Optimization of Boron-10 Concentration in Cancerous Tumors for Boron Neutron Capture Therapy Using a Mixture of Boron Agents

T. A. El Malky, M. El Ghazaly, and A. El-M Eghawry
College of Medicine and Medical Science, Taif University, KSA

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ABSTRACT

In the current investigation an attempt is carried out to increase the Boron-10 in liver tumor. In the first phase of the experiment, an induction of cancer using the well known carcinogens was carried out. Afterthere the rats are injected by a mixture of Boron-10 carriers was carried out using Boronophenylalanine (BPA) alone and with Sodium tetraborate Na$_2$B$_4$O$_7$. It is found that the concentration of boron in a tumor was increased by about 10-20% when rats are injected by a mixture of BPA and Sodium tetraborate. This increasing should enhance the performance of the Boron–Neutron Capture therapy. The optimized conditions, namely injected concentration and half life time, which achieve maximum concentration of Boron within tumor, have been determined.

Key Words: Boron Neutron Capture Therapy / Sodium tetraborate (STB)/ Boronophenylalanine (BPA).

INTRODUCTION

Boron Neutron Capture Therapy (BNCT) is being used successfully for the treatment of malignant tumors. The first component of BNCT is a stable isotope of Boron-10 that should be concentrated in tumor cells by attaching it to tumor seeking agents. Boron concentration within tumor cells should be higher than normal cells by a considerable factor \textsuperscript{(1-4)}. The second component is a beam of thermal neutrons (less than 0.025 eV). After thermal neutron is captured by Boron-10, excited Boron-11 is formed which undergoes a fission in 10\textsuperscript{-12} Sec. Boron-11 in/or adjacent to the tumor cells disintegrates into Lithium-3 with energy about 0.84 MeV and Helium nucleus (alpha particles) with energy 1.47 MeV and gamma ray of energy 0.48 MeV according to the following equations,

$$^1_0 n + ^{10}_5 B \rightarrow ^7_3 Li + ^4_2 He + 2.79 \text{ MeV} \quad (6\%)$$ \hspace{1cm} (1)

$$^1_0 n + ^{10}_5 B \rightarrow ^7_3 Li + ^4_2 He + 0.48 \text{ MeV(\gamma)} + 2.31 \text{ MeV} \quad (94\%)$$

This energy is deposed along the particles trajectory in tissue, which in order of some microns, results in selectively destroying the cells in close proximity to its track, primarily cancer cells, leaving adjacent normal cells largely unaffected \textsuperscript{(5-8)}. This reaction has not toxic effect on the normal cells since Lithium and alpha particles have range in some microns which are comparable with the cell dimensions\textsuperscript{(9)}. Fig. (1) shows Bragg curve which is describing the Linear Energy Transfer (LET) along the trajectory of charged particles is calculated for alpha particle emitted after decay of B-11 in liver tissue \textsuperscript{(7)}. Maximum energy deposition within tumor takes place at the end of the range of alpha particle which is in order of some microns less that the dimension of cells. One of the most important issues regarding BNCT is the boron delivery to the tumor cells. Concentration ratios exceeding 2:1 and extended to 10:1 between tumor and healthy brain have been considered as a curial prerequisite for successful BNCT, similarly this condition could be extended and applied to liver treatment using BNCT\textsuperscript{(11,12)}. 
Many drugs separately and/or in a combination such as BPA, BSH have been used. Despite the long history of clinical use of L-BPA worldwide, nobody has been used BPA in a combination with STB. Fundamental studies on bio-distribution of boron after intravenous (i.v.) injections of L-BPA and Sodium tetraborate into tumor bearing animals were carried out based on the typical dose employed in clinical NCT. In the current work an attempt has been carried out to increase the boron concentration within liver tumor using a mixture of boron drugs, namely boronophenylalanine (BPA) and with Sodium tetra Borate STB.

**Fig. (1):** Bragg curve (Linear Energy Transfer) for alpha particles of energy 1.47 MeV emitted after decaying of excited B-11 in liver tissue\(^{(7)}\).

**MATERIALS AND METHODS**

Boronophenylalanine (BPA) was purchased from Ryscor Science Inc. (North Raleigh, NC, USA) and converted to BPA-fructose (BPA-f), shortly BPA and fructose were combined in water, the pH was adjusted to 10.0 with NaOH, the mixture was stirred until all solids dissolved, and the pH was readjusted to 7.4 with HCL\(^{(11,12)}\). 40 adult male Sprgue-Dawley rats, aged about 16 weeks of age and weighting (200±20) g were housed in a temperature-controlled environment (25 °C) with 12-hr. light-dark cycle. Food and water were freely available. The experiments were conducted in accordance with national animal protection guidelines and performed at the animal house in college of medicine, Taif University, KSA.

