Study on the micro-distribution of platinum anticancer drug-containing polymeric micelles in tumor tissue

Horacio Cabral[a]. Kazue Mizuno[b], Sachiko Kaida[a], Yasuko Terada[c], Kazunori Kataoka[a,b]

[a] Faculty of Medicine, The University of Tokyo, [b] Faculty of Engineering, The University of Tokyo, [c]SPring-8

The intratumoral disposition of nanoparticles therapeutics will determine the efficiency of the anticancer treatment. Herein, we utilized μ-X ray fluorescence (XRF) to determine the microdistribution of micelles-delivered metal-drugs in healthy and malignant tissues and the effect of the size of those micelles in the resulting tissue disposition. The 30-nm micelles deeply penetrated the tumors while 80-nm micelles were retained near the vasculature in pancreatic cancers. Thus, the penetration of the micelles and the delivery of platinum drugs in the different tumors were affected by the tumor architecture and the size of the nanocarriers.

Keywords: drug delivery, anticancer drugs, μ-X ray fluorescence

Introduction: Recently, there has been an explosive development of chemotherapy for cancer; however, the efficacies of these drugs are still insufficient. To improve the efficiency of those drugs several types of nanoparticled therapeutics have been developed to selectively deliver drugs to the tumors[1]. The tumor targeting of these nanocarriers is based on the enhanced permeability and retention (EPR) effect[2], i.e. the increased accumulation of macromolecules in tumor tissue due to the leaky of tumor blood vessels and the retention of these macromolecules because of the impaired lymphatic drainage at the cancer site.

We have developed polymeric micelles incorporating platinum anticancer drugs, i.e. cisplatin and (1,2-diaminocyclohexane)Pt(II) (DACHPt; the parent complex of the potent drug oxaliplatin), [3,4]. Figure 1A shows the schematic formation of platinum drug loaded micelles. The outstanding *in vivo* performance of these micelles lead to the undergoing clinical studies (NC6001 and NC4016, Nanocarrier).

Since there is an imperative need to directly assess the effect of the nanocarrier size on the tumor distribution for their rational design, the distribution of platinum drug-loaded micelles in tumors presenting different characteristics was studied by elemental mapping of tumor sections with μ-X ray fluorescence (XRF). The 30-nm micelles presented deep tumor penetration regardless of the tumor type, while the permeability of 80-nm micelles is restricted to the areas near the vasculature in hypovascular fibrotic tumors. Moreover, the tumor distinctive features can be studied by the combination of μ-XRF and small molecular imaging metal drugs. Thus, the necrotic areas in the tumor tissues were visualized by mapping the Cu atoms of Cu-ATSM, shown in Figure 1B, a positron emission tomography imaging agent for tumor necrosis.

In addition, in the clinical situation, the ability to visualize the accumulation of the nanocarriers at the cancer will allow to determine the drug distribution inside the tumor in real-time and to optimize the treatment protocol. Thus, we provided diagnostic function to DACHPt-loaded micelles by the loading of Gd-DTPA, a widely used MRI contrast agent, as shown in Figure 1A. The dispositions of Pt and Gd for these micelles, studied by μ-XRF, showed that both drugs co-localized at the tumor site while the accumulation in healthy tissue was negligible, suggesting that the MRI imaging of these micelles can be associated with the distribution of the Pt drug.

Experiment:

Micelles preparation: The micelles were prepared as reported previously [3,4]. Briefly, 5mM of Pt drugs, plus 5mM Gd-DTPA for the MRI micelles, were mixed with poly(ethylene

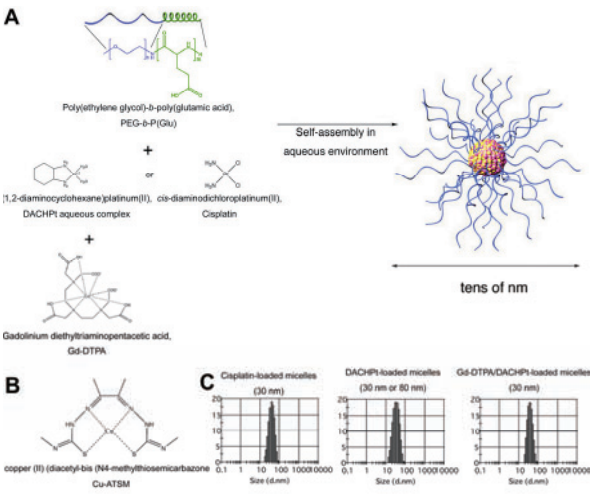

Fig. 1. A. Schematic diagram of micelles assembly; B. Chemical structure of Cu-ATSM; C. Size distribution of the micelles utilized in this study.

glycol)-b-poly(glutamic acid) at 37 °C. The size of the micelles was determined by dynamic light scattering. The results are shown in Figure 1C. The drug loading was studied by inductively coupled plasma-mass spectroscopy.

