Editorial

Teachings of our translational studies on boron neutron capture therapy (BNCT): thinking "outside the box"

Amanda E. Schwint[1,2], Marcela A. Garabalino[1], Andrea Monti Hughes[1,2], Emiliano C. C. Pozzi[1], Elisa M. Heber[1], Mónica A. Palmieri[3], Verónica A. Trivillin[1,2]

[1]Comisión Nacional de Energía Atómica (CNEA), San Martin, Provincia Buenos Aires, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina; [3]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

Correspondence to: Amanda E. Schwint, PHD. Head Radiation Pathology Division, Department of Radiobiology, Comisión Nacional de Energía Atómica, Avenida General Paz 1499, (B1650KNA) San Martín, Provincia Buenos Aires, Argentina. Email: mandyschwint@gmail.com.

Received: 12 February 2019; Accepted: 21 May 2019; published: 11 June 2019.
doi: 10.21037/tro.2019.05.03
View this article at: http://dx.doi.org/10.21037/tro.2019.05.03

Basic principles of boron neutron capture therapy (BNCT)

BNCT is a technique for the treatment of solid tumors. BNCT is considered a binary technique because it involves two components that exert little or no action individually but induce a significant effect when they combine. BNCT is based on the combination of neutron irradiation and the administration of ^{10}B compounds that are incorporated selectively by tumor tissue via different mechanisms, depending on the boron carrier. The capture reaction between a thermal neutron and a boron-10 nucleus gives rise to the formation of a ^{7}Li nucleus and an alpha particle. These densely ionizing particles have high linear energy transfer (LET), high relative biological efficacy (RBE) and travel only a short distance in tissue (5–9 micrometers). The fact that high LET particles have a short range in tissue makes the microdistribution of boron relative to a sensitive target of great relevance to therapeutic efficacy. Damage is largely circumscribed to tumor tissue where ^{10}B atoms are preferentially localized (1,2). Thus, BNCT targets tumor tissue selectively, sparing healthy tissue. In the case of standard radiotherapy, selective tumor targeting is based on identification of the tumor volume and delimitation of its boundaries. However, in the case of BNCT, selective tumor targeting is biological/biochemical, relying on preferential incorporation of boron to tumor. In this way, BNCT would be adequate to treat infiltrating cells or micrometastases that cannot be identified by imaging techniques (3).

BNCT involves a mixed irradiation field composed of the boron dose component that affects tumor selectively and a background dose that affects tumor and healthy tissue alike. The background dose includes the low-LET gamma rays that contaminate the neutron beam and those resulting from the capture of a thermal neutron by a hydrogen atom, high LET protons resulting from the scattering of fast neutrons and high-LET protons that originate in the capture of a thermal neutron by a nitrogen atom. The design of BNCT protocols strives to maximize the ratio between the tumor selective boron component of the dose and the non-selective background dose (1).

BNCT clinical trials

BNCT clinical trials for the treatment of glioblastoma multiforme, melanoma, recurrent head and neck tumors, and liver metastases performed in the United States, Japan, Europe, Argentina and Taiwan have been performed employing mostly nuclear reactors (4-7). Recent clinical BNCT trials in Japan have explored the potential therapeutic advantage of BNCT to treat other tumors and localizations such as recurrent hepatic and gastrointestinal cancer, lung cancer and extra-mammary Paget's disease (8-10). Overall clinical results to date show a therapeutic advantage for BNCT associated to an improvement in quality of life and prolonged survival (11). Nevertheless, optimization of BNCT is clearly necessary to improve therapeutic efficacy and reduce associated toxic side-effects. The use of hospital-based accelerators as the neutron source

rather than nuclear reactors will promote new clinical trials for existing and new treatment targets.

