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Biodistribution of calcium fructoborate as a targeting agent for boron neutron capture therapy in an experimental model of MDA-MB-231 breast cancer cells

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ABSTRACT

Boron neutrons capture therapy (BNCT) involves optional agglomeration of 10B carriers in further neutron irradiation to the tumor tissue. The possibility of a Calcium Fructoborate (CFB) mediated by liposome has been proposed previously as boron carriers for the transfer of boron compound to the tumor tissue. Furthermore, for developing the use of a carrier, folate has been considered as an appropriate substrate with the potential to attach to tumor receptors on the surface of certain cancer cells compared with normal cells. The main problem associated with breast cancer is that the tumor and normal cells are mixed without a map of the boron accumulation. The present study aimed to examine boron biodistribution in the breast cancer model in BALB/c mice employing PEGylated liposome-encapsulated CFB formulations that were previously obtained. The predetermined boron concentration was obtained to be 20 mg 10B/g to 35 mg 10B/g. Also, the samples of the tumor tissue, such as the liver, muscle, heart, lung, spleen, skin, stomach, brain, bone, and kidney were taken as post-administration at different times to measure the boron content by inductively coupled plasma (ICP) analysis. The results demonstrated the existence of GLUT-5 expression as an erythrocyte-type glucose transporter protein in a wide range of tumor cells. In summary, the results of the present study suggest the therapeutic potential of boron-bearing liposomes given large boron biodistribution values.

1. Introduction

Boron chemists and pharmacists have a very important role and responsibility for the future of BNCT. In pharmaceutical and medical aspects, the advancement of selective anti-cancer agents has become one of the major roles in cancer treatment. Furthermore, novel drug delivery systems such as polymers, liposomes, and mAbs have opened new views in cancer treatment.

Recently, a remarkable therapeutic success of BNCT mediated by liposome-encapsulated CFB molecules has been evidenced. Also, based on the correlation between the results of thermal analysis and the elemental analysis, the correct molecular formula of CFB was obtained as Ca[(C6H10O6)2B]24H2O (Pirouz et al., 2019). One significant aspect is developing compounds that can deliver a high boron payload to the tumors. Hence, considerable successes have been obtained on the liposomal delivery system as a new targeting fact. Liposomes are profitable drug delivery carriers which are able to selectively transfer a large amount of a broad range of 10B agents to the tumor tissue. In this article, the application of BNCT following liposomal delivery of a 10B polyhedral carborane was investigated. For this purpose, a protein called folate was considered as an appropriate substrate with the potential to attach to tumor receptors on the surface of certain cancer cells compared with normal cells.

For the successful implementation of the BNCT technique, experiments need a suitable neutron source. Important factors in the selection of neutron sources include the flux and energy of the neutron beam. In most centers that use BNCT treatment, the reactor is a neutron source, and appropriated neutrons are very high (Gambarini et al., 2014). During the investigation of its behavior, we found that the breast cancer in BALB/c mice model possesses a unique advantage for treatment of tumors (Sato et al., 2018) (Wyzlic et al., 1994), (Chen et al., 1997), (Moss, 2014).

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The treatment of cancer involves destroying as many cancerous cells as possible, extending the survival of patients, and maintaining their quality of life. The main problem with breast cancer is that the tumor and normal cells are mixed without a map of the boron accumulation. As a result, in different tissue samples, the boron concentration and/or tissue composition measured is not accurate. The purpose of this study was to explore the long-term potential inhibitory effect of BNCT protocols and to perform biodistribution studies of CFB in breast cancer in BALB/c mice model in a research reactor located in Tehran. The optimization of selective boron delivery to cancer cells is a great area of research and should be considered as a starting point to enhance and optimize its therapeutic efficacy. In other words, it is important to be able to compare and predict the biological effects of mixed radiation fields at different kinds of centers. A quantitative comparison of the beams across centers leads to obtaining more robust analyses of clinical data. The aim is to verify the BNCT effects on the tumor and the surrounding healthy organs. To this end, we examined different sites in a BALB/c mice model. In this model, a predetermined concentration of the CFB was delivered to a tumor site. Specific method of inductively coupled plasma (ICP) analysis was applied for samples of the tumor, normal tissue, liver, muscle, heart, lung, spleen, skin, stomach, brain, bone, kidney, and blood (Ceberg et al., 1993). Use of a novel drug-containing 10B compound in smart release and administering it to a patient is a novel method in the treatment of the cancerous tumors. Low production cost as well as minimum side effects in treatment are other advantages of the applied drug and method.

