2008B1965 Study on the macro- and micro- distribution of platinum anticancer drug-containing polymeric micelles in tumor tissue

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Beamline: BL37XU

Research Purpose and Background

Oxaliplatin is a widely used and very potent anticancer drug. Nevertheless, it distributes rapidly to the whole body leading to undesired side effects [1]. Consequently, enormous effort has been dedicated to develop drug delivery systems that target the drug to solid tumors by taking advantage of the improved accumulation and retention of macromolecules (enhanced permeability and retention effect) at the tumor site. [2]. However, successful formulations have not been developed yet due to unfavorable properties of platinum drugs.

A novel approach to the design of nanocarriers for oxaliplatin has been utilizing polymeric micelles. These polymeric micelles incorporate the oxaliplatin parent complex, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt), by the complexation of DACHPt with poly(ethylene glygol)-poly(glutamic acid) block copolymer (PEG-b-P(Glu)) (Fig. 1A). DACHPt-loaded micelles (DACHPt/m) have shown more than 20-fold greater accumulation in tumor tissue and a strong antitumor activity compared to free oxaliplatin without any side effect [3] suggesting that DACHPt/m are exceptionally promising carriers for oxaliplatin.

In addition to the enhanced accumulation of polymeric micelles at the cancer site, the improved penetration of the drugs in the tumor tissues can be also responsible for the superior activity of DACHPt/m. Thus, we studied for the first time the tissue distribution of oxaliplatin and DACHPt/m with different diameters (Fig. 1B) by XRF in intractable human pancreatic cancer, which are known to have low drug permeation due to their high fibrosis and very low vascularization. Accordingly, in order to see the penetration efficiency of the Pt drugs into the cancer tissue, the Pt distribution in tumor sections was analyzed. Moreover, the tissue attributes were determined by the distribution of K, Fe, Zn and Ca

Experimental/Analytical Methods

DACHPt/m with 35 nm-diameter were prepared by mixing DACHPt (5mM) and PEG-b-P(Glu) ([COOH]= 5mM) in water. DACHPt/m with 80 nm-diameter were prepared likewise, but with the addition of p(Glu) homopolymer ([COOH]= 0.5mM). The micelles were purified by ultrafiltration. The diameter of the micelles was determined by dyamic light scattering and the amount of loaded DACHPt was determined by ICP-MS.

To determine the plasma clearance of the micelles, Balb-c nu/nu (female, n=5) were injected intravenously with DACHPt/m with 35 nm or 80 nm-diameter. The blood was collected, the plasma was separated by centrifugation and the amount of DACHPt was determined by ICP-MS.

To study the tumor distribution, Balb-c nu/nu mice (n=5) were inoculated subcutaneously with BxPC3 cells (1×10^7 cells/ml) and allowed to grow for 3 weeks. Subsequently, oxaliplatin or DACHPt/m of 35 and 80 nm were administered at 10mg/kg (on a platinum base). The tumors were excised 24 h later and kept at –80°C. Then, tissue sections were set on propylene sheets. The XRF studies were performed at the BL37XU beamline. The spectral acquisition time was set to 1s and the step size varied depending on the sampling size. Other experimental conditions, including the excitation energy, were similar to

previous micro-XRF imaging measurements.

Research Results

All the DACHPt-loaded micelles presented similar pharmacokinetics (Fig. 1C). Thus, it is reasonable to compare the distribution in tumor tissue of the different DACHPt/m.

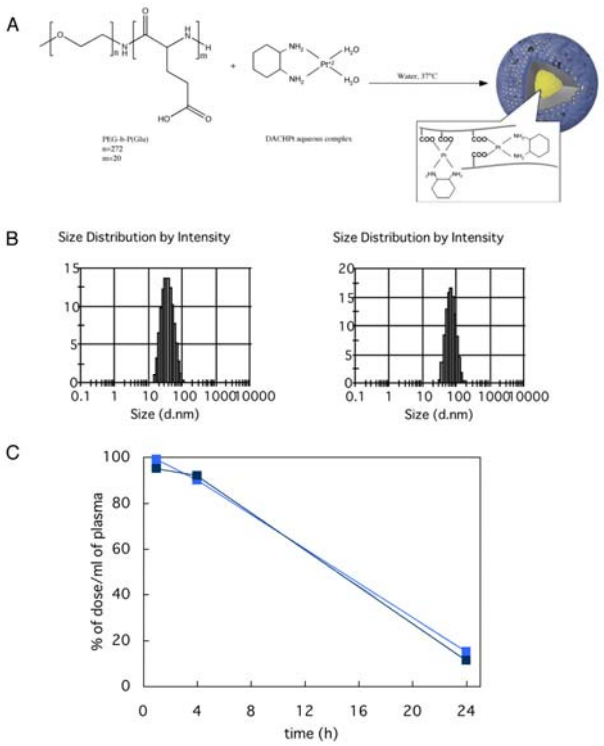

Figure 1. A. Scheme of DACHPt-loaded micelles (DACHPt/m) formation. B. Size distribution of DACHPt/m with 35 nm and 80 nm-diameters. C. Plasma clearance of DACHPt/m with 35nm and 80 nm-diameters.

