Boron Neutron Capture Therapy (BNCT): experimental aspects and clinical applications in the isolated liver

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BNCT is based on the cytotoxic effects of neutron irradiation of ^{10}B. The cell damage is due to the highly ionising particles released in the nuclear reaction:

$$^{10}B + n \rightarrow {}^{4}He + {}^{7}Li + 2.792\ MeV.$$

Both α (^{4}He) particles and ^{7}Li have a high Linear Energy Transfer (LET) and have a cytotoxic effect within their range of action of 18 μm which is the same as the cellular diameter.

Thus if tumour cells can capture ^{10}B at a higher concentration than surrounding hepatocytes, a highly specific cellular therapy can be achieved.

This research is part of a program called *PAVIA BNCT project* for the treatment of unresectable hepatic tumour by neutron irradiation.

Liver metastases are the most frequent kind of malignancy in Western countries (Europe and North America) and represent the most frequent site of recurrence of any primary tumour. In our geographic area diffused liver metastases from colorectal cancer represent the a highly frequent kind of tumour liver involvement.

Among colorectal cancer patients, 50-60% of the cases develop metachronous liver metastases while 20-30% have synchronous ones; furthermore only 10-25% of metastatic patients are suitable surgical candidates.

In different Authors' series it is reported that survival of patients with liver metastases depends primarily from the stage of the primary tumour, nevertheless untreated patients invariably have a poor prognosis. In particular, as regards liver metastases from colorectal cancer, all the series reported in the last 30 years show that median survival time of untreated patients is 6 to 12 months.

The optimization of surgical techniques has yielded a remarkable improvement in the results of hepatic resection which is still to be considered the elective treatment of hepatic metastases from colorectal cancer. In particular 5 year survival of patients with radically resected cancer, that underwent adjuvant systemic and/or loco-regional chemotherapy, is about 25-40%.

If we consider that the extent of resection depends on the site, diameter and proximity to main vascular and biliary vessels; that a free resection margin of 1-2 cm must be preserved; that liver resection should never permanently compromise the residual hepatic function, we must conclude that deep, multiple, huge lesions cannot be resected. Unfortunately other therapeutic options have not demonstrated good results:

- systemic chemotherapy can yield just a slight improvement of median survival time of unresected patients (up to 12-18 months) and a reduction of symptoms is registered only in 15-20% of the cases;
- loco-regional arterial chemotherapy seems to present some advantages in comparison with systemic chemotherapy, in particular it has demonstrated a better control on the tumour growth, a remarkable reduction of systemic toxicity as well as an improvement of the quality of life Nonetheless a significant lengthening of survival has not been registered. Furthermore a variability in the response has been noted in different patients;
- local ablation of the lesions include the use of ethanol, radiofrequency, cryotherapy, laser. These methods present the advantages of reduced surgical trauma but the results depend from the ability of the operator and have limited efficacy mainly in the lesions with diameter

greater than 3 cm as well as in the lesions located in critical sites. Chemo-embolization seems to have a local therapeutic effect due to the combination of ischemia with the cytotoxic effect of chemotherapy, unfortunately this therapeutic option can be employed only for tumour masses located in one hepatic segment or lobe;
- traditional photonic radiotherapy cannot be performed on liver tissue owing to its lack of selectivity with related risk of actinic hepatitis and related fibrosis and portal hypertension;
- finally liver allo-transplantation is not generally performed in metastatic patients because the life expectancy is too short, furthermore the anti-reject treatment in patients with advanced tumour facilitates the progression of the disease, thus the procedure results highly expensive and scarcely effective.

Our project started in 1986 and involves researchers from different disciplines such as medical doctors, biologists, and nuclear physicists. The research consisted of several phases:

- the original structure of the thermal column of the nuclear reactor of the University of Pavia was modified in order to realize a channel with a well-moderated neutron field with a strong reduction of gamma background;
- we have adopted an experimental model of liver metastases from colorectal cancer in the rat;
- we demonstrated both *in vitro* and in the experimental model, that BPA is selectively captured by cancer cells;
- we demonstrated that in the experimental model the concentration ration of ^{10}B in tumour and healthy liver is higher from 4 up to 10 and that the maximum value is obtained 2 hours after the i.v. injection of ^{10}B;
- we demonstrated in the experimental model remarkable damages in tumour cells after BNCT while healthy liver was preserved.

Our clinical experience

We report 2 patients who presented with inoperable diffuse hepatic metastases due to Ca Colon and who had not responded to standard chemotherapy regimen after radical resection of the primary tumour.

After the approval of the Bioethical Committee of IRCCS Policlinico S. Matteo (Pavia) and the Ministry of Health, the candidate patients underwent Boron Neutron Capture Therapy (BNCT), the first of its kind in the whole world.

