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FULL PAPER

Selective boron delivery by intra-arterial injection of BSH-WOW emulsion in hepatic cancer model for neutron capture therapy

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Objective: Boron neutron-capture therapy (BNCT) has been used to inhibit the growth of various types of cancers. In this study, we developed a ¹⁰BSH-entrapped water-in-oil-in-water (WOW) emulsion, evaluated it as a selective boron carrier for the possible application of BNCT in hepatocellular carcinoma treatment.

Methods: We prepared the ¹⁰BSH-entrapped WOW emulsion using double emulsification technique and then evaluated the delivery efficacy by performing biodistribution experiment on VX-2 rabbit hepatic tumour model with comparison to iodized poppy-seed oil mix conventional emulsion. Neutron irradiation was carried out at Kyoto University Research Reactor with an average thermal neutron fluence of $5 \times 10^{12} \text{ n cm}^{-2}$. Morphological and pathological analyses were performed on Day 14 after neutron irradiation.

Results: Biodistribution results have revealed that ¹⁰B atoms delivery with WOW emulsion was superior

compared with those using iodized poppy-seed oil conventional emulsion. There was no dissemination in abdomen or lung metastasis observed after neutron irradiation in the groups treated with ¹⁰BSH-entrapped WOW emulsion, whereas many tumour nodules were recognized in the liver, abdominal cavity, peritoneum and bilateral lobes of the lung in the non-injected group.

Conclusion: Tumour growth suppression and cancer-cell-killing effect was observed from the morphological and pathological analyses of the ¹⁰BSH-entrapped WOW emulsion-injected group, indicating its feasibility to be applied as a novel intra-arterial boron carrier for BNCT.

Advances in knowledge: The results of the current study have shown that entrapped ¹⁰BSH has the potential to increase the range of therapies available for hepatocellular carcinoma which is considered to be one of the most difficult tumours to cure.

INTRODUCTION

Hepatocellular carcinoma (HCC) is considered to be one of the most difficult tumours to cure by operation, chemotherapy or radiotherapy.^{1–3} In the treatment of HCC, only 30% patients can be operated on due to complications, including liver cirrhosis or multiple intrahepatic tumours. The usefulness of iodized poppy-seed oil (IPSO) for treating HCC was first reported by Nakakuma et al⁴ by taking advantage of the fact that IPSO selectively enters HCC cells. In the clinical setting, anticancer agents are usually administered by mixing with IPSO and are delivered intra-arterially.^{5–9} Problems still remain, since the anticancer agents easily separate from the IPSO within a short time period, with the result that the anti-cancer agents do not accumulate effectively in tumour cells and fail to affect cancer tissues adequately.

In water-in-oil-in-water (WOW) emulsions, fine vesicles of the aqueous solution of an anticancer drug are dispersed in IPSO. Higashi et al^{10–12} previously reported the preparation of a long-term inseparable WOW emulsion containing 8–60 mg epirubicin to use in arterial-injection therapy of patients with HCC, in which the WOW emulsion was prepared by a membrane emulsification technique using controlled pore glass membranes.^{13,14} It was also reported that the tumour size of HCC was reduced in patients, and a >50% decrease in initial alpha-fetoprotein levels within 14 days was observed.^{15,16}

Boron neutron capture therapy (BNCT) has been used to inhibit the growth of various difficult-to-treat cancers.^{17–19} The cytotoxic effect of BNCT is due to the nuclear reaction between Boron-10 (¹⁰B) and thermal neutrons, producing high linear energy transfer of alpha particles and lithium recoil. These particles (α and ⁷Li) destroy cells within an approximately 10- μ m path length from the site of the capture reaction. Therefore, it is very important to develop selective boron delivery systems for effective BNCT treatment.²⁰ As one of the boron carriers, we have reported that ¹⁰BSH-entrapped immunoliposomes showed cytotoxic effect on human pancreatic carcinoma cells *in vitro* through BNCT²¹ and that intratumour injection of boronated immunoliposomes resulted in suppression of tumour growth *in vivo* through BNCT.²² We have also previously prepared polyethylene-glycol-binding liposomes, which effectively delivered the boron compound ¹⁰BSH [The sodium salt of mercapto-undecahydro-closo-dodecaborate ($\text{Na}_2^{10}\text{B}_{12}\text{H}_{11}\text{SH}$)].^{23,24}

In order to increase the range of therapies available for patients with HCC, in this study we developed a ¹⁰BSH-entrapped WOW emulsion and evaluated it as a selective boron delivery carrier in the VX-2 hepatic tumour model, in order to investigate the possible application of BNCT in HCC treatment.

