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学位の種類	博　士　（医　学）
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学位授与の要件	学位規則第４条第１項該当
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学位論文題目	MRI-detectable polymeric micelles incorporating platinum anticancer drugs enhance survival in an advanced hepatocellular carcinoma model. (MRI により検出可能なプラチナ抗癌剤含有高分子ミセルは進行肝細胞癌モデルにおける生存率を向上する)
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別紙様式３

論 文 内 容 要 旨

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学位論文題目	**MRI-detectable polymeric micelles incorporating platinum anticancer drugs enhance survival in an advanced hepatocellular carcinoma model** （MRI により検出可能なプラチナ抗癌剤含有高分子ミセルは進行肝細胞癌モデルにおける生存率を向上する）		

Purpose:

Nanoparticle drug carriers have been demonstrated as an effective method of targeting drugs to malignant tumors with permeable neovasculature and impaired lymphatic drainage. Polymeric micelles are generated by the self-assembly of amphiphilic block copolymers into core-shell nanostructures with drug loaded cores and biocompatible shells showed advantages as nanoparticle drug carriers. We have developed MRI-detectable polymeric micelles by incorporating the T1-weighted MRI contrast agent Gd-DTPA and the platinum anticancer drug DACHPt within the core of micelles. In this study, we examined the feasibility of using these Gd-DTPA/DACHPt-loaded micelles to simultaneously diagnose and treat a rat model of hepatocellular carcinoma (HCC).

Methods:

The Gd-DTPA/DACHPt-loaded micelles were created by self-assembling as follows. Gd-DTPA was converted to sodium salt and lyophilized. DACHPt in water was mixed with the sodium salt of Gd-DTPA, and then PEG-b-P(Glu) was added to this solution ([DACHPt]/[Glu] =1.0) and are allowed to react for 120 hours at 37°C to prepare Gd-DTPA/DACHPt-loaded micelles. The size distribution of the Gd-DTPA/DACHPt-loaded micelles was evaluated by dynamic light scattering measurement. Morphology of Gd-DTPA/DACHPt-loaded micelles was observed by transmission electron microscopy.

HCC rat model was generated by injection of 1×10^6 N1S1 cells to subserosal liver. Seven days after cell implantation, MR images of hepatic tumors were obtained using a 1.5T GE MRI system before and after hepatic arterial injection of GD-DTPA/DACHPt-loaded micelles. ROI's gray scale value was measured on MR images using ImageJ software.

Tumor cytotoxicity was evaluated by TUNEL assay after treating with Gd-DTPA/DACHPt-loaded micelles and with free oxaliplatin or normal saline for control. Therapeutic efficacy was assessed by measuring tumor size using MRI at 3, 6, 10, 14 and 21 days after treatment.

Hepatic and renal functions were tested for partial evaluation of adverse reactions after treatment with micelles, oxaliplatin, or saline.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等で印字すること。

２．※印の欄には記入しないこと。

Results:

Characterization of Gd-DTPA/DACHPt-loaded micelles:

The Gd-DTPA/DACHPt-loaded micelles were 33 nm in diameter. The micelles incorporated 0.42 mg of DACHPt/mg polymer and 0.04 mg Gd-DTPA/mg polymer, corresponding to 45% and 5% of the carboxylic groups in PEG-b-P(Glu), respectively. No Gd3+ was detected in these micelles, indicating that the safety of Gd-DTPA chelates remained stable.

Gd-DTPA/DACHPt-loaded micelle mediated tumor enhancement: Tumor intensity of T1-weighted MR images increased considerably after hepatic arterial injection of Gd-DTPA/DACHPt-loaded micelles and remained stable for up to 3 h investigation whereas signal intensity of healthy liver remained near the initial values. The identical injection of free Gd-DTPA or DACHPt micelles showed no tumor contrast enhancement.