2. Material and methods

2.1. Synthesis of liposome-encapsulated CFB

The synthesis of CFB was performed according to Miljkovic's procedure using boric acid as mentioned by Pirouz et al. (Pirouz et al., 2019). The synthetic procedure of liposome-encapsulated CFB using Poly Ethylene Glycol (PEG) as a precursor mentioned in the literature (Ito et al., 2009) allows solubility of the drug in the blood and binding of a ligand to the liposome. Hence, in this article, a new design with two sizes of PEG was used. Note that folate ligand bound to the larger size of PEG compared with other PEG is not repulsed when connected to their respective receptors at the surface of cancerous cells (Kanemitsu et al., 2019). As demonstrated in Fig. 1, the liposome can encapsulate aqueous solutions of sodium salts anions and join lipophilic boron-containing nanodrug components embedded within the bilayer membrane (Nakamura et al., 2009). For this purpose, 1 M solution of fructose in water or ethanol and 1 M solution of Boric Acid containing 10Boron were provided. Also, the solution pH was adjusted and controlled around 3–4. Then, some carbonate salt of solid calcium (dissolved in a little amount of deionized water) was added to the solution. In response to this process, a biphasic solution was formed where the lower phase was separated (Fig. 1). The nano drug has several boron atoms in each molecule thereby facilitating the penetration across the membrane of the tumor cell (Fig. 2).

2.2. Model of cancer: biodistribution and boron analysis studies

The digestion of animal samples was carried out according to Pirouz et al. procedure as discussed in the literature (Zaboronok et al., 2015). Also, the conducted experiments were analyzed to monitor the distribution of boron in the tumor, blood, and normal tissues over time to identify the optimal injection protocol for delivery of boron to the tumor tissue. Following appropriate experiments, the data were presented and boron concentration was analyzed individually.

In this research, several samples of tumor and cancerous tissues around tumor tissue were taken from each animal to evaluate the degree of homogeneity of boron distribution in different portions of these critical tissues (Trivillin et al., 2014). The cell line of MDA-MB-231 used in this project is a prominent sample of breast cancer cells. Boron concentration values in the tumor, blood, and normal tissues were obtained as post-administration to calculate the ratio of boron concentration in the tumor/blood, normal tissue/blood, and tumor/normal tissue for each compound (Fig. 3) (Miyabe et al., 2019). Fig. 3 shows an overall schematic view of the entrance process of the drug and the process of Boron Neutron Capture Therapy (BNCT) used in the investigation (Fig. 4).

3. Results and discussion

In this section, experiments associated with the in-vivo and in-vitro conditions of the biological tests are carried out. The effects of drug-containing 10B were tested on the malignant cancerous cell line of breast along with the corresponding dose-response curves. The predetermined concentration ranged from 20 mg 10B/g to 35 mg 10B/g. The experiment was performed on the biologic distribution of the designed drug at different times of injection, biologic distribution of various doses of the drug, and sufficient accumulation of the designed drug-containing 10Boron for effective treatment of the tumor. As mentioned above, the drug with concentrations of 15, 25, 35 and 45 mg was selected to be injected into the animal. The results of ICP data indicated that a drug concentration of 35 mg in cell culture medium maximized the accumulation of the drug within 24 h incubation of the drug being introduced to the environment. The reason can be attributed to the representation of toxicity of the synthesized composition using MTT assay method.

To perform the in-vivo treatment in the questioned animal, the toxicity of the liposome-encapsulated CFB was analyzed. Fig. 5 displays the diagram of the toxicity of the liposome-encapsulated CFB as determined by MTT assay method (Shu et al., 2019). As can be seen, initially, the number of live cells has not changed considerably because of using the unit dietary compound for drug synthesis. However, over time, the number of tumor cells gradually decreased. These reductions were 79, 64, 50, 80 and 36% for various boron compounds at concentrations of 5, 10, 25, 50 and 100 mg/L, after 24 h respectively. After 48 h, the results were similar to those obtained after 24 h with only a slight difference (Heber et al., 2006).

