Gene Therapy (1997) 4, 80–81
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Editorial

Nitroreductase activation of CB1954 – an alternative 'suicide' gene system

Despite showing an impressive antitumour effect against the rat Walker tumour model, the dinitrophenylaziridine CB1954 was not effective in human clinical trials and therefore was labelled as 'a drug in search of a human tumour to treat'.[1] This lack of an antitumour response in humans was due to species differences in CB1954 reduction, catalysed by the nitroreductase enzyme DT-diaphorase,[2] generating the 4-hydroxylamine metabolite. This molecule then reacted with thioesters, such as acetyl CoA, to produce a highly cytotoxic difunctional alkylating agent capable of cross-linking DNA.[2]

Recently, new possibilities for the use of CB1954 have arisen with the advent of antibody-directed enzyme prodrug therapy (ADEPT)[2] and gene-directed enzyme prodrug therapy (GDEPT).[3] In these approaches either *E. coli* nitroreductase (NTR) which can also catalyse CB1954 reduction,[2] or the gene encoding this enzyme are delivered to the tumour site where they locally activate prodrug. Two articles in this issue of *Gene Therapy* show the potential of the latter approach.[4,5]

Both groups have limited expression of the bacterial enzyme to specific cellular targets by placing the gene under the transcriptional control of tissue-specific elements. In the work of Clark *et al*[4] the ovine β-lactoglobulin promoter limited expression to the luminal cells of the mammary gland whereas in Drabek *et al*'s studies[5] control elements of the human CD2 locus restricted nitroreductase expression to T cells. This targeted destruction of particular cell types allowing the ablation of selected tissues offers a powerful tool for many types of studies. Though, as both groups point out, it is the possibility of using the nitroreductase (NTR) gene in anticancer therapy which appears to offer greatest promise. However, while transgenics provide a model for studying the *in vivo* efficacy of NTR/prodrug therapy, they may not be particularly predictive of the clinical situation where levels of gene transfer are unlikely to be as high.

None the less, in several human and murine cell lines, following either plasmid or retroviral gene delivery,[4–7] the differential toxicity to CB1954 between cells expressing the NTR gene and nonexpressing control cells has reached 10–100-fold and therefore is as impressive as that observed with other 'suicide genes' like the HSV*tk*.

CB1954 appears to act more rapidly than some other suicide gene prodrugs such as ganciclovir, and *in vitro* effects have been noted when as little as 4-h drug exposures have been used, although 36 h was reported to be optimum.[7] *In vivo*, in a transgenic model, the cytotoxic effects of the prodrug have been observed relatively shortly after prodrug administration, ie by 48 h.[4] The rapid action of CB1954, and the short exposure times required, should facilitate the clinical application of this 'suicide gene' system as an antitumour therapy. One reason why CB1954 may act more rapidly is that, unlike HSV*tk*, it does not require cells to be in the S phase of growth for its activity. This has been demonstrated both *in vitro*[7] and *in vivo*[4] and suggests that, as long as tissue specificity of expression is achieved to prevent the targeting of normal somatic cells, this prodrug may be used to eliminate nondividing neoplastic cells; a not infrequent component of many human cancers. Following drug administration *in vitro* Drabek *et al*[5] reported morphological changes in murine L cells, including the generation of enlarged, multinucleate cells. The authors proposed that these changes could be explained by a block in DNA synthesis with continued RNA and protein synthesis. We too have observed similar morphological changes in V79 Chinese hamster cells (Bailey *et al*, unpublished data) and found that cytotoxic doses of CB1954 result in a block in the G2/M phase of the cell cycle. DNA strand breaks and interstrand cross-links were observed in CB1954-treated cells,[4] suggesting that these may be involved in the mechanism of cytotoxicity. Possibly as a consequence of these lesions in the genome the cells appear to undergo apoptosis. Thus Drabek and co-workers,[5] who targeted NTR expression to T cells using the CD2 locus control region (LCR), reported an increased proportion of apoptotic thymocytes in CB1954-treated mice relative to controls. Thymocytes readily undergo apoptosis but this does not appear to be an idiosyncratic lineage-specific response since Clark *et al*[4] also observed high apoptotic levels in targeted mammary luminal cells. Whether this cell response to CB1954-induced damage is a universal phenomenon remains to be determined. What is clear is that CB1954, when activated by NTR, exerts the bystander phenomenon *in vitro*. This characteristic, thought to be such an important component of potential prodrugs utilisable for 'suicide gene therapy', has been documented in tissue culture experiments.[7] Whether such a phenomenon always occurs in targeted tissues in transgenic mice, and whether it has any effect upon tissue-specific ablation, remains rather uncertain at this point. Thus Drabek and colleagues suggest that the killing of nonexpressing B cells was a consequence of an *in vivo* bystander response[5] while in Clark's studies mammary luminal cells were ablated completely leaving closely associated myoepithelial cells unaffected.[4]

Clearly the route by which the bystander effect occurs will be of primary importance in determining the extent,

or otherwise, of this phenomenon. Certainly it is predicted that eradication of tumour deposits using this technology will, given the likely efficiency of gene transfer, require the existence of a bystander response. While the two studies using transgenic models are elegant in conception and performance, they underline the fact that what may be a desirable attribute under one set of circumstances need not be so desirable under other circumstances. Thus in cell-lineage ablation studies the aim might be to minimise the bystander effect whereas in tumour destruction the aim would be to maximise the response. In this regard, perhaps it is disappointing that in cell culture experiments with 5–10% of murine L cells expressing NTR, a figure which might be at the upper end of achievable tumour transduction efficiencies *in vivo*, Drabek *et al*[5] observed no such bystander response. However, cell density is likely to play a substantial part in the manifestation of this response and two-dimensional monolayer cultures might be poor models of the situation likely to obtain in three-dimensionally growing tumour masses.

Obviously the CB1954/NTR GDEPT system offers a potent means of killing targeted cell types. Given the different modes of action of CB1954/NTR and HSV*tk*/ganciclovir treatments it could be that combinations of these two approaches offer a means of obtaining additive, and potentially synergistic, effects.[7] Future work aimed at evaluating the possible efficacy of this mode of tumour destruction is required. It is apparent though that the work of Clark *et al*[4] and Drabek *et al*[5] has shown how potentially useful is this means of eliminating cells and their reports should inspire future efforts with this system.

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