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Boron absorption imaging in rat lung colon adenocarcinoma metastases

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Abstract. The encouraging results we obtained with BNCT treatment of two explanted livers affected by multiple metastases, convinced us to study the possibility to extend our technique to lung metastases. An essential requirement for BNCT application is the possibility to gain higher ¹⁰B concentrations in the metastases compared to those in healthy tissue. For this reason we have developed a rat model with lung methastases in order to study the temporal distribution of ¹⁰B concentration in tissues and tumoral cells. Some rats with induced lung methastases from colon adenocarcinoma, were sacrificed two hours after Boronphenylalanine infusion. Thin slices were cut from lung tissues, that were taken and frozen in liquid nitrogen, and underwent neutron autoradiography in the nuclear reactor Triga Mark II, University of Pavia. The images clearly demonstrate an higher Boron uptake in tumoral nodules compared to health lung parenchima.

1. Introduction

Lung carcinoma is the first cause of death for tumour in the west world; current techniques are not effective, new approaches have to be experimented.

The very encouraging results obtained with BNCT application to liver metastases through autotransplant [1], convinced us to explore the possibility to apply BNCT to the cure of diffuse lung metastases. One of the principal requirements for BNCT applications to lung tumours is represented by the possibility to obtain higher ^{10}B concentrations in metastases compared with those in lung healthy tissue. For this reason we have developed a rat model with lung metastases in order to study the temporal distribution of ^{10}B concentration.

2. Animal model

To obtain pulmonary metastases in the animal model we used DHDK/K12/TRb colon-carcinoma cells obtained from a 1,2dimethylhydrazine-induced colon adenocarcinoma in syngeneic BDIX rats, selected and cloned for its capacity to induce progressive and metastatic tumours in the syngeneic host [2].

The cells can be maintained at confluence for long periods of time without any apparent change in cell biology, including tumorigenicity. This cell line grows as confluent, polygonal cells in monolayer in tissue culture flasks in Ham's F10 medium supplemented with 10% fetal calf serum. Pulmonary metastases in the rats (male, 250 g) are induced by intra-splenic injection of $20 \cdot 10^6$ colon-carcinoma cells under general anaesthesia. After injection, splenectomy is performed.

Twenty eight days after tumour induction in the rats (Fig.1), intra-peritoneal Boronphenylalanine [3] (14M solution of BPA-fructose complex; 300 mg/Kg) was administered and the animals were sacrificed 2 hours after BPA infusion.

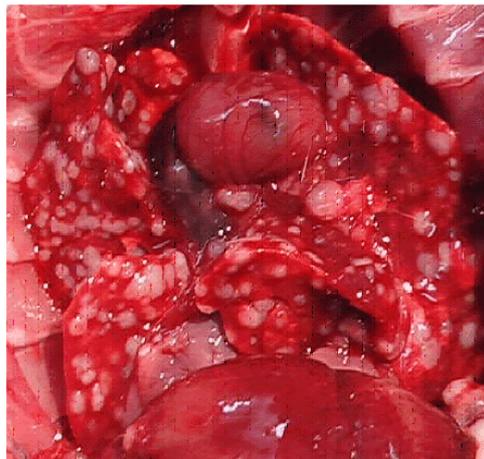


Figure1 Rat lungs in which metastatic nodules (clear spots) appeared after intrasplenic injection of $20 \cdot 10^6$ colon-carcinoma cells.

The lung were taken and frozen in liquid nitrogen. Several couples of neighbouring samples were cut using a Leica cryostat: a section of $10 \mu\text{m}$ deposited on glass for morfological analysis by standard ematoxilin-eosin stining, the next one of $70 \mu\text{m}$ to submit to neutron autoradiography.

3. Boron imaging

The samples for neutron autoradiography were put directly on a Cellulose Nitrate film (CN85 by Kodak Pathè).

The CN85 is only sensitive to high LET radiation and is not influenced by γ background possibly present in the irradiation position, nor by visible light; this characteristic makes it easily handling without the need of a dark-room.

The samples containing ^{10}B , deposited on the described film, are irradiated with thermal neutrons; the α particles coming from $^{10}\text{B}(n, \alpha)^7\text{Li}$ reaction leave latent tracks on the film, which become visible by an appropriate etching method.

The irradiation of our samples was performed in a position inside the Thermal Column of the Triga Mark II reactor of Pavia University. The thermal neutron flux in the irradiation position was $2 \cdot 10^9 \text{ cm}^{-2} \text{ s}^{-1}$; with irradiation times between 10 and 100 minutes, ^{10}B concentration between 1 and 100 ppm can be pointed out (1 ppm = $1 \mu\text{g}$ of ^{10}B / 1 g of tissue). After irradiation the films were etched in a NaOH solution at 10% in weight, at a temperature of 60°C for 20 minutes. The images of histological samples and of the etched films of neutron autoradiography were acquired by a Leica stereomicroscope with digital camera..

4. Results

Fig.2 shows the image of a rat lung tissue section, with metastases from colon Adenocarcinoma.