Boron biodistribution studies are essential to preclinical design and

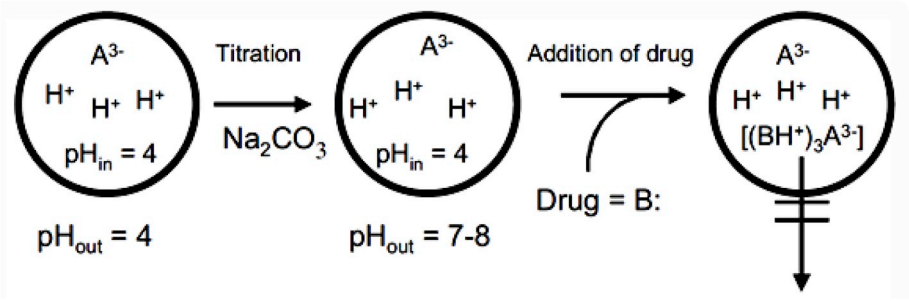

Fig. 1. Schematic view of the loading process of the nanodrug in the liposome.

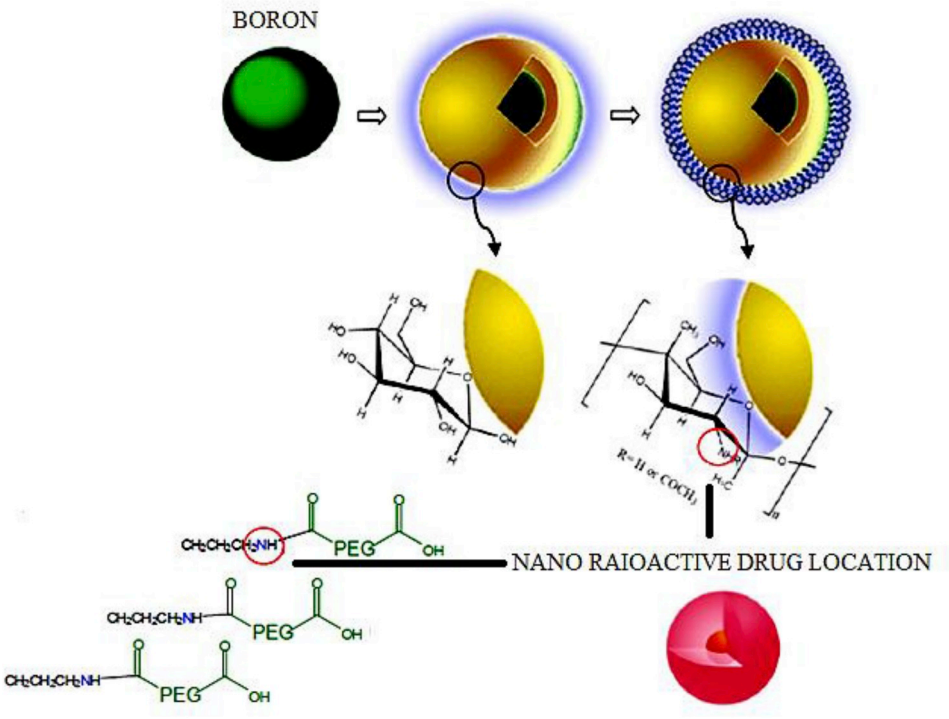

Fig. 2. Schematic view of the liposome-encapsulated CFB.

