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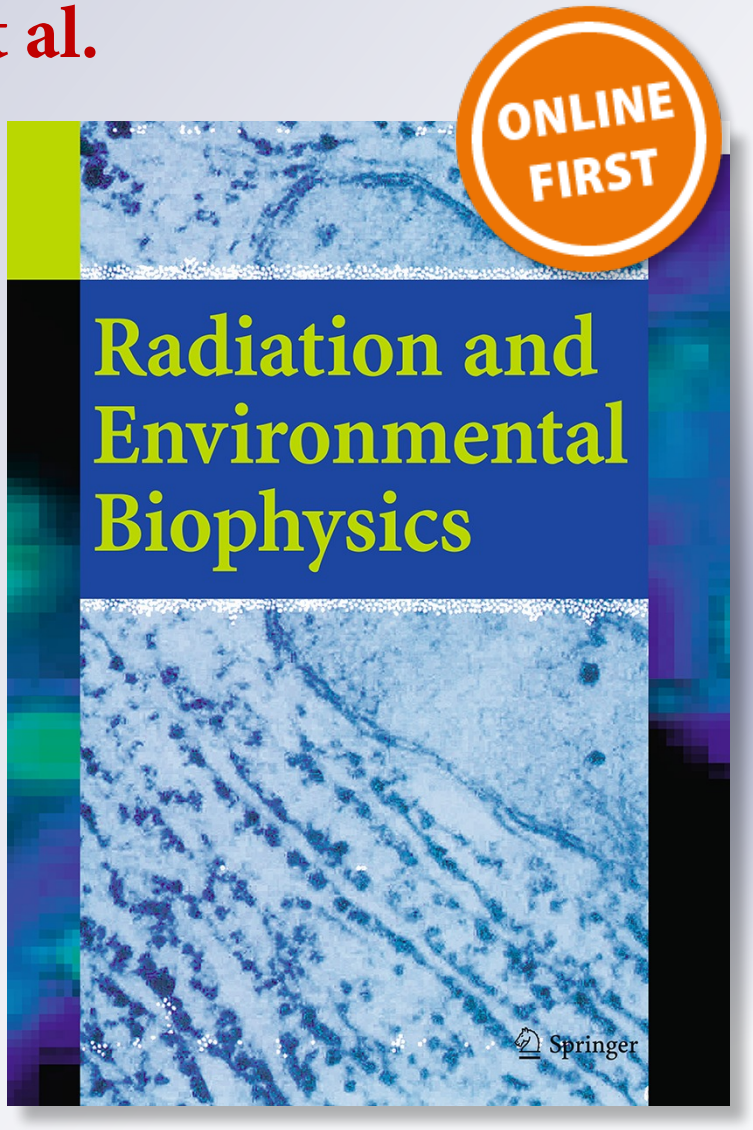

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ORIGINAL ARTICLE

Boron neutron capture synovectomy (BNCS) as a potential therapy for rheumatoid arthritis: radiobiological studies at RA-1 Nuclear Reactor in a model of antigen-induced arthritis in rabbits

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Abstract Rheumatoid arthritis is a chronic autoimmune pathology characterized by the proliferation and inflammation of the synovium. Boron neutron capture synovectomy (BNCS), a binary treatment modality that combines the preferential incorporation of boron carriers to target tissue and neutron irradiation, was proposed to treat the pathological synovium in arthritis. In a previous biodistribution study, we showed the incorporation of therapeutically useful boron concentrations to the pathological synovium in a model of antigen-induced arthritis (AIA) in rabbits, employing two boron compounds approved for their use in humans, i.e., decahydrodecaborate (GB-10) and boronophenylalanine (BPA). The aim of the present study was to perform low-dose BNCS studies at the RA-1 Nuclear Reactor in the same model. Neutron irradiation was performed post intra-articular administration of BPA or GB-10 to deliver 2.4 or 3.9 Gy, respectively, to synovium (*BNCS-AIA*). *AIA* and healthy animals (*no AIA*) were used as controls. The animals were followed clinically for 2 months. At that time, biochemical, magnetic resonance imaging (MRI) and histological studies were performed. *BNCS-AIA* animals did not show any toxic effects, swelling or pain on palpation. In *BNCS-AIA*, the post-treatment levels of TNF-α decreased in four of six rabbits and IFN-γ levels decreased in five of six rabbits. In all cases, MRI images of the knee joint in *BNCS-AIA* resembled those of *no AIA*, with no necrosis or periarticular effusion. Synovial membranes of *BNCS-AIA* were histologically similar to *no AIA*. BPA-BNCS and GB-10-BNCS, even at low doses, would be therapeutically useful for the local treatment of rheumatoid arthritis.

