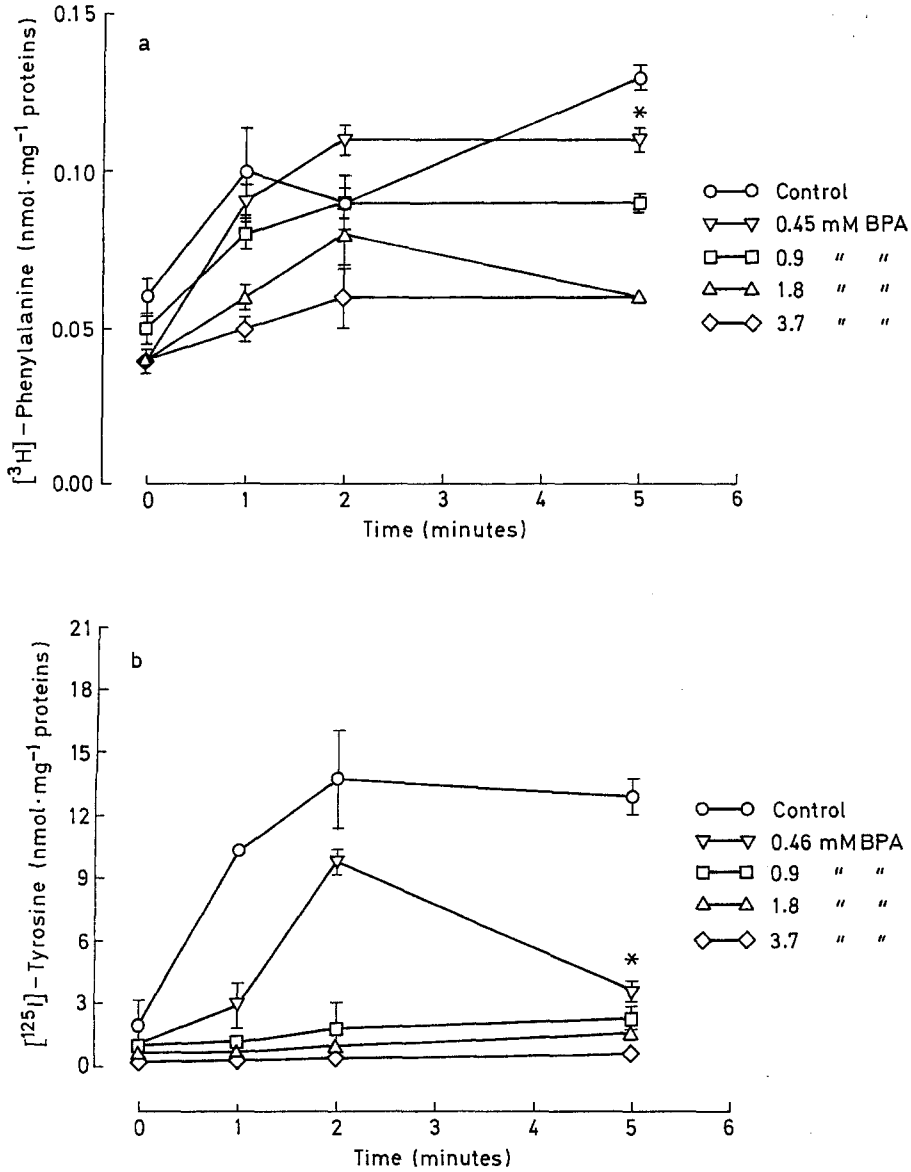


Fig. 3. Uptake of [^{3}H]-Phe (**a**) and [^{125}I]-Tyr (**b**) in the absence or the presence of BPA. Each point represents the mean of 4 determinations ± the standard deviation. * $P < 0.01$ vs control. Previous control experiments had shown the similar biochemical behaviour in these experiments of Tyr labelled with ^{3}H or ^{125}I

Table 1. Inhibition of [^{3}H]-Phe and [^{3}H]-Tyr transport in MK-T cells by BPA. Km values are expressed in μM, Vmax in nmoles · mg^{-1} proteins · min^{-1}. The incubation conditions are explained in the Materials and methods section