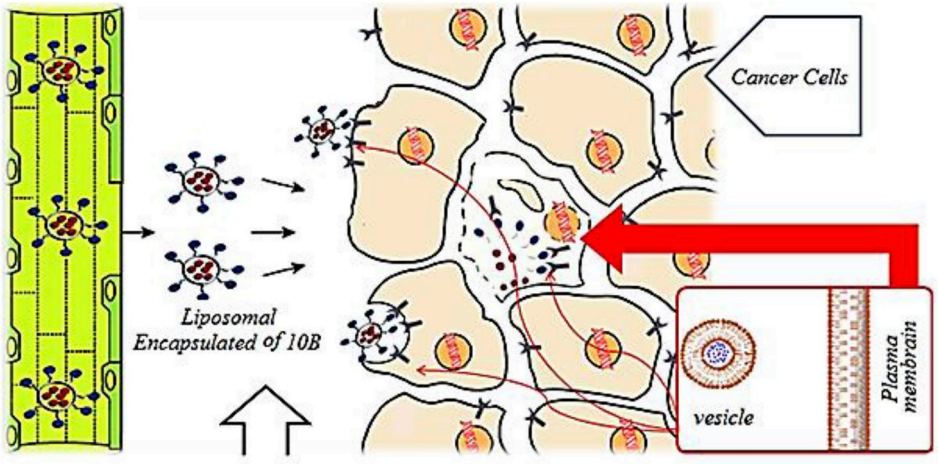

Fig. 3. Schematic view of the entrance of the drug and the process of BNCT used in the investigation.

subsequent clinical research protocols of BNCT. In particular, this analysis identifies the optimum time of post-administration of the boron carrier to perform neutron therapy (Watabe et al., 2017). In recent research, dose calculations have been based on boron content values in the blood, tumor, and normal tissues as obtained from biodistribution studies. Generally, in the case of animal models, calculations are based on the mean values obtained from biodistribution studies in separate sets of tissues (Sauerwein et al., 2011). The tabulated diagrams and data corresponding to the mean value ± standard deviation of the ratio for each sample are shown in Fig. 6.

The results indicate that the maximum concentration of drug in the tumor tissue occurred within 45 min to 3 h. Also, after drug injection, the concentration in the blood initially reached the maximum value, but it reached the lowest value within 24 h. Additionally, based on the results obtained above, the amount of fructose transmitter was high in the normal tissue, resulting in the accumulation of drug reaching the maximum value after 3 h (Lee et al., 2018).

Generally, the results of boron concentration in the tumor tissue showed that after 3 h of drug injection to the tumor, the drug concentration reached its maximum level (27 mg/L) due to the excessive amount of sugar transmitters such as GLUT5 in the tumor cells. It can be concluded that the transfer of CFB to the tumor occurred by the GLUT5 transmitter (Farhood et al., 2018). Since the number of these transmitters in the cancerous tissue is greater than in other tissues, selective accumulation of drugs to the cancerous tissue occurs.

The drug concentration in the tumor tissue increased over time because of the drug release to the tumor tissue through the blood (Maruyama et al., 2004). Upon the accumulation of the drug in the tumor, its concentration increased after 6 h of injection due to the consumption of the drug sugar by tumor cells after which the amount of boron in the tumor tissue diminished. These results are inconsistent with the results obtained by researchers regarding the excessive amount of

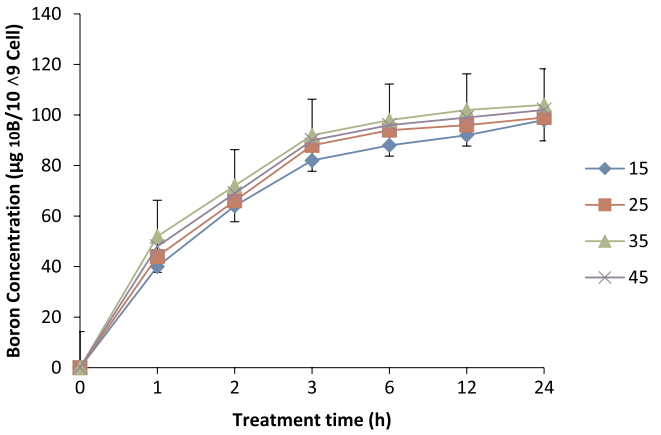

Fig. 4. Results of drug ICP for the obtained optimum boron concentrations (µg) vs. treatment time (h).

