Interdyscyplinarne **Studia Doktoranckie**
RadFarm – Radiofarmaceutyki dla ukierunkowanej molekularnie diagnostyki i terapii medycznej

APPLICATION OF MASS SPECTROMETRY TECHNIQUES IN THE DETERMINATION OF BORON CONCENTRATION IN CELLS

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Introduction

^{10}B isotope of boron plays a key role in boron-neutron capture therapy (BNCT) - a method of cancer treatment that utilizes a nuclear reaction occurring between this isotope and thermal neutrons from the external beam [1]. BNCT offers great application potential in the treatment of many different cancers, including lung cancer, also being a promising tool for the treatment of metastatic cancers. Boron is delivered to tumour sites by a carrier, that should be characterized with specific requirements - one of the currently used carriers is 4-borono-L-phenylalanine (L-BPA), which transport to tumour cells is governed by the active mode of L-amino acid transporters, mainly LAT-1 (overexpressed in various types of cancers [2]). Some reports suggested that the accumulation of L-BPA in tumour can be affected by earlier pre-loading with other L type amino acids [3].

Aim of the research

Aim of this study was to investigate if L-BPA uptake in cells can be affected by pre-loading with amino acid like phenylalanine, using mass spectrometry techniques. Uptake of L-BPA was assessed in tumour (A549) and normal (V79) cell lines pre-treated with L-phenylalanine (L-Phe) and boron determination was performed by inductively coupled plasma mass spectrometry (ICP-MS). A new method for boron concentration measurement was explored with the use of *single-cell* inductively coupled plasma mass spectrometry (SC-ICP-MS).

Materials and methods

Cell cultures

A549 cells were cultured in F-12K medium (Gibco) supplemented with 10% fetal bovine serum and 1% penicilin/streptomycin. V79 cells were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum and 1% penicilin/streptomycin. Both cell lines were maintained in 37 ºC, 95% humidity, 5% CO_2.

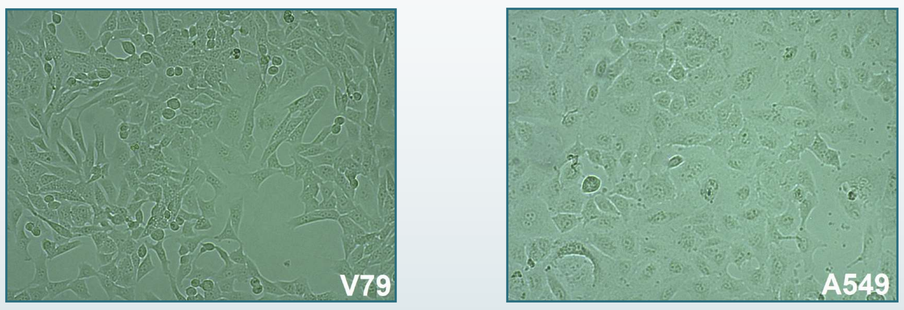

Fig 1. Microscopic image of V79 and A549 cell lines - images taken with an inverted light microscope (NIKON eclipse TS 100).

L-BPA and L-Phe exposure

L-BPA solution was prepared as L-BPA-D-fructose complex to a concentration of 2 mM. L-Phe solution was prepared by dissolution in MiliQ water to a concentration of 5 mM. Prior to the experiment both solutions were filtered through 0,22 µm pore membranes. Exposure conditions: 37 ºC, 95% humidity, 5% CO_2, 1 h for L-Phe pre-treatment, 2 to 6 h for L-BPA exposure.

ICP-MS and *Single-Cell* ICP-MS

Parameter	ICP-MS	SC-ICP-MS
Instrument	NexION 300D (PerkinElmer)	NexION 2000 (PerkinElmer)
Measured isotopes	^{10}B ^{11}B	^{10}B ^{11}B
Calibration range	(10–100) µg/L	(1–5) µg/L
Sample preparation	Digestion with 65% HNO_3, ultrasonification (50°C, 30 min), dilution with MiliQ water	Medium change and dilution with 10% PBS to ~100K cells/mL
Measurement Mode	Standard	Standard
Nebulizer	Mainhardt	HEN NEB
Spray chamber	Quartz cyclonic	Quartz Asperon
NEB gas flow	0,88 L/min	0,40 L/min
Sample introduction	Manually with peristaltic pump	SC Micro DX with syringe pump
Sample Flow Rate	0,2 mL/min	10 µL/min
Dwell time	100 ms	50 µs
AMS gas flow	0 L/min	0,7 L/min