The XRF results showed the distinctive aspect of BxPC3 pancreatic cancers, i.e. cancer cell nests surrounded by fibrotic tissue and very low vasculature. These nests presented high concentration of K and Zn due to cellular activity but they were depleted of Fe because of the restricted blood flow inside the cancer cellular nests (Fig. 2A). Moreover, those nests were surrounded by Fe-rich areas suggesting vascularization.

The accumulation of oxaliplatin in the tumor was not enough to be detected since it is cleared very rapidly from the bloodstream. Conversely, the DACHPt/m presented much higher accumulation. Moreover, the Pt concentration for DACHPt/m was higher in the areas surrounding the cancer cell nests and gradually diminished as moving to the center of the nests (Fig. 2A and 2B).

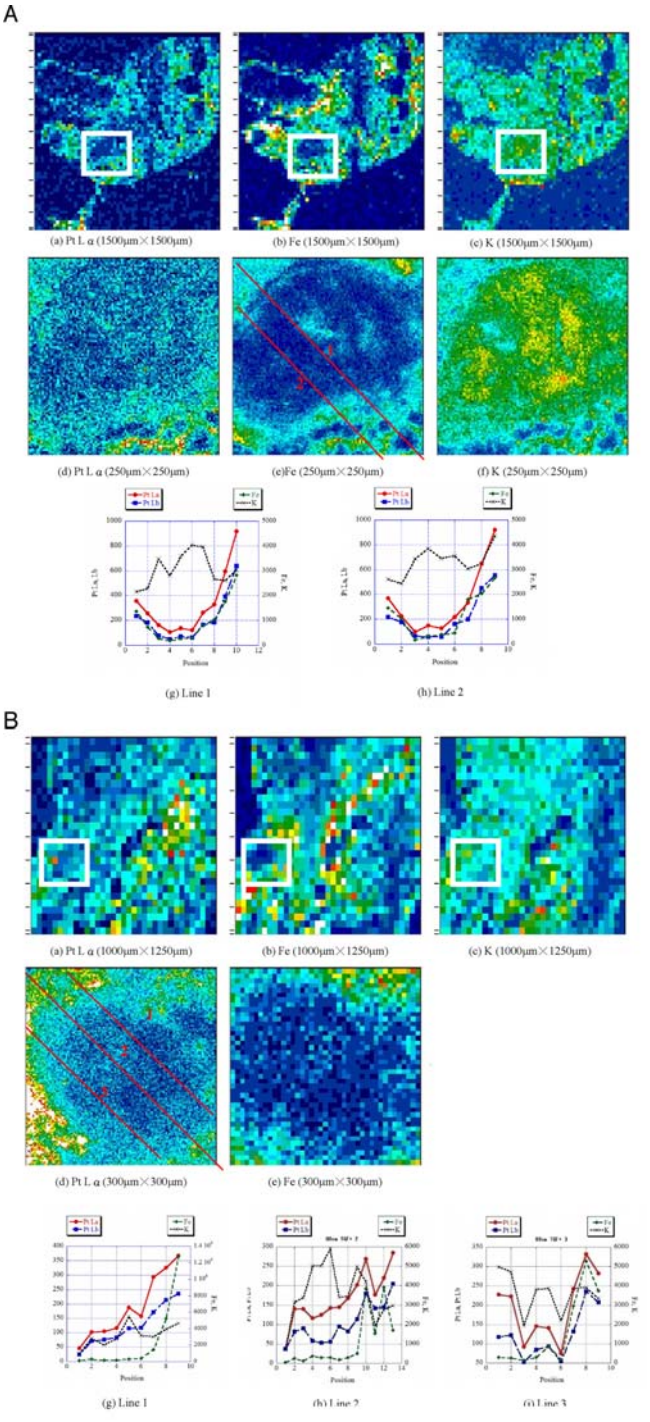

Figure 2. A. Tissue distribution of Pt, Fe, K, for DACHPt/m with 35 nm-diameters. B. Tissue distribution of Pt, Fe, K, for DACHPt/m with 80 nm-diameters.

Additionally, the ability of the micelles to penetrate inside

the nest seemed to be dependent on the micelle size as the DACHPt/m with 35 nm-diameter showed a more homogeneous distribution than the micelles with 80 nm-diameter (Fig. 2B).

Current and Future issues

These results suggest XRF is a very powerful technique to study the macro and micro-distribution of platinum drugs as well as other metallocompounds in tumor tissues. Moreover, this study will provide a deeper comprehension in the mechanism of the unprecedented tumor-growth suppression of DACHPt-micelles against pancreatic cancers but also will give new insights for the design of drug delivery systems that can penetrate deeper inside tumor tissues with low vascularization.

References

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Status of publication: Preliminary results

Keywords: drug delivery system, polymeric micelles, chemotherapy, oxaliplatin, intratumor distribution