In vivo cytotoxicity against tumor cells: The mean tumor apoptosis percentages at 3 days postinjection were 92.4 ± 3.4% with micelles, 45.3 ± 20.8% with oxaliplatin, and 9.65 ± 5.9% with saline. These results indicated that Gd-DTPA/DACHPt-loaded micelles induced tumor cell death much more efficiently than free oxaliplatin and saline.

Tumor growth suppression and animal survival: At 3 day postinjection, the mean tumor volume of micelle group decreased 74% in compared with preinjection and tumor suppression continued until 10 days postinjection. Then tumors continued to grow and were abdominally disseminated by 3 weeks postinjection. In contrast, there was no tumor suppression after oxaliplatin or saline injection. Survival of micelle-, oxaliplatin-, saline-treated rats was 28, 20, and 18 days, respectively.

Systemic toxicity: Micelle treatment caused less severe hepatic disorders and body weight loss than oxaliplatin treatment.

Discussion:

Gd-DTPA/DACHPt-loaded micelles provided strong and specific tumor contrast enhancement and induced high rates of tumor cell death, significantly reduced tumor size and growth rate. The administration of micelles did not caused severe adverse reactions and improve survival outcome.

After injecting micelles, intensity of tumors was greatly enhanced from hypointense to markedly hyperintense while there was minimal change in signal intensity in healthy liver parenchyma, indicating that micelle did not penetrate through normal vessels in healthy tissue. Furthermore, the high rate of tumor apoptosis and tumor growth suppression following micelle treatment supports the theory of high penetration, enhanced retention, and persistent and broad distribution within HCC tissue.

The Gd-DTPA/DACHPt-loaded micelles may improve the sensitivity of clinical HCC diagnoses, particularly in cases of multiple small tumours or satellite tumours in a cirrhotic liver, which may be overlooked by other imaging diagnostic modalities. Moreover, the concomitant incorporation of contrast molecules and anticancer agents in the micelles permits real-time evaluation of drug distribution in the tumour by MRI, thus enabling practitioners to adjust the dose to ensure proper efficacy with minimal adverse reactions and to anticipate treatment response. The use of micelles that provide selective MRI-contrast tumour enhancement also facilitates disease management as it allow patients and practitioners to avoid unnecessary exposure to ionizing radiation.

Conclusions:

Gd-DTPA/DACHPt-loaded micelles are an excellent candidate for clinical translation for the diagnosis and treatment of HCC.

別紙様式８（課程・論文博士共用）

学位論文審査の結果の要旨

整理番号	７６１	氏　名	NGUYEN QUOC VINH
論文審査委員			

（学位論文審査の結果の要旨）（明朝体１１ポイント、６００字以内で作成のこと。）

本論文では、MRI 造影剤および活性型 oxaliplatin 含有高分子ミセル（Gd-DTPA/DACHPt ミセル）のラット肝細胞癌モデルにおける治療・診断薬としての可能性明らかにすることを目的として研究を行った。そのため、Gd-DTPA/DACHPt ミセルを経肝動脈的に投与されたラット肝癌（N1S1）モデルを対象として、MRI 造影効果と投与効果を確認した。抗腫瘍効果は、同剤、oxaliplatin、saline を各投与後の 3 群で、腫瘍サイズと TUNEL アッセイで評価した。副作用として、肝・腎機能を解析した。結果、以下の点を明らかにした。

１）同剤の肝動注後、MRI の T1 強調画像において Tumor intensity の増強を認めるが、正常肝部分には認めない。

２）同剤の肝動注後の肝癌組織におけるアポトーシス細胞の割合は、oxaliplatin と比べて高い傾向にある。また、同剤の肝動注後の腫瘍体積は投与前の約 74％であり、生存期間が延長する。

３）同剤による肝・腎機能障害、体重減少は oxaliplatin に比べて軽い傾向にある。

本論文は、Gd-DTPA/DACHPt ミセルのラットモデル肝細胞癌における診断、治療応用の可能性の一端を明らかとし、その臨床開発に向けた新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数５９９字）

（平成２８年１月２５日）