METHODS AND MATERIALS

Chemicals

The sodium salt of mercapto-undecahydro-closo-dodecaborate ($\text{Na}_2^{10}\text{B}_{12}\text{H}_{11}\text{SH}$; ¹⁰BSH) was purchased from Katchem spol. s.r.o (Prague, Czech Republic). IPSO (Lipiodol Ultra-Fluid, Kodama, Co. Ltd., Tokyo, Japan) is composed of iodized ethyl esters of the fatty acids obtained from poppy-seed oil and contains 37% iodine.

Preparation of tumour-bearing rabbit

The VX-2 cells (a Shope-virus-derived squamous-cell-carcinoma cell line) and the female New Zealand white rabbits were obtained from Nihon SLC Ltd (Shizuoka, Japan). The rabbits were used at a mean body weight of 2 kg, and the VX-2 cells were inoculated into the left lobe of the liver following the procedures of tumour implantation and sacrifice in accordance with the approved guidelines of Meiji Pharmaceutical University's animal ethics committee and with the Declaration of Helsinki.²⁵

Preparation of ¹⁰BSH-entrapped water-in-oil-in-water emulsion

500 mg ¹⁰BSH was dissolved in 5 ml of a 5% glucose solution, which was filtered through a controlled pore glass membrane and then emulsified in 5 ml IPSO containing 500 μ g polyoxyethylene 40 stearate to form the water-in-oil emulsion (WO). The WO emulsion was then emulsified again with an aqueous phase containing 5 ml saline solution and 500 μ g polyoxyethylene 60 stearate using a double membrane emulsifying machine (SPG Techno Ltd, Miyazaki, Japan). The ¹⁰BSH-entrapped WOW emulsion was prepared by a double emulsifying technique using a fine-pore glass membrane of equal pore size, to obtain monodisperse lipid microdroplets containing aqueous fine.^{6,10}

Characterization of ¹⁰BSH-entrapped water-in-oil-in-water emulsion

The shape of the WOW emulsion was observed using an optical microscope BHS-323 (Olympus Optics Co., Tokyo, Japan), and the particle size distribution of the WOW vesicles and IPSO microdroplets was determined using a SALD-2000 laser-diffraction particle-size analyser (Shimadzu Corp., Kyoto, Japan). To evaluate the amount of ¹⁰B compound entrapped in the WOW emulsion, quantitative analysis was performed using inductively coupled plasma mass spectroscopy (ICP-MS) at the Juntendo University School of Medicine, Tokyo, Japan.

In vivo biodistribution experiments

2 weeks after tumour inoculation, ¹⁰BSH-entrapped WOW emulsion was administered by intra-arterial injections *via* the hepatic artery proper in VX-2 rabbit hepatic tumour models. To evaluate the delivery efficacy, we measured ¹⁰B concentration in tumour or normal liver tissue by preparing three rabbits for each of the three groups: ¹⁰BSH-entrapped WOW emulsion-injected, ¹⁰BSH-IPSO mix conventional emulsion-injected and non-injected groups. All three groups received interventional therapy 14 days after tumour inoculation. The animals were sacrificed on Day 1, Day 3 and Day 7 after injection. The tumours and normal liver tissues were removed and analysed using ICP-MS at the Juntendo University. Tissues were weighed immediately after dissection and recorded as "wet weights", and the results were expressed as micrograms of boron per gram of tissue.

Pathological findings of tumours after boron compound injection

Electron microscopy was performed on liver sections of rabbits sacrificed on Day 1, Day 3 and Day 7 after intra-arterial injection of the ¹⁰BSH-entrapped WOW emulsion or the ¹⁰BSH-IPSO mix conventional emulsion. Livers were resected and stored in optimal cutting temperature compounds frozen at -80°C .

Figure 1. (a) Microphotograph of the ^{10}BSH -entrapped water-in-oil-in-water (WOW) emulsion, revealing numerous spherical iodized poppy-seed oil (IPSO) microdroplets suspended in the aqueous phase. The IPSO microdroplets were smooth on the surface and contained many vesicles of aqueous solution of ^{10}BSH . (b) The curve of the relative particle ratio from laser-diffraction particle-size analyser measurement results indicates that the median particle size of the IPSO microdroplets in WOW was $70\text{ }\mu\text{m}$.