Keywords Boron neutron capture synovectomy BNCS · Rheumatoid arthritis · Boronophenylalanine (BPA) · Decahydrodecaborate (GB-10) · Antigen-induced arthritis (AIA) · RA-1 Nuclear Reactor

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Introduction

Rheumatoid arthritis (RA) is a painful and disabling polyarticular autoimmune pathology that affects up to 1 % of the population worldwide. RA is characterized by the proliferation and inflammation of the synovium, the membrane lining the inner surface of the joint capsule of all articulating joints. This process leads to the formation of the pannus tissue which causes the progressive degradation of adjacent articular cartilage, ligaments and bone (Firestein 2003;

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Garnero et al. 2002; Kim et al. 2010). Some temporary relief is afforded by drugs that reduce synovial inflammation, but one or more joints often remain refractory to this type of treatment (Smolen and Steiner 2003). In the past few years, biological agents, especially inhibitors of tumor necrosis factor, have afforded some therapeutic benefit, although even with these drugs the frequency and degree of responses are restricted (Smolen et al. 2007; Malviya et al. 2013). Because chronic synovial inflammation can lead to destruction of the joint, unresponsive joints require surgical removal of the pathological synovium. Although both open synovectomy and arthroscopic synovectomy allow for partial removal of inflamed tissue and alleviate symptoms transiently, it is difficult to remove all of the synovium from the nooks and crannies of the joint, the synovial membrane is replenished within 6 months after surgery and inflammation recurs (Shortkroff et al. 2004). Furthermore, prolonged hospitalization and rehabilitation are often required. A less invasive approach is radiation synovectomy using beta-emitting radionuclides injected directly into the joint space. The beta particles that deposit most of their energy within a few millimeters destroy the diseased synovium. Although success rates of up to 80 % for 2–5 years have been reported, the procedure is not widely used due to potential damage to healthy tissues caused by leakage of the beta emitter from the joint (Yanch et al. 1999).

The pathological synovium is the main target in RA. This tissue and a local malignancy have several features in common (Trivillin et al. 2014) such as increased proliferation rate (Sweeney and Firestein 2004), angiogenesis (Szekanecz and Koch 2009), mutations and impaired apoptosis (Mor et al. 2005). Within this context, boron neutron capture therapy (BNCT), classically described for the treatment of tumors, has been proposed as an alternative to surgical or radiation synovectomy (Watson-Clark et al. 1998) and has been termed boron neutron capture synovectomy (BNCS). BNCT for the treatment of tumors is classically described as a binary treatment modality that involves the selective accumulation of ^{10}B carriers in tumor followed by neutron irradiation. The high linear energy transfer (LET) α particles and recoiling ^{7}Li nuclei emitted during the capture of a thermal neutron by a ^{10}B nucleus (Locher 1936) are known to have a high relative biological effectiveness (RBE). Their short path length in tissue (6–10 μm) limits their effect mostly to cells containing ^{10}B atoms, providing a strategy to damage tumor cells while protecting healthy tissue (e.g., Coderre and Morris 1999; Heber et al. 2012). In addition to the high LET particles that give rise to the tumor-specific boron dose component, the nonspecific background dose delivered by the neutron beam and the interactions with the nuclei of the elements in the tissue (hydrogen and nitrogen) affect tumor and normal tissue similarly. Thus, BNCT protocols seek to maximize the boron radiation dose and minimize the background dose (Trivillin et al. 2006). The fact that BNCT is based on biological/biochemical rather than geometric targeting makes it adequate to treat undetectable micrometastases (Garabalino et al. 2011; Pozzi et al. 2012, 2013), infiltrating cells and foci of malignant transformation in field-cancerized tissue (Monti Hughes et al. 2009, 2011, 2013). In this sense, BNCS would be ideally suited to treat the pathological synovium in the nooks and crannies of the RA joint (Shortkroff et al. 2004).