Patient selection was based on a rigid selection criteria: history of radical resection of primitive tumour, absence of extra-hepatic metastases, good general condition, sero-negative for hepatitis B and C, normal hepatic function, Galactose Elimination Capacity > 50%, negative response to standard chemiotherapy, and age < 50.

BNCT is carried out in 4 phases

1) Selection of candidate patient based on BNCT protocol for Liver Metastases.
2) First Surgical Phase; Hepatectomy
3) Radiotherapeutic Phase: involves neutron radiation of isolated liver in thermal column of the Triga Mark II Nuclear Reactor of the University of Pavia.
4) Second Surgical Phase; Auto-transplantation of treated liver.

The Radio-Surgical procedure began with hepatic perfusion via a tributary of the Portal vein with ^{10}BPA-Fructose solution (300mg/kg body weight) for a period of 2 hours. At an hourly interval biopsies were taken from metastatic tissue (T) and apparently normal tissue (H) for measurement of the actual concentration of ^{10}Boron (C) and the percentage of tumour cells in each specimen. This permitted the determination of ^{10}Boron uptake in tumour cells and the ratio of Boron uptake of tumour cells in respect to normal cells (C_T/C_H.). Pre-established cut-off values of C_T > 45ppm and C_T/C_H ratio > 8 were mandatory for the continuation of the procedure. After cholecystectomy, the liver was mobilised sparing the vascular connection (Hepatic A and V, Portal V, Inf and Sup. Vena

Cava) and the Bile duct. Extra-corporeal veno-venous circulation was initiated by shunting the Lt. Femoral Vein and Inferior Mesenteric Vein to the Lt. Axillary Vein using a Bio-pump after clumping the Sup. and Inf. Vena Cava. At this point hepatectomy was completed. The extracted liver was immediately subjected to cold ischemia and irrigated with ice-cold Belzer Solution. It was then wrapped in specially prepared sterile Teflon bags, placed in a specially prepared rigid transport container and then transferred to the nearby Nuclear Reactor.
Neutron radiation lasted 11 and 9 minutes in the two patients respectively. During the first half of the respective time period the liver was rotated through 180°.
The liver was brought back to the operation theatre for auto-transplantation that involved the vasculare and bile duct re-anastomosis.

In both patients a vascular prothesis (Teflon) was interposed between the vascular stumps of the inferior Vena Cava due to a resulting gap during the vascular re-anastomosis of the liver.
The second patient presented with an abnormality of the hepatic artery, where the Rt. Hepatic artery originated from the Superior Mesenteric artery.
Normal blood circulation was reinstated after 5 hours of extra-corporeal circulation. During this period the 1st patient lost copious amounts of blood from the surgical site. The entire procedure lasted 21hours and 19 hours in the 1st and 2nd patients respectively.
The post-operative period of the 1st patient was characterised by the following complications: renal failure that lasted 10 days, hepatic failure with resulting haemoperitoneum due to coagulation defects. The haemoperitoneum was evacuated on the 8th day Post-Op; jaundice with peak value on the 10th day Post-Op (20mg%); dorsiflexion paralysis of Lt. foot due to compression trauma of the external sciatic-popliteal nerve. On discharge the patient refused to enrol for further regimens of chemotherapy. During the follow-up period the patient presented with a rise in serum tumour marker values with 2 nodules of peritoneal carcinosis, one in the surgical scar and the other located on the parietal peritoneum beneath the II hepatic segment. They were surgically removed and the serum tumour maker values returned to normal by the 10th day Post-Op. At present, 2 years after BNCT, the patient have no recurrence of hepatic metastases.
The 2nd patient initially had a similar post-operative course with initial and subsequent reversal of renal and hepatic failure. On the 15th day Post-Op, the patient went into cardiac failure. He had a positive history of compromised cardiac function due to previous chemotherapy. He died on the 33rd day Post-Op due to ARDS. Post mortem and histological results demonstrated selective targeting of hepatic metastases with 99% necrosis of tumour cells by BNCT.

On the basis of our experience , BNCT combined with adjuvant chemotherapy is efficient in the treatment of hepatic metastases form Colorectal cancer with an important impact on life expectancy. This treatment protocol requires organ neutron radiation outside the hospital setting (in nuclear stations like L.E.N.A in Pavia, ENEA in Frascati, Stusvik in Sweden), or in research centres furnished with proton accelerators (Berkeley, USA).
Further trials are needed to demonstrate the reproducibility of our results with even the participation of other centres in Italy and Europe and the eventual broadening of the application involving other tumours and other transplantable organs like the lungs.