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NOVEL ANALOGUES OF CB 1954: THEIR POTENTIAL USE IN ANTIBODY DIRECTED ENZYME PRODRUG THERAPY (ADEPT)

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CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] was the most cytotoxic against the Walker tumour of a series of N-substituted 5-aziridinyl-2,4-dinitrobenzamides, and also the most hydrophilic [Khan and Ross, *Chem. Biol Interac.* (1971/72) 4:11]. CB 1954 becomes a difunctional alkylating agent following reduction of its 4-nitro group [Knox et al., *Biochem. Pharmacol.* (1992) 43:1249]. A suitable enzyme to undertake this activation in an ADEPT strategy [Bagshawe, *Br. J. Cancer* (1987) 56:531] is *E. Coli* B nitroreductase which metabolises CB 1954 to the 2- and 4-hydroxylamine regioisomers in 1:1 ratio but only the 4-hydroxylamine isomer is significantly cytotoxic. In order to avoid the unnecessary reduction at the 2-position, the nitro group was replaced by alkanesulphonyl groups with varying hydrophobicity. The starting material in the multi-step synthesis of each analogue was 2-amino-5-chlorobenzoic acid. The 2-amino group was first diazotised and displaced by the appropriate alkyl thiol, followed by oxidation to the sulphone. The aromatic ring was nitrated at C-4 by nitronium ion and the carboxy group amidated via the acid chloride. Finally, the aziridinyl group was introduced by nucleophilic chloride displacement at C-5. The novel analogues were assayed against human, *E. Coli* and Walker nitroreductases using high pressure liquid chromatography to quantify substrate disappearance. The rate of reduction by the human and Walker nitroreductases was CB 1954 > butanesulphonyl > trifluoroethanesulphonyl > propanesulphonyl > ethanesulphonyl > methanesulphonyl. Reduction by *E. Coli* nitroreductase showed a different trend; propanesulphonyl = butanesulphonyl > ethanesulphonyl > trifluoroethanesulphonyl > CB 1954 > methanesulphonyl. The relationship between % reduction by *E. Coli* nitroreductase and the hydrophobicity constant (π) of the 2-alkanesulphonyl substituents is inversely parabolic with the 2-propanesulphonyl analogue showing the greatest propensity for reduction. The latter therefore appears to be the optimal drug of this type, at least in terms of reduction by the non-mammalian enzyme.

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TUMOUR REGRESSIONS WITH NOVEL PRODRUGS IN ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY (ADEPT).

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ADEPT is a two-phase system in which a non-toxic prodrug is converted to a potent cytotoxic agent by an enzyme targeted to the tumour site with a monoclonal antibody. In the ADEPT system, we have used a $F(ab'')_2$ fragment of the antibody A5B7 (raised against CEA), conjugated to the bacterial enzyme carboxypeptidase G2 (A5B7-CPG2), to convert selectively a monomesyl benzoic acid mustard-glutamate prodrug (IC_{50} > 1000 μM: 1h exposure) to an active drug (IC_{50} 200 μM). This prodrug was converted by CPG2 with K_m = 3 μM; K_{cat} = 600 s^{-1}. An ADEPT clinical trial using this system is currently on-going at the Royal Free Hospital, London. One goal in ADEPT is to design potent active drugs with short half-lives so that systemic toxicity by leakback of drug from the tumour may be prevented. Novel prodrugs were synthesised which released much more potent phenol mustard- and aniline mustard-derived active drugs. Structural modifications were made to the mustard arms, to the aromatic ring and to the glutamate moiety. Surprisingly, these prodrugs remained substrates for the CPG2 enzyme (K_m 0.5-100 μM; K_{cat} 10-100 s^{-1}). The cytotoxicity of the active drugs (IC_{50} <0.1-10 μM) released by cleavage with CPG2 was considerably enhanced over that of the benzoic acid drug. These novel drugs were >50 fold more toxic than their corresponding prodrugs. Administration of the A5B7-CPG2 conjugate (2000 U enzyme/kg) followed by the aniline mustard-glutamate prodrug (3 x 40 mg/kg) led to regressions and a significant growth delay in human LoVo xenografts of colorectal tumours in nude mice. Controls of A5B7-CPG2 alone, prodrug alone, active drug or vehicle alone had little or no effect on tumour growth. As illustrated by these studies, colorectal cancer treatment may be improved by the use of ADEPT.

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SOMATOSTATIN RECEPTOR SUBTYPE GENE EXPRESSION IN BREAST EPITHELIAL CELL-LINES.

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In vitro studies using chemical cross-linking assay provided evidence that human breast tumours contain specific receptors for somatostatin and a somatostatin analogue, BIM23014C. Five somatostatin receptors (SSTR1-5) were recently cloned. By northern hybridization, we investigated the gene expression of SSTR1, SSTR2, and SSTR3 subtypes in nine epithelial breast cell-lines derived from *(i)* the milk of an apparently healthy woman (HBL100), *(ii)* sclerocystic benign breast lesions (NPM14T, NPM21T4), *(iii)* a ductal carcinoma (Hs578T), and *(iv)* pleural effusions of breast adenocarcinomas (MDAMB-231, T47D, MCF7 and *Ha-ras* transfected cells : MCF7-*ras*, MCF7-TCM). Both, the SSTR1 and SSTR3 transcripts were present in all cell-lines. The size of these transcripts (4.8 kb) was similar to that previously reported for SSTR1 in the stomach and the jejunum and for SSTR3 in the cerebrum. SSTR2 mRNAs — a 8.5 and a 2.5 kb molecular species found in the cerebrum and the kidney — were absent in these cell-lines. A densitometric study revealed no correlation between the various SSTR mRNAs and the tumorigenicity of the cells, the presence of steroid-hormone receptors, or the malignancy of the original tissue.