Hydrogels for Protease-Responsive Chemotherapy

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Introduction: Glioblastoma Multiforme (GBM) is the most invasive and advanced grade of brain tumor. GBM is especially difficult to treat due to diffuse infiltration of tumor cells leading to a high probability of recurrence even after treatment [1]. Invasion and metastasis require degradation of the extracellular matrix which is mediated by a family of enzymes called matrix metalloproteases (MMPs) [2]. MMP-2 and MMP-9 have been found to be expressed and overactive in GBM, while being minimally expressed in normal brain [1, 3, 4]. Platinum analogues have been found to be effective in treating brain tumors [5] despite their inability to fully penetrate the blood brain barrier. In the current study, platinum was complexed to peptide substrates of MMP-2 and MMP-9 which have been incorporated in poly(ethylene glycol) diacrylate (PEGDA) hydrogels. In the presence of MMPs, the peptide substrates are cleaved releasing the active drug from the hydrogels. This study investigates the hypothesis that MMP-2 and MMP-9 can be used to deliver active platinates from PEGDA hydrogels. Since cleavage of peptide requires diffusion of MMPs into the hydrogel, a comparison of different chain lengths of PEG was made to evaluate the effect of chain length on release and activity of platinum.

Methods: Hydrogels were synthesized from PEG(4000)DA and PEG(8000)DA by incorporating a pendant MMP substrate, K*PAGLLGC, via Michael addition of cysteine to acrylate. Platinum was complexed via amine group of lysine on the peptide. Lysine was labeled with fluorescence (*) for detection purposes. These hydrogels were characterized with respect to swelling ratio and mesh size. Release of Pt from the hydrogels was determined in the presence and absence of MMP-2 and MMP-9. Activity of the individual components of the system and the intact system was studied in a malignant glioma cell line, U-87 MG.

Results/Discussion: PEG(8000)DA and K*PAGLLGC produced hydrogels with substantially higher mesh size (126 Å) compared to PEG(4000)DA (85 Å). This suggested that MMPs could diffuse more freely in the hydrogels synthesized from PEG(8000)DA and show higher activity. In the presence of MMP-2 and MMP-9, both hydrogels showed a significantly higher release of Pt. However, release from PEG(4000)DA hydrogels showed a plateau after 48 hours and did not respond to addition of more MMP. PEG(8000)DA hydrogels showed a release profile which did not plateau and responded to additional MMPs with increased Pt release (Figure 1). Non-specific release of Pt from hydrogels was low, suggesting a strong complexation between Pt and amine groups. Low non-specific release permitted almost complete control of release to when MMPs were present. The peptides and cleaved fragment were not toxic to U-87 MG cells, however the Pt-complexed fragment showed significant activity (EC_{50} =100.8 μM). This was slightly lower than that of potassium tetrachloroplatinate (EC_{50} = 69.91μM) indicating that Pt released from the hydrogels would be active, though slightly attenuated. Hydrogels without platinum were not toxic to cells. However, there was significantly higher activity of Pt-complexed hydrogels with addition of MMP-2 or MMP-9. Due to higher release of Pt from PEG(8000)DA hydrogels, there was higher anti-neoplastic activity observed than with PEG(4000)DA hydrogels. Platinum release from PEG(8000)DA hydrogels thus showed good sensitivity to presence of MMP-2 and MMP-9.

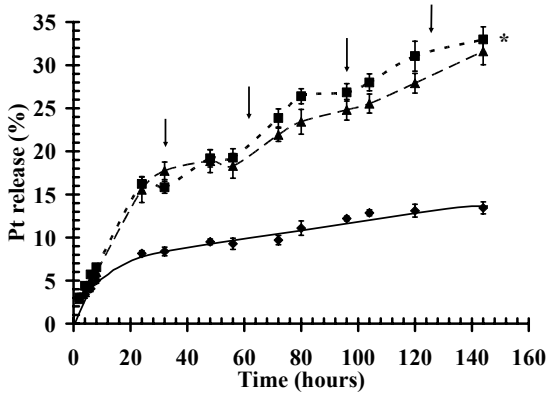

Figure 1. Release of Pt from PEG(8000)DA hydrogels in the absence (◆) and presence of MMP-2 (■) and MMP-9 (▲).

Conclusions: PEG(8000)DA hydrogels had sufficient mesh size for diffusion of MMP-2 and MMP-9 into the hydrogels to release active chemotherapeutics. These results prove that diffusion of MMPs through hydrogels can be controlled by varying the chain length of the macromers used to create the hydrogels. MMP-2 and MMP-9 were able to cleave the peptide substrate K*PAGLLGC from the hydrogels to release platinum. The platinum released from the hydrogels by MMPs retained anti-neoplastic activity for U-87 MG cells. Hence, PEG(8000)DA hydrogels with K*PAGLLGC proved to be sensitive to the presence of MMPs expressed in GBM and should be further explored as a delivery device for chemotherapy. Biological control of the release of drug as required may help in the successful delivery of effective concentrations of active platinates thus prolonging the survival of GBM patients.

References:

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