論文の内容の要旨

論文題目 A Study on Pharmacological Activity of Platinum Anticancer Drug-loaded Micellar Nanomedicines against Scirrhous Gastric Cancer and its Lymph Node Metastasis

（スキルス胃癌とそのリンパ節転移に対する白金制がん剤内包高分子ミセルの薬理作用に関する研究）

氏名： ラフィ　モハマド
RAFI MD

Conventional chemotherapeutic agents rapidly distribute nonspecifically in the body affecting the normal tissues as well as cancer tissue leading to toxicity. Nano-scaled medicines are being developed for improving the treatment of solid tumors while decreasing the toxicity [Nishiyama et al. Pharmacol Ther. 112:630–648(2006); Kataoka et al. Adv Drug Deliv Rev. 47:113–131(2001)]. These nanomedicines effectively accumulate in solid tumors due to the hyperpermeability of blood capillaries to circulating macromolecules and the impaired lymphatic drainage of these macromolecules, which is known as the Enhanced Permeability and Retention (EPR) effect [Matsumura et al. Cancer Res. 46:6387–6392(1986)]. Indeed, several nanomedicine formulations have been approved for clinical use against hypervascular cancers such as ovarian cancers, HIV-associated Kaposi's sarcoma and breast cancers [Gottlieb et al. Lancet 350:1363–64(1997); Gradishar et al. J Clin Oncol. 23:7794–7803 (2005)]. However, in some intractable cancers such as pancreatic and gastric cancers, the hypovascularity and fibrosis of tumors may compromise the extravasation and tissue penetration of nanomedicines [Kano et al. Proc Natl Acad Sci. U S A. 104:3460–3465(2007)].

Gastric cancers cause 1 million deaths per year worldwide being the 2nd leading cause of cancer-related death following lung cancer [Crew et al. World J Gastroenterol. 12:354–362(2006)]. Among gastric cancers, diffuse-type scirrhous gastric cancer (SGC) affects younger patients and presents the highest mortality [Otsuji et al. Am J Surg. 188:327–32(2004)]. SGC is characterized by hypovascularity, extensive stromal fibrosis and metastasis to the lymph nodes [Hippo et al. Cancer Res. 61:889-95(2001)]. These characteristics impair the therapeutic efficacy of chemotherapy as well as nanomedicine-mediated targeting chemotherapy. Moreover, the targeting chemotherapy against lymph node metastasis involve intralymphatic or local administration of nanomedicine-encapsulated antitumor agents [Hagiwara et al. Anticancer-Drugs. 8:666–670(1997); Oussoren et al. Adv Drug Deliv Rev. 50:143-56(2001)]; however, these approaches may not target all draining lymph nodes due to the inappropriate position of the injection and the obstruction of lymphatic vessels in advanced stages of cancer [Yamagata et al. Jpn J Clin Oncol. 28:104-106(1998)]. Hence, the improved targeting against the lymph node metastasis is strongly needed to eradicate the lymph node metastasis.

Polymeric micelles, self-assemblies of block copolymers, are characterized by core-shell structures with drug-loaded core surrounded by hydrophilic poly(ethylene glycol) (PEG) shell, and have shown great potential as tumor-targetable nanomedicines [Kataoka et al. Adv Drug Deliv Rev. 47:113–131(2001); Nishiyama et al. Pharmacol Ther. 112:630–648(2006)]. PEG shell of the micelles prevents

the protein adsorption and the recognition by the phagocyte system, leading to prolonged blood circulation time. The substantial advantages of polymeric micelles include relatively small size ranging from 20 to 100 nm, controllable drug loading and release, and favorable biodistribution and enhanced tumor accumulation [Kataoka et al. Adv Drug Deliv Rev. 47:113–131(2001); Nishiyama et al. Pharmacol Ther. 112:630–648(2006)]. Moreover, functional properties can be integrated into polymeric micelles such as stimuli-responsiveness and target recognition capability. Accordingly, micelle formulations incorporating doxorubicin [Matsumura et. al Br J Cancer. 91:1775–1781(2004)], SN-38 [Matsumura et al. Adv Drug Deliv Rev. 63:184-92(2010)], paclitaxel [Hamaguchi et al. Br J Cancer. 97:170–6(2007)], cisplatin [Plummer et al. Br J Cancer. 104: 593–598(2011)], and (1,2-diaminocyclohexane)platinum(II) (DACHPt) (an active form of oxaliplatin) exerted significant efficacy against several tumor models with appreciably lowered toxicity compared to free drugs, and are currently under clinical evaluation. Particularly, DACHPt-loaded polymeric micellar nanomedicines (DACHPt/m), are prepared by the polymer-metal complex formation between DACHPt and the carboxylic acid moieties in the poly(ethylene glycol)-*b*-p(glutamic acid) (PEG-P(Glu)) copolymer. These micelles are characterized by the small size (ca. 30 nm) [Cabral et al. J Control Release. 101:223–232(2005)], achieving high penetration into tumor mass and remarkable antitumor activity against poorly permeable tumors such as pancreatic tumors [Cabral et al. Nat Nanotechnol. Oct 23(2011); Kaida et al. Cancer Res. 70:7031-41(2010)] and oxaliplatin resistant tumors [Murakami et al. Sci. Transl. Med. 3,64ra2(2011)].

