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Boron biodistribution study in colorectal liver metastases patients in Argentina

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ABSTRACT

Ex-situ BNCT for multifocal unresectable liver metastases employing whole or partial autograft techniques requires knowledge of boron concentrations in healthy liver and metastases following perfusion and immersion in Wisconsin solution (W), the procedure employed for organ preservation during ex-situ irradiation.

Measurements of boron concentration in blood, liver and metastases following an intravenous infusion of BPA-F in five colorectal liver metastases patients scheduled for surgery were performed. Tissue samples were evaluated for boron content pre and post perfusion and immersion in W. Complementary histological studies were performed. The data showed a dose-dependent BPA uptake in liver, a boron concentration ratio liver/blood close to 1 and a wide spread in the metastases/liver concentration ratios in the range 0.8–3.6, partially attributable to histological variations between samples.

Based on the boron concentrations and dose considerations (liver $\leqslant$ 15 Gy-Eq and tumor $\geqslant$ 40 Gy-Eq) at the RA-3 thermal neutron facility (mean flux of about $(6\pm1)\times10^9$ n cm^{-2} s^{-1}), ex-situ treatment of liver metastases at RA-3 would be feasible.

1. Introduction

Excluding skin tumours, colorectal cancer represents the third more frequent cancer worldwide, and it is the third cause of cancer deaths in both sexes Local and distant failure, following initial surgery, is the ultimate cause of death. Near 50% of colon cancer patients will be diagnosed with hepatic metastases, including advanced disease at the presentation and relapses.

Conversely to other distant localizations, hepatic metastases are considered a locoregional disease, as a local treatment can improve the overall survival in this group. In spite of the exciting developing of new systemic therapies in colorectal cancer, metastasectomy still is the most useful treatment for advanced disease, and actually, is the way to get the best long-term survival in this group of patients (Nordlinger et al., 2007). Surgery, when possible, could be a curative treatment as recommended by Chong and Cunningham (2005). Resectability rates are low, and chemotherapy and new "target" therapies allow to resect 10–30% of initially unresectable patients. The remaining patients would be candidates for palliative chemotherapy, and some of them for other palliative techniques such as radiofrequency ablation or chemoembolization, both being limited especially by the number of lesions or by the residual functional liver parenchyma after treatment. Taking into account these points, BNCT could be an attractive approach. The fact that BNCT is a biological targeting technique would confer on it the potential of treating undetectable micrometastases.

The selective ^{10}BPA-F uptake in liver metastases was evaluated by Roveda et al. (2004).

The TAOrMINA project developed and employed a new method for BNCT treatment of multifocal unresectable liver metastases, based on ex-situ irradiation and whole liver autograft by Zonta et al. (2006). The surgeons of the Roffo Institute in Argentina (JEC) propose a new technique based on partial liver autograft (Cardoso et al., 2007). In situ BNCT treatment of liver metastases is also being considered in the BNCT community. In all three scenarios boron biodistribution is pivotal to deliver the necessary doses to achieve tumor control ($\geqslant$40 Gy-Eq) and spare healthy liver ($\leqslant$15 Gy-Eq) (Pinelli et al., 2001). In addition, both whole and partial autograft techniques involve liver perfusion with

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Table 1
Characteristics of the patients.

P♯	Weight (kg)	Height (m)	Age	Gender	Adenocarcinoma	Chem.	Surgery type ♯ tumors sampled—localization
1	60	1.73	60	M	ND	Yes	Hepatic tissue ND tumor
2	95	1.80	70	M	MD/WD	No	Left hepatectomy 1-II/III
3	84	1.60	66	M	MD/WD M_1–M_2	Yes	Metastasectomy 2-II, III
4	77	1.76	74	F	M_1: MD/WD M_2:PD	No	Right hepatectomy 2-V, VII
5[a]	82	1.57	53	F	M_1 WD M_2: PD	Yes	Metastasectomy 2-V, III

ND: Not detected; P: patients; Chem: chemotherapy; W: well; M: moderately; P: poorly; D: differentiated.
[a] Heavy chemotherapy pretreatement.

