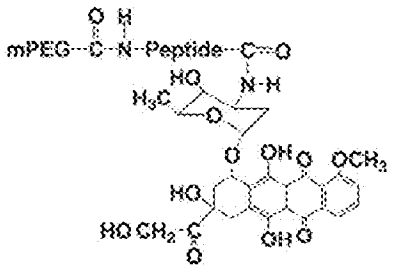

Figure 1. The structure of PEG-peptide-doxorubicin conjugates

Experimental methods: PEG-peptide-doxorubicin conjugates were prepared using peptides with the specificity for the matrix metalloproteinases MMP-2 and MMP-9. The secretion of MMPs from cancer cells was analyzed *in vivo* for the cell lines of LLC, MCF-7, and Head-Neck SCC by zymograpy. Using in situ zymography using gelatin-coated film, the expression of MMPs from tumor tissue inoculated in C57Bl/6 was observed. The distribution of MMPs in tumor tissue was observed immunohistochemically. The degradation behavior of our conjugates was observed by HPLC with the incubation time with enzyme and the concentration of active MMP-2. The cytotoxic activity of the conjugates compared to free doxorubicin was investigated by MMT assay.

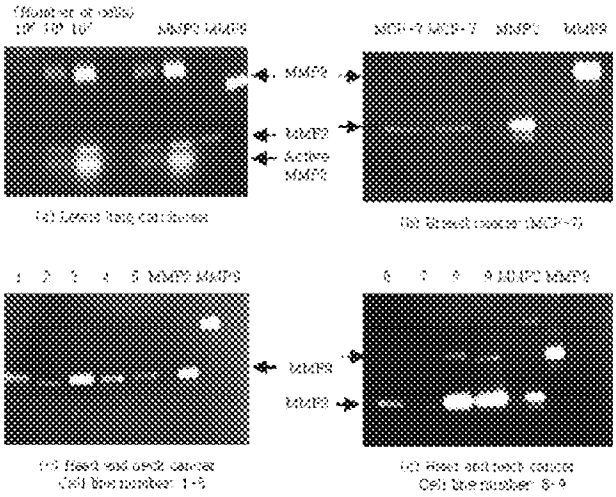

Figure 2. Screening of MMPs released from various cancer cell lines

Results and Discussion: Cancer cell lines were secreted two types of MMPs such as pro- and active-MMPs. Among the cell lines, LLC cells released pro- and active-MMP-2 and only pro MMP-9 (Fig. 2). It was observed that MMP-2 and MMP-9 distributed around the tumor from the immunohistogram of tumor tissue (Fig. 3) and the result from in situ zymography of tumor tissue tumor was showed similar tendency.

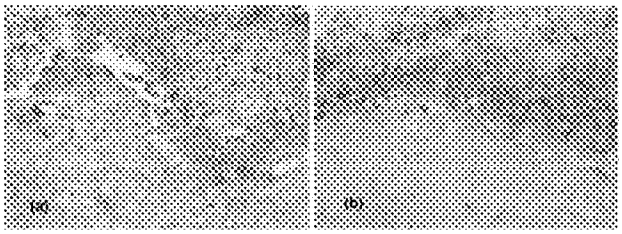

Figure 3. Ditribution of Lewis Lung. MMPs around Carcinoma tissue in C57BL/6 mice. (a) and (b) showed pro MMP-2, pro MMP-9 around tumor site, respectively.

We demonstrated by HPLC analysis that PEG-peptide-doxorubicin conjugates were degraded by the active MMP-2 to maximum 90 % depending on the concentraion of doxorubicin and the incubation time. Also the conjugates were less toxic in MMT assay because of their micelle form.

Conclusion: PEG-peptide-doxorubicin showed the selective degradation by the active MMP-2. Also the conjugates showed the reduced toxicity compare to free doxorubicin in MMT assay. As results, it is expected for PEG-peptide-doxorubicin conjugates to be degraded selectively by MMP-2 or MMP-9 in tumor region and then show the anti-cancer activity. Therefore, They are available to apply our conjugates as an anti-cancer drug to target cancer.

