serum cortisol value 1 h and 8 h after ACTH stimulation either on day 1 or on day 7. In summary, the lower dose of CsA produced increases in both baseline and ACTH stimulated cortisol levels, whereas the higher dose produced an increase in ACTH stimulated plasma cortisol but not in baseline levels. Possible mechanisms include an induction of cortisol biosynthesis, stimulation of cortisol secretion, inhibition of cortisol excretion or inhibition of cortisol catabolism. The role of P-gp in this effect is not yet clear because the CsA levels achieved at the first two doses are not sufficient for complete inhibition of the transporter. Moreover, the 6-day treatment regimen may have induced either P-gp or metabolic pathways independent of any acute inhibitory effect on the transporter. It is planned to explore the underlying mechanism further by reducing the number of doses of CsA to two and increasing the dosing progressively.

39
In vitro testing of MDR chemosensitizers in polarized HCT-8 human intestinal adenocarcinoma monolayer and suspension cultures

J Zacherl, G Hamilton, EP Cosentini and E Wenzl

University Clinic of Surgery, AKH, A-1090 Vienna, Austria.

Chemosensitizers (CSs) reverse multidrug resistance (MDR) by inhibiting P-glycoprotein (P-gp) action competitively, but *in vivo* usage is limited by toxic side effects in effective concentrations. In solid tumors typical microenvironmental conditions such as hypoxia and acidosis could play a role in reducing the clinical efficacy of CSs. The purpose of the present study was preclinical testing of CSs with an improved therapeutic index, namely dexverapamil (RVP, 1–10 μM), dexniguldipine (RNG, 0.25–5 μM), SDZ PSC 833 (PSC, 50–250 ng/ml) S 9788 (0.3–2.5 μM) and tamoxifen (TMX, 1–10 μM) in comparison to racemic verapamil (RSVP) in polarized cell layers. For representation of the effects of the above CSs we used (1) highly resistant ($R > 1400$ W.cm^2) HCT-8 monolayers, exhibiting directed P-gp-mediated transepithelial vinblastine (VIN) transport and (2) carried out flow cytometric rhodamine 123 efflux studies in HCT-8 suspension cells both under different extracellular pH (pHo: 6.8, 7.0, 7.5, 7.8) conditions known to prevail in solid tumors. (3) Overall CS-induced modulation of VIN toxicity in HCT-8 cells was shown in chemosensitivity assays (ChA) by [^{3}H]thymidine incorporation (HCT-8 IC_{50}: 6 μg/ml VIN). RVP, RNG, PSC, S 9788 and TMX inhibited transepithelial [^{3}H]VIN transport up to 50, 53, 55, 57 and 30% respectively. Resulting cell-associated drug content was increased about 3.4, 2.6, 3, 2.8 and 2.2 times, respectively. In contrast to RNG, PSC, S 9788 and TMX we found a 32% and 47% loss of P-gp inhibition potency of RVP at pHo 7.0 and 6.8, respectively, when compared to pHo 7.5. ChA revealed a 5-, 2.7-, 3.7-, 3.1- and 10-fold increase of VIN toxicity against HCT-8. In conclusion the newer CSs specifically developed as P-gp-directed drugs show high MDR-reversing activity in comparatively low concentrations in drug transport assay systems. Epithelial-like cell formations and acidic extracellular conditions, representative of solid tumors, induced no impairment of their activity, but the high residual drug resistance of CS-modulated HCT-8 cells (approximately 1 μg/ml VIN) indicates the major contribution of non-P-gp-based mechanisms to the overall resistance of carcinomas.

40
Inhibition of carboxylesterases activity and reversal of MDR

Olivera Markovic, Nenad Markovic and Svetomir Markovic

Medical College of Pennsylvania, Philadelphia, PA 19129, USA.

It has been demonstrated that several chemosensitizers such as chlorpromazine, quinidine, quinine and chloroquine have one common denominator: they are all potent inhibitors of intracellular carboxylesterases. This effect was obtained on a number of freshly isolated and cultured malignant and normal cells using Image Analysis technology for intracellular measurement of enzyme activity. This inhibitory effect was confirmed on purified porcine liver carboxylesterase. In a preliminary study, we have also demonstrated an increase of carboxylesterase activity in P-glycoprotein (P-gp) positive, doxorubicin resistant KB-A1 cell line cells in comparison with the sensitive parent cell line,[1] as well as the augmentation of carboxylesterase activity in the K562 cell line, along with the acquisition of resistance to doxorubicin. This work suggests that new chemosensitizers might be expected among inhibitors of detoxifying carboxylesterases.

1. Both KB cell lines were kindly provided by Dr Michael Gottesman, NCI, NIH, Bethesda, MD, USA.

Supported in part by Sterling Winthrop, Parmaceuticals Research Division, Collegeville, PA and the Eberhard Foundation, Malvern, PA.

41
Approaches to reverse anthracycline (AX) resistance due to pleiotropic multidrug resistance (MDR1) in the human lymphoblastic leukemia line CCRF-CEM-DAC

S Bielack, K Beer, G Looft, P Kapaun, D Grabowski, K Winkler and R Erttman

Department of Pediatric Hematology and Oncology, University of Hamburg, 20246 Hamburg, Germany.

MDR1 is an important cause of AX treatment failure. CCRF-CEM-DAC (donated by H Diddens, Med.Laserzentrum