21
Low expression of topoisomerase II contributes to drug resistance of prostatic cancer-derived cell lines

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Following failure of surgical, androgen suppression and radiation therapy the prognosis of patients with advanced prostatic cancer is dismal due to the lack of effective chemotherapeutic treatment. Apart from the limited activity of Vinca alkaloids, estramustine and anthracyclines, most other drugs exhibited very low clinical response rates. It has been demonstrated[1] that P-gp mediated multidrug resistance (MDR) is of minor importance for the resistance of the PC-3 and DU-145 hormone-insensitive prostate adenocarcinoma cell lines to doxorubicin. In the present study we have investigated the *in vitro* sensitivity of these cell lines to amsacrine (AMSA) and the level of topoisomerase II expression in comparison with normal human prostatic tissue. With IC_{50} values of 3.8 ± 1.8 μg/ml AMSA for PC-3 and 3.7 ± 1.4 μg/ml for DU-145, respectively, both cell lines show high resistance to this topoisomerase II-directed cytostatic drug in thymidine incorporation assays (48 h). The hormone-sensitive LNCaP prostate cancer cell line displays higher sensitivity to both doxorubicin (IC_{50}: 31±22 ng/ml) and AMSA (0.41±0.09 μg/ml). Expression of topoisomerase II was investigated in Western blots of cell lysates using an anti-serum (Cambridge Technologies, UK) and found to be low in comparison to normal human prostatic tissue (< 10%). These results obtained with representative cell lines support the existence of an 'atypical MDR' associated with decreased expression of topoisomerase II in prostatic cancer and therefore alternative non-P-gp directed treatments may improve the clinical results.

1. Theyer G. *J Urol* 1993; 150: 1544.

Preclinical studies: detection

22
Non-invasive monitoring of MDR with positron emission tomography (PET) and C-11 labeled daunorubicin (C-11 DNR): synthesis and pharmacokinetics

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In vivo measurement of pharmacokinetics by PET with a positron-emitting MDR cytostatic agent may determine the presence of P-gp efflux pumps in human tumors and may also evaluate effects of MDR modulators on the efflux of the anti-cancer drug. For this purpose C-11 DNR was prepared by the reaction of [C-11] diazomethane with the corresponding trifluoroacetyl-protected aldehyde (gift from Farmitalia, Carlo Erba) yielding [C-11] trifluoroacetyl-daunorubicin. Mild alkaline hydrolysis of the trifluoroacetyl group, followed by RP-HPLC afforded C-11 DNR (200 μCi), with a specific activity > 2000 Ci/mmol at 55 min after end of bombardment. The pharmacokinetics of C-11 DNR were studied in P-gp negative respectively positive human ovarian cancer cell lines A2780 and 2780^{AD}. Steady-state intracellular concentration of C-11 DNR was 2.3-fold lower in 2780^{AD} than in A2780. The pharmacokinetics of a tracer dose and a pharmacological dose (10 mg/kg) of C-11 DNR were studied in Wistar rats. The results below were expressed as differential absorption ratios (counts/g organ: total injected counts/g body weight) 60 min post-injection (ns: not significant; *: $p < 0.05$).

Tracer dose	*ca* 100 ng/kg ($n = 3$)	10 mg/kg ($n = 4$)	
Heart	0.62 ± 0.07	0.78 ± 0.19	ns
Kidney	3.20 ± 0.73	1.83 ± 0.34	*
Liver	2.50 ± 0.76	0.64 ± 0.18	*
Lung	1.11 ± 0.21	1.51 ± 0.13	*
Muscle	0.45 ± 0.13	0.20 ± 0.08	*
Bone	0.75 ± 0.24	0.39 ± 0.05	*
Spleen	1.54 ± 0.33	2.57 ± 0.22	*
Urine	4.55 ± 1.80	9.29 ± 0.94	*
Brain	0.48 ± 0.06	0.12 ± 0.02	*
Blood cells	0.52 ± 0.08	0.10 ± 0.02	*
Plasma	0.91 ± 0.13	0.17 ± 0.04	*

Since the pharmacokinetics of C-11 DNR are dose dependent, the specific activity of the tracer may influence the measurement of tumor accumulation and tumor efflux of C-11 DNR with PET. Studies are in progress to evaluate the pharmacokinetics of C-11 DNR in animals bearing MDR positive and sensitive tumors.

23
Optimization of P-glycoprotein detection in formalin fixed paraffin embedded tissues utilizing antigen retrieval methods

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A study was carried out to determine the optimum immunohistochemistry (IHC) procedure for the detection of P-glycoprotein (P-gp) in tissues undergoing different conditions of fixation as might be encountered in a surgical pathology practice. Two cell lines, KB 3-1 and KB V-1 (low and high expressers of P-gp respectively) were harvested, fixed for 4, 8, 12, 24 and 48 h in formalin, placed in nylon bags and embedded into a single paraffin block. Multiple cuts of this block