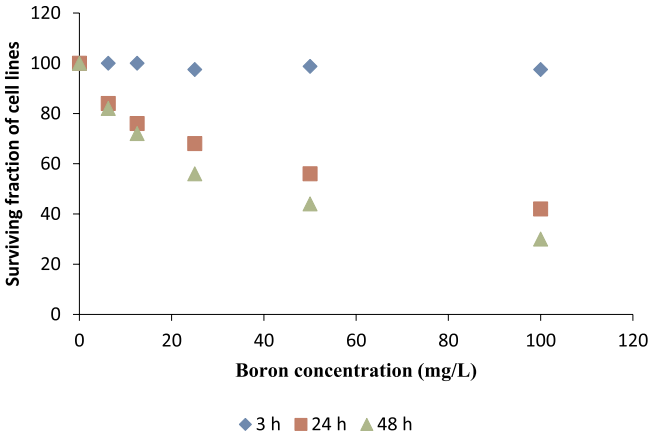

Fig. 5. Diagram of toxicity for the in-vivo treatment in the animal.

sugar transmitters in the tumor tissue (Carpano et al., 2015).

Furthermore, Table 1 reports the absolute boron concentration values for the blood, tumor, and normal tissues. The boron concentration (BC) in the blood, compared with normal tissue, is often used as the cited phrase to calculate the BC in the tumor for BNCT. Once the drug was injected, the concentration in the blood was maximum at the initial time. However, as to the drug transferred to other tissues and organs over time, its concentration decreased and ultimately reached the minimum level within 24 h post-injection (Woollard et al., 1990). In this article, the ratio of BC in normal tissue to that in blood, i.e. N/B ratio, remained about ~1.6 at 45 min post-administration (Hassanein et al., 2018). Table 1 tabulates the data corresponding to the mean value of the ratio for each sample.

Fig. 7A-I show a graphical representation of the biodistribution of 10B-Drug in different tissues of the animal body model at different times post injection (41.1 mg of 10Boron complex per every 30 gr of the animal weight). The concentration of boron in the kidneys tissue increased initially reaching the maximum of 17 mg/L within 3 h post injection. The kidneys contain glomeruli that are considered as blood filters. Hence, these data are consistent with the results of Edward's study indicating the expression of GLUT5 in the kidney glomerular tissue (Fig. 7A). In the liver tissue, the level of fructose transmitters was high and after 3 h, the accumulation of the drug reached the maximum value (Fig. 7B). The results of the ICP analysis for the heart tissue are similar to the results obtained for the muscle tissue due given the muscular structure of the heart (Fig. 7C). The results of drug distribution studies on spleen and lung tissues were interesting. The results in Fig. 7D and E show that the drug concentration was high at initial time post injection

