133.12

Role of nitric oxide synthase and cyclooxygenase2 on cyclophosphamide-induced cystitis, in rats

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The role of inducible nitric oxide synthase(iNOS) and of cyclooxigenase2(COX2) on the pathogenesis of cyclophosphamide(CYP)-induced cystitis was investigated in rats. CYP induced marked cystitis with plasma protein extravasation(PPE). Rats received one of these treatments: a)saline; b)CYP 75 mg/kg; c)CYP+methylthiourea (MITU,10mg/Kg; 2 doses, 30min before and 3hrs after CYP);d) meloxicam(MEL,10mg/kg 12hrs before CYP and 5 mg/kg 30min before CYP); e)Rofecoxib(ROF,15mg/kg, 30min before CYP)and g)CYP+MITU+MEL. Bladder-PPE was measured by Evans blue technique,6hrs after CYP.

Results (PPE as μgEB/g tissue): ***p<0.0001; **p<0.005 to CYP alone Control:30±2.5; CYP:328±30.5; CYP+MITU:51±15.9 ***; CYP+MEL: 166±30.5** CYP+ROF: 290±73.0; CYP+MITU+MEL:84±25.0***

We have previously shown that blockade of NK1 receptors ameliorate CYP-induced cystitis. Increased NO formation due to iNOS induction seems to be the major factor producing the inflammatory changes. Inhibition of COX also protects; a combination of the iNOS inhibitor plus MEL, did not further enhance the effects of the iNOS inhibitor. These results suggest that activation of NK1 receptors by sP and/or increases in NO production may stimulate COX activity contributing to inflammation. The greater protection of MEL over ROF suggests that COX1 may also play a role.

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133.13

Sodium nitroprusside up-regulates heme oxigenase-1 via protein kinase A pathway in RAW 264.7 cells

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Heme oxygenase (HO) is the rate-limiting enzyme of heme degradation, and its induction is considered to be protective against oxidative stress. Sodium nitroprusside (SNP) is generally used as NO donor in many experiments. NO directly activates the effector cells and/or produces cGMP in the target cells which, in turn, elicits biological actions. In macrophage cells, the involvement of cyclic nucleotides in the induction of HO is not entirely clear. The aim of the present study was to know the signal(s) involved in the induction of HO-1 by SNP in RAW 264.7 cells. SNP induced time- and concentration-dependent HO-1 expression, in which optimal condition was showed as 500 μM SNP and 6 h incubation time. Expression of HO-1 mRNA by SNP was inhibited by PKA inhibitor, H89 (30 μM) but not soluble guanylate cyclase inhibitor, LY83583 (30 μM), indicating that cGMP is not involved in inducing HO. Forskolin and isoproterenol, concentration-dependently, up-regulated HO-1 protein which was inhibited by H89 and propranolol, respectively, confirming that cAMP plays a crucial role in the induction of HO-1 expression in RAW 264.7 cells. Not deferoxamine, iron chelator, but carboxy-PTIO, NO scavenger, partially blocked HO-1 protein expression, suggesting that NO may not be the sole factor contributing HO induction by SNP. We concluded that SNP transcriptionally regulates HO-1 expression via PKA pathways, which requires further investigation.

PULMONARY PHARMACOLOGY/TOXICOLOGY (134.1 – 134. 41)

134.1

Interspecies Dosimetry Models for Pulmonary Pharmacology

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Inhalation exposures tests with surrogate animals can be performed to estimate the therapeutic effects of pharmacologic drugs. However, it is difficult to extrapolate the findings of animal tests to human conditions. In this work, interspecies dosimetry models especially designed for implementation with pharmaceutical protocols are presented. The mathematical model was tested with data from surrogate (rat) simulations, and theoretical predictions agreed well with experimental particle deposition measurements. For human subjects, appropriate algorithms for morphologies and ventilatory parameters were used as subroutines in the validated model. We conducted a comprehensive series of interspecies computer simulations describing the behavior of inