Despite their broad spectrum activity, the clinical usefulness of cyclophosphamide and ifosfamide is limited by the formation of toxic byproducts. Both agents generate acrolein, a metabolite that has been implicated in kidney and bladder toxicity. In addition, ifosfamide gives rise to chloroacetaldehyde, a metabolite believed to cause CNS toxicity. To avoid toxicologic problems associated with the formation of such byproducts, we have investigated alternative strategies to deliver PM's into cells. Various prodrug formulations of PM's were prepared including compounds that are activated by carboxylate esterase, β-glucuronidase and β-galactosidase. GRAPH. In the absence of activating enzymes, most of the prodrugs were fairly stable and showed a low order of biological activity. In the presence of activating enzymes, however, the prodrugs were rapidly converted to PM's. The increased toxicity of the prodrugs to human tumor cells in the presence of activating enzymes varied from as little as 10-fold to as much as 500-fold. In addition to providing an alternative cell-delivery strategy for PM's, some of these prodrugs offer potential for use in conjunction with gene therapy approaches to tumor-selective drug activation. (Supported by grant CA RO1 89386).

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Design of a DNA damaging molecule "programmed" to release multiple high affinity inhibitors of EGFR tyrosine kinase under hydrolytic conditions: A novel antitumour drug combination strategy

R. Banerjee, B. Jean-Claude. *McGill University/Royal Victoria Hospital, Medicine, Montreal, Canada*

The altered protein expression and activity of receptor tyrosine kinases (TK) are implicated in the progression of various types of cancers. One such dysfunction is the overexpression of the epidermal growth factor receptor (EGFR) that correlates with aggressive tumor progression and poor prognosis. Recently, we developed a novel strategy that seeks to combine DNA damaging properties and EGFR TK inhibitory activities into single molecules termed "combi-molecules" designed to kill EGFR-expressing tumour cells (Matheson et. al., J. Pharm. Exp. Ther, 296, 832-840, 2001 Brahimi et al., ibid, 2002, in press). In order to enhance the EGFR inhibitory potency and stability of these compounds, we designed a novel strategy termed "cascade release" (CR) that seeks to mask the combi-molecule into a stable carrier "programmed" to release the antitumour species by hydrolytic cleavage. Since these molecules henceforth referred to as "cascade release molecules" (CRM) are also designed to retain EGFR affinity on their own, this principle leads to molecular systems whereby three generations of inhibitors can arise from the hydrolysis of the parent CRM. To study this model, we recently designed and synthesized RB24 (IC_{50} competitive binding=130 nM), which was a masked form of RB14 (IC_{50}=100 nM), a hydrolabile triazene capable of generating the combi-molecule ZR08 (IC_{50}=44 nM). The latter was found to further degrade into RB10, another potent inhibitor of EGFR (IC_{50}=40 nM). Kinetic studies using UV spectrophotometry demonstrated that the parent CRM, RB24, was hydrolyzed with a t1/2=42 min. Western blot analysis demonstrated potent inhibition of EGFR autophosphorylation by the CRM in the carcinoma of the vulva cell line, A431 (IC_{50}=2 uM). Studies on serum stimulated growth using a pair of isogenic cells [NIH3T3 and HER14 (engineered to overexpress EGFR)] showed that RB24 selectively induced approximately 5-fold stronger growth inhibitory activity in the EGFR-transfectant when compared with its parent NIH3T3, indicating significant EGFR selectivity. The results in toto suggest that RB24 is the first ever molecule capable of being an EGFR TK inhibitor (I), while being the parent of two other EGFR inhibitors (I2) and (I3), the latter being the precursor of another stable inhibitor (I4) + a DNA damaging fragment. Further studies are ongoing in our laboratory to determine the effects of the CR system on the sustainability and reversibility of EGFR TK inhibition.

Drug delivery

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Taxane-monoclonal antibody covalent conjugates for targeted chemotherapy of cancer

A. Safavy[1,2], K.P. Raisch[1,2], K. Safavy[2,3], H.W. Waksal[4], M.B. Khazaeli[1,2], D.J. Buchsbaum[1,2], J.A. Bonner[1,2]. *[1]University of Alabama at Birmingham, Radiation Oncology, Birmingham, USA; [2]University of Alabama at Birmingham, Comprehensive Cancer Center, Birmingham, USA; [3]University of Alabama at Birmingham, Medicine, Birmingham, USA; [4]ImClone Systems, Inc., Somerville, USA*

In efforts to develop taxane derivatives capable of tumor-specific drug delivery (Safavy, US Patent 6,191,290 B1), we previously reported the synthesis and cytotoxicity results of a paclitaxel (PTX) monoclonal antibody (MAb) C225 (ErbituxTM, ImClone Systems, Somerville, NJ) conjugate (Figure 1) (Safavy et al., Eighth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, 2000) with enhanced cytotoxicity of PTX against A431, UM-SCC-1, and UM-SCC-6 human cell lines.

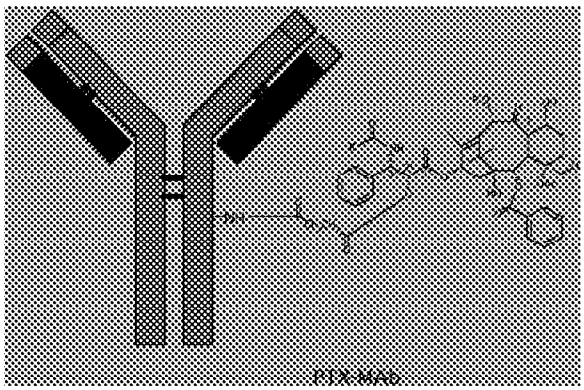
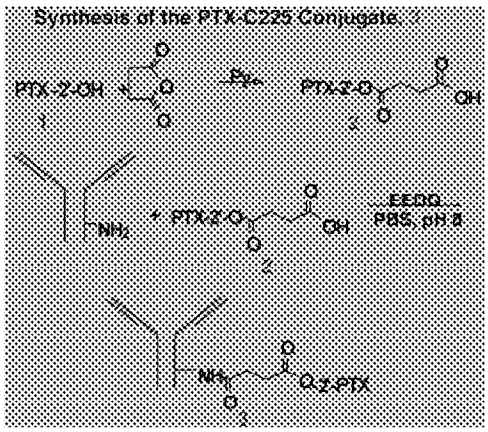


Figure 1

Here, conjugates of PTX with the anti-epidermal growth factor receptor antibody, C225 and anti HER2/neu antibody, Herceptin (Her, Trastuzumab, Genentech, South San Francisco, CA) were synthesized by the procedure shown in Scheme 1. The purity and number of drugs per antibody (PTX: MAb) were evaluated by HPLC and MALDI-TOF mass spectrometry, respectively.



Scheme 1

Purities of *98% and PTX: MAb of 2.5 were detected for both conjugates. These conjugates were then tested in cell binding and cytotoxicity experiments using MDA-MB-468 (human breast carcinoma) and LNCAP and DU145 (human prostate carcinoma) cell lines to determine the effect of receptor-targeted delivery in enhancing the drug efficacy. To demonstrate the retention of antigen-binding ability, the parent MAb and conjugates were radiolabeled with 125I and screened in binding inhibition assays. The percent cell binding (%B) of the PTX-C225 conjugate, as compared to the unconjugated MAb (parenthesized values) were 49 (88), 41 (87), and 40 (87) in MDA-MB 468, LNCAP, and DU145 cells, respectively. Herceptin and the PTX-Her conjugate showed a %B of 21 (36) to LNCAP cells with no appre-