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Detection of NQO1 (NAD(P)H:Quinone oxidoreductase, EC 1.6.99.2) C609T polymorphism in archived human tumour tissue using PCR-RFLP

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NQO1 is a cytosolic flavoprotein whose physiological function is believed to be the detoxification of quinone based xenobiotics. Paradoxically however, NQO1 also plays a central role in the activation of quinone based bioreductive drugs. The gene encoding for NQO1 is polymorphic (C to T transition at position 609 of human cDNA). Cells which are homozygous for the polymorphic variant are devoid of NQO1 enzyme activity leading to suggestions that individuals may be either predisposed to cancer (as a result of loss of a detoxification enzyme) or be resistant to quinone based anti-cancer therapies such as Mitomycin C. The C609T polymorphism can be detected by PCR-RFLP techniques designed to exploit the fact that the C to T base pair mutation introduces a Hinf 1 restriction site. Whilst current methods are successful using genomic DNA isolated from fresh tissues, the generation of 'clean' PCR products from DNA isolated from archival formalin fixed human tumour tissue is unsatisfactory. In this study, genomic DNA was isolated from 12 archived paraffin embedded human bladder tumour tissue (following approval by local research and ethics committee) and subjected to two rounds of PCR amplification using a nested primer strategy. In the first round of PCR, a 334 bp product was amplified following 35 cycles (94C: 1 min, 60C: 30s, 72C:45s) using forward (GAGACGCTAGCTCTGAACTGAT) and reverse (CTGCCTGGAAGTTTGGTCA) primers. In the second round of PCR using forward (ATTTGAATTCGGGCGTCTGCTG) and reverse (TCTAGTGTGCCTGAGGCCTCC) primers and 30 cycles of PCR (94C:1min, 63C:30s and 72C:30s), a clean PCR product of 217bp was obtained for all specimens tested. Digestion of this PCR product with Hinf 1 generated fragments of 217bp (wild type), 217bp, 161bp and 56bp (heterozygotes) and 161bp and 56bp (homozygous mutant) following separation on a 3% agarose gel. The method provides reliable and reproducible information on the NQO1 genotype status of genomic DNA isolated from archived formalin fixed, paraffin embedded human tissues. This method together with the use of archival tumour material should enable the design of retrospective studies to critically assess the role of the NQO1 polymorphic genotype in predicting tumour response to quinone based agents such as mitomycin C.

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DT diaphorase and cytochrome P450 reductase protein localisation in archived bladder tumour specimens: correlation with clinical response following mitomycin therapy

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Mitomycin C (MMC) is an adjuvant intravesical therapy used to prevent recurrences of superficial bladder tumours. Many enzymes have been implicated in the metabolic activation of MMC particularly DT Diaphorase (DTD) and Cytochrome P450 reductase (P450R). Recent studies have shown that response of human bladder tumour histocultures to MMC can be predicted based on analysis of DTD and P450R gene expression (Gan et al, Clin Cancer Res 7; 1313, 2001). The role of DTD in the activation of MMC is however controversial and the aim of this study therefore is to determine whether or not clinical response to MMC therapy can be predicted based on immunohistochemical analysis of DTD and P450R protein. In this retrospective study, 91 formalin fixed, paraffin embedded transitional cell carcinomas (TCC) of the bladder (from patients diagnosed more than 3 years ago) were collected following local research and ethical committee approval. Tumours used were representative of all grades (G1 [n=25]; G2 [n=42]; G3 [n=23]) of both superficial (pTa [n=40]; pT1 [n=32]; Cis [n=1]) and muscle-invasive (pT2 [n=18]) stages of TCC. Tissue arrays were constructed, sectioned and stained with antibodies for human DTD and P450R.The intensity of staining was scored (by 3 independent observers) using a scale of no stain, moderate stain and heavy stain. An inverse relationship exists between the stage of tumour and intensity of staining for DTD with 73% (n= 40) and 39% (n = 19) of Ta and T2 specimens recorded as positive for DTD respectively. In contrast, DTD staining intensity was independent of tumour grade. In the case of P450R, staining intensity increased with both tumour grade and stage although the majority of tumours were scored as moderate for expression of P450R protein. These findings are consistent with previous studies based on analysis of gene expression patterns and demonstrate that superficial TCC of the bladder have elevated levels of DTD. Clinical response criteria employed were time to first recurrence following treatment with single dose MMC post transuretheral resection. A broad spectrum of responses was observed (ranging from 2 to 36 months). Clinical response was independent of both DTD and P450R levels in tumours. Specific examples exist of poor response (time to recurrence = 3 months) with high DTD (moderate P450R). This study suggests that whilst DTD is a target for new drug development, prediction of response to MMC on the basis of DTD and P450R may be premature.

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Product development of fenretinide, NSC 374551, intravenous formulation

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Purpose: It is not possible to prepare an injectable formulation of fenretinide owing to its poor aqueous solubility. The objective of these studies was to study the solubility profile of fenretinide in various solvents leading up to an intravenous formulation suitable for human clinical trials.
Methods: Diluent 12 is a mixture of equal volume of absolute alcohol and Cremophor EL, polyoxyethoxylated castor oil, USP. The drug solubility was studied in alcohol as a function of cremophor concentration. The concentrated solutions of the drug were prepared in alcohol/cremophor EL and diluted in NS and D5W at various dilution factors. The short term and long term stability of the concentrates and diluted solutions were followed. We also developed a lyophilized formulation of fenretinide and studied its long-term stability.
Results: The drug can be easily dissolved in diluent 12 at a concentration of 10 - 15 mg/mL. The drug solutions are stable at refrigerated and room temperature for up to 3 months with no significant loss in potency or build up of impurities. The Diluent 12 formulations can be conveniently diluted in NS or D5W to yield 1 mg/mL solution, which is stable for 4 days with no loss in potency of the solution. It is also possible to lyophilize the fenretinide formulations from t-butanol/water solutions. The concentration of t-butanol can be varied from 5% to 95%. The resulting amorphous powder can be reconstituted using diluent 12 and further diluted in normally used infusion solutions such as normal saline and 5% dextrose. The lyophilized powder is stable at room temperature.
Conclusions: A stable, lyophilized and ready-to-use liquid formulation of a fenretinide was developed.

Antimetabolites

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Gene expression profiling of colorectal cancer cell lines exposed to 5-fluorouracil (5-FU) and 5-FdUrd

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Although, 5FU is the mainstay of treatment for colorectal cancer, its precise molecular mechanism of action is undefined. It is already known that 5FU treatment results in inhibition of thymidylate synthase and misincorporation of its metabolites into both DNA and RNA. Microarray technology allows for the measurement of global gene expression and is poised to revolutionise the discovery and use of new anticancer agents. This project involved the analysis of gene expression patterns in three human colorectal cancer cell lines exposed to 5FU and one of its important metabolites 5FdUrd. The complex mechanisms of action of 5FU were expected to challenge the microarray technology. The three cell lines were exposed to five times their IC_{50} concentrations of 5FU or 5FdUrd and cell cycle distribution analysed over 2-72 hours. Messenger RNA was also extracted from cells exposed to the same drug concentrations and time course. In addition one of these cell lines, HCT116 was exposed to an increased dose of 5-FU, 375uM.