Histological observations were also carried out on the same liver samples. $6\text{-}\mu\text{m}$ -thick sections were stained with haematoxylin and eosin (H&E) and Oil Red O for light microscopy.

Neutron capture autoradiography

In order to examine the biodistribution of the ^{10}BSH -entrapped WOW emulsion in the liver after intra-arterial injection, we

carried out a neutron capture autoradiography (NCAR) measurement on $40\text{-}\mu\text{m}$ -thick section of frozen liver samples. The liver samples were cut sagittally and mounted on thin 3-M Scotch tape, freeze dried at $-20\text{ }^{\circ}\text{C}$ for 2 weeks and air dried further for a week to prepare dried samples. Liver sections were put in close contact with CR-39 (poly-allyl-diglycol carbonate) plates (HARZLASTM; Fukuvi Chemical Industry) using thin

Table 1. ^{10}B concentration in hepatic tumours and normal liver tissue of a VX-2 hepatic tumour bearing rabbit model after intra-arterial injection of ^{10}BSH -water-in-oil-in-water (WOW) emulsion or ^{10}BSH -iodized poppy-seed oil (IPSO) mix

^{10}BSH -containing delivery system	Tumour	Normal liver	Blood	Heart	Lung	Kidney	Spleen
WOW							
Day 1	141.80 ± 6.87	6.05 ± 4.60	1.20 ± 0.57	0.20 ± 0.28	4.35 ± 4.74	6.95 ± 3.04	1.55 ± 2.19
Day 3	52.63 ± 48.19	4.32 ± 3.28	0.07 ± 0.12	0.27 ± 0.31	1.80 ± 0.85	6.63 ± 3.12	1.93 ± 1.26
Day 7	28.80 ± 12.02	3.67 ± 2.42	0.13 ± 0.23	0.27 ± 0.46	0.53 ± 0.16	3.30 ± 3.35	1.23 ± 1.25
IPSO mix							
Day 1	58.0 ± 21.05	24.00 ± 8.52	0.40 ± 0.57	0.75 ± 0.07	1.40 ± 1.13	11.65 ± 3.18	1.25 ± 0.92
Day 3	24.5 ± 33.02	2.57 ± 3.38	0.20 ± 0.28	0.20 ± 0.28	0.40 ± 0.28	2.95 ± 0.64	0.90 ± 0.28
Day 7	6.0 ± 10.33	1.20 ± 0.57	0.20 ± 0.28	ND	0.50 ± 0.42	2.70 ± 0.14	0.85 ± 0.21

ND, Not Done.

We prepared one group treated with ^{10}BSH -containing WOW emulsion ($n = 3$ rabbits), and the other group treated with ^{10}BSH -IPSO mix conventional emulsion ($n = 3$ rabbits). These groups received interventional therapy 14 days after tumour inoculation. The animals were sacrificed 1, 3 and 7 days after injection and the tumours and normal liver tissues were removed to determine the ^{10}B concentration using ICP-MS at the Juntendo University, Tokyo, Japan. Tissues were weighed immediately after dissection and recorded as "wet weights".

Results are expressed as micrograms of boron per gram of tissue (ppm).

adhesive tape. Boron-containing standard samples were also prepared using dried filter paper sheets wetted with ^{10}BSH solutions of four different ^{10}B concentrations (10, 50, 100 and 500 ppm ^{10}BSH). ^{10}B concentrations of the ^{10}BSH solutions were determined by ICP-MS at the Juntendo University.

The set of liver samples were simultaneously exposed in the JRR3 reactor of the Japan Atomic Energy Research Institute with an average thermal neutron fluence of $2.0 \times 10^{12} \text{ n cm}^{-2}$ for imaging,^{26–30} with the etching of NCAR images performed in NaOH solution.³¹ In α -autoradiographic imaging, CR-39 detector plates were etched in a 7 N NaOH solution at 70°C for 2 h to reveal tracks. However, imaging results would include the recoiled proton tracks produced by the biogenically abundant nuclide, ^{14}N (n, p). In order to selectively desensitize and eliminate undesirable proton tracks, we also applied PEW solution (15 wt% KOH + 65 wt% $\text{C}_2\text{H}_5\text{OH}$ + 20 wt% H_2O) at 50°C for 8 min to the etching of the CR-39 detector plates. The tracks were automatically measured using a track analysis system of the Radiation Medical Institute, Chiba, Japan, which is capable of accurately measuring the dimension, shape, grey-level intensity and position of individual tracks of different origin on a large CR-39 plate.

