Applied Radiation and Isotopes 106 (2015) 260–264

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Reprint of Application of BNCT to the treatment of HER2+ breast cancer recurrences: Research and developments in Argentina

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HIGHLIGHTS

- A new proposal of BNCT for HER2+ breast cancer treatment is introduced.
- The proposal considers development of immunoliposomes as boron carrier nanovehicles.
- Locoregional recurrences after treatment were identified as candidates for initial BNCT studies.
- First analysis show acceptable neutron flux distributions provided by RA-6 BNCT facility.

ARTICLE INFO

Article history:
Received 7 February 2015
Received in revised form
13 May 2015
Accepted 2 July 2015

Keywords:
HER2-enriched breast cancer
Post treatment recurrences
Immunoliposomes

ABSTRACT

In the frame of the Argentine BNCT Project a new research line has been started to study the application of BNCT to the treatment of locoregional recurrences of HER2+ breast cancer subtype. Based on former studies, the strategy considers the use of immunoliposomes as boron carriers nanovehicles to target HER2 overexpressing cells. The essential concerns of the current stage of this proposal are the development of carriers that can improve the efficiency of delivery of boron compounds and the dosimetric assessment of treatment feasibility. For this purpose, an specific pool of clinical cases that can benefit from this application was determined. In this work, we present the proposal and the advances related to the different stages of current research.

1. Introduction

HER2-enriched breast cancer subtype (HER2+) is characterized by overexpression of HER2 transmembrane protein. This macromolecule is normally expressed in healthy cells and is related to regulation of cell growth and differentiation. As it has been determined from clinical studies this breast cancer subtype is associated to reduced overall survivals and short times to relapse, thus associated to a poor prognosis to patients (Slamon et al., 1989; Yarden and Sliwkowski, 2001).

Protein overexpression allows treatment with targeted therapy by administration of anti-HER2 monoclonal antibodies. Trastuzumab, a humanized monoclonal antibody, is currently the most commonly used for this purpose. However, a group of patients do not show a satisfactory response to this treatment and, consequently, new research focuses in different strategies to overcome this situation, including the development of new monoclonal antibodies (Valabrega et al., 2007; Pohlmann et al., 2009). In Argentina, accordinsg to statistical data derived from the 'National HER2 test Program', the prevalence of HER2+ breast cancer is 13.2% from a population of 34,640 cases analyzed between 2005 and 2010 (Cáceres et al., 2012).

A new research line stimulated by previous studies (Bohl Kullberg et al., 2005; Mundy et al., 2006; Sztejnberg and Jevremovic, 2009) has been started in the National Atomic Energy Commission as part of the Argentine BNCT Project. This research line aims at addressing certain selected cases of those resilient HER2+ breast cancers that oncologists would consider an appropriate match for BNCT.

Our proposal consists of the application of BNCT using immunoliposomes as boron carrier nanovehicles to target HER2 overexpressing cells. Immunoliposomes labeled with the monoclonal antibody trastuzumab results in specific binding to HER2 overexpressing breast cancer cells and would allow to enhance both selectivity and concentration levels of boron compounds in tumor microenvironment.

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http://dx.doi.org/10.1016/j.apradiso.2015.10.009
0969-8043/

The essential concerns of this stage of the proposal are: (a) identification of specific pool of clinical cases that could benefit from a potential application of BNCT; (b) development of boron nanocarriers that could improve the selectivity of boron compounds; and (c) feasibility assessment from dosimetric point of view aimed at evaluating biodistribution requirements and irradiation conditions taking into account the identified clinical cases.

This work introduces CNEA's new research line together with the determination of clinical cases currently being considered for the feasibility study for the application of BNCT, advances in the development of liposomes and some initial considerations regarding the dosimetric analysis.

2. Clinical cases

The study of application of BNCT to the treatment of HER2+ breast cancer includes among its objectives to establish the conditions for which this therapy can be offered. Special focus is paid to cases that do not satisfactorily respond to current therapeutics or whether BNCT would mean a lower morbidity. Considering this scenario and based on their practical experience, local medical doctors have identified as general selection criteria those locoregional recurrences that may arise after breast cancer treatment as candidate cases (Gadan, 2014). From surgical treatment standpoint, locoregional recurrences can be classified as arising after breast conserving therapy (BCT) or after mastectomy. Local presentation of recurrences after BCT includes the ipsilateral breast (which stands for the treated breast) and the surgery scar. Local presentation after mastectomy concerns mastectomy bed and surgery scar too. Regional recurrence presentation concerns axillary and supraclavicular lymph nodes independently from the surgery treatment applied (Moran et al., 2014).