Wisconsin solution (W) and hypothermal preservation in W during neutron irradiation. Thus, the boron concentration in liver and metastases following W perfusion and immersion in will reflect a clinical scenario more adequately, issue not been addressed to date.

The aim of the present study was to determine boron concentration in blood, liver and tumor tissue, both pre and post perfusion and immersion in W, following an intravenous infusion of L-p-boronophenylalanine complexed with fructose (^{10}BPA-F) in patients scheduled for colorectal liver metastases surgery.

2. Materials and Methods

2.1. Patients

From August 2007 to March 2008, five patients were enrolled in this trial, three males and two females; Table 1 shows the patient data. These trials were performed with approval ♯ 5178/00 addenda July/03/05 from the Argentine National Agency of Drugs, Food and Clinical Technology (ANMAT).

All the patients described in this study were scheduled to undergo surgery for colorectal liver metastases. In each of the patient (P) a diagnosis of adenocarcinoma was confirmed by histopathological analysis and liver CT scans. All P gave informed consent to participate in the study.

Protocol eligibility criteria included normal renal function, absence of cardiovascular disease and phenylketonuria. Blood pressure, temperature and pulse rate were within normal limits in all P at the start of the BPA infusion. P5 was heavily pre-treated with chemotherapy up to six months before surgery.

BPA was administered intravenously (iv) at a dose of 100 mg/kg $_{bw}$ ($n = 3$) or 300 mg/kg $_{bw}$ ($n = 2$), infused over 80–90 min. Blood samples for boron analyses were taken during surgery concomitantly with tissue resection, 80–220 min after the end of the infusion depending on surgical procedures.

2.2. BPA-F infusion, boron analysis

^{10}BPA isotopically enriched (>99 at%) was obtained from Glyconix (New York, NY). A fresh solution of BPA-F was prepared for each P. Boron measurements for blood, liver and tumor tissues were performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) following the same procedure as described previously (Liberman et al., 2004).

2.3. Tissue sampling and processing

One set of samples was processed for measurement of boron concentration. A second set was fixed in 10% buffered formalin for histological analysis and a third set was stored in liquid nitrogen for future boron microdistribution studies. Tissue samples were evaluated pre and post perfusion and about 2 h immersion in W to simulate the clinical scenario of ex-situ irradiation.

Degree of perfusion varied with the surgical procedure and was not always representative of perfusion in an autograft scenario. In particular, P5 had inefficient W. Sets of paired adjacent tumor samples were taken for P4 and P5 to explore the potential association between boron uptake and histology. There were no detectable metastases (ND) in P1 (due to complete response to preoperative chemotherapy), one set of metastases samples (M_1) was taken for P2 and two sets for P3, P4 and P5 (M_1,M_2).

3. Results

Table 2 summarizes the information of the BPA dose infused to each P, over 80–90 min, tissue extraction time after infusion ended (Te), boron uptake in blood (B), liver (L), metastases (M_1, M_2) and all tissues perfused and immersed in W and boron concentration ratios before L/B, M/L and after W. Standard deviation of the data is indicated when the number of samples $n \geqslant 3$. P1–3 received a BPA dose 100 mg/kg $_{bw}$, while P4–5 had 300 mg/kg $_{bw}$, clinical dose in the TAOrMINA trial (Zonta et al., 2006)

Fig. 1 shows data from individual boron concentration M/L ratios without W. The histological analysis of the tumor samples showed heterogeneity and areas with varying degrees of necrosis, even within the same metastases. Taking into account the individual samples in Fig. 1 we found some correlation between boron uptake and histology; i.e. lower boron content was frequently associated to predominantly necrotic samples and poorly differentiated areas were mostly associated to higher boron values.

In Fig. 2 data normalized to 100 mg/kg $_{bw}$ BPA dose, for B, L, M_1 and M_2 ($\pm$SD) are plotted for all patients, at Te. The data is consistent with the heterogeneity shown in Fig. 1.