Neutron irradiation of VX-2 rabbit hepatic cancer model

To prepare the neutron irradiation experiment, rabbit VX-2 cells were inoculated into the left lobe of the liver using the same procedure as for the biodistribution experiment. 2 weeks post tumour inoculation, ^{10}BSH -entrapped WOW emulsions were administered by intra-arterial injections *via* the hepatic artery proper in VX-2 rabbit hepatic tumour models. The tumour-bearing rabbits were irradiated with thermal neutrons of $5 \times 10^{12} \text{ n cm}^{-2}$ average fluence at the Kyoto University Research Reactor Institute. The thermal neutron irradiation mode was set to OO-0000-F with a Cd ratio of approximately 9.4. The reactor power was 1 MW, and irradiation was carried out for 45 min. Neutron fluence was measured with gold foil at 2 points on the frontal side of the abdomen and rear side of the rabbit while the gamma ray dose was measured by thermoluminescent dosimeter at the same points.

Morphological and pathological analysis after neutron irradiation

Tumour sizes and the status of the abdomen and peritoneum were investigated on Day 14 after neutron irradiation by sacrificing the rabbits for morphological observation. Pathological analysis was performed on tumour samples resected after neutron irradiation. Harvested tumour samples were sliced into 6- μm sections with a cryostat and deposited on glass slides before being stained with H&E. Tumour samples of the same slice thickness were also analysed using the *Wako* apoptosis *in situ* detection kit to detect apoptotic cells. The kit is based on the terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labelling procedure and involves the addition of fluorescein-dUTP to the 3'-terminal ends of apoptotically fragmented deoxyribonucleic acid with terminal deoxynucleotidyl transferase followed by immunochemical detection using anti fluorescein antibody conjugated with horseradish peroxidase and DAB as substrate. Negative controls for both irradiated and non-irradiated groups were also prepared.

Figure 2. (a) Haematoxylin and eosin (H&E) and Oil Red O staining pathological findings of VX-2 tumour in liver on Day 1 post-intra-arterial injection for ^{10}BSH -entrapped water-in-oil-in-water (WOW) emulsion, (b) H&E and Oil Red O staining pathological findings of VX-2 tumour in liver on Day 1 post-intra-arterial injection for iodized poppy-seed oil (IPSO) mix solution, (c) Oil Red O staining pathological findings of VX-2 tumour in liver on Day 3 after injection for both WOW emulsion and IPSO mix solution.

(a) WOW Emulsion (Day 1)

(b) IPSO mix solution (Day 1)

(c) WOW Emulsion (Day 3)

IPSO Mix Solution (Day 3)

Figure 3. Electromicroscopic findings after intra-arterial injection of the ^{10}B BSH-entrapped water-in-oil-in-water (WOW) emulsion and iodized poppy-seed oil mix solution at (a) Day 1, Day 3 and Day 7. The accumulation of lipid capsules on the WOW emulsion in the VX-2 cancer cells were recognized by electron microscopical investigations. Original magnification $\times 4000$. (b) At Day 3 (day of irradiation) with higher magnification of $\times 4000$ and $\times 10000$.

RESULTS

Characterization of the ^{10}B BSH-entrapped water-in-oil-in-water emulsion

Microscopy examination of the WOW, as shown in Figure 1a, revealed numerous spherical IPSO microdroplets suspended in the aqueous phase. IPSO microdroplets were also observed to be smooth on the surface and to contain many vesicles of the aqueous solution of ^{10}B BSH. The vesicles in aqueous solution of ^{10}B BSH in WOW were measured to be 500 nm on average; the curve of relative particle ratio in Figure 1b shows that the median particle size of the IPSO microdroplets in the WOW was 70 μm . The separation of the emulsion into oil and water and the breakdown of the IPSO microdroplets did not occur for at least 120 days when kept at 4 $^{\circ}\text{C}$. The mean concentration of ^{10}B

prepared in the ^{10}B BSH-entrapped WOW emulsion was found to be 13,000 ppm.