	Control		1.2 mM BPA	
	Km	Vmax	Km	Vmax
[H]-Phe	120	26	517	28
[H]-Tyr	61	4	268	3

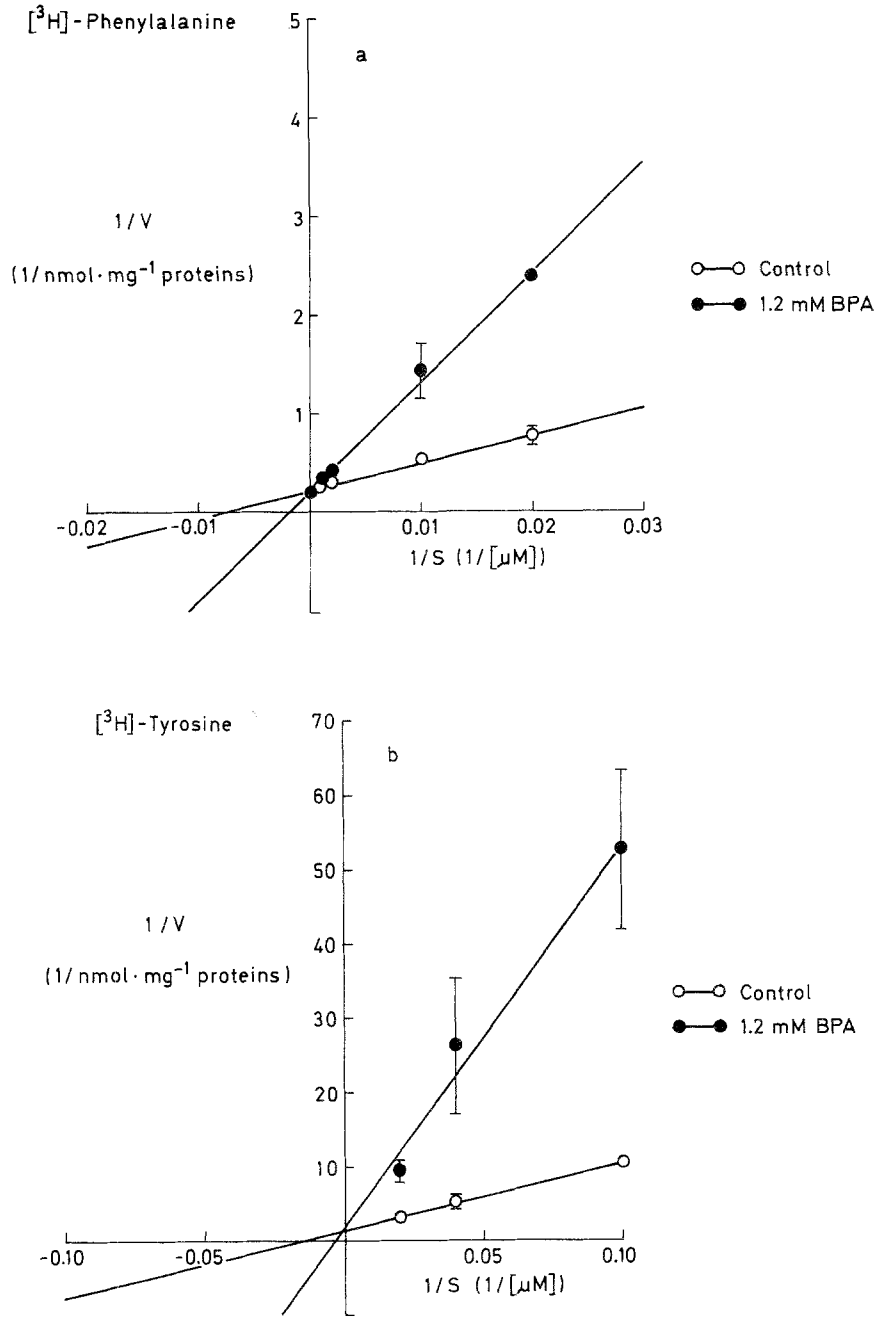


Fig. 4. Kinetics of the transport of [^{3}H]-Phe (**a**) and [^{3}H]-Tyr (**b**) in the absence or the presence of BPA. Each point represents the mean of 4 determinations ± the standard deviation. The values of the kinetic parameters are given in Table 1

Moreover, regardless of the extraction procedure, a similar amount of boron is consistently found in the supernatant while protein content varied according to the pH of the extraction solution. A decrease in boron content was observed in the pellet in parallel with a decrease in protein content, hence suggesting the possible binding of boron (or of BPA) to ionized proteins. The presence of NaCl (Scheme 2) did not further change the extraction of boron from the pellet (results not shown).

