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研究課題名（和文）次世代型ホウ素中性子捕捉療法の実現に向けた多角的アプローチ

研究課題名（英文）Multilateral approach toward realization of next generation boron neutron capture therapy

研究代表者

松本　孔貴（Matsumoto, Yoshitaka）

筑波大学・医学医療系・助教

研究者番号：７０５１０３９５

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研究成果の概要（和文）：本研究ではがんで高発現する葉酸受容体に着目し、シクロデキストリンに葉酸を標識した葉酸修飾シクロデキストリン（ND201）を用い、既存ホウ素化合物の腫瘍特異的かつ能動的な集積性の向上を目指した。BSHをND201で包接した結果、高い結合安定度を示した。葉酸受容体高発現Colon-26腫瘍を移植したマウスにおいて、BSHは血中および腫瘍内で投与後の時間依存的に減少したのに対し、BSH-ND201投与群では投与後24時間で高いホウ素集積を確認し、その集積は72時間まで継続した。腫瘍/血液比（T/B ratio）はBSHの1.1に対し、BSH-ND201では10.6と極めて高値を示した。

研究成果の概要（英文）：The stability constants Kc was 1.4×104 (/ M) in BSH and the value suggests that ND201 and BSH shows stable complex in culture medium and human blood. The stoichiometry of a host-guest complex was determined by the continuous variation plot method. The plots made by monitoring the fluorescence intensity change gave a maximum peak at 0.5, indicating that ND201 forms an inclusion complex with BSH at a 1:1 molar ratio. Next, the boron concentration in tumors and blood was measured by ICP-MS. The concentration in blood showed similar time course kinetics after BSH and BSH-ND201 without depending on the tumor type. On the other hand, the concentration in tumor showed drastic decrease immediately after BSH administration, whereas it increased to 24 hours and showed high value at 72 hours after BSH-ND201 administration. The T/B ratio when the intratumoral boron concentration was peak was calculated and BSH-ND201 showed high T/B ratio (10.6) for Colon-26 tumor.

研究分野：　放射線治療生物学

キーワード：　BNCT　DDS　Folate receptor　Cyclodextrin　high LET

１．研究開始当初の背景

Boron neutron capture therapy (BNCT) is a next-generation radiation therapy that irradiates thermal neutrons to boron compounds accumulated in tumor cells and selectively irradiates tumor cells with high LET radiation by the generated α-rays and Li nuclei. The therapeutic effect of BNCT greatly depends on the boron compound which collects boron at the tumor site, and existing boron compounds, L-p-Boronophenylalanine (L-BPA) and disodium mercaptoundecahydrododecaborate (BSH) have limitation with regard to the adaptive cancer type and its sufficient and specific accumulation to tumor. Therefore, new boron compounds and carrier with higher tumor cell accumulation without normal tissue accumulation are being searched. BSH don't have the active accumulation to tumor cells, but it has 12 x ^{10}B in one molecule, BSH induces a strong biological effect even with small accumulation. Folate receptor-α (FR-α) is highly expressed on the many tumor (ovarian, kidney, colorectal, et al.), and it is useful as a target for drug delivery system (DDS) against cancer. It has been reported that the compound which is a cyclic oligosaccharide cyclodextrin modified with folic acid, has improved tumor accumulation and therapeutic effects of paclitaxel (PTX) and doxorubicin (DOX), which are anticancer drugs.

２．研究の目的

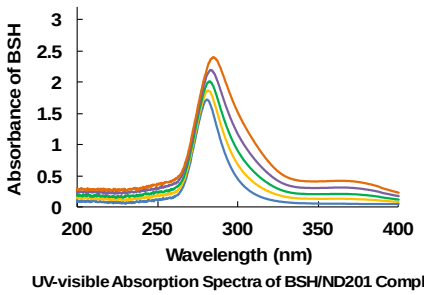
In this study, we aimed to construct BSH inclusions with folate-modified cyclodextrin (ND 201) and to realize active accumulation of BSH against folate-targeted tumor and its usefulness.