**EXPERIMENTAL DESIGN**

The rats were randomized and divided into 2 groups consists of 20 rats each.

**Group I** served as control and was given daily olive oil only.  
**Group II** Rats were injected with one dose of DEN (100 mg/kg/bw). 4-(methylnitrosamine)-1-(3pyridyl)-1-butanone was purchased from Sigma-Aldrich Co. for cancer induction in liver.  
Group II is divided into two subgroups; subgroup one is injected intervenesly with BPA of dose 1000 mg/kg BPA. Meanwhile subgroup two Rats were injected with both of BPA of dose 1000 mg/kg in a combination with Sodium tetraborate (STB) of dose 200 mg/kg to avoid toxic effect caused by high doses of STB.
Both groups were injected with Boron-10 agents namely, BPA and Sodium Tetra Borate STB. The inductively coupled plasma atomic emission spectrometric (ICP-AES) method was used in Boron analysis in tissues. A weighed sample of rats tissue or homogenate (typically 100–200 mg) was hermetically digested with HClO$_4$ (0.6 mL) and H$_2$O$_2$ (1.2 mL) for 24–48 hours at 75 °C. The resulting solution was diluted with ultra pure water to 5 mL, followed by filtration with a 0.45 mm disposable syringe filter unit.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PREPARATIONS

Rats were sacrificed to investigate their liver tissues on the same day after weeks 14 of DEN administration. Representative samples of tumors and tissues were histologically examined. The specimens were fixed in formalin 10% and embedded in paraffin. Slices of 5 µm were stained with hematoxylin and eosin for microscopic examination and other for determination of marker using immunostaining technique. Five-micrometer tissue sections were cut, mounted on poly-L-lysine coated glass slides and incubated overnight at 37°C. Immunohistochemical staining was performed using an avidin–biotin peroxidase complex. Briefly, samples were treated with 0.6% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. Staining of formalin-fixed tissues requires boiling tissue sections in 10 mM citrate buffer, pH 6.0, (NEOMARKERS’ Cat. #AP-9003), for 10-20 min followed by cooling at RT for 20 min. The slides were preincubated with normal goat serum (1:10) (NEOMARKERS, USA) for 10 min and then with human specific Mouse Monoclonal Ki67 (Clone SP 6) antibody (Ab-1, Lab. Vision, NeoMarkers, USA), Using antibody dilution at 1:200 for 30 min at RT. The sections were further incubated with biotinylated secondary antibody (NEOMARKERS, USA) for 10 min, followed by incubation with peroxidase- conjugated streptavidin diluted 1:3000 in phosphate- buffered saline for 15 min. The peroxidase reaction was performed using 0.02% 3, 3-diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide and counterstaining was performed with hematoxylin for 1min. As negative control, the primary antibody was omitted. Each section was counted manually at high power (400X) after identifying at low power (100X) the representative areas with the highest concentration of stained cells according to the recommendation of Cohen et al., (1993), about 1000 cells/slide were counted in each of five microscopic fields from well-labeled areas to determine the average of Ki67 labeling index (Ki67 LI). Ki67 LI was expressed as number of labeled cells (positive for Ki67) as a percentage of the total number of cells counted in each specimen. All identifiable staining was regarded as positive. The results are expressed as mean plus or minus standard deviation (Ki67 LI = mean±SD) the positive results for proliferation marker Ki67 is brown nuclear stain.

RESULTS AND DISCUSSIONS

Liver cancer induction:

The present results demonstrated the normal polyhedral hepatocytes with granular cytoplasm. Each cell has a centrally located nucleus with one or two nucleoli in addition to a number of chromatin particles. Sometimes, binucleated hepatocytes are seen. The hepatocytes arranged in cords or strands forming a network around the central vein. The liver strands are alternating with narrow blood sinusoids also radially extending along the liver lobules. The boundaries of the sinusoids composed of a single layer of fenestrated endothelial cells and kupffer cells (Figure 2A). Boron treated rats showed normal liver tissue as previously seen in normal liver of control rats (Figure 2B). Liver of rats treated with NNK showed obvious fatty degeneration with displacement of the nucleus. Hydrophobic degeneration (oedema) was also seen in severe injured hepatocytes. The nuclei of the hepatocytes were apparently hyperchromatic and displayed some features of pyknosis. Inflammatory lymphocytic infiltration was clearly visible in these rats, also tumor in some area was arranged in cords and in others in a duct-like pattern (Figures 2C, D and E).
Figure (2): Photomicrographs of liver from different groups. (A) The normal liver. (B and C) the hepatocellular carcinoma with high proliferation. Immunohistochemical staining photomicrographs show rat liver sections were stained with anti-Ki67 with Avidin-Biotin technique. (D) Normal liver with very weak immunostaining. (E and F) DEN group show the strong positive brown staining with the proliferating marker Ki67.
In the immunostain investigation, the liver sections of control and boron treated rats stained with Ki67 showed very weak positive stained nuclei indicating the mild cell division of some hepatocytes Figure 2A. However, sections in liver of rats treated with DEN were showed strong positive stained nuclei in most of the hepatocytes (Figure E and F). Moreover, the hepatocytes of rats treated with Boron and DEN were demonstrated that the positive stained nuclei less than that of the DEN treated animal (Figure 2F). The changes in liver Ki67 labeling index were observed. Rats fed on Spirulina containing diet were showed a nonsignificant when compared with control group. While, DEN treated group was displayed a very high significant. Rats protected with Spirulina and treated with DEN were illustrated a high significant increase compared with control rats and a very high significant when compared with DEN treated group.

