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# BNCT of 3 cases of spontaneous head and neck cancer in feline patients

Mónica Rao[a], Verónica A. Trivillin[b], Elisa M. Heber[b], María de los Angeles Cantarelli[a], María E. Itoiz[b,c], David W. Nigg[d], Raúl Jiménez Rebagliati[e], Daniel Batistoni[e], Amanda E. Schwint[b,*]

[a] *Veterinary Oncology Center, Avenida del Libertador 15013, (1642) Acassuso, Prov. Buenos Aires, Argentina*
[b] *Department of Radiobiology, National Atomic Energy Commission, Avenida General Paz 1499, (1650) San Martín, Prov. Buenos Aires, Argentina*
[c] *Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, Argentina*
[d] *Idaho National Engineering and Environmental Laboratory (INEEL), USA*
[e] *Department of Chemistry, National Atomic Energy Commission, Argentina*

**Abstract**

Having demonstrated BPA-BNCT induced control of experimental squamous cell carcinomas (SCC) of the hamster cheek pouch mucosa with no damage to normal tissue we explored the feasibility and safety of treating spontaneous head and neck tumors, with particular focus on SCC, of terminal feline patients with low dose BPA-BNCT employing the thermal beam of the RA-1 Reactor within a preclinical context. The biodistribution studies showed that, in all three cases evaluated, BPA delivered absolute boron values to tumor in the range that proved therapeutically useful in the experimental model of SCC. BPA-BNCT studies showed no radiotoxic effects, partial tumor control in terms of impaired growth and partial necrosis, an improvement in clinical condition and prolonged survival beyond the terminal condition of the feline patients at the time of recruitment.


*Keywords:* Preclinical BNCT; Squamous cell carcinoma; Feline

## 1. Introduction

We proposed and validated the use of the hamster cheek pouch oral cancer model to explore new applications of BNCT, investigate the radiobiology of BNCT (Coderre and Morris, 1999), analyze the behavior of clinically relevant, dose-limiting, normal tissues and test the potential therapeutic advantage of different boron agents (Barth et al., 2003), alone or administered jointly (Kreimann et al., 2001a, b, 2003; Heber et al., 2004). Having demonstrated BPA-BNCT induced control of experimental squamous cell carcinoma (SCC) of the hamster cheek pouch mucosa with no damage to normal tissue and only slight, reversible mucositis in precancerous tissue around tumor (Kreimann et al., 2001b), we decided to explore the feasibility and safety of treating spontaneous head and neck tumors of feline patients with BPA mediated BNCT employing the thermal beam of the RA-1 Reactor within a preclinical context. Within the context of head and neck tumors, we were particularly interested in the study of SCC. The study of spontaneous tumors of the same histological type that we induce experimentally in the hamster cheek pouch would allow us to examine, to a certain degree, the clinical relevance of our experimental data.

SCC is a common tumor involving the skin and accounts for approximately 15% of cutaneous tumors in cats. SCCs are usually found in unpigmented or lightly

*Corresponding author. Tel.: +54-11-6772-7149; fax: +54-11-6772-7121.
*E-mail address:* schwint@cnea.gov.ar (A.E. Schwint).

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pigmented skin and the most common cutaneous locations in the cat are the sparsely haired areas of the nasal planum, eyelids and pinnae. Generally, SCCs involving the facial skin of cats are locally invasive but late to metastasize. The degree of local invasion can be quite severe and significantly impairs therapeutic success. Surgery or cryosurgery are most commonly used. Radiotherapy, photodynamic therapy and chemotherapy are also used with varying efficacy. Tumors of higher stages consistently respond poorly to all therapeutic modalities.

The aim of the present study was to perform biodistribution studies of BPA and BPA-BNCT at a low total radiation dose in virtually terminal feline patients with head and neck cancer, with a particular focus on SCC. This study was performed on feline patients that were not eligible for standard treatment due to the degree of local invasion of the tumor or that had been refractory to standard treatments. The specific aim of the study was to assess the feasibility of treating spontaneous head and neck cancer safely with BPA-BNCT, evaluate potential radiotoxic effects on normal tissue and, if possible, monitor potentially therapeutic effects on tumor tissue, improve the clinical condition of virtually terminal feline patients and prolong their survival.