In vivo experiments: Hypervascular Murine colon adenocarcinoma C-26 murine melanoma and B16-F10, and hypovascular BxPC3 human pancreatic cancer cells were inoculated to mice, and the tumors were allowed to grow for 2 weeks. Then, the free cisplatin, the Pt drug-loaded micelles and the Gd-DTPA/DACHPt-loaded micelles were intravenously injected at 10 mg/kg in a Pt-base. Cu-ATSM was injected at 50 mg/kg. The tumors were collected at defined time periods and frozen in liquid nitrogen. The tissues were sectioned with a cryostat, fixed on a polypropylene sheet and attached on a sample holder.

μ-XRF: The μ-XRF was performed at the 37XU beam line. The sample on the acryl board was mounted on an x-y translation stage. The fluorescence X-ray intensity was normalized by the incident X-ray intensity, I0, to produce a two-dimentional elemental map. The Pt, Gd, Cu were evaluated to determine the drug distribution while Fe, K and Zn were studied for the tissues characteristics.

Results and discussion: The mapping of Pt, Gd, Cu clearly showed the distribution of the drugs in the tumor tissue. The 30-nm cisplatin micelles presented a deep tumor penetration. One hour after the injection the cisplatin micelles accumulated near the Fe-rich vascular areas; however, the cisplatin micelles permeated the tumor tissues and distribution of platinum extended for 4 h and 24 h covering the whole tumor section as observed in Figure 2A. The tumor characteristics were assessed combining μ-XRF and Cu-ATSM. Thus, the distribution of Cu-ATSM in Figure 2 identified the necrotic areas in B16-F10 tumors at nearly 100 μm from the blood vessels while in C-26 tumors there were not defined necrotic regions.

The distribution of 30-nm micelles and 80-nm micelles in pancreatic tumors, showed in Figure 2B, present an accumulation for the bigger micelles confined to the vascular areas surrounded by thick fibrosis while the smaller micelles penetrated deep in the tumor nests suggesting that the architecture of the cancer strongly affects the penetration of nanoparticles.

The analysis of tumor distribution of 30-nm Gd-DTPA/DACHPt-loaded micelles allowed us to determine that the micelles accumulated only at the tumor site since the drug concentration in the

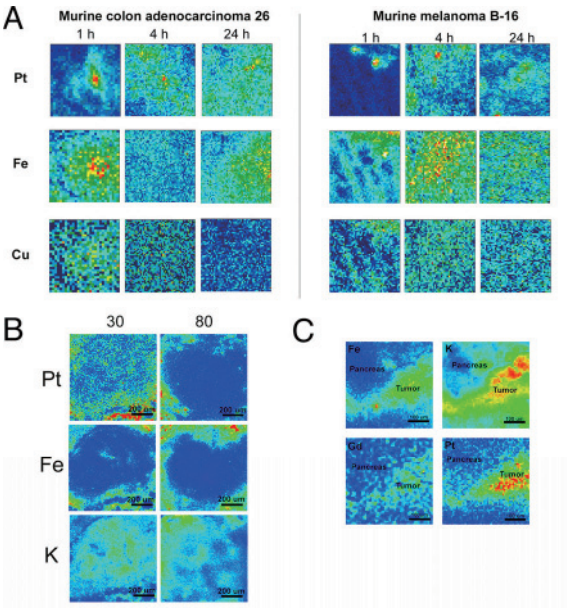

Fig. 2. Elemental mapping of tumors. A. Time dependent intratumoral distribution of 30-nm cisplatin micelles in C26 and B16 tumors; B. Tumor penetration of 30 and 80-nm DACHPt micelles in hypovascular pancreatic tumor; C. Penetration of 30-nm GD-DTPA/DACHPt micelles

normal pancreas is considerable low as shown in Figure 2C. The co-localization of the Gd and the Pt suggests that these micelles can be used to determine the distribution of the platinum drug by MRI given that Gd-DTPA is a strong MRI contrast agent.

Conclusion:

The μ-XRF is a potent technique to determine the microdistribution of metal-drugs in healthy and malignant tissues and to study the attributes of those tissues. The penetration of the micelles and the resulting delivery of platinum drugs were affected by the tumor architecture and the size of the nanocarriers. Moreover, the μ-XRF results supported the strategy for the co-delivery of imaging and therapeutic drugs for the real-time visualization of the drug delivery.

References:

1. Davis, M.E., Chen, Z., Shin, D.M., Nature Rev. Drug Discov. **7** (2008) 771
2. Matsumura, Y., Maeda, H. Cancer Res. **46** (1986) 6387
3. Nishiyama, N., Okazaki, S., Cabral, H., Kataoka, K. Cancer Res. **63** (2003) 8977
4. Cabral, H., Nishiyama, N., Okazaki, S. Koyama, H., Kataoka, K., J. Control. Rel. **101** (2005) 223