Biodistribution after intra-arterial injection of ^{10}B BSH

From the ICP-MS measurement results, the mean concentration of ^{10}B in VX-2 tumours was found to be 141 and 52 ppm at Day 1 and Day 3, respectively, for the ^{10}B BSH-entrapped WOW emulsion, whereas for the IPSO mix conventional solution, the ^{10}B concentration in tumours was measured to be 58 and 24 ppm at Day 1 and Day 3, respectively. Concentrations of ^{10}B in other organs of tumour-bearing rabbit are shown in Table 1. These results indicate that the WOW emulsion has the potential to be an effective drug delivery system in accumulating a more than sufficient amount of ^{10}B for BNCT treatment.

Figure 4. (a) Image of neutron capture autoradiography using CR-39 alpha-detector for water-in-oil-in-water (WOW) emulsion and iodized poppy-seed oil (IPSO) mix solution on Day 3 and Day 7 after injection, both for tumour site and normal liver. NCAR image of BSH standard with different concentration was also presented. (b) Alpha and proton track etch pits distributions of the tumour on Day 3 after injection with NaOH and PEW etched for WOW emulsion and IPSO mix solution, showing tumour or normal liver site of a neutron-capture radiograph from a set of VX-2 hepatic cancers bearing after intra-arterial injection of ^{10}B compound.

The H&E and Oil Red O staining results indicate that accumulation of the WOW emulsion in VX-2 tumour cells was observed at Day 1 following intra-arterial injection. It was observed that the groups treated with the WOW emulsion (Figure 2a) or IPSO mix conventional emulsion (Figure 2b) showed accumulation at the tumour site. The accumulation of IPSO microdroplets from the WOW emulsion was also seen in the cytoplasm of VX-2 cancer cells in tumour tissues at Day 3 after injection following Oil Red O staining as shown in Figure 2c.

Electron microscopy images of VX-2 cancer cells after arterial injection of the WOW emulsion (Figure 3a) also revealed that the microdroplets of IPSO were retained in the cytoplasm of cancer cells in the same conformation after Day 1, Day 3 and Day 7, whereas no accumulation of fat droplets in the IPSO mix conventional emulsion-injected group was observed. A more detailed image at Day 3 is shown in Figure 3b for both the WOW emulsion and the IPSO mix conventional emulsion. As can be seen, higher amounts of fat droplets accumulated more effectively in the WOW emulsion-injected group. These results demonstrate that ^{10}B -entrapped WOW emulsion can be taken up selectively by cancer cells, delivering and retaining the boron atoms to tumour tissues.

Image results for NCAR measured by the microscopic track analysis system³² of liver sections from VX-2 hepatic cancer-bearing rabbits that had been given intra-arterial injections of 13,000 ppm ^{10}B -containing WOW emulsion or ^{10}B IPSO mix conventional emulsion are shown in Figure 4a. Images from boron-containing standard samples are also presented in Figure 4a for comparison. Images for Day 3 post-intra-arterial injection with etching technique using PEW solution, which are shown in Figure 4b, demonstrated that the selective accumulation of the ^{10}B compound with the ^{10}B -entrapped WOW emulsion was superior than that for the ^{10}B IPSO mix solution. This result also indicates that the majority of proton tracks disappeared and only α -tracks were observed. It was readily apparent that the α -etch pits revealed the existence of ^{10}B atoms delivered by the WOW emulsion to the VX-2 tumour.

Morphological and pathological analysis after neutron irradiation

Considering that the accumulation of ^{10}B atoms in tumours using the WOW emulsion was superior than in the group injected with the ^{10}B -IPSO mix conventional emulsion, neutron irradiation was performed on the ^{10}B -entrapped WOW emulsion and non-injected groups only. Irradiation was carried out at Day 3 following compound injection after taking into account that ^{10}B was measured to be higher than 50 ppm, which is more than sufficient than the recommended value for the clinical condition of BNCT. The fluence of thermal neutrons was measured to be $5 \times 10^{12} \text{ n cm}^{-2}$ on the surface of the beam port.