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Microsphere-encapsulated 4OH-Tamoxifen: a new sustained release delivery system with antitumour activity against DMBA-induced mammary carcinoma in sprague-dawley rats

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Introduction: Tamoxifen (TAM), a synthetic non-steroidal anti-estrogenic compound, is considered as the standard treatment of hormonodependent advanced breast cancer. One of its metabolites, 4-hydroxytamoxifen (4OH-TAM), may be responsible for a major part of the *in vivo* effects of TAM. However, the use of 4OH-TAM is limited by its very low solubility.

Aim: To investigate the efficacy of one single subcutaneous (sc) injection of 4OH-TAM encapsulated in microspheres (MS) that induce a sustained release. This experiment was done in comparison with daily repeated administrations of free 4OH-TAM (sc) and TAM (per os) in the model of 7,12-dimethybenz(a)anthracene (DMBA)-induced rat mammary tumours. Material & Methods: Biodegradable PLGA microspheres were obtained by solvent extraction method. Sprague-Dawley rats received TAM (10.0 mg/kg daily per os for 28 consecutive days), free 4OH-TAM (0.1, 1.0, 10.0 mg/kg daily sc for 28 consecutive days) and MS/4OH-TAM (single sc injection of 28.0 mg/kg) or were ovariectomised (OVX) 9 weeks following DMBA administration.

Results: No major toxicity was observed in all groups, except for animals treated with free 4OH-TAM, where a severe local necrosis due to the ethanol/water (65:35) vehicle was observed. The total number of tumours/animal and the sum of tumour volumes/animal were significantly ($p<0.01$) lower in TAM, MS/4OH-TAM groups and in OVX rats as compared to control. Moreover, the percentage of tumour reduction was significantly ($p<0.01$) higher in MS/4OH-TAM (67%) than in TAM (33%) treated-groups. MS formulation allowed obtaining a 4-weeks release of 4OH-TAM without any toxicity.

Conclusion: Our data demonstrated that this sc single administration MS delivery system gives an antitumour activity of 4OH-TAM equal/better than that of TAM. Therefore MS may be a promising new class of polymers suitable for 4OH-TAM targeted drug delivery.

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Interference with TGF-beta1 and -beta3 in tumor stroma lowers tumor interstitial fluid pressure independently of growth in experimental carcinoma

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Solid tumors are characterized by a high interstitial fluid pressure (IFP) which constitute a hydrodynamic barrier resulting in a low uptake of anticancer drugs. The aim of the present investigation was to study the role of members of the transforming growth factor (TGF)-beta family on the generation of a high tumor IFP. We used an *in vivo* model of human anaplastic thyroid carcinoma (ATC) and a specific inhibitor of TGF-beta1 and -beta3. Treatment of KAT-4 ATC tumors grown in athymic mice with 10 mg/kg of the TGF-beta inhibitor for 10 days resulted in a 48% reduction in tumor IFP compared to untreated control tumors. The mice that received the inhibitor had initially a higher tumor growth rate. However, at day 10 the apoptotic index, as well as the protein level of the cell cycle inhibitor p27Kip1, were higher in tumors from treated mice compared to the control mice. This was followed by a decreased tumor growth rate between days 15 to 29 in the treated mice. Since the KAT-4 cells *in vitro* did not respond to TGF-beta1 stimulation, measured as phosphorylation of Smad2 protein and growth inhibition, the effects observed in the tumors by the inhibitor are believed to be caused by the effects on the tumor stroma. Taken together, the present data indicate that members of the TGF-beta family are involved in the generation of the high tumor IFP observed in the ATC model, as well as in regulation of tumor growth, by changing the properties of the tumor stroma. These results identify TGF-beta1 and -beta3 as potential targets for novel anticancer treatment directed to the tumor stroma.