4. Discussion

The pharmacokinetics of boron values in blood given in Table 2, within the usual spread for P, is in agreement with Liberman et al. (2004) and Wittig et al. (2008).

Table 2
Infusion parameters and boron uptake in tissues with and without W.

P #	Te (min)	Blood (μg/g)	Liver (μg/g)	Liver/blood	Metastases (μg/g)	Metastases/liver	Liver $_W$ (μg/g)	Metastasis $_W$	(Metastases/liver)$_W$
1	75	5.9±0.1 $n=2$	6.2±0.6 $n=7$	1.0±0.1	ND	–	4.3±0.4 $n=11$	–	–
2	220	3.8±0.3 $n=2$	3.9±0.7 $n=11$	1.0±0.2	8.0±02 $n=3$	2.0±0.2	2.9±0.4 $n=8$	6.7±0.9 $n=7$	2.3±0.2
3	127	3.4±0.1 $n=2$	4.2±0.2 $n=6$	1.2±0.1	M_1: 10.2 $n=1$ M_2: 12±3 $n=4$	M_1 :2.4 M_2: 2.9±0.	3±1 $n=2$	M_1:8±2 $n=5$ M_2: 8.7 $n=1$	M_1: 2.7±0.4 M_2: 2.9
4	165	14.7±0.3 $n=9$	15±1 $n=16$	1,0±0.1	M_1: 28±3 $n=7$ M_2: 31±10 $n=14$	M_1 : 1.9±0.1 M_2 : 2.1±0.3	9.3±0.9 $n=12$	M_1: 14±4 $n=10$ M_2:14±7 $n=10$	M_1 : 1.5±0.3 M_2 :1.5±0.6
5[a]	169	12.0±0.4 $n=6$	11.8±0.9 $n=3$	1.0±0.1	M_1:12.6±0.7 $n=5$	M_1 : 1.1±0.1	12±1 $n=4$	M_1: 11±1 $n=3$	M_1: 0.9±0.1
	187	12.5±0.4 $n=9$	13.5±0.7 $n=4$	1.1±0.1	M_2:19±2 $n=4$	M_2 : 1.4±0.1	11.8±0.6 $n=3$	M_2: 11.6±0.6 $n=3$	M_2: 1.0±0.1

P: patients; P1–3: 100 mg/kg $_{bw}$; P4–5: 300 mg/kg $_{bw}$ BPA; Infusion time 80–90 min; Te: time of sample extraction after end of infusion; W: perfused with and immersed in Wisconsin solution; M_1, M_2: metastases 1 and 2.
[a] Inefficient W.

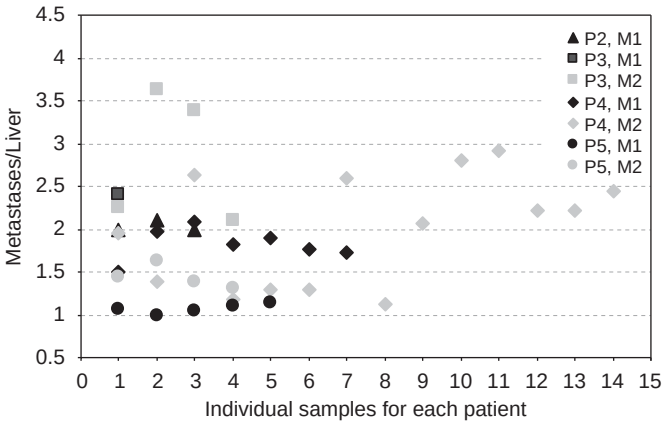

Fig. 1. Metastases/liver individual boron concentration ratio for patients (P) and metastases M_1 and M_2 without W.