## 2. Materials and methods

### 2.1. Feline patients

Eligibility criteria included a pathological diagnosis of head and neck cancer, and a clinical assessment of the case indicating that standard therapy was no longer a valid option. Three cases of felines which presented with terminal head and neck cancer were included in the study. Diagnosis was confirmed by histopathological analysis at the time of the study. When possible, laboratory blood tests were performed to determine the clinical status of the animal. Informed consent forms were signed by the owners. The study was carried out in strict compliance with local regulations to protect animal subjects.

*Patient 1*: Female, adult (age unknown), with a highly aggressive, locally invasive tumor in the eyebrow area, diagnosis: neurofibroma/neurinoma, time of evolution: approximately 2 years. The cat had been submitted to surgical removal of the tumor three times (following subsequent recurrences) and ablation of one eye approximately one year earlier. The tumor had recurred and standard therapy was no longer a valid option.

*Patient 2*: Female, 13 years old, with an ulcerative, bleeding, locally invasive tumor in the nose area, diagnosis: SCC, time of evolution: approximately 1.5 years. The animal exhibited asthenia, difficulty to breathe and lack of appetite. Local tumor invasion impaired olfaction and had led to blindness. The cat had received no prior treatment.

*Patient 3*: Female, 12 years old, with an ulcerative, bleeding, locally invasive tumor in the nose area, diagnosis: undifferentiated SCC, time of evolution: approximately 5 years from detection of the first lesion. The cat had received 2 cycles of chemotherapy 9 months earlier. Tumor control had been only slight.

### 2.2. BPA biodistribution studies

A 0.14 M solution of L-BPA-fructose (>98% $^{10}$B-enriched) was administered intravenously over approximately 15–20 min at a dose of 300 mg/kg body weight. Blood samples (duplicate samples of 0.2 ml) were taken prior to infusion, at the end of the infusion, and 30 min, 1, 2 and 3 h after the end of the infusion under light ketamine-xylazine anesthesia. At 3 h after the end of the infusion the animals were anesthetized with ketamine-xylazine. The surgeon took one or more (if possible) samples of tumor, one sample of lip mucosa and/or one sample of dorsum skin and, in one case (Patient 2), one sample of tumor infiltrated pinna. In the case of Patient 1 we also took a sample of saliva and two samples of urine. The samples were processed for Boron analysis by atomic emission spectroscopy with inductively coupled plasma (ICP-OES) as previously described (Kreimann et al., 2001a).

### 2.3. In vivo BNCT

Having demonstrated selective uptake of absolute amounts of boron in tumor that have previously been proved therapeutically useful in experimental SCC (Kreimann et al., 2001a), we proceeded to perform BNCT approximately 2 weeks after the biodistribution study. Irradiations were performed with the thermal beam of the RA-1 Reactor of the Constituyentes Atomic Center at a flux of approximately $3 \times 10^8$ n/cm$^2$ s to the tumor area in keeping with preliminary flux wire measurement data of Nigg et al. (personal communication). The present geometric set-up involves no body shielding. Irradiations lasted 10 min, resulting in an approximate fluence of thermal neutrons of $1.8 \times 10^{11}$ n/cm$^2$ and a total gamma dose of $0.28 \pm 0.06$ Gy to the tumor area. The animals were irradiated under ketamine-xylazine anesthesia. Patient 3 was treated with BNCT a second time, 7.5 months after the first treatment. Follow-up of tumor evolution and clinical status was performed periodically in all cases. A biopsy of the tumor area post-BNCT was taken at a representative time-point. Autopsies of the three cats were performed following euthanasia as indicated in the results section. Radiotoxic effects were evaluated in terms of clinical signs such as presence of gastrointestinal

syndrome, symptoms of neurological involvement such as convulsions, ataxia and lack of coordination, behavioral disturbances, regional loss of hair and peripheral congestion and findings at autopsy compatible with radioinduced toxicity such as gastric and intestinal ulcerations, ascitis, fatty deposits in liver and lung edema.

## 3. Results

### 3.1. BPA biodistribution studies

Tables 1, 2 and 3 show the boron values for each patient, and each of the samples and time-points evaluated. In each case we took the number of samples that was compatible with clinical practice. Thus, the data for some of the time-points and tissues are lacking. In all cases pre-infusion values were below the detection limit. Tumor/blood boron ratios were 2.7/1 in the case of Patient 1 (Table 1), 4.3/1 in the case of Patient 2 (Table 2) and 2.3/1 (Table 3A) and 2.6/1 (Table 3C) in the case of Patient 3. In the case of Patient 3, the cat for which three sets of data were available, we observed reasonable reproducibility of the data before treatment and on the day of treatment although it was not possible to exactly match the samples and time-points (Tables 3A and B). The biodistribution data obtained 7.5 months after the first treatment with BNCT were slightly higher, overall, than the first set of data (Tables 3A and C). However the tumor/blood boron ratios remained almost constant. Absolute and relative boron values warranted performing BNCT in all three cases.

### 3.2. In vivo BNCT

*Patient 1*: The cat did not evidence any radiotoxic effects evaluated as described in Materials and methods. In this case which involved a measurable tumor mass, 20 days post-BNCT we detected a 12% reduction in tumor volume followed by a halt in tumor growth. One month post-BNCT the histopathological analysis of a biopsy taken of the tumor area showed areas of necrosis intermingled with viable areas. The light microscopy image of the tumor area pre-BNCT showed only viable, proliferative nuclei. Five months post-BNCT the animal was euthanized due to generalized decline. The autopsy failed to show any lesions attributable to radioinduced toxicity.

*Patient 2*: The cat did not evidence any radiotoxic effects evaluated as described in Materials and methods. Twenty-four hours post-BNCT the tumor area exhibited a scab. One week post-BNCT the cat's clinical status improved in terms of mobility, appetite and breathing. Before BNCT, the lesion had impaired breathing and eating (Fig. 1A). At 17 days post-BNCT, tumor control allowed for the formation of healthy epithelium in the tumor area (Fig. 1B) . One and a half months

Table 1
Boron concentration (ppm) in blood and tissue samples at different times after administration of BPA at a dose of 300 mg/kg to Patient 1, 2 weeks before treatment with BNCT

| Time post-administ. (h) | Blood | Tumor | Skin over tumor | Face skin | Urine | Saliva |
|---|---|---|---|---|---|---|
| 0 | 32.2 | | | | | |
| 0.5 | 16.4 | | | | 182.5 | |
| 1 | 16.6 | | | | | |
| 1.5 | 11.0 | | | | | |
| 2 | 12.8 | | | | | |
| 3 | 7.9 | $21.6 \pm 3.0$ ($n = 12$) | 18.3 | 13.3 | 368.8 | 0 |

Zero hours indicates the end of the infusion. The number of samples is indicated in brackets when appropriate.

Table 2
Boron concentration (ppm) in blood and tissue samples at different times after administration of BPA at a dose of 300 mg/kg to Patient 2, 2 weeks before treatment with BNCT

| Time post-administ. (h) | Blood | Tumor | Tumor-infiltrated pinna |
|---|---|---|---|
| 1 | 20.4 | | |
| 2 | | | |
| 3 | 9.2 | 39.2 | 35.4 |

Table 3
Boron concentration (ppm) in blood and tissue samples at different times after administration of BPA at a dose of 300 mg/kg to Patient 3

| Time post-administ. (h) | Blood | Tumor | Lip mucosa | Skin dorsum |
|---|---|---|---|---|
| (A) 2 weeks before the first treatment with BNCT | | | | |
| 0 | 40.3 | | | |
| 0.5 | 19.5 | | | |
| 1 | 14.1 | | | |
| 2 | 11.1 | | | |
| 3 | 7.9 | $18.2 \pm 0.4$ ($n = 3$) | 12.4 | 11.2 |
| (B) On the day of the first treatment with BNCT | | | | |
| 2 | $9.7 \pm 0.4$ ($n = 4$) | | | |
| 2.5 | | | | 18.1 |
| 3.5 | $12.6 \pm 1.0$ ($n = 4$) | | | $15.5 \pm 4.7$ ($n = 4$) |
| (C) 2 weeks before the second treatment with BNCT | | | | |
| 0 | 55.4 | | | |
| 0.5 | 20.2 | | | |
| 1 | 13.6 | | | |
| 2 | 9.5 | | | |
| 3 | 9.2 | $24.0 \pm 2.8$ ($n = 6$) | 17.3 | 14.6 |

Zero hours indicates the end of the infusion. The number of samples is indicated in brackets when appropriate.

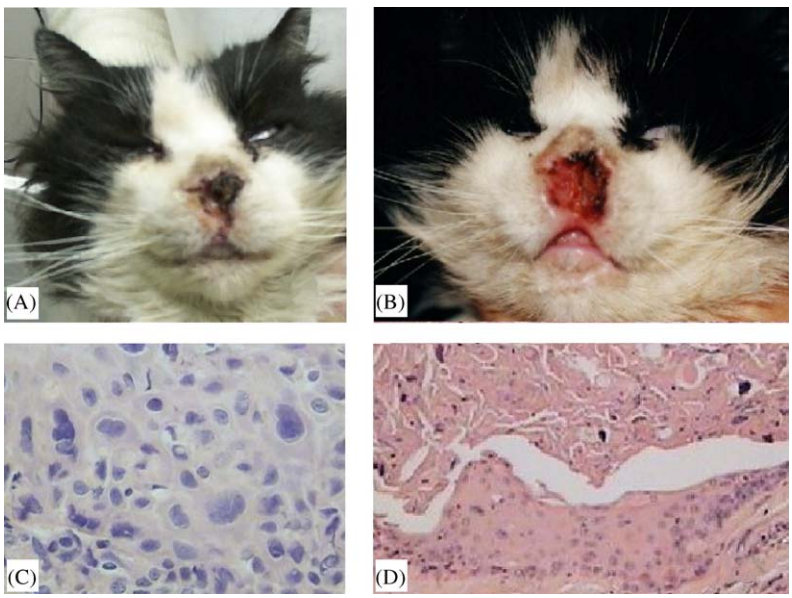


Fig. 1. (A) Macroscopic view of the tumor area of Patient 2 pre-BNCT. The proliferative, bleeding tumor is covered by a scab that periodically fell off and was replaced by a new one. (B) Macroscopic view of the tumor area of Patient 2, 17 days post-BNCT. Tumor control was sufficient to allow for complete epithelialization of the tumor area. (C) Characteristic light microscopy view of tumor area of Patient 2 pre-BNCT showing viable, proliferative tumor cells (magnification: $\times 40$, hematoxylin-eosin stain). (D) Characteristic light microscopy view of tumor area of Patient 2, 1.5 months post-BNCT exhibiting areas of necrosis interspersed with viable tumor cells (magnification: x40, hematoxylin-eosin stain).

post-BNCT a biopsy of the tumor area exhibited areas of necrosis interspersed with viable areas (Fig. 1D) which contrasted with the light microscopy images pre-BNCT that evidenced only viable, proliferative nuclei (Fig. 1C). At 2.5 months post-BNCT the cat was euthanized due to general decline. The autopsy failed to show any lesions attributable to radioinduced toxicity.

*Patient 3*: The cat did not evidence any radiotoxic effects evaluated as described in Materials and methods after the first treatment with BNCT. Over the first month post-BNCT, macroscopic follow-up revealed partial tumor control in terms of a halt in gross tumor growth and the development of areas of necrosis. Macroscopic observations correlated with biopsies taken 8 and 33 days post-BNCT that showed areas of necrosis surrounded by viable tumor cords. Three months post-BNCT the cat continued to be in good clinical condition as evaluated subjectively in terms of mobility, appetite, and overall comfort. However, no further tumor control was observed. A course of chemotherapy was performed at that time but the tumor failed to respond. Seven months post-BNCT we performed a second biodistribution study as previously described. The potentially therapeutic boron concentration values (Table 3C), the fact that no other therapeutic option could be made available to the animal, and the potentially contributory nature of the data that could be obtained, prompted us to perform a second treatment with BNCT 7.5 months after the first treatment. Two days after the second treatment with BNCT the animal exhibited a clinical decline. Steroids were administered. Four days post-BNCT the cat had recovered mobility and appetite and the tumor exhibited macroscopically necrotic areas. Twenty days post BNCT the cat began climbing trees. A radiograph of the lungs showed no radioinduced lesions at that time. The only change attributable to radiotoxicity was loss of hair in the area surrounding the tumor. Tumor control was only partial and at 2 months after the second treatment with BNCT marked tumor invasion was detected clinically in the neck lymph nodes. Over the period after the first treatment with BNCT, lab tests indicated slight anemia and renal disorders that were present prior to treatment. Before the second treatment with BNCT the anemia had worsened but responded to iron therapy. The animal was euthanized 2 months after the second treatment with BNCT and 9.5 months after the first treatment because no further tumor control was observed and further tumor invasion and clinical decline were anticipated. The autopsy showed lung metastasis and failed to reveal any lesions attributable to radioinduced toxicity.

## 4. Discussion

The present study showed that BPA delivers boron to spontaneous feline head and neck cancer in amounts that proved therapeutically useful in experimental oral cancer in hamsters (Kreimann et al., 2001a). Herein we showed that it is feasible to treat spontaneous head and neck carcinomas in feline patients with BNCT with the thermal beam of the RA-1 Reactor, achieving a therapeutic effect with no significant damage to normal tissues. This is the first BNCT study in spontaneous feline cancer worldwide.

Given that the thermal fluence was low, the main endpoint to be assessed was safety. In this sense, no radiotoxic effects were observed as evaluated in terms of clinical symptoms, macroscopic observation, lab tests and histopathological analysis of autopsy material. Preliminary characterization of the thermal beam of RA-1 precludes an accurate calculation of the actual dose delivered to tumor. However, a rough estimation of physical dose delivered to tumor affords values in the range of 0.6 Gy with a 30% uncertainty (David Nigg, personal communication). Ongoing studies will contribute to further beam characterization that may reduce the uncertainty in dose calculation. The low dose is in keeping with the primarily safety assessment aim of this study. However, almost surprisingly, this dose elicited partial tumor control in terms of impaired growth and partial necrosis. Within this context, we may speculate that compound biological effectiveness (CBE) values (to date undetermined) for these tumors may be higher than expected. The fact that BNCT improved the clinical condition of the animals and prolonged their survival beyond their terminal condition at the time of recruitment is clearly an asset. The present study also demonstrated, within the present conditions, the possibility of performing two treatments with low-dose BNCT, 7.5 months apart, with no significant radiotoxic effects on the animal. The two cases of SCC described herein allowed us to extrapolate to a preclinical context, within the constraints of the present study, certain aspects of our biodistribution and BNCT studies on experimentally induced SCC in the hamster cheek pouch (Kreimann et al., 2001a, b).

The present data warrant future dose-escalation studies and the inclusion of feline patients with SCC at an earlier stage of evolution. Given the encouraging data on in vivo BNCT mediated by a combination of GB-10 and BPA in the hamster cheek pouch oral hamster model (Trivillin et al., 2004), future preclinical studies on spontaneous feline head and neck cancer may address the potential of BNCT mediated by a combination of GB-10 and BPA.

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