each treatment but the expression of TRAIL (TNF-related Apoptosis inducing ligand) was dramatically increased by the classic retinoids not by 4-HPR.

But the cells were treated with human recombinant TRAIL there was no significant induction of apoptosis and the Bcl-XL transfected cells were registant to the retinoids. So we concluded that the retinoids induced apoptosis in NIH:OVCAR-3 cell is mediated by the paracrine action of TRAIL only with the disruption of Bcl-XL:Bax ratio.

LB242

Molecular Targets in Colon Cancer:Cellular Efflux Pathways for Cyclic GMP

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Intracellular cGMP is influenced by receptor-guanylate cyclases (R-GC) and cGMP-phosphodiesterases (PDE). However, efflux of cGMP from cells is another fundamental mechanism. Uroguanylin (UGN) activates intestinal R-GC, elicits apoptosis in colon cancer cells and decreases tumors in the Min mouse. Sulindac sulfone (SSo) also raises cGMP and causes apoptosis. Herein, we report that SSo is a potent inhibitor of cGMP efflux (i.e. IC50-0,01mM) from T84 and CaCo-2 colon cancer cells. SSo also inhibits cGMP-PDEs in T84 cells with an IC50 of 1mM and potentiates the cGMP responses to UGN, which is accompanied by SSo-mediated inhibition of cGMP efflux. In prostate PC-3 cancer cells, cGMP is increased by ANP and CNP, and SSo markedly reduces cGMP efflux from stimulated cells. Two ABC proteins, MRP-4 and MRP-5, are cGMP pumps and mRNAs for the transporters are expressed in T84, CaCo-2, PC-3 and other cancer cells. Thus, SSo raises cGMP in cancer cells by inhibition of cGMP efflux and cGMP-PDEs and both actions may contribute to cGMP-induced apoptosis. MRP-5/4 are cellular targets for development of novel therapeutic/preventive agents that raise cGMP levels in cancer cells.

CARDIOVASCULAR PHARMACOLOGY/TOXICOLOGY

LB243

A Pharmacological Dissection of Canine and Rat Ventricular Ito

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It has been suggested that Ito in canine Purkinje fibers is pharmacologically distinct from Ito in canine ventricular myocytes (Nattel, 2000). In this study we evaluated the functional differences in canine ventricular Ito and compared them to rat ventricular Ito using standard microelectrode techniques at 37o C in Tyrode's [K+= 4mM]. Syncytial preparations included canine Purkinje fibers, ventricular papillary muscle, and ventricular mid-myocardial "M" cells, and rat ventricular papillary muscle. Tetraethyl ammonium (TEA), 2.5 mM, and 4-aminopyridine (4-AP), 100uM, resulted in positive block of Ito in both canine Purkinje and rat papillary preparations. This effect was absent in both canine ventricular "M" cells and papillary muscles. It is important to note that these agents, at the concentrations tested, have been reported to have no blocking effects on any of the Ito candidate subunits (Kv1.4, 4.2, and 4.3) in heterologous expression systems. Conclusion: Canine Purkinje Ito is pharmacologically distinct from other canine ventricular Ito, but similar to rat ventricular Ito. This may reflect a difference in subunit stoichiometry or a novel subunit interaction in the respective currents.

LB244

Pravastatin Preserves Vasomotor Response in Atherosclerotic Arteries After Balloon Angioplasty

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It has been shown that pravastatin, enhances endothelial cell nitric oxide production. This study was conducted to evaluate the effect of pravastatin on vasomotor response following balloon angioplasty (BA) in normal and atherosclerotic rabbit arteries. Three normal and three atherosclerotic NZW rabbits were sacrificed and carotid arteries were dissected and placed in a dual perfusion chamber. Arteries were perfused with PBS at 37oC and 60 mmHg. One artery was exposed to pravastatin (100 mM) and one was control. BA was performed in arteries and challenged with acetylcholine (2'10-5 M) and nitroprusside (2'10-5 M) in norepinephrine (2'10-6 M) preconstricted arteries. In normal arteries, acetylcholine did not demonstrate significant difference in percent lumen dilation between control and pravastatin (25.5±10.4 vs 16.6±7.5, p=ns) while atherosclerotic arteries had preserved vasomotor response with pravastatin (16.9±7.2 vs 33.6±18.2, p<0.005). Similar results were noted with nitroprusside in normal arteries (29.0±14.5 vs 18.0±10.5, p=ns) and atherosclerotic arteries (18.6±7.4 vs 38.4±19.8, p<0.003). Pravastatin preserved vasomotor response in atherosclerotic arteries following BA when compared to normal arteries. This effect may be due to an enhanced production of nitric oxide in atherosclerotic arteries. However, pravastatin also appears to influence vasomotor response by either non-endothelial dependent or a combination of endothelial and non-endothelial dependent mechanism.

LB245

2D-Analysis of myocardial protein expression following myocardial ischemia and reperfusion in rabbits.

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Myocardial ischemia and reperfusion injury (MI/R) can be related to leukocyte activation, release of cytokines and activation of the complement system.

In the present study we analyzed the myocardial protein expression following MI/R with and without complement inhibition (FUT-175) in a rabbit model of 60min I + 180min R. FUT-175 significantly reduced myocardial injury, i.e. creatine kinase release (p<0.05). Similar, histological analysis demonstrated reduced myocardial tissue injury and reduced leukocyte accumulation following FUT-175 treatment. Further, the myocardial protein expression was analyzed by two-dimensional gel electrophoresis following MI/R. The protein patterns were evaluated by MELANIE III a computer-assisted gel analysis system. The biochemical identification of the proteins of interest was achieved using nanoHPLC/ESI-MS/MS. On average, 509±25 protein spots were found on the gels. We analyzed 10 spots which were significantly altered, by using massspectrometry. Superoxide dismutase and aB-crystallin were identified. Expression of the two identified proteins decreased by half in the vehicle group when compared to sham treated animals. Treatment with Fut-175 preserved significantly superoxide dismutase and aB-crystallin protein expression when compared to vehicle treated animals.

The results present profound differences in myocardial protein expression after ischemia and reperfusion and following treatment with the complement inhibitor FUT-175.

LB246

Cardioprotective Effects of Translation-Inhibitor Puromycin in Rabbit Myocardial Ischemia and Reperfusion

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Myocardial tissue injury following ischemia (I) and reperfusion (R) is to a large amount related to neutrophil accumulation with inflammatory responce. These events trigger translocation of nuclear transcription factors (i.e., NFkB), mRNA synthesis and de novo protein expression.

We studied the effect of puromycin, known to block translation in a rabbit model of 60min. myocardial I followed by 180min of R. Puromycin (0,4mg/kg KG) or its vehicle were injected 5 minutes prior to reperfusion. Myocardial injury following Puromycin treatment was significantly reduced compared to vehicle treated animals (11%±2.7% vs 29%±2.1% necrosis related to ischemic myocardium, p<0.05). Plasma creatine kinase (CK) activity, another marker for myocardial injury, increased from 2.5±0.4 IU/g protein at baseline to 33.3±2.3 IU/g protein following 3 hours of reperfusion in the vehicle group. Administration of Puromycin significantly decreased plasma CK release (14.2 ± 1.5 IU/g protein, p<0.05). In the necrotic zone MPO activity, a marker for PMN accumulation, was significantly decreased in Puromycin treated animals compared to the vehicle group (p<0.01). In the myocardium of vehicle treated animals histologic analysis demonstrated increased leukocyte accumulation. In contrast, Puromycin treatment significantly decreased PMN accumulation (p<0.01).