Clinical trials of BNCT for the treatment of glioblastoma multiforme, melanoma, head and neck tumors, liver metastases and mesothelioma have shown a potential therapeutic advantage for this technique (e.g., Barth et al. 2012; Trivillin et al. 2014). The application of BNCT to the treatment of arthritis (BNCS) has previously been explored (e.g., Yanch et al. 1999; Watson-Clark et al. 1998; Shortkroff et al. 2004; Zhu et al. 2006; Wu et al. 2007; Van Lent et al. 2009). Both BNCS and radiation synovectomy have several advantages over surgery, i.e., increased potential for complete destruction of the inflamed synovium, reduced risk of blood clots and infection, the need for only local anesthesia and the fact that the procedure is carried out on an outpatient basis with no associated pain and would require no rehabilitation. However, BNCS avoids the dangers associated with the leakage of local emitters from the joint in radiation synovectomy. Yanch et al. (1999) explored the biodistribution of the boron carrier $K_2B_{12}H_{12}$ for BNCS in the rabbit model. Watson-Clark et al. (1998) explored the biodistribution of a boronated liposome in an experimental model of arthritis in rats, and Van Lent et al. (2009) explored the biodistribution of another boronated liposome in murine normal knee joints and assessed the effect of BNCS on macrophages 5 days post-treatment. While these boron compounds have advantages and disadvantages for BNCS, none of them have been approved for use in humans. If and when a new ^{10}B carrier is identified as promising from cell culture studies, it still faces many hurdles, beginning with biodistribution studies in appropriate animal models and in vivo evaluation of toxicity. Translation of experimental biodistribution data to clinical biodistribution studies is costly, is of no direct benefit to participants and must comply with the stringent requirements of regulatory agencies (Barth 2009; Schwint and Trivillin 2015). In this context, an excellent short- and medium-term strategy is to optimize the delivery of boron compounds currently authorized for use in man employing adequate experimental models.

We previously developed a model of antigen-induced arthritis (AIA) in female New Zealand rabbits (Abramson et al. 2014; Mortarino et al. 2016). Employing this model, we performed a biodistribution study with the boron carriers boronophenylalanine (BPA) and sodium

decahydrodecaborate (GB-10), both boron carriers authorized for use in humans. We showed that the intra-articular administration protocols at <40 min post-administration for BPA and GB-10 exhibited no manifest toxicity, and yielded therapeutically useful absolute boron concentrations (≥20 ppm) in the pathological synovium, boron concentration ratios between target tissue (pathological synovium) and normal dose-limiting tissue (cartilage) ≥1 and a long enough residence time in the synovium for irradiation to be completed (Trivillin et al. 2014). These results were in keeping with our guidelines for potential therapeutic efficacy (Garabalino et al. 2011), and dosimetric estimations suggested that BNCS would be able to achieve a therapeutically useful dose in pathological synovium without exceeding the radiotolerance of normal tissues in the treatment volume (Trivillin et al. 2014). Within this context, the aim of the present study was to perform low-dose BNCS studies mediated, for the first time, by BPA or GB-10, at the RA-1 Nuclear Reactor in the AIA model in rabbits to assess the safety of the procedure, potential toxicity and therapeutic efficacy.