**Boron Concentration**

The concentration of Boron-10 in different organs, such as blood, kidney, liver and liver tumor as a function of elapsed time for both BPA and Sodium Tetra Borate Na₂B₄O₇ were measured. The curves of blood boron are shown in Fig. 3. Which are fitted using exponential decay functions of second order, Eq. (2), from which the elimination half life time, \( t_{1/2} \) measured in minutes, was calculated by multiplying 0.693 by \( \frac{C_1 \times C_2}{C_1 + C_2} \),

\[
C_B = C_{B1} \times \exp\left(-\frac{t}{C_1}\right) + C_{B2} \times \exp\left(-\frac{t}{C_2}\right) + C_{B0}
\]  

where \( C_{B0} = 1.4 \) mg/mL, \( C_{B1} = 83.9 \) mg/mL, \( C_{B2} = 17.99 \) mg/mL, \( C_1 = 336.1 \) min, and \( C_2 = 41.8 \) min. The half life time of Boron concentration for i.v. single injection with BPA is 36.5 minutes mean while for single injection with BPA and STB simultaneously was 44.6 minutes. Boron concentration is decaying exponentially; therefore it will be described by a second order exponential decay function, which is written as,

\[
C_k = C_{k0} + C_{k1} \times \exp\left(-\frac{t}{Ck1}\right) + C_{k2} \times \exp\left(-\frac{t}{Ck2}\right)
\]  

Fig. (3): Boron concentration in blood following start of boronophenylalanine (BPA) administration in a combination with Sodium tetrab
Fig. (4): Boron concentration in the kidney as a function of elapsed time for BPA administration alone and administration with Boronophenylalanine (BPA) in a combination with BPA plus Sodium tetraborate.

Where $C_{K0} = 0.275 \text{ mg/mL}$, $C_{K1} = 32.1 \text{ mg/mL}$, $C_{K2} = 76.6 \text{ mg/mL}$, $CK1 = 379 \text{ min}$, and $CK2 = 64.77 \text{ min}$. The half life time of Boron concentration for i.v. single injection with BPA is 55.3 minutes mean while for single injection with BPA and STB simultaneously was 63.7 minutes, which is in a good agreement with data reported by Ichikawa et. al. \(^5\). Boron concentration has been measured in a normal tissue after a single BPA i.v. administration, the boron concentration increased with increasing the time reaching it maximum after 200 minutes and then decaying exponential with elapsed time as shown in Fig. (4). The half live time for boron concentration with normal liver tissue is about (350±31) minutes, which is higher with some order than the half life time in blood and kidney.

Fig. (5): Concentration of Boron in normal liver tissue as a function of elapsed time for i.v. single administration with BPA.
Boron concentration within a liver cancer tissue, which has inducted as shown in liver cancer induction part, is measured as a function of the elapsed time as shown in Fig. 5. For both a single i.v. administration with BPA alone and BPA with STB simultaneously. First point to observe in Fig. 5 is that the increasing of accumulated boron within tumor for i.v. administration uses BPA with STB simultaneously and BPA alone, giving longer opportunity for irradiation time with thermal neutrons and treatment. The half-life times, for both injection with BPA and STB simultaneously, doesn’t show a significant change when BPA used alone, they amount to (340 ±43) for BPA and (345±33) for BPA plus STB which results in a constant elimination constant for both born agents. The boron concentration reaches its maximum value after 200 minutes for both, therefore it is recommended to start the irradiation process after elapsed time 200 minutes. The maximum boron concentration in the liver tumor is increase by about 10-20% for injection with BPA plus STB than BPA alone; this however should enhance the boron accumulation in liver tumor by a factor 3:1 for normal liver tissue. This should improve and increased the performance and efficiency of Boron Neutron Capture therapy against liver cancer\(^{14}\).

CONCLUSION

Boron concentration in a liver tumor is increased by a factor 10-20% when STB and BPA are administrated simultaneously than administration of BPA alone; this could enhance the performance of Boron neutron capture therapy against liver cancer.

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