Regarding clinical presentation, local relapses after BCT can affect the glandular parenchyma but can also involve the skin. This later condition is associated with an increased risk of progression to metastatic disease. The relapse rate in patients treated on local medical institutions varies between 9% and 10.2% (Núñez de Pierro et al., 2004; Ghiraldo et al., 2012). The most common site for post-mastectomy local relapse is the mastectomy bed of the ipsilateral chest wall, either skin or sub-dermal deposits, and may occur not only as a single phenomenon but also may indicate the development of distant metastases (Buchanan et al., 2006). The relapse rate on local medical institutions can vary between 3 and 5%. Regional recurrences presentation is rare compared to local relapse. Patients with this type of recurrence have a poor prognosis because they are linked to a higher chance of developing distant metastases. In Argentina, some retrospective studies report a rate of regional relapse of 0.7% with an expectation range between 0.3% and 2% (Núñez de Pierro et al., 2004).

Prognosis for patients with local recurrence after BCT is better than that of patients with chest wall recurrence after mastectomy. Radical mastectomy is the treatment of choice for this relapses. In the same way, the primary local treatment for post-mastectomy chest wall recurrence is the surgery. Alternatively, systemic therapy is administered only for control of a potential spread of the disease. Concerning regional recurrences, since they are not a frequent event, the possibility of establishing an optimal management of the disease is difficult and varies according to medical institution criteria (Pedersen et al., 2011). In general, attention should be paid to patients that have already received radiotherapy for the treatment of primary tumor since the first radiotherapy treatment limits future irradiations. A new application of radiotherapy could compromise the already irradiated healthy tissues, presenting higher morbidity. Reducing the amount of fields and controlling the total dose to avoid toxicity effects do not allow adequate treatment of the disease.

Considering these aspects, cases of patients who relapse after primary breast cancer treatment represent a group with a real need for treatment for which the application of BNCT could mean a potential benefit. These cases should be addressed and be included in the feasibility analysis of this proposal treatment.

3. Liposomes

Current work also involves the evaluation of boron–phenylalanine–fructose (BPA–Fru) encapsulation in liposome testing different lipids formulations. Neutral charge liposomes were obtained by the combination of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), cholesterol and phosphoethanolamine- [methoxy(polyethyl-eneglycol)-2000 (DSPE-PEG) at 61.8:37:1.2 M ratio respectively, whereas cationic liposomes were obtained adding 3.7% of dimethyldioctadecylammonium bromide (DDAB) to the mentioned formulation (60.5:36.3:1.25:1.95). Liposomes were performed using the lipid film hydration technique. Briefly, lipids were previously dissolved in chloroform (Mallinckrodt Baker, Inc., Paris, KY) and dried under a steady stream of nitrogen for 2 h. Films were hydrated with a solution of 8 mg/ml BPA–Fru in phosphate buffered saline (PBS). Multilamellar liposomes were subsequently extruded through 800 nm, 400 nm, 200 nm and 100 nm polycarbonate membranes using an extruder (Avanti Polar Lipids, USA). Subsequently, unilamellar liposomes were purified by size exclusion chromatography column (Sepharose CL-4B). Aliquots that contain liposomes were identified by absorbance measurements at two wavelengths (265 nm and 280 nm) using a UV–vis spectrophotometer (Nanodrop 2000 UV, Thermo Scientific) and were concentrated using 10,000 MWC concentrators (VivaSpin6, GE Healthcare). Mean size distribution and polydispersity index (PI) of liposomes were determined by dynamic light scattering (Zetasizer Nano; Malvern, UK).

Encapsulation efficiency of different lipids formulations was evaluated by determination of the boron to phosphorus ratio (B/P). In order to obtain boron concentration, samples were digested and appropriately diluted for subsequent measurement of boron by Optical Inductive Couple Plasma (ICP-OES, Jobin Yvon). To determine phosphorus concentration, phospholipids were previously extracted by Bligh and Dyer protocol and phosphorus was quantified by Bartlett-modified method (Bartlett, 1959).

Neutral charge liposomes size distribution has a mean value of 127 ± 25 nm with a polidispersity index (PI) value 0.187 while cationic liposomes size distribution has a mean value of 120 ± 20 nm with PI value 0.177. The B/P ratio of neutral charge liposomes was 0.2, while an increment of 65% of B/P ratio was achieved in cationic with respect to neutral liposomes formulation.

4. Neutron flux study and dosimetry considerations

The dosimetric feasibility assessment considers as a first option the use of existing irradiation facilities in our country, such as the new RA-6 BNCT facility. This facility has been designed for the treatment of cutaneous melanoma (Longhino and Blaumann, 2010). The new beam, named B2, has a mixed thermal and epithermal composition, which allows a deeper penetration in tissue compared to a pure thermal beam, and a low fast neutron component.

As a starting point in this part of the research, numerical characterizations of the beam and neutron flux distribution were performed using anthropomorphic computational models, taking into account the selected clinical cases and an a neutron beam impinging “en face” (as recommended in Horiguchi et al. (2011),