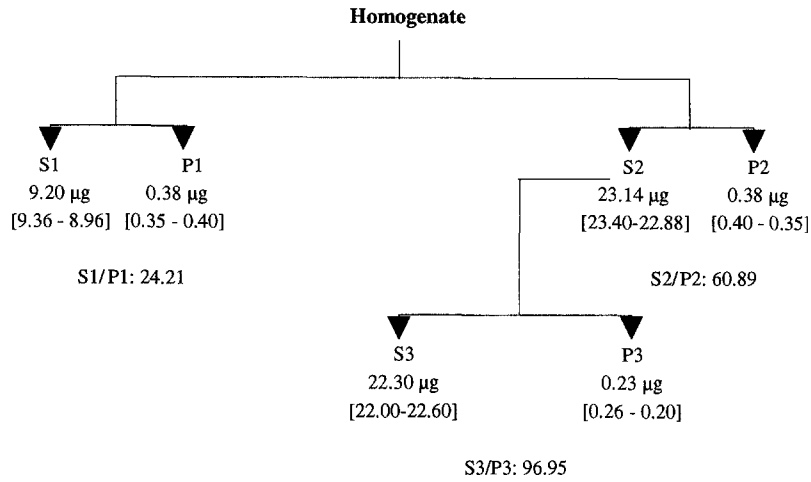


Fig. 5. Boron content in MK-T subcellular fractions obtained as explained in Scheme 1. The values are the average (total boron content) of two separate experiments (single values are given in square brackets). *S/P* Ratios of supernatant boron content compared to the corresponding pellet boron content

Table 2. Boron and protein extraction in the supernatant and pellet obtained from homogenates extracted at different pH's. After cell homogenization and centrifugation, as specified in Scheme 2, boron content was measured by Ionisation Coupled Plasma Mass Spectrometry and proteins according to Lowry et al. (1951)

pH	Homogenate						
	Supernatant			Total Bore	Pellet		
	B	Pr.	B/Pr.	S+P	B	Pr.	B/Pr.
5.0	6.60 (6.80–6.40)	5.70 (4.60–6.80)	1.16	8.08	1.48 (0.71–2.25)	13.10 (12.90–13.30)	0.11
7.4	4.60 (4.00–5.20)	7.80 (8.70–6.90)	0.59	5.97	1.37 (1.60–1.13)	13.05 (10.70–15.40)	0.10
8.6	6.20 (6.00–6.40)	8.90 (9.70–8.10)	0.70	6.54	0.34 (0.45–0.23)	8.40 (9.20–7.60)	0.04

B total boron content in µg; *Pr* total protein content in mg; *S* supernatant; *P* pellet. The values represent the mean of two separate experiments (single values are given in brackets)

Discussion

The interest of BNCT in the treatment of low depth tumors has been increasing in the past years as a potential target technique for killing cancer cells. The technique takes advantage of the ^{10}B (n, α) ^{7}Li nuclear reaction to deliver an α particle after absorption of a boronated molecule into the cancer cell following the equation:

$$^{10}B + {}^{1}n_{th}\ (0.025eV) \Rightarrow [^{11}B] \Rightarrow {}^{4}He + {}^{7}Li + 2.79\ MeV\ (6\%)$$
$$\Rightarrow {}^{4}He + {}^{7}Li + \gamma 0.48\ MeV + 2.31\ MeV\ (94\%)$$

One of the main limitations to the clinical applications of BNCT has been the selectivity of the boron containing compounds for tumoral cells (Barth et al., 1992; Hawthorne, 1993). This has now been circumvented by the development of boronated molecules which have been shown to be accumulated specifically in tumoral tissue with varying ratios (3–6: 1) depending on the tumor localization and on the organ tissue concerned (Coderre et al., 1987). One of these molecules, BSH, has reached phase III in an European Clinical Trial (Moss et al., 1992; Stragliotto and Frankenhauser, 1992). The interest in BPA has been raised because of its role as precursor for melanin via its transformation to Tyr and to indolequinone, BPA acting then as an analog of either Phe, or Tyr, or both amino acids (Scheme 3). However, only one biochemical study (Ichihashi et al., 1986) has been performed, to our knowledge, on the uptake of BPA, in particular, and none on human ocular melanoma cells. The present experiments show that BPA is rapidly incorporated into a melanoma cell line in competition with both amino acids (Table 1). The carrier(s) for the amino acids recognize(s) BPA which may be accumulated inside the cell. After 2 hours of incubation with 0.45 mM BPA it is possible to calculate that 8×10^7 atoms of boron were incorporated per MK-T cell. This value is close to the theoretical one (10^9 atoms $\cdot$ cell^{-1}) considered to be necessary for the full effect of the nuclear reaction in BNCT (Soloway et al., 1986).