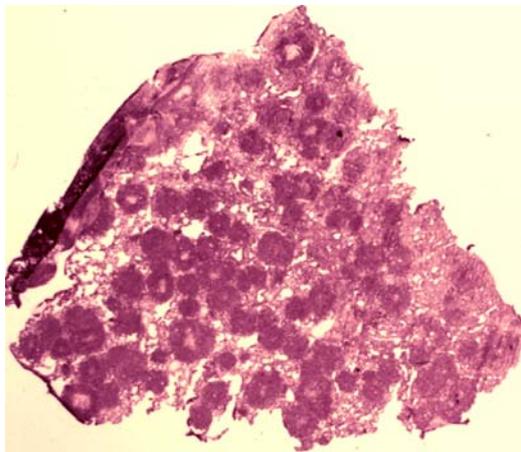


Figure 2. Histological section of a rat lung after standard ematoxilin-eosin staining. The circular structures are metastatic nodules invading the lungs.

Fig.3 presents two neighboring sections of rat lung tissue: the left one is the histological sample, the right one is the neutron autoradiography. The dark zones of the histological preparation correspond to tumoral nodules; in the autoradiography the dark zones correspond to the parts of sample with higher ^{10}B concentrations. A perfect correspondence between tumoral areas and zones with higher ^{10}B concentration can be noted comparing the two images.

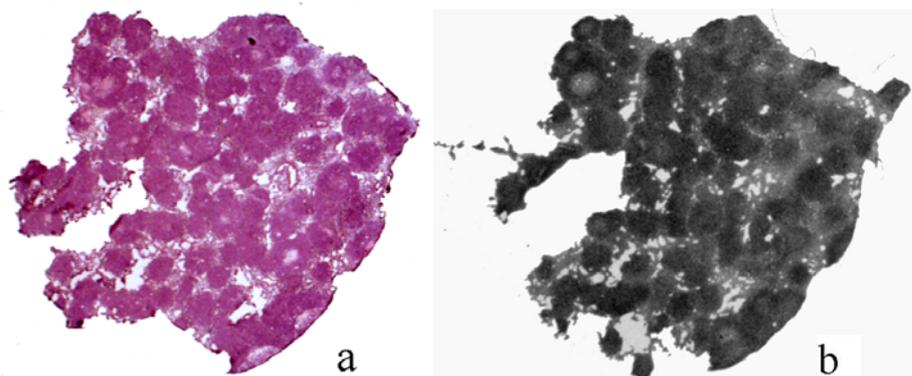


Figure 3. Comparison between two neighboring section of a rat lung: **a.** histological sample, **b.** neutron autoradiography. The geometrical correspondence between tumoral nodules (circular, dark fractions on the left) and darker zones on the right, representing tissues with high ^{10}B concentration, is clearly visible.

Fig.4 shows another example of neutron autoradiography, once again the agreement between the dark areas in the histological preparation and in the radiography is very good.

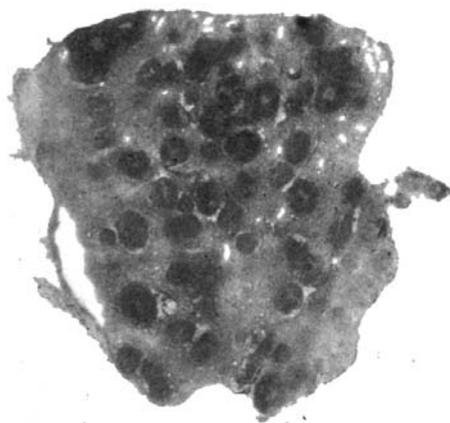


Figure 4. Another example of neutron autoradiography of a section of rat lung with tumour. The dark zones clearly demonstrate an higher ^{10}B uptake by tumoral cells comparing to healthy ones.

5. Conclusions

The results obtained in this work clearly show the selectivity of ^{10}B uptake in lungs of the animal model. After two hours from intraperitoneal BPA infusion the ^{10}B concentration is higher in the tumour comparing to healthy tissue. The quantitative analysis of ratios of ^{10}B concentrations in lung metastases and in healthy lung parenchima for different times after BPA infusion is now in progress.

References

- [1] T.Pinelli, A.Zonta, S.Altieri, S.Barni, A.Braghieri, P.Pedroni, P.Bruschi, P.Chiari, C.Ferrari, F.Fossati, R.Nano, S.Ngnitejeu Tata, U.Prati, G.Ricevuti, L.Roveda and C.Zonta TAOOrMINA: From the first Idea to the Application to the Human Liver Research and Development in Neutron Capture Therapy 1065-1072 Edited by Sauerwein W., Moss R., Wittig A. Monduzzi Editore 2002A
- [2] Cagnard A., Martin M.S. Michel M.F. and Martin F. Interaction between two cellular sub-population of a rat colonic carcinoma when inoculated to the syngeneic host. *Int.J.Cancer*, 36,273-279, 1985
- [3] Coderre JA, Bergland R, Chada M et al. Boron Neutron Capture Therapy of Glioblastoma Multiform using the p-Boronphenylalanine-Fructose complex and epithermal neutrons. *Neutron Capture Therapy for Cancer*. Y.Mishima Ed. Plenum Press, New York; 1996. p.553-561.