３．研究の方法

Colon-26 cells derived from murine colorectal cancer and A549 cells derived from human lung cancer were purchased from RIKEN BioResource Research Center. Colon-26 cells show the overexpression of FR and A549 cells show low expression level of FR. BALB/c nu/nu mice were used for in vivo kinetics experiments. BSH was purchased from Stella Pharma in powder form and dissolved in a phosphate buffer at the appropriate time before the experiment. ND201 was purchased from NanoDex corporation and dissolved in 0.1 mol/l carbonic acid/bicarbonate buffer (pH9-10). The solution was neutralized with a phosphate buffer (pH 6.8-7.2) and stocked at -30°C freezer. The interaction between BSH and ND201 was evaluated from stability constants and stoichiometric ratio. BSH and BSH containing ND201 (BSH-ND201) were administered from the tail vein to mice at concentrations of 100 and 5 mg/kg, respectively. Boron concentration (ppm) in the tumor and blood was measured with ICPS-8100 (Shimadzu Corporation) and the tumor/blood (T/B) ratio was calculated with each value.

４．研究成果

The stability constants Kc was 1.4×10^4 (/ M) in BSH and the value suggests that ND201 and BSH shows stable complex in serum-containing culture medium and human blood. The stoichiometry of a host-guest complex was determined by the continuous variation plot method. The plots made by monitoring the fluorescence intensity change gave a maximum peak at 0.5, indicating that



UV-visible Absorption Spectra of BSH/ND201 Complex

System	K_c (M^{-1})
BSH/ND201 complex	$1.4 \times 10^4 \pm 0.2 \times 10^4$

Fig 1. Stability of CD201-BSH complex

ND201 forms an inclusion complex with BSH at a 1:1 molar ratio.

Next, the boron concentration in tumors and blood of BALB/c nu/nu mice was measured by ICP-MS. The concentration in blood showed similar time course kinetics after BSH and BSH-ND201 without depending on the tumor type. On the other hand, the concentration in Colon-26 tumor showed drastic decrease immediately after BSH administration, whereas it increased to 24 hours and showed high value at 72 hours after BSH-ND201 administration. The T/B ratio when the ratio (10.6) for Colon-26 tumor, and this value satisfied the T/B ratio > 10 required for clinical safety in BNCT. On the other hand, the ratio was too low (1.6) for A549 tumor.

It was suggested that chemical modification targeting folate receptor to existing boron compounds may contribute to improvement of therapeutic effect of BNCT.

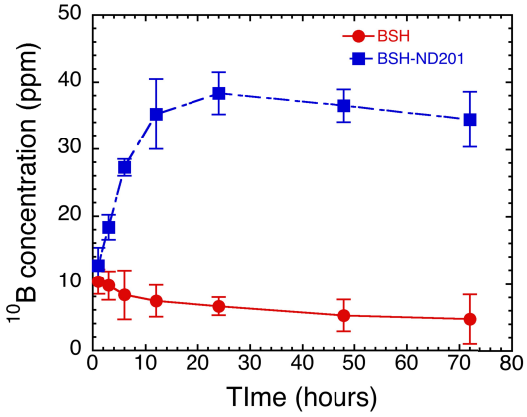


Fig 2. Boron concentration of Colon-26 tumor after BSH and BSH-ND201 compounds.

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〔産業財産権〕

〔その他〕

６．研究組織

(1)研究代表者

松本　孔貴（MATSUMOTO, Yoshitaka）
筑波大学・医学医療系・助教
研究者番号：７０５１０３９５

(2)研究分担者

増永　慎一郎（MASUNAGA, Shin-ichiro）
京都大学複合原子力化学研究所・放射線生命科学研究部門・教授
研究者番号：８０２３８９１４

(3)連携研究者

熊田博明（KUMADA, Hiroaki）
筑波大学・医学医療系・准教授
研究者番号：３０３５４９１３

福光延吉（FUKUMISTU, Nobuyoshi）
筑波大学・医学医療系・准教授
研究者番号：４０２７７０７５

櫻井良憲（SAKURAI, Yoshinori）
京都大学複合原子力化学研究所・放射線生命科学研究部門・准教授
研究者番号：２０２７３５３４

(4)研究協力者

服部憲治郎（HATTORI, Kenjiro）

櫻井英幸（SAKURAI, Hideyuki