Herein, pharmacological activity of DACHPt/m were evaluated against SGC. The experimental model of SGC was prepared by orthotopic inoculation of bioluminescent OCUM-2MLN-Luc cells [Fujihara et al. Clin Exp Metastasis. 16:389–98(1998)]. In this model, the induction of lymph node metastasis occurs in all mice, while the tumor microenvironment shows hypovascularity and thick fibrosis similar to SGC in the patients, indicating the clinical relevancy of this model [Fujihara et al. Clin Exp Metastasis. 16:389–98(1998); Yashiro et al. Cancer Microenviron. 3:127-35(2010)]. Results demonstrated that DACHPt/m efficiently inhibited the growth of orthotopic gastric tumors. In addition, systemically injected DACHPt/m can target the metastatic lymph nodes, leading to reduction of the metastatic tumor growth. These findings suggest that DACHPt/m may provide a promising therapeutic approach for SGC and their metastasis to the lymph nodes. While the micelles accumulation to the tumor is based on the enhanced permeability and retention effect, the mechanisms of the accumulation of the micelles in the metastatic lymph nodes remain to be clarified yet. Two mechanisms for the accumulation of the micelles in the metastatic lymph nodes can be proposed: (i) the micelles accumulate in the orthotopic tumors, followed by migration and accumulation in the metastatic lymph nodes via the lymphatic vessels, (ii) the micelles can directly accumulate in the metastatic lymph nodes via blood vessels in the metastatic niche probably due to the enhanced permeability of these blood vessels. Further studies to determine the mechanism are undergoing.

Nanomedicines with diameters lower than 100 nm have been recently recognized as important from the standpoint of therapeutic efficacy while avoiding recognition of the reticuloendothelial system

[Weissig et al. Pharm Res. 15:1552-6(1998)]. Recent studies have shown that the size of nanomedicines in the sub-100 nm range can greatly affect the efficacy of the targeting and the therapy [Cabral et al. Nat Nanotechnol. Oct 23(2011); Perrault et al. Nano Lett. 9: 1909-1915(2009)]. In this regard, the accumulation and antitumor effect of nanomedicines in hypovascular tumors is a critical issue [Kano et al. Proc Natl Acad Sci. U S A. 104:3460–3465(2007)]. We have previously reported that DACHPt/m with 30-nm diameter can accumulate in pancreatic cancer models [Cabral et al. Nat Nanotechnol. Oct 23(2011); Kaida et al. Cancer Res. 70:7031-41(2010)] which shares histological characteristics with OCUM-2MLN tumors, such as hypovascularity and very thick fibrotic stroma [Yashiro et al. Cancer Microenviron. 3:127-35; (2010)]. Conversely, the accumulation of nanocarriers with larger size, i.e. DACHPt/m larger than 50 nm, 60-nm doxorubicin(Dox)-loaded micelles and 100-nm PEGylated liposome incorporating Dox, was considerably reduced leading to poor antitumor effect against these pancreatic tumors [Cabral et al. Nat Nanotechnol. Oct 23(2011); Kano et al. Proc Natl Acad Sci. U S A. 104:3460–3465(2007)]. As the optimal size for the treatment of SGC and its lymph node metastasis remains poorly understood, I studied the penetration, accumulation and antitumor activity of DACHPt/m having different sub-120 nm diameters.

DACHPt/m with 70- and 120-nm diameters were prepared by the addition of poly(glutamic acid) homopolymer to the mixture of PEG-P(Glu) and DACHPt. The tumor penetration, accumulation and therapeutic effect of these micelles were evaluated. Even though in the orthotopic model of OCUM-2MLN-Luc, DACHPt/m with 30 nm showed remarkably high antitumor activity, 70- and 120-nm DACHPt/m failed to show any antitumor effect. Our results also demonstrated that only 30-nm micelles achieved high penetration and accumulation in the metastatic lymph nodes enhancing the antitumor efficacy against lymph node metastasis. Low accumulation of 70- and 120-nm micelles in the metastatic lymph nodes led to poor antitumor activity.

The accumulation and antitumor activity of nanomedicines can be enhanced with the low dose of an inhibitor of transforming growth factor (TGF)-β (LY364947). TGF-β has been shown to play an important role in both the regulation of the growth of tumor cells and the tumor stroma [Miyazono et al. Cancer Sci. 94:230-4(2003)]. The transient inhibition of TGF-β results in a tumor-specific decrease of the pericyte coverage of the endothelium without reducing the endothelial area specifically in the neovasculature of tumors. This effect augmented the extravasation of nanomedicines in hypovascular tumors [Kano et al. Proc Natl Acad Sci. U S A. 104:3460–3465(2007)]. Therefore, the effect of TGF-β inhibitor on the penetration, accumulation and antitumor activity of the size-modulated DACHPt/m was evaluated in SGC tumors. The results demonstrated that combination of 70-nm DACHPt/m with TGF-β inhibitor improved the antitumor activity against SGC tumors and their lymph node metastasis by augmenting the penetration and accumulation of these micelles in the tumor tissue. Conversely, TGF-β inhibitor slightly improved the accumulation of 120-nm DACHPt/m but there was no enhancement of the antitumor activity. These results suggest that TGF-β inhibitor is a highly promising strategy for the treatment of scirrhous gastric tumors with nanomedicines larger than 30 nm.

Our results highlight that systemically injected DACHPt/m can extravasate and penetrate in orthotopic scirrhous gastric tumors and lymph node metastasis, eliciting significantly potent antitumor activity. Enhanced drug delivery to the lymph node metastasis by polymeric micelles can reduce the metastatic growth and may improve the morbidity of the patients with SGC. DACHPt/m can also be useful for the adjuvant therapy of SGC, that is, the administration of the micelles before surgery, by improving the lymph node status while controlling the tumor volume, which may lead to the downgrading of unresectable SGC. Control of distant lymph node metastasis by DACHPt/m can also impede further dissemination of the disease. Moreover, our findings suggest that TGF-β inhibition is a viable strategy to increase the extravasation and accumulation of large nanomedicines in SGC and its lymph node metastasis. In conclusion, this study not only will provide new insights for the therapy of SGC and their lymph node metastasis, but also indicates that DACHPt/m may have considerable clinical benefits against SGC.