Day 14 post-neutron irradiation, tumour growth suppression in the group injected intra-arterially with the ^{10}B -entrapped WOW emulsion was observed, where the tumour size was smaller than in the non-injected group or the group that received thermal neutron irradiation only (Figure 5a). There was no dissemination in the abdomen or lung metastasis in the groups treated with BNCT using the ^{10}B -entrapped WOW

Figure 5. (a) Morphological findings at Day 14 after BNCT. Tumour growth suppression was observed after BNCT treatment on the group injected intra-arterially with the ^{10}B -entrapped water-in-oil-in-water (WOW) emulsion compared to the group receiving neutron irradiation only. (b) Pathological findings with haematoxylin and eosin staining indicated the efficacy of cancer cells killing after BNCT treatment on ^{10}B -entrapped WOW emulsion-injected group compared to the neutron-irradiated only group. Original magnifications are $\times 20$ and $\times 200$. (c) TUNEL staining results of the VX-2 tumour slice after BNCT treatment for the group injected with ^{10}B -entrapped WOW emulsion compared to the group receiving neutron irradiation only. Original magnification $\times 200$.

emulsion, whereas many tumour nodules were recognized in the liver, abdominal cavity, peritoneum and bilateral lobes of the lung in the non-injected group. H&E staining results shown in Figure 5b indicate the efficacy of cancer-cell killing after BNCT treatment. These results were also supported by the observation of brown-stained cells, which correlates with the degree of apoptosis in the VX-2 tumours treated with neutron capture therapy after intra-arterial injection of the ^{10}BSH -entrapped WOW emulsion (Figure 5c).

DISCUSSION AND CONCLUSION

Higashi et al^{10,11} previously reported a new drug delivery system for HCC using a long-term inseparable WOW emulsion. In this system, the WOW emulsion is prepared by a membrane emulsification technique, using a glass membrane with a large numbers of pores of almost equal diameter. Through this membrane, IPSO enclosing minute vesicles of an aqueous solution of epirubicin (WO emulsion) is separated and dripped into a physiological saline solution to make the WOW emulsion. When producing WOW emulsions, IPSO microdroplets can be controlled to yield a range of sizes from 1 to 70 μm using different glass membranes of an appropriate pore size.¹⁵ In accordance with the technique used by Higashi et al, we developed a ^{10}BSH -entrapped WOW emulsion for application in BNCT and evaluated the emulsion as a selective carrier to deliver boron to cancer tissues in a rabbit hepatic cancer model. In this current work, IPSO microdroplets were found to be almost equal in size and showed remarkable stability. Electron microscopy and histological staining results also showed superior accumulation of fat droplets from the WOW emulsion at the tumour site compared with the IPSO mix emulsion.

After performing α -autoradiographic measurements of ^{10}B biodistribution in sections of VX-2 tumour-bearing rabbit hepatic samples using CR-39 plastic track detectors, NCAR results of tumour and normal liver following intra-arterial injections of 13,000 ppm ^{10}BSH -entrapped WOW emulsion or IPSO mix solution revealed that ^{10}B atoms had been delivered by the WOW emulsion into the VX-2 tumour. When the ^{10}BSH IPSO mix solution was injected into tumour-bearing rabbits, the accumulation of ^{10}B atoms in tumours was lower than those

injected with the WOW emulsion, indicating the superior efficacy of the WOW emulsion as a drug delivery system. The α -track densities of normal livers were not detected on Day 3 after intra-arterial injection, suggesting the rapid clearance of the WOW emulsion and IPSO mix solution in healthy tissue. In the case of etching using PEW solution, the majority of proton tracks disappeared and only one peak associated with the α -tracks was observed. Consequently, undesirable proton tracks of α -autoradiographic imaging were effectively desensitized.^{26–28} The true α -tracks, shown in Figure 4b, indicate that accurate boron accumulation in the VX-2 tumours was achieved using the WOW emulsion with selective accumulation of the ^{10}B compound.

The clinical administration of WOW emulsion-encapsulated anticancer reagent in inner droplets was reported to be surprisingly effective for both terminal and multiorigin HCC.^{33–35} And in this current study, we observed tumour growth suppression after neutron irradiation of the ^{10}BSH -entrapped WOW emulsion-injected group compared with the non-injected group receiving thermal irradiation only, indicating the efficacy of BNCT treatment in the killing of cancer cells. The biodistribution results confirmed that the ^{10}BSH -entrapped WOW emulsion can deliver and retain boron atoms in cancer cells in tumour tissues, suggesting that WOW emulsions could represent a novel boron delivery carrier for BNCT of cancer using intra-arterial techniques. We plan to carry out a clinical study of BNCT for patients with HCC and hope to perform the first BNCT trial with WOW emulsion in the near future.

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