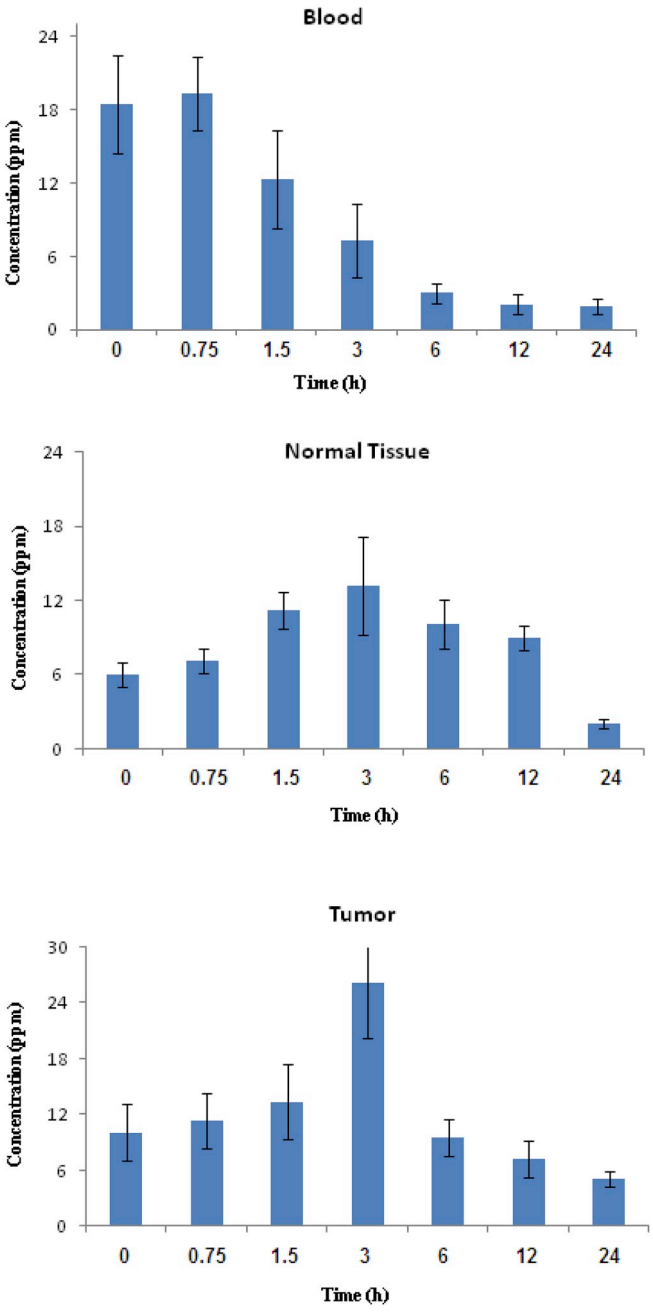

Fig. 6. Boron concentration (mg/L) in the blood, normal tissue, and tumor tissue for the drug at different times.

Table 1
T/N, T/B, and N/B boron concentration ratios in CFB administered mice.

Ratio	Time after administration (h)						
	0	0.75	1.5	3	6	12	24
T/N	0.33	0.37	0.91	1.81	3.37	4.29	1.05
T/B	0.55	0.59	1.09	3.59	3.17	3.43	2.69
N/B	1.68	1.59	1.20	1.98	0.94	0.80	2.55

T: Tumor; N: Normal tissue (muscle); B: Blood.

to the lung and spleen tissues due to the high rate of blood perfusion in the tissues. Within 24h, it is observed that the concentration of drug in the tissues diminished significantly and reached its minimum value (2 mg/L). The concentration of drug in the skin tissue at the initial time was maximum but it dropped rapidly to 1.5 mg/L over 3 h. This result