Materials and methods

Model of antigen-induced arthritis

Six three-month-old female New Zealand rabbits (approximately 3 kg in weight) were housed in individual cages and given standard rabbit chow (ACA Cooperativas Argentina), and water ad libitum. All experimental procedures were performed in keeping with the guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures and in accordance with local laws and regulations of the Bioethics Committee of the Faculty of Medicine of the Universidad Nacional de Rosario and the Institutional Committee of the Comisión Nacional de Energía Atómica for the Care and Use of Experimental Animals. The animals were submitted to induction of antigen-induced arthritis (AIA) in the knee joints in keeping with a modification of a standard protocol (Sanchez-Pernaute et al. 2003). Briefly, AIA was induced by two successive intradermal immunizations, 15 days apart, with ovalbumin emulsion (OVA, Sigma Chemicals, USA, 5 mg/ml in 0.9 % NaCl), 1:1 in complete Freünd's adjuvant (Difco, USA). Ten days later, two successive injections, 10 days apart, of 1 ml of OVA (5 mg/ml in 0.9 % NaCl) were performed in the knee articulation of both hind legs of each rabbit. Approximately 50–60 days after the first immunization, the onset of the disease was verified by clinical examination (swelling, pain or tenderness on palpation). In addition, magnetic resonance imaging (MRI) studies were performed as previously described 70 days after the first immunization. Briefly, we employed a magnetic imaging MRI scanner general electric "Vectra" 0.5 T, coronal coil, with the following sequence: Inversion Recovery TR 2000, TE 60, NEX 3, time of acquisition 6 min (Abramson et al. 2014).

Previous histopathological studies revealed that the clinical and MRI signs of disease were associated with the presence of inflammatory infiltrate, synovial hyperplasia, fibrosis and/or pannus (Mortarino et al. 2016; Toledo et al. 2009). In addition to the pre-treatment and post-treatment data in each of the six AIA rabbits (12 articulations) treated with BNCS, we included in the analysis the corresponding information from previous studies in AIA and normal rabbits performed by members of the group (Abramson et al. 2014; Mortarino et al. 2016).

In vivo BNCS

Three AIA rabbits (six articulations) were injected intra-articularly in the knee joint with 0.5 ml of 0.14 M BPA–fructose (BPA-f) (Glyconix Corp., USA) (0.7 mg ^{10}B). Another set of three rabbits (six articulations) was injected similarly with 0.5 ml of GB-10 (Neutron Therapies, L.L.C., USA) (5 mg ^{10}B). BPA and GB-10 were >99 % enriched in ^{10}B and were prepared as previously described (Garabalino et al. 2011). Intra-articular injections were performed under anesthesia with ketamine (35 mg/kg), xylazine (18 mg/kg) and acepromazine maleate (0.1 mg/kg). The boron compound administration protocols were selected from our previous biodistribution studies in this model (Trivillin et al. 2014). Fifteen minutes post-administration of the boron compounds, irradiations were performed with the thermal beam of the RA-1 Reactor of the Constituyentes Atomic Center (Provincia Buenos Aires, Argentina) at a thermal neutron flux of approximately $(1.6 \pm 0.1) \times 10^8$ n/cm^2 s to the target area (knee joint). The actual thermal neutron flux within the joint would be higher due to in-depth thermalization of higher-energy neutrons. The geometric setup involves no body shielding. Still under anesthesia after intra-articular administration of the boron compounds, the rabbits were placed on a device designed ad hoc (Fig. 1) to transport the rabbit to the irradiation position using pulleys. The animals were placed face up, with their hind legs toward the core of the reactor. Positioning took approximately 5–7 min and was performed while the time post-administration of 15 min elapsed. Irradiations lasted 10 min, resulting in an approximate fluence of thermal neutrons of $(9.6 \pm 0.5) \times 10^{10}$ n/cm^2. Table 1 presents the boron concentration values used for dose calculations corresponding to each of the clinically relevant tissues as indicated (Trivillin et al. 2014), the irradiation dose components and total absorbed dose for each protocol.

