論 文 の 内 容 の 要 旨

論文題目 Development of Nanoparticles Incorporated with T_1 Contrast Agents for Enhanced Magnetic Resonance Cancer Imaging

（ガンの MRI 診断用 T_1型造影剤を内包したナノ粒子の開発）

氏　　名　　米 鵬

Cancer became one of the top threats to people's health causing high death rate in Japan and all over the world. Early detection of neoplastic lesions is critical for success in cancer treatment and recovery health. Several cancer diagnostic imaging methods such as X-ray, computed tomography (CT), magnetic resonance image (MRI), ultrasound and position emission tomography (PET) are widely used in clinical. Including, magnetic resonance imaging (MRI) provides a powerful diagnostic imaging modality of cancer, because of its non-invasiveness, high definition and precise three-dimensional position ability. Several kinds of contrast agents are used in clinical to enhance the signal of MR images, such as T_1 type contrast agents like Mn(II)-based agents and Gadolinium (III)-based agents which make the enhanced parts became white, and T_2 type contrast agents like iron oxide materials which make the enhanced parts black. But those commercial used contrast agents are small molecules has short blood circulation time and spreading systemically in the body, which could increase the signal of background tissues to make tumor detection become a challenge.

Nanodevices have been recently developed to selectively deliver imaging agents to solid tumors by passively leaking out from circulation and accumulate in the tumor positions due to the enhanced permeability and retention effect (EPR effects). Among promising nanodevice systems for MRI, block copolymeric micelles, i.e. nanostructures consisting of a drug-loaded core and poly(ethylene glycol) (PEG) protective shell, present exceptional advantages including their relatively small sizes, ability to engineer drug loading mechanisms, controlled release of their cargo, prolonged life in the bloodstream and enhanced accumulation in solid tumors after intravenous administration. Therefore,

this research aimed to develop a series of novel T_1 contrast agent loaded nanocarriers, which could target cancer and cancer metastasis and enhance the contrast of magnetic resonance imaging (MRI) for precise diagnosis.

Firstly, Nanodevices for Magnetic Resonance Imaging of cancer were self-assembled to core-shell micellar structures by metal chelation of Gd-DTPA, a clinical widely used T_1-contrast agent, to poly(ethylene glycol)-*b*-poly(N-{N'-(2-aminoethyl) -2-aminoethylaspartamide) copolymer via Pt(IV) in aqueous solution. Gd-DTPA-loaded polymeric micelles (Gd-DTPA/m) showed a hydrodyamic diameter of 45 nm and a core size of 22 nm. Confining Gd-DTPA inside the core of the micelles increased the relavity of Gd-DTPA more than 13 times (45 $mM^{-1}s^{-1}$). Moreover, in physiological conditions, the ligand exchange reaction of Pt(IV) from the carboxylic group of Gd-DTPA to chloride ions results in sustained release of free Gd-DTPA, which can be rapidly excreted from the body. The improved tumor accumulation of Gd-DTPA loaded micelles (Gd-DTPA/m) enhanced the T_1 weighed contrast of the malignancies in mice. On the contrary, free Gd-DTPA could not enhance the signal in tumor position. μ-synchrotron radiation-X-ray fluorescence results confirmed that Gd-DTPA was delivered to the tumor sites by micelles.

Calcium phosphate is a non-toxic and biocompatible materials demonstrating its widly application as biomaterials in tissue engineering and drug delivery system. Here, PEGlyated calcium phosphate hybrid nanoparticles incorporating Gd-DTPA with enhance relaxivity for MRI has been developed by hydrothermal synthesis method. This Gd-DTPA loaded CaP nanoparticles (Gd-DTPACaP) have a diameter of 75 nm and a ξ-potential of -0.3 ~ -0.7 mV. The relaxivity of Gd-DTPACaP was increased 6 times (18 $mM^{-1}s^{-1}$) higher than free Gd-DTPA. Moreover, non-cytotoxicity was investigated from MTT assay both on C-26 tumor cell lines and HUVEC normal cell lines after 72 hours incubation. *In vivo*, Gd-DTPACaP has longer circulation time in plasma and passively accumulated in tumor position by EPR effects leading to contrast enhancement about 40% in tumors position according to MRI measurements. Finally, the tumor position was sliced and scanned by μ-Synchrotron radiation-X-ray fluorescence in SPring-8 conforming lots of Gd-DTPA was delivered to tumor position.

Mn^{2+} is another T_1-contrast agent of MRI as it could conjugate with some protein as albumin to increase the relaxivity, but its toxicity limited further application. Therefore, we prepared MnCaP nanoparticles with crystal core of MnCaP and PEGylated surface. The MnCaP nanoparticles has a diameter of 80 nm and a ξ-potential of -2 ~ -5 mV.

MnCaP showed reduced toxicity from *in vitro* cytotoxicity test on HUVEC normal cell lines and C-26 tumor cell lines, and *in vivo* toxicity test on nude mice. The LD50 of MnCaP was enhanced 5 times higher than $MnCl_2$ of 0.22 mmol/kg. MnCaP is pH responsive, stable at pH 7.4 of physiological condition and brake at pH lower than 6.7 releasing Mn^{2+}, which could bonding albumin to increase the relaxivity. MnCaP nanoparticles was intravenous injected to C-26 subcutaneous tumor bearing Balb/c nude mice at a dose of 0.22 mmol/kg, then take the MR images every 30 min using 1 T MRI machine. Gd-DTPA was used as control sample for MRI measurement with the same dose. Cancer could be fast detected on the mice administrated with MnCaP and there was no signal enhancement of injection Gd-DTPA. Moreover, MnCaP also showed a higher contrast enhancement than $MnCl_2$ at a dose of 0.045mmol/kg, because the LD_{50} of $MnCl_2$ is 0.22 mmol/kg. Moreover, liver metastasis tumor model was made on Balb/c nude mice by implant C-26 tumor cells to into mesenteric vein. Then, 9 days later, the liver metastasis position was diagnosed by MRI administration of MnCaP to liver metastasis bearing mice at a dose of 0.22 mmol/kg. Even quite small liver metastasis could be detected by MRI with the contrast of MnCaP.

Those T_1-contrast agent loaded nanoparticles exhibited low toxic, high stability, increased relaxivity, extended plasma circulation time and f high ability for MR cancer imaging. Those nano-scaled contrast agents may be further applied in clinical for cancer and cancer metastasis diagnosis, which could give cancer incidents effective and in time therapy. This study also provides a novel and facile strategy for incorporating contrast agents, dyes and bioactive molecules into nanodevices for developing safe and efficient drug carriers for further clinical applications.