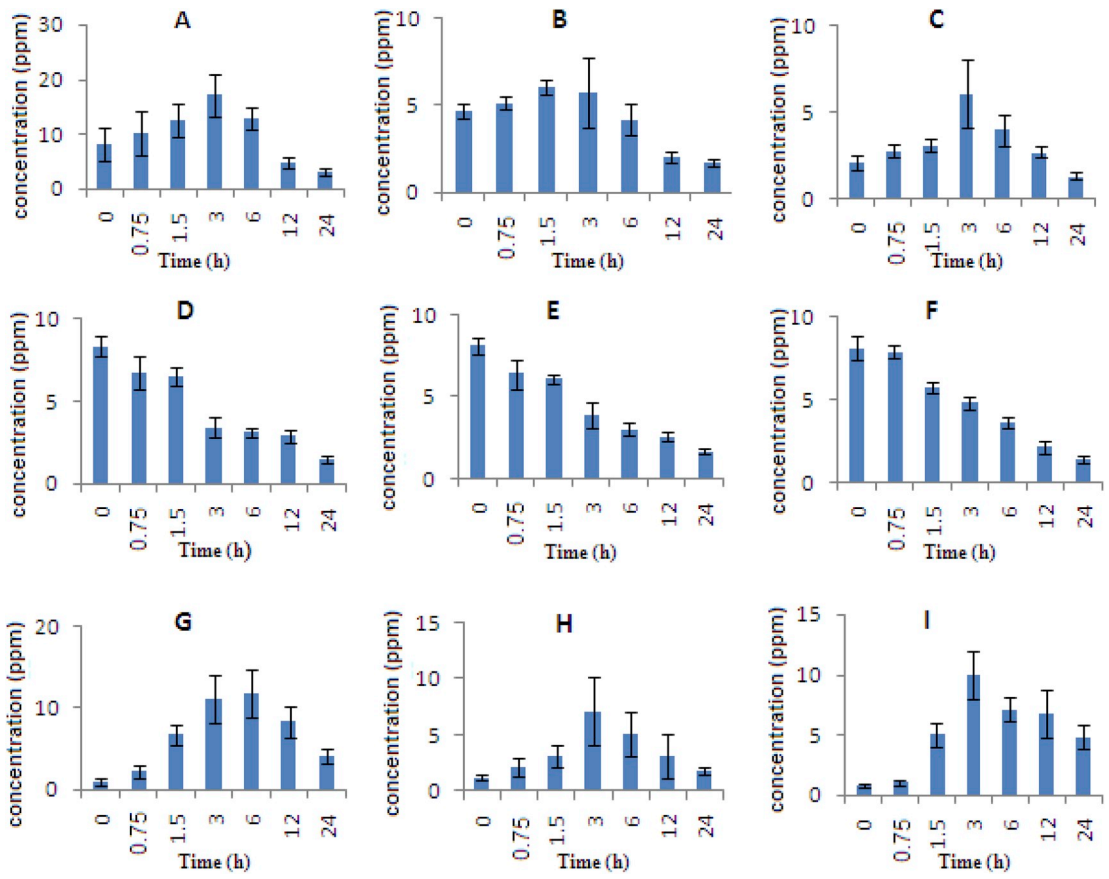

Fig. 7. Boron concentration (mg/L) in several tissue samples for the drug at different times.

can be attributed to the high distribution of blood capillary network inside the skin tissue (Marone et al., 2016). At the initial time post injection, the concentration of boron in the skin tissue increased and then decreased with blood circulation (Fig. 7F). The study of drug concentration in stomach tissue in Fig. 7 G reveals its highest concentration after 3 and 6 h of injection after which is shows a descending trend to the value of 4 mg/L within 24 h after injection. The reason for high concentrations of boron in the stomach tissue can be attributed to its muscle structure and high blood perfusion within a time interval of 3 to 6 h. In addition, the stomach tissue has a large number of fructose transmitters in its membrane cells. The highest amount of boron in the brain tissue occurred within 3 h post injection (Fig. 7H). The concentration of boron in the bone tissue increased to 10 mg/L after 3 h with a high rate (Fig. 7I). The drug concentrations in different periods of time, in different tissues, and organelles are shown in Table 2. Considering the high importance of the tissues in the case of breast cancer, it can be concluded that the mean concentration of boron decreased considerably, and its value fell within the range of 6 to 10 mg/L similarly for the tumor, lung, spleen, and skin tissues.

The results of the present study suggest the therapeutic potential of boron-bearing liposomes given large boron biodistribution values (Rotaru et al., 2010), (Musacchio González and Martín Hernández, 2017), (Ciani et al., 2013), (Alberti et al., 2015).

Table 2
Boron concentration (mg/L) in blood and tissue samples for the drug at different times post-administration.

Tissue (mg/L)/Time (h)	0	0.75	1.5	3	6	12	24
Tumor	10.1	11.3	13.4	26.2	9.5	7.2	5.1
Normal Tissue	6	7.1	11.2	13.2	10.1	9	2
Blood	18.4	19.3	12.3	7.3	3	2.1	1.9
Liver	4.7	5.2	6.1	5.8	4.2	2.1	1.8
Heart	1.1	2.8	3.1	6.1	4	2.7	1.3
Lung	8.3	6.7	6.5	3.4	3.1	2.9	1.5
Spleen	8.1	6.4	6.1	3.9	3	2.6	1.7
Skin	8.1	7.9	5.7	4.8	3.6	2.1	1.4
Stomach	0.9	2.3	6.8	11.2	11.8	8.3	4.1
Brain	1.2	2.1	3.1	7.1	5.1	3.1	1.8
Bone	0.8	1	5	10	7.1	6.7	4.8
Kidney	8.1	10.1	12.4	17.1	12.8	4.7	3.1

4. Conclusion

In the present study, the time-course biodistribution of boron delivered by liposome-encapsulated CFB in the BALB/c mice breast cancer model was reported for the first time. In summary, it was observed that the combined administration of CFB offered the greatest similarity in practical targeting of different tumors as these boron carriers have different uptake mechanisms and properties. Furthermore, it was possible to transfer the contents of therapeutic boron to BALB/c mice tumor selectively and achieve ratios between tumor and blood that was compatible with the treatment. ICP data indicated that a drug concentration of 35 mg in the cell culture medium maximized the accumulation of drugs for 24 h incubation after the drug was introduced into the environment. Also, the maximum concentration of the drug (27 mg/L) in the tumor tissue occurred within a period of 45 min to 3 h due to the excessive amount of sugar transmitters such as GLUT5 in the tumor cells. The results can be considered as a guideline for further understanding boron targeting processes associated with the agents studied.

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