Fig. 1 The *upper panel* shows a view of RA-1 Nuclear Reactor. The *lower four panels* show a device designed ad hoc to transport the rabbit to the irradiation position using pulleys. The animals were placed face up, with their hind legs toward the core of the Reactor

Table 1 Absorbed dose (Gy) for the different experimental protocols

Tissue	ppm boron for BPA*	ppm boron for GB-10*	Neutrons (Gy)	Gamma-ray photons (Gy)	Boron (Gy/min per ppm B)	Total dose BPA-BNCS 10-min exposure	Total dose GB-10-BNCS 10-min exposure
Synovium	159 ± 65	378 ± 256	0.68 ± 0.13	0.57 ± 0.10	$(7 \pm 0.4) \times 10^{-4}$	2.36 ± 0.49	3.89 ± 1.81
Skin	2.5 ± 1.2	157 ± 62	0.68 ± 0.13	0.57 ± 0.10	$(7 \pm 0.4) \times 10^{-4}$	1.27 ± 0.16	2.35 ± 0.47
Cartilage	128 ± 61	206 ± 103	0.68 ± 0.13	0.57 ± 0.10	$(7 \pm 0.4) \times 10^{-4}$	2.15 ± 0.46	2.69 ± 0.74
Muscle	1.7 ± 0.9	6.8 ± 2.9	0.68 ± 0.13	0.57 ± 0.10	$(7 \pm 0.4) \times 10^{-4}$	1.26 ± 0.16	1.30 ± 0.16

* Taken from Trivillin et al. (2014)

Follow-up

The animals were followed clinically for 2 months. At that time, MRI studies of the knee joints were performed as described above. A semiquantitative score was established for each joint based on the following end points: degree of hydrarthrosis in the joint space (0: none, 1: slight, 2: moderate, 3: severe), alterations in the subchondral bone (hyperintensity scored from 0 to 3) and alterations in the peri-articular soft tissue scored from 0 to 3. The final score (0–9) was computed for each group by adding the scores corresponding to the three end points.

Table 2 Serum concentration values of TNF-α and IFN-γ, pre- and post-treatment for each of the rabbits as indicated

Rabbit/BNCS Protocol	TNF (pg/ml)		INF (pg/ml)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
17/BPA	6.3	2.9	6.4	2.4
18/BPA	1.8	3.4	5.9	2.9
19/BPA	4.9	2.5	8.3	6.2
20/GB-10	8.5	5.6	7.5	5.7
21/GB-10	4.5	7.2	3.8	4.6
22/GB-10	8.8	5.6	3.8	2.6

Serum levels of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) were assessed by the enzyme immunoassay technique (Quantikine laboratories from R&D Systems) as previously described (Mortarino et al. 2016), pre-treatment and 2 months post-treatment. Determinations were done in triplicate.

Following blood extraction for cytokine assessment, the animals were euthanized by an overdose of anesthesia. Samples of synovial tissue were taken, fixed in formaldehyde 10 % in PBS and routine processed for anatomopathological studies. The severity of the pathological status of the synovium was determined by semiquantitative light microscopy assessment of histological features of RA, i.e., inflammatory infiltrate, synovial hyperplasia, angiogenesis and edema. Three independent observers evaluated 50 high-power fields per sample. A semiquantitative scale was employed to grade histological response according to the following criteria: normal synovial tissue when no inflammatory cells were observed, low inflammation tissue for less than 5 inflammatory cells/field, moderate inflammation tissue for 5–10 inflammatory cells/field and edema and severely inflamed tissue for more than 15 inflammatory cells/field, angiogenesis and edema.