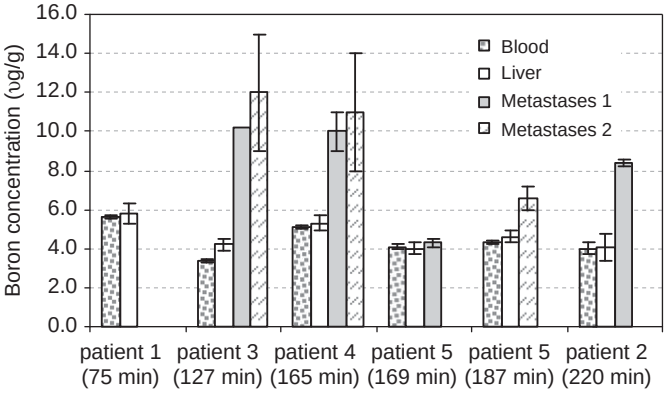

Fig. 2. Mean boron concentrations (±SD) normalized to 100 mg/kg BPA$_{bw}$, for all patients ($n=1$ for M_1–P3). Time after infusion is shown for each patient.

Mean L/B ratio: 1.05±0.08 ($n=6$), calculated from Table 2 shows an equivalence between blood and liver boron concentration values for all doses and dose-dependent absolute boron uptake by liver within the conditions of the study.

This L/B constant ratio is markedly relevant in the clinical trials involving whole or partial liver autograft, when ICP is used for boron measurements. Within this context, tissue boron content values can be obtained from the blood measurements that are much faster to perform.

Average M/L ratios in Table 2 are 2.0±0.6 and 2.2±0.8 for intact and W samples respectively. These results show no difference between these groups, within the precision of the measurements.

The boron loss by the W calculated from Table 2 (disregarding P5 due to inefficient W) ranges from 26 to 38 and from 15% to 55% for the liver and metastases respectively. This % lost from the liver vascular system was within the 30% range, consistent with the data reported for liver by Blustajn et al. (1997). The higher dispersion values for the boron loss in the metastases might be partially due to their heterogeneity.

The M/L boron concentration ratios, with and without W were $1.5 \leqslant (M/L)_W \leqslant 2.9$ or $1.1 \leqslant M/L \leqslant 2.9$, respectively (Table 2). Those values are strongly dependent on the histology of the sample. For the individual samples in Fig. 1: $0.9 \leqslant M/L \leqslant 3.6$.

The analysis of the present samples and previous studies by Roveda et al. (2004) and Wittig et al. (2008) suggest that M/L ratios would be considerably higher if the values reported herein are corrected for % of metabolically active tumor.

Further microscopic analyses of the samples should be performed, disregarding necrotic and connective tissue, to improve the accuracy of actual effective M/L ratios.

The present data allow us to define the boron concentration conditions, for M and L to achieve tumor control with no L damage.

A preliminary attempt for dose calculation (D) for L and M (D_L and D_M) is done assuming the higher M/L range in Fig. 1 $M/L \sim 3.5$ and $L = 15$ μg/g for 300 mg/kg $_{bw}$ BPA dose. For the healthy liver the tolerance dose assumed is $D_L \leqslant 15$ Gy-Eq as a conservative value, while the dose for tumor control considered is $D_M \geqslant 40$ Gy-Eq, in agreement with (Pinelli et al., 2001).

For $D_L \leqslant 15$ Gy-Eq, $D_M \geqslant 53.4$ Gy-Eq is calculated for the irradiation in the thermal column of the RA-3 reactor Gadan et al., 2008, with a mean flux at the irradiation position of about $(6 \pm 1) \times 10^9$ n cm^{-2} s^{-1} and a fluence after 12 min irradiation $\sim 4 \times 10^{12}$ n cm^{-2}. Assuming 20% boron losses in L and M by W, after 12.4 min irradiation $D_L \leqslant 15$ and $D_M \geqslant 49.6$ Gy-Eq. These estimated doses make the ex-situ treatment of liver metastases at RA-3 feasible.

5. Conclusions

Under the conditions of this study we conclude:

(a) $L/B = 1$ for the samples, showing an equivalence between blood and liver values for all the doses.
(b) The spread in M/L ratios is strongly dependent on the histology of M.
(c) W does not show a significant effect on M/L.
(d) The RA-3 reactor thermal facility provides an adequate neutron flux for ex-situ treatment of liver metastases.

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