The incorporation of BPA into the cells, however, does not inevitably lead to its metabolism as suggested by the experiments showing the presence of boron essentially in the cell supernatant and poorly in the protein (pellet) fractions (Fig. 5). Small changes in boron concentration were in fact found concomitantly with increasing amount of either soluble or membrane bound proteins. This suggets that BPA remains mostly unaltered in the cytoplasm and, as shown by the experiments at different pH extractions, that it may bind, but in a very small proportion, to ionically charged proteins. The presence of the borane terminal in the para– position of the molecule is an impediment for its metabolism into proteins, as a possible inhibitor of the specific amino acyl tRNA synthetase active site, and into melanin (Hawthorne, 1993). The lack of BPA incorporation into melanin has also been reported elsewhere (Coderre et al., 1987). It is known that for the metabolism of BPA to melanin the removal of the 4-hydroxyboryl– group is a necessary prerequisite (Roberts et al., 1980). The latter may then remain in the form of boric acid, free in the cytoplasm. The possible diffusion of this free molecule across the plasma membrane might explain the fall in boron concentration observed in Fig. 2a. If we assume that the incorporation of BPA is linear with time, similarly to the analog amino acid, the cytoplasmic hydrolysis of borane will allow the diffusion of this molecule out of the cell giving, as a result, a decrease in cytoplasmic boron content after an initial peak of incorporation. Between 10 and 24 hours of incubation, possibly an equilibrium between the cytoplasm and the medium may be established and, as a consequence, the incorporation may continue. This suggests that for an efficient BNCT treatment the cells should be incubated with BPA for a short time (two hours) or for a relatively long time (more than 24 hours). However, even under unfavourable con-

ditions (preincubation for 16 hours with 0.45 mM BPA), we have previously shown that in MK-T cells a neutron fluence of 6×10^9 n $\times$ cm^{-2} has a lethal effect of 30% (Belkhou et al., 1992).

As a tentative conclusion we suggest that melanoma cells may transport BPA as an analog of Phe and Tyr but that its therapeutic effects, in terms of BNCT, should take into consideration the possible presence of free borate.

Acknowledgements

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References

Anisuzzam AKM, Alam F, Soloway AH (1990) Synthesis of a carboranyl nucleoside for potential use in neutron capture therapy of cancer. Polyhedron 9: 891–892

Barth RF, Soloway AH, Fairchild RG, Brugger RM (1992) Boron neutron capture therapy for cancer. Cancer 70: 2995–3007

Belkhou R, Mykita S, Meyer L, Sahel J, Abbé J-Ch, Dreyfus H, Massarelli R (1992) Lethal effect of the boron neutron capture reaction on human uveal melanoma cells in culture incubated with borophenyalanine. C R Acad Sci (Paris) 315: 485–491

Coderre JA, Glass JD, Fairchild RG, Roy U, Cohen S, Fand I (1987) Selective targeting of borophenylalanine to melanoma in BALB/c mice for neutron capture therapy. Cancer Res 47: 6377–6383

Coderre JA, Glass JD, Packer S, Micca P, Greenberg D (1990) Experimental boron neutron capture therapy for melanoma: systemic delivery of boron to melanotic and amelanotic melanoma. Pigm Cell Res 3: 310–318

Harper JF (1984) Peritz "F" test: basic program of a robust multiple comparison test for statistical analysis of all differences among group means. Comput Biol Med 14: 437–445

Hatanaka H (1975) A revised boron-neutron capture therapy for malignant brain tumors: II. Interim clinical result with the patients excluding previous treatments. J Neurol 209: 81–94