Results

Throughout the follow-up period of 2 months, the rabbits did not exhibit any clinical signs of toxicity. At 2 months post-BNCS, the hind leg knee joints of all the rabbits treated with BPA-BNCS or GB-10-BNCS were no longer swollen or painful on palpation. Table 2 shows the serum concentration values of TNF-α and IFN-γ, pre- and post-treatment for each of the rabbits as indicated. The reference values in healthy rabbits for TNF-α and IFN-γ serum levels were 1.5 ± 0.8 and 1.8 ± 0.8 pg/ml, respectively (Sanchez-Pernaute et al. 2003, Mortarino et al. 2016). No statistically significant differences (Wilcoxon's test) were observed between mean pre-treatment and post-treatment values. However, a trend toward a reduction in TNF-α levels was observed in four of the six rabbits treated with BNCS. Likewise, five of the six rabbits treated with BNCS exhibited a trend toward a reduction in IFN-γ serum levels.

The MRI studies of the knee joints showed that in 100 % of the cases the post-BNCS images were similar to those of the control joints (*no AIA*), i.e., with no areas of necrosis or peri-articular effusion, and markedly different from AIA joints. The semiquantitative score corresponding to AIA joints was 8 ± 1. In contrast, the score for AIA articulations treated with BNCS (*BNCS-AIA*) was 0, similarly to control articulations (*no AIA*) which also exhibited a score of 0.

The histological analysis of the synovial membranes obtained postmortem at 2 months post-treatment revealed that in 70–100 % of the fields corresponding to cases of AIA joints treated with BNCS (*AIA-BNCS*), the histological features were similar to those of normal joints (*no AIA*), i.e., with no synovial hyperplasia, scarce (less than 5 inflammatory cells per field) or no lymphoplasmacytic infiltrate and no alterations in vascularization. In three of the six rabbits treated with BNCS, we observed fields with areas of fibrosis. As illustrated in Fig. 2, the histological appearance of AIA joints treated with BNCS was similar to that of normal joints and very different to that of untreated AIA joints which exhibited synovial hyperplasia, angiogenesis, edema and abundant inflammatory infiltrate (scored as severe inflammation) as previously described (Sanchez-Pernaute et al. 2003). No conspicuous differences were detected between the rabbits treated with BNCS mediated by BPA and those treated with BNCS mediated by GB-10 for any of the end points assessed.

Discussion

In a previous biodistribution study (Trivillin et al. 2014), we identified boron administration protocols that fulfilled our guidelines for therapeutic potential (Garabalino et al. 2011). The present study explores the actual therapeutic efficacy of low-dose BNCS for the treatment of AIA joints in a RA model in rabbits using intra-articular boron compound administration protocols. The possibility of using intra-articular administration protocols is an asset because

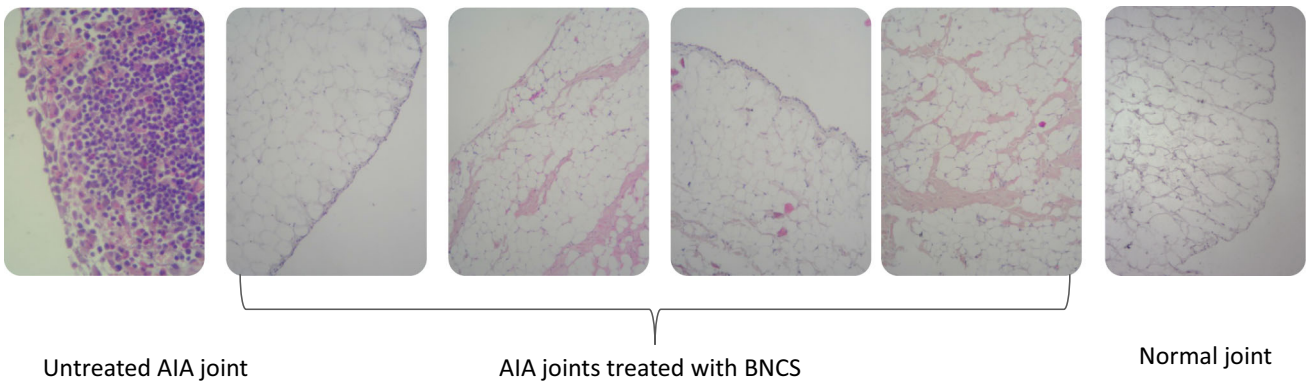

Fig. 2 The histological appearance of AIA joints treated with BNCS (*middle panels*) was similar to that of normal joints (*right panel*) and very different to that of untreated AIA joints (*left panel*) which exhibited synovial hyperplasia, angiogenesis, edema and abundant inflammatory infiltrate. Original magnification: ×100

it allows for boron uptake to be maximized in the target volume, while reducing the dose administered to healthy tissues not included in the target volume. In addition, the fact that most of the articular joints are located far from the body's most radiosensitive organs is an additional advantage. The leakage of boron into the systemic blood stream is minimum, i.e., <4 ppm as revealed by our previous biodistribution study (Trivillin et al. 2014). The selected protocols employ BPA and GB-10, two of the three boron compounds (BPA, GB-10 and sodium borocaptate [BSH]) approved for their use in humans (Barth et al. 2012), thus contributing to bridge the gap between research and clinical application (Schwint and Trivillin 2015).

To the best of our knowledge, this is the first study that includes a long enough follow-up with appropriate end points to assess clinical response. Shortkroff et al. (2004) showed synovial ablation in the AIA rabbit stifle joint with no adverse effects to skin or extra-capsular tissues at 72 h post-treatment with BNCS mediated by potassium dodecahydrodecaborate. Van Lent et al. (2009) demonstrated selective depletion of macrophages in the synovial lining of normal murine knee joints 5 days post-treatment with BNCS mediated by boronated liposomes. However, it must be stressed that the effects shown in normal joints may not hold true in AIA joints due to tissue volume and diffusion considerations (Shortkroff et al. 2004). The diameter of a human finger is approximately the same as the diameter of the arthritic rabbit knee. In this sense, the rabbit experimental model is better suited for BNCS studies than murine models. The follow-up in the present study was enough to show reversal of the clinical symptoms (swelling, pain on palpation), MRI and histological features of AIA, with no evidence of toxicity. The reduction in serum levels of pro-inflammatory cytokines post-BNCS can be described as a consistent trend in most of the rabbits. This trend would not be due to mere variations over time given that a previous study by members of the group at 5 months after the first immunization (Mortarino et al. 2016) and the present pre-treatment data (2 months after the first immunization) showed that there are no statistically significant variations in the serum levels of TNF-α and IFN-γ in nontreated AIA animals over a period of 3 months. However, the difference in pre- and post-treatment mean values did not reach statistical significance, conceivably because BNCS is a local treatment and a robust systemic effect might take longer to achieve.

Both BPA-BNCS and GB-10-BNCS were therapeutically effective. Our experience with the boron carriers BPA and GB-10 in BNCT for experimental oral cancer showed that while BPA would target malignant tissue on a cell-by-cell basis, GB-10 would mainly target aberrant tumor blood vessels while preserving normal blood vessels in healthy tissue (Trivillin et al. 2006). It is well known that neovascularization of the rheumatoid synovium is essential to perpetuate an angiogenic disease such as rheumatoid arthritis (Szekanecz and Koch 2001; Taylor and Feldmann 2004). Within this context, GB-10 as a boron carrier for BNCS, alone or in combination with BPA, would be particularly suited to treat RA. However, the present findings do not evidence an ostensible therapeutic advantage for GB-10 over BPA, conceivably due to a higher CBE factor for BPA versus GB-10 (Trivillin et al. 2006) and/or the fact that the extremely high boron concentration values in synovium are enough to exert a cell-by-cell effect as described for BPA. In future studies, it would be contributory to explore the therapeutic efficacy of BNCS mediated by BPA and GB-10 administered together, profiting from the advantages of the combined administration of boron compounds (e.g., Molinari et al. 2011).

Our previous dose estimations to assess the therapeutic potential of our boron compound administration protocols (Trivillin et al. 2014) were based on the findings of Deutsch et al. (1993) that suggested that the minimum dose to effectively ablate synovium with radiation synovectomy

was 100 Gy. Since it is essential to spare articular cartilage from permanent damage, cartilage was considered a healthy dose-limiting tissue. Because acute doses of 50 Gy resulted in no observable effect in cartilage (Takahashi et al. 1992) and considerable damage to cartilage was only observed in AIA rabbit joints exposed to BNCS mediated by an experimental boron compound at a dose of 810 Gy-eq, we considered that dose to cartilage should ideally be below 50 Gy. We also considered that skin doses should not exceed 8 Gy to avoid skin erythema (Nias 1990). Furthermore, dose restrictions were imposed based on the fact that while moderate/severe side effects might be acceptable when treating a malignant disease, safety is paramount in the case of a disease that is not life-threatening.

Because irradiations were performed at RA-1, it was not possible to shield the body of the animals (Rao et al. 2004). We decided to perform a low-dose study employing intra-articular administration of the boron compounds (Trivillin et al. 2014), relying on boron retention in the joint to exert a selective effect in the pathological articulation. The fact that the boron concentration in the target tissue was high enabled us to deliver the same boron dose to the target tissue with a shorter irradiation time. In this way, it was possible to reduce background dose and maximize the target-specific boron component of the dose (Coderre and Morris 1999). As indicated above, the absorbed doses (in Gy) delivered in the present study are presented in Table 1. However, it is well known that alpha and lithium particles and neutrons are more biologically effective in inducing damage than photons and high-energy beta particles. Thus, RBE factors should be applied to individual dose components to calculate Gy-eq dose values to enable a comparison with standard radiotherapy dose levels. In the case of the boron component of the radiation dose, the compound biological effectiveness factor (CBE) for the corresponding boron carrier must be used for dose calculation (Coderre and Morris 1999). Using the RBE and CBE values cited in Trivillin et al. (2014) as rough estimations, the dose for BPA-BNCS and GB-10-BNCS to synovium was estimated to be approximately 8 Gy-eq and the estimated dose to cartilage was approximately 6 Gy-eq. The Gy-eq dose to pathological synovium is well below what we estimated would be a therapeutically useful dose based on radiation synovectomy studies (Deutsch et al. 1993). Considering absorbed dose values (Table 1), the dose to synovium is also well below the therapeutic absorbed dose of 13 ± 9 Gy administered by Van Lent et al. (2009) with BNCS mediated by boronated liposomes to deplete macrophages selectively in the synovial lining of normal murine knee joints. However, in this case, a comparison of absorbed doses may be biased due to differences in the spectrum of the neutron source employed and the difference in the CBE values between the boron carriers used in each of the experiments. In addition, the study of Van Lent et al. (2009) was performed in normal joints making a comparison even more difficult. Our previous study at RA-1 that involved the treatment of spontaneous squamous cell carcinomas in cats with BNCT mediated by BPA showed that unexpectedly low absorbed dose levels (even lower that those used herein) were therapeutically effective (Rao et al. 2004).

Our study suggests that considerably lower doses to target tissue than anticipated from radiation synovectomy studies would be necessary to achieve a therapeutic effect with BNCS mediated by intra-articular administration of BPA or GB-10. This is definitely an asset and minimizes (or altogether prevents) associated toxicity to healthy tissues such as cartilage and would allow for re-treatment if necessary. The recent initiation of BNCT clinical trials employing hospital-based accelerators rather than nuclear reactors as the neutron source will favor new and more numerous clinical trials for different tumors (Schwint and Trivillin 2015). Conceivably, the development of dedicated accelerators installed in a medical environment will also favor BNCS studies. For a 1-mA accelerator current, therapy times to treat a human finger joint and a human knee would range from 4 to 14 min and 8 to 31 min, respectively (Yanch et al. 1999).

The present study provides proof of principle that low-dose BNCS mediated by intra-articular administration of BPA and GB-10 is clinically effective to treat arthritic joints with no evidence of toxicity at 2 months post-treatment. The use of boron carriers approved for their use in humans facilitates the extrapolation of these findings to a clinical scenario.

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