GDEPT Pharmacokinetics Studied with 19F-MRS for Validating Gene and Prodrug Delivery and Prodrug Activation

L. Mancini[1,2], L. Davies[3], F. Friedlos[3], A. S. Dzik-Jurasz[1,4], C. J. Springer[3], M. O. Leach[1], G. S. Payne[1]

[1]Cancer Research UK Clinical Magnetic Resonance Research Group, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom, [2]University College London Hospitals, London, United Kingdom, [3]Center for Cancer Therapeutics, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom, [4]Epix Pharma, Boston, Mass, United States

Introduction

The novel anticancer strategy GDEPT (Gene-directed enzyme prodrug therapy; (1-3)) is a two-step therapy in which tumour cells are transfected with a gene expressing an exogenous enzyme, which subsequently converts a non-toxic prodrug to an active anti-cancer agent. The strategy limits toxicity to normal tissues and is effective with various enzyme systems in cells and animal tumour models, providing a systemic low-toxicity treatment. It is currently being assessed in humans.

Several different enzyme systems are being evaluated for use with the GDEPT strategy. One example is carboxypeptidase (CPG2). For each of these enzymes specific substrates are being developed that become toxic on activation. Successful development and implementation depends on effective gene delivery and expression, as well as drug delivery and activation. In this study we show

(i) that activation of several lead substrates of CPG2 leads to a large change (~ 1ppm) in the chemical shift of their ^{19}F resonance, suggesting that ^{19}F MRS can be used to detect both drug delivery and activation;

(ii) that non-toxic analogues can be made with a similar chemical shift change on cleavage, and therefore may be used to evaluate gene delivery/ expression prior to administration of the therapeutic agent, and

(iii) that in cells transvected with CPG2 the ^{19}F MRS linewidth and chemical shift change on activation are such that both enzyme expression and drug delivery/activation can be monitored, and with a sensitivity sufficient for use in vivo.

Thus ^{19}F MRS can be used as an important tool in assessing and optimising the different steps of the GDEPT strategy, and can also be extended for use in clinical trials.

Methods

Several 1.5 mM solutions of six ^{19}F-containing compounds were analysed at 500MHz and 37°C. The compounds were two prodrug substrates of the carboxypeptidase (CPG2) enzyme, the two corresponding activated drugs, one analogue of the pro-drugs and the corresponding non-toxic cleaved compound. The solutions were made with 2.5ml of buffer containing 0.2, 1, 4% of BSA (bovine serum albumin) or 10% FBS (fetal bovine serum). 0.015unit/ml of CPG2 were added to the solutions containing the prodrugs and prodrug analogue.

Two tumoural cell lines expressing CPG2 (MDAMB361 and WiDr) were studied in confluent monolayers at 300MHz and 35°C. 1mM of prodrug analogue was added to the medium and the time evolution was followed with a home-made 1cm diameter surface coil (rectangular pulse, TR = 1 s, 128 acquisitions).

Results and Discussion

All prodrug-drug pairs and derived compounds showed a chemical shift difference of ~1ppm (see Fig1, showing the metabolism of one prodrug-analogue in WiDr cells).

The CPG2-mediated metabolism of prodrug to drug, observed with MRS, took 10 min in water, 110 min in FBS-containing buffer, and 16 hours in cells.

The narrow linewidths of the prodrug analogue and its product (~27Hz at 300MHz in cells) make them suitable as markers for localising the gene-delivery/enzyme-expression in vivo.

In the cells studies, a SNR~3.5 was given by 1mM prodrug analogue in 3.5-minute scan. MRS data showed that the prodrug analogue is reduced to 50% of its initial value after ~500min in MDAMB361 cells, and after 326 min in WiDr cells.

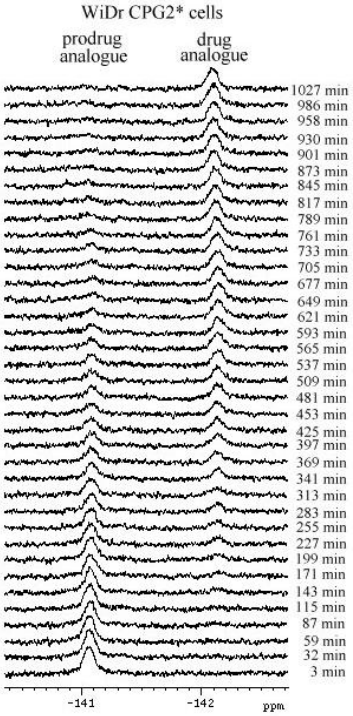

Fig.1 Metabolism of one ^{19}F-labelled prodrug-analogue in WiDr cells

Conclusions

It has been demonstrated that ^{19}F MRS measurements of ^{19}F-labelled substrates of CPG2 can be used to separately assess gene delivery/expression, drug delivery and drug activation for GDEPT with a sensitivity suitable for use in vivo, allowing each of the stages in this complex but promising therapy to be validated in vivo. Being non-invasive the method can also be used in subsequent clinical studies.

Acknowledgements

Cancer Research UK, for funding the work.

1)Niculescu-Duvaz I et al.,Curr.Opin.Mol.Ther.,1999;1(4):480-6 2)Blaese,RM et al.,Science,1992;258:1960. 3)Pandha,HS et al. J.Clin.Oncol,1999;17:2180-2189.