Translational radiobiological BNCT studies for head and neck cancer

Translational radiobiological studies in appropriate *in vivo* experimental models are essential to the advancement of BNCT for different pathologies. Our first BNCT studies were devoted to explore what was then a new application of BNCT, i.e., the treatment of head and neck cancer. We provided evidence of the success of BNCT employing the boron compound boronophenylalanine (BPA) as the boron carrier to treat head and neck cancer in an oral cancer model in the hamster cheek pouch (91% tumor response [partial remission + complete remission] prescribing 5 Gy absorbed dose to tumor). Mucositis in the radiosensitive precancerous tissue surrounding tumors (precancerous tissue mimics field-cancerized tissue in human oral cancer) was reversible but limited the dose that could be applied to tumor. The dose of 5 Gy prescribed to tumor was based on pilot studies to establish the maximum dose that could be administered to tumor without exceeding the radiotolerance of precancerous tissue, the dose-limiting tissue. In later studies we prescribed dose to precancerous tissue, considered as the "organ at risk" within the treatment volume. This study suggested, for the first time, that BNCT could induce a robust tumor response in head and neck cancer with acceptable toxicity in dose-limiting precancerous tissue (12,13). These translational studies contributed to the first clinical BNCT trial for head and neck cancer (14).

Seeking to improve BNCT therapeutic efficacy, we envisioned the potential value of combining the boron agents BPA and decahydrodecaborate (GB-10). Homogeneous tumor boron targeting is essential to the success of BNCT because those cells that are loaded with insufficient ^{10}B atoms ($<10^9$ ^{10}B atoms) will fail to respond to BNCT and cause the failure of treatment. A combination of boron carriers that are incorporated to cells by different mechanisms will favor tumor boron targeting homogeneity and concomitant improved therapeutic efficacy of BNCT (15,16). Merely as a control group, we explored the boron biodistribution of GB-10 in the oral cancer model in the hamster cheek pouch. GB-10 is a largely diffusive agent that was proposed as a boron carrier for the treatment of brain tumors with BNCT because it does not traverse the intact blood brain barrier (BBB) but can enter tumors surrounded by a disrupted BBB. We thus did not expect GB-10 to target tumor preferentially in the case of tumors outside brain and assumed that it would not serve as a boron carrier used alone for oral cancer. In effect, our boron biodistribution studies showed that GB-10 did not target hamster Squamous Cell Carcinomas (SCC) selectively (17). However, counter-intuitively, we showed that even when GB-10 is not incorporated selectively by tumor, when used as a boron carrier for BNCT in our oral cancer model, it leads to selective tumor damage with no concomitant precancerous and normal tissue toxicity. GB-10-BNCT induced a 70% tumor response at approximately 8 Gy absorbed dose to tumor, associated to only mild and reversible mucositis in dose-limiting precancerous tissue. The fact that GB-10-BNCT induced milder toxicity than BPA-BNCT in dose-limiting precancerous tissue allowed us to escalate tumor dose. This new paradigm in BNCT would be based on a selective effect of GB-10-BNCT on the more radiosensitive aberrant tumor vasculature while precancerous and normal tissues that do not have aberrant vasculature are preserved (18). If we had relied only on biodistribution studies (17), we would have discarded GB-10 as a boron compound administered alone for BNCT of tumors of the head and neck due to its lack of tumor selectivity. However, actual *in vivo* BNCT studies showed that a selective lethal effect on tumor can occur as a result of a selective effect of GB-10-BNCT on aberrant blood vessels in tumor instead of the selective incorporation of the boron agent by tumor tissue. In addition, the other side of the coin of a lack of selective tumor *vs.* blood, precancerous and normal tissue uptake of GB-10, is homogeneous boron targeting within the tumor, a "plus" for therapeutic success. Conversely, BPA is taken up selectively by tumor by active transport using the amino acid LAT1 transport system that is overexpressed in metabolically active cells. The down-side of BPA's selective tumor uptake is that, because it is associated to enhanced metabolic activity, boron targeting within the tumor is heterogeneous, admittedly a "minus" for therapeutic success (16,18).

The assessment of BNCT mediated by BPA + GB-10 administered together did in effect enhance therapeutic effect, conceivably by bringing together in a single treatment the selective effect of GB-10-BNCT on aberrant blood vessels and the "cell by cell" therapeutic effect of BPA-BNCT and, additionally, by improving tumor boron targeting homogeneity. Also, an enhanced tumor response rate (93% at approximately 8 Gy absorbed dose to tumor), was associated to milder mucositis in precancerous

tissue than in the case of BNCT mediated by BPA (18). Mucositis is a particular concern associated to BNCT mediated by BPA. This is not a surprising finding because mucositis originates in damage to the basal layer (19), exactly the site where BPA would accumulate (13). The largely diffusive boron compound GB-10 does not target the basal layer preferentially and GB-10-BNCT (delivered at therapeutically useful doses), induces only mild, reversible, mucositis in precancerous tissue. Boron targeting homogeneity in tumor, achieved by administering combinations of boron compounds, or a largely diffusive boron compound such as GB-10, also improved the complete remission rate induced by BNCT in the larger tumors, the most difficult tumors to treat (18).

Because optimizing tumor boron targeting is the best way to maximize the therapeutic efficacy of BNCT, widespread international efforts are focused on the development of the "ideal" boron compound for BNCT, i.e., it will be non-toxic at therapeutic dose levels, it will be tumor selective, it will enter all tumor cells and will be an efficient carrier of ^{10}B to tumor, placing ^{10}B in the vicinity of radiosensitive intracellular targets. BNCT literature typically stresses the importance of a high (≥3/1) tumor/normal tissue and tumor/blood boron concentration ratio. While selective tumor uptake is an asset to BNCT therapeutic advantage, it is only part of the story. For example, adhering to this concept would have led us to rule out the use of GB-10 as a boron compound to be used in BNCT of head and neck cancer (20,21). Absolute boron concentration in tumor is of paramount importance to response. If boron content falls below approximately 10^9 atoms ^{10}B/cell, the $^{10}B(n,\alpha)^7Li$ capture reactions will be insufficient to yield a lethal effect. Also, high absolute tumor boron concentration will allow for shorter irradiation times to reach the same absorbed dose in tumor. Shortening irradiation time will lead to a reduction in the background dose that affects normal tissue, regardless of its low boron concentration, and a concomitant increase in the proportion of the boron component of the dose that is tumor selective. Finally, because α and lithium particles travel short distances (approximately the diameter of a cell), the microlocalization of boron relative to radiosensitive subcellular targets will condition the outcome of BNCT (2). The "ideal" boron compound has yet to be developed—definitely no easy task. Furthermore, its extrapolation to a clinical scenario would be extremely costly and time-consuming. In the US for example, only 5 of the 10,000 general medicinal compounds that are developed enter clinical trials. Finally, only one of these is typically approved for treatment by FDA. The process "from bench to bedside" takes typically 10 years and costs over 1,000 million US dollars (22).

Although several of our translational studies have been devoted to the assessment of novel boron compounds (e.g., 23,24), our efforts have been largely focused on developing strategies to improve boron uptake and distribution employing compounds already approved for their use in humans such as BPA, GB-10, sodium borocaptate (BSH) and boric acid (BA). This approach can be used to shorten the distance between translational research and clinical evaluation. In addition, the findings can be then applied to a more ideal boron compound in the future (20).

Our strategy of "thinking out of the box" that led us to show, for the first time, the therapeutic efficacy of BNCT for head and neck cancer and the selective effect of BNCT mediated by GB-10, a boron compound that is not incorporated to tumor tissue selectively, prompted us to search for ways to improve the incorporation and distribution of boron in tissue employing the boron compounds that are approved for their use in humans, knowing they are not ideal.

We developed Sequential-BNCT, a novel strategy based on the sequential application of BPA-BNCT and GB-10-BNCT with an interval of 24–48 hours between applications (25). This treatment modality is based on radiobiological findings of our group and others. The first application would reduce interstitial fluid pressure and give rise to void space as a result of cell death. These effects would favor intratumoral delivery of GB-10 in the second application, improving tumor boron targeting homogeneity. This strategy also profits from the benefits of combining boron compounds. The brief interval between applications would avoid tumor re-population but would favor targeting of tumor cell populations that failed to respond to the first application. Sequential BNCT enhanced tumor response (partial remission + complete remission) *vs.* a dose-matched single application of BNCT mediated by (GB-10 + BPA) from 75% to >90%, with an improvement in complete remission from 50% to 70%. This potentiation of the therapeutic effect of BNCT employing Sequential-BNCT did not exacerbate mucositis in precancerous tissue, the toxic effect that limits the dose that can be applied to tumor.

Pursuing strategies to improve tumor boron targeting, we identified the need to fix the defective vascular system in tumors. The abnormal, faulty structure of aberrant tumor blood vessels leads to deficient distribution of blood-borne therapeutic agents. We performed transient normalization

of defective tumor blood vessels in the oral cancer model in the hamster cheek pouch. We employed thalidomide (also approved for use in the humans) as an anti-angiogenic drug, striving to improve boron distribution in the tumor. We administered BPA in the normalization window. Our working hypothesis was that boron concentration in tumor would rise as a result of blood vessel normalization before the administration of BPA. Surprisingly, ICP-MS gross boron concentration measurements did not show the expected increase in tumor boron concentration. Perplexed, we turned to boron microdistribution studies and actual radiobiological BNCT experiments searching for an answer. Pre-treatment to achieve transient normalization of tumor blood vessels before administering BPA enhanced tumor response from 67% to 84%, with an increase in complete tumor response from 43% to 56%. Our boron biodistribution studies showed that this effect could not be ascribed to an increase in gross boron content. However, neutron autoradiography studies revealed that a normalized vascular system improved boron targeting homogeneity in tumors (26). This would be the mechanism behind enhanced tumor response. Rather than increase total boron uptake, normalization would lead to improved distribution of boron to a larger proportion of tumor cells.

We then went on to combine normalization of aberrant tumor blood vessels and Sequential-BNCT in the hamster oral cancer model. This combined treatment strategy yielded a remarkable tumor response (100% tumor response and 87% complete tumor remission). Neither associated toxicity in normal tissue nor severe mucositis in dose-limiting precancerous tissue were observed (27).

Further seeking to favor boron delivery and distribution in tumor, employing boron compounds approved for their use in humans, we examined the potential capacity of electroporation (EP) to optimize the uptake and microdistribution of GB-10 in the model of oral cancer in hamster. EP involves the localized application of pulsed fields and was approved by the European Community to improve the delivery of chemotherapeutic drugs in cutaneous and subcutaneous tumors. EP was performed individually in each pouch tumor 10 minutes after administering GB-10 and irradiation was performed 2 hours and 50 minutes after EP, matching neutron fluence with the GB-10-BNCT protocol that did not include EP. BNCT mediated by GB-10 prescribing 4 Gy absorbed dose to tumor induced 48% tumor response (complete remission + partial remission) with only mild mucositis in precancerous tissue. EP induced a significant rise in tumor response from 48% to 92%, with a remarkable improvement in complete remission in small tumors (<10 mm^3) from 7% to 65%. The enhancement in therapeutic efficacy induced by EP correlated with the finding of a significant increase in gross boron concentration in tumor and variations in the relative microdistribution of GB-10 in parenchyma and stroma. The fact that gross boron content and microdistribution did not show EP-induced variations in precancerous tissue would explain why an improved tumor response would not bear a cost in terms of toxicity in precancerous tissue (28).

Another issue that requires attention is the feasibility of re-treatment in the case of insufficient tumor control, local recurrence and second primary tumors that develop from field cancerized tissue. In principle, if the first treatment was applied prescribing the maximum dose tolerated by the "organ at risk" (or the tissue that limits the dose that can be applied to tumor), re-treatment would not be possible. However, we showed in the oral cancer model in hamster, that a double application of BNCT (that involves re-treatment at full dose) mediated by BPA, BPA + GB-10 or MAC-TAC boronated liposomes with an interval of 4–6 weeks between applications was therapeutically effective and did not exceed radiotolerance of the dose-limiting precancerous tissue (23,24,29). This would be possible due to the dose gradient that can be achieved with BNCT for tumor *vs.* normal tissue.

Novel applications of BNCT: translational research

We went on to show the therapeutic efficacy of BNCT mediated by BPA and BPA+GB-10 in models of liver metastases (30) and lung metastases of colon carcinoma in BDIX rats (31), providing evidence that the findings for head and neck cancer also held true for other pathologies and localizations.

Changing gears, we showed the efficacy of boron neutron capture synovectomy (BNCS) mediated by the intra-articular administration of GB-10 or BPA to treat Rheumatoid Arthritis employing an antigen induced arthritis (AIA) model in New Zealand rabbits. While biodistribution studies showed that synovium boron targeting selectivity *vs.* dose-limiting healthy cartilage was only marginal, follow-up after BNCS showed that the clinical symptoms, MRI and histological features of AIA had reverted 2 months after treatment. A selective effect of BNCS on pathological synovium *vs.* articular cartilage was observed despite a failure of GB-10 and BPA to target

synovium selectively. This phenomenon could be attributed to the fact that articular cartilage is relatively radioresistant (32,33).

Having demonstrated the direct effect of BNCT on tumor in different *in vivo* animal models employing different treatment strategies, we showed, for the first time, the abscopal effect of BNCT in an ectopic model of colon carcinoma in BDIX rats (34). The abscopal effect is described as the inhibitory action of standard radiotherapy on tumor growth in an area that was not irradiated and would be mediated by radiation-induced immune responses. Our studies provided, for the first time, proof of principle of the abscopal effect of BNCT.

Clinical-veterinary BNCT studies

Our long and fruitful, albeit sometimes zigzagging, road of translational *in vivo* BNCT studies led us to our ongoing clinical-veterinary BNCT studies at the RA-6 Nuclear Reactor in terminal cats and dogs with head and neck cancer with no other therapeutic alternative. Our data to date in 6 cats (35,36) and 5 dogs (ongoing studies) reveal that BPA-BNCT can improve the clinical condition of the veterinary patients, prolong survival with good quality of life and partially control tumors with only slight-moderate and reversible associated toxicity. Also, in a clinical-veterinary scenario, we were able to show the safety and therapeutic effect of full dose re-treatment with BNCT in a case of tumor recurrence.

Conclusions

Our translational research has been devoted to optimize BNCT for different pathologies. In an attempt to pave the road to clinical evaluation, we have largely used novel approaches that combine boron compounds and techniques approved for their use in humans, i.e., (I) the combination of boron compounds with different properties and uptake mechanisms (GB-10 + BPA) to improve boron targeting homogeneity in tumor; (II) the use of GB-10, a boron carrier that is not tumor selective, that achieves homogeneous tumor boron targeting and a selective effect on tumor associated with only mild mucositis in precancerous tissue; (III) Sequential BNCT, a strategy based on the sequential application of BPA-BNCT and GB-10-BNCT with an interval of 24–48 hs between applications; (IV) transient normalization of aberrant tumor blood vessels prior to the administration of BPA to fix the flawed delivery system and improve boron microdistribution in tumor; (V) combination of aberrant blood vessel normalization and Sequential BNCT; and (VI) EP to optimize the uptake and microdistribution of boron in tumor.

Translational BNCT studies in *in vivo* animal models are essential to design novel, safe and effective clinical BNCT protocols for existing or new targets for BNCT. Team-work is equally essential to the advancement of BNCT.

Acknowledgments

Funding: Our studies were supported by Comisión Nacional de Energía Atómica and grants from Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas and Instituto Nacional del Cáncer, Argentina, and Missouri University and Idaho National Laboratory, USA. The authors wish to acknowledge the valuable and generous contributions of the co-authors on each of our published papers.

Footnote

Provenance and Peer Review: This article was a standard submission to the editorial office, *Therapeutic Radiology and Oncology*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tro.2019.05.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All our studies employing animals were carried out with protocols approved by the Argentine National Atomic Energy Commission Animal Care and Use Committee (CICUAL-CNEA) in keeping with the National Institutes of Health (NIH, USA) guide for the care and use of Laboratory animals.

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doi: 10.21037/tro.2019.05.03
Cite this article as: Schwint AE, Garabalino MA, Monti Hughes A, Pozzi ECC, Heber EM, Palmieri MA, Trivillin VA. Teachings of our translational studies on boron neutron capture therapy (BNCT): thinking "outside the box". Ther Radiol Oncol 2019;3:20.