Hatanaka H, Kamano S, Amano K, Hojo S, Sano K, Egawa S, Yasukochi H (1986) Clinical experience of boron neutron capture therapy for gliomas. A comparison with conventional chemo-immuno-radiotherapy. In: Hatanaka H (ed) Boron neutron capture therapy for tumors. Nishimura Co., Niigata, pp 349–379

Hawthorne MF (1993) The role of chemistry in the development of boron neutron capture therapy of cancer. Angew Chem Int 32: 950–984

Ichihashi M, Mishima Y, Ueda M, Hayashibe K, Hatta S, Funasaka Y, Fujiwara H, Yoshino K (1986) Selective lethal effects of ^{10}B-para-borophenylalanine on mouse and human melanoma cells in thermal neutron capture therapy enhanced by tyrosine and phenylalanine deficiency. In: Hatanaka H (ed) Neutron capture therapy. Nishimura Co., Niigata, pp 237–246

Kahl SB, Joel DD, Nawrocky MM, Micca PL, Tran KP, Finkel GC, Slatkin DN (1990) Uptake of a *nido*-carboranylporphyrin by human glioma xenografts in athymic nude mice and by syngeneic ovarian carcinomas in immunocompetent mice. Proc Natl Acad Sci USA 87: 7265–7269

Leukart O, Caviezel M, Eberle A, Escher E, Tun-Kyi A, Schwyzer R (1976) Carboranylalanine, a boron analogue of phenylalanine. Helv Chem Acta 59: 2184–2187

Locher GL (1936) Biological effects and therapeutic possibilities of neutrons. Am J Roentgenol Radium Ther 36: 1–13

Lowry OH, Roseborough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Manthorpe M, Fagnani R, Skaper SD, Varon S (1986) An automated colorimetric microassay for neuronotrophic factors. Dev Brain Res 25: 191–198

Massarelli R, Belkhou R, Dunel-Erb S, Chevalier C, Abbé J-Ch, Pignol J-P, Moutaouakkil M, Sahel J (1994) Morphological characterisation of human uveal melanoma cell lines. C R Acad Sci 317: 25–33

Mishima Y, Honda C, Ichihashi M, Obara H, Hiratsuka J, Fukuda H, Karashima H, Kobayashi T, Kanda K, Yoshino K (1989) Treatment of malignant melanoma by single thermal neutron capture therapy with melanoma-seeking ^{10}B-compounds. Lancet ii: 388–389

Moss RL, Stecher RA, Asmussen F, Huiskamp R, Dewit L, Mijnheer B (1992) The Petten BNCT project. In: Allen BG, Moore D, Harrington B (eds) Progress in neutron capture therapy for cancer. Plenum Press, New York, pp 7–12

Packer S, Coderre J, Saraf S, Fairchild RG, Hansrote J, Perry H (1992) Boron neutron capture therapy of anterior chamber melanoma with p-borophenylalanine. Invest Ophtalmol Vis Sci 33: 395–403

Roberts DC, Suda K, Samanen J, Kemp DS (1980) Pluripotential amino acids. I. L-p-dihydroxyborylphenylalanine as a precursor of L-Phe and L-Tyr containing peptides: specific tritiation of L-Phe containing peptides at a final step in synthesis. Tetrahedron Lett 21: 3435–3438

Schinazi RF, Prusoff WH (1978) Synthesis and properties of boron and silicon substituted uracil or 2′-deoxyuridine. Tetrahedron Lett 50: 4981–4984

Soloway AH, Barth RF, Alam F, Carey WE (1986) Boron compounds and boronated antibodies for neutron capture therapy. In: Hatanaka H (ed) Boron neutron capture therapy for tumors. Nishimura Co., Niigata, pp 47–58

Spielvogel BF, Good A, Hall IA, Fairchild RG, Micca PL (1989) Synthesis, antineoplastic activity and biodistribution of boron analogues of amino acids and related compounds. Strahlenter Onkol 165: 123–125

Strasgliotto G, Fankhauser H (1992) Biodistribution of boron sulphydral (BSH) in patients with intracranial tumors. In: Allen BG, Moore D, Harrington B (eds) Progress in neutron capture therapy for cancer. Plenum Press, New York, pp 551–556

Taylor HJ, Goldhaber M (1935) Detection of nuclear disintegration in a photographic emulsion. Nature 135: 341–348

Tjarks W, Gabel D (1991) Boron-containing thiouracil derivatives for neutron capture therapy of melanoma. J Med Chem 34: 315–319

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