Results

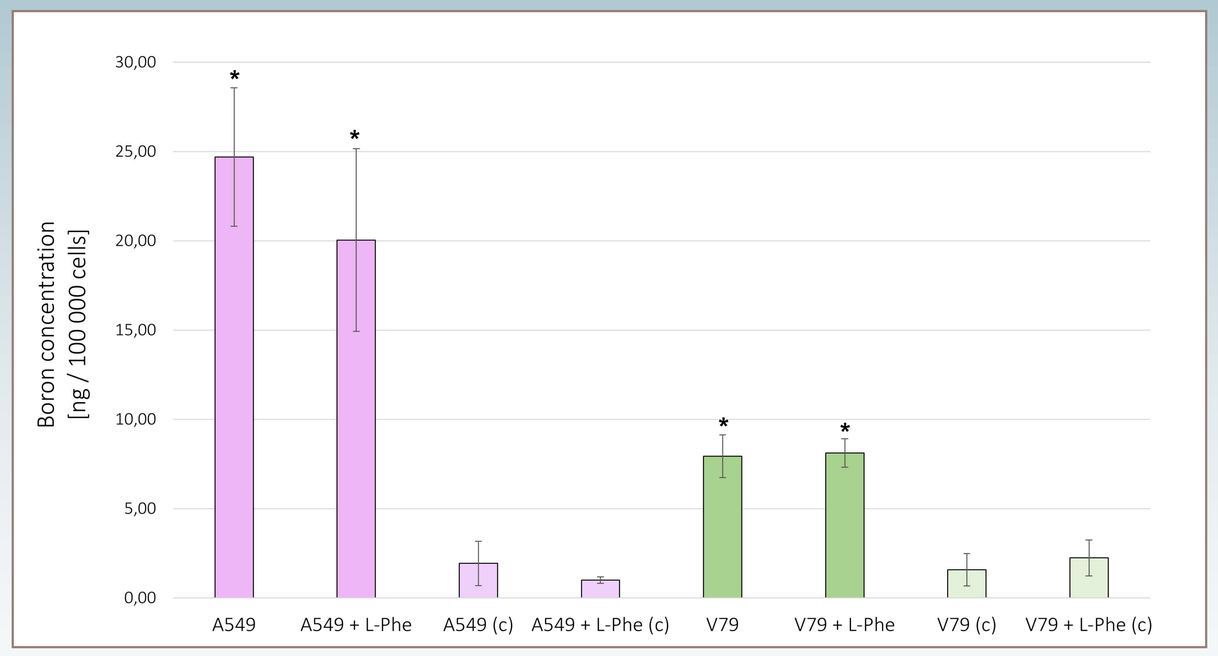

Fig 2. Boron concentration in cells assessed by the ICP-MS measurements for V79 and A549 cells treated with L-BPA for 2 h and pre-treated with L-Phe (marked as + L-Phe) for 1 h. Control groups are marked with (c). Data are expressed as the mean ±SD from two independent experiments performed in triplicate. Statistical significance: *p<0.05.

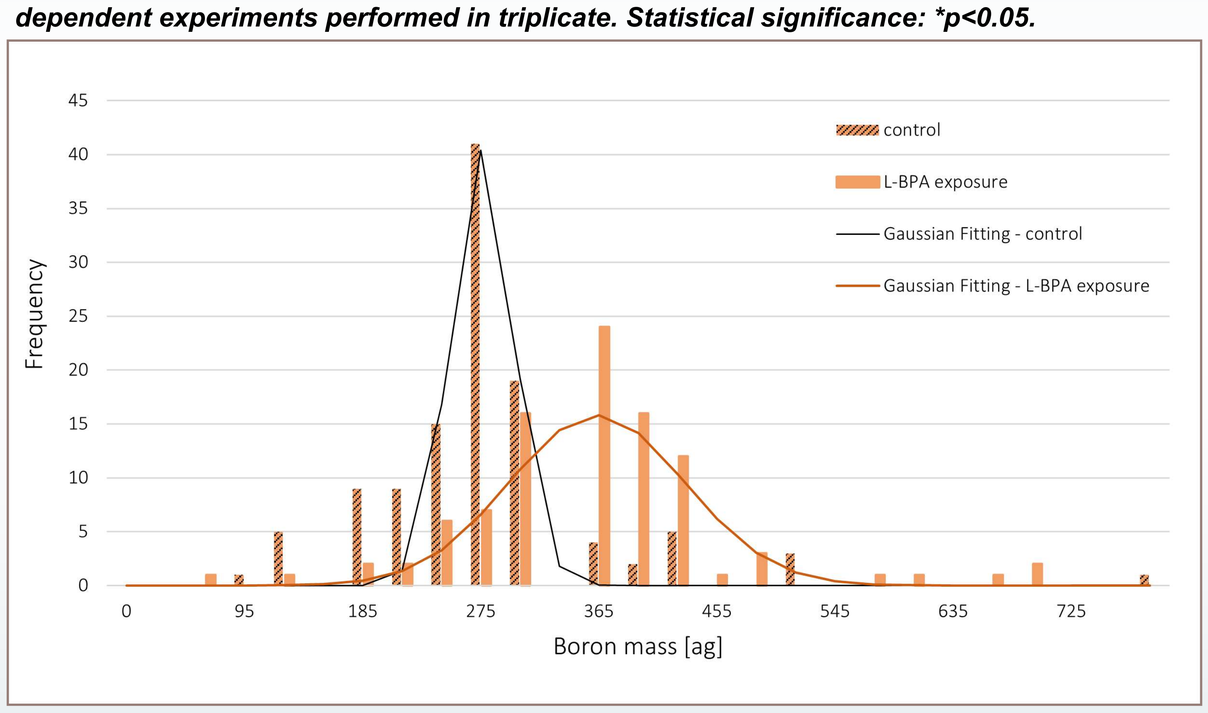

Fig 3. SC-ICP-MS measurements results for V79 cells treated with L-BPA for 2 h and for control group with fitted Gaussian distributions.

Conclusions

The experiments have shown that short-term exposure increased boron concentration in cells and no significant difference in boron uptake was observed within the range of 2-6 hours exposure time. In the measurements involving L-phenylalanine pre-loading, an inhibiting influence of pre-treatment was observed for the A549 cell line which is in accordance with the previously described results of similar measurements [4]. More experiments have to be conducted in order to formulate conclusive results. Mass spectrometry techniques can be successfully applied in such studies and a new method for the assessment of boron uptake in cells was successfully established with the use of SC-ICP-MS, which offers a possibility of calculating boron concentration in individual cells rather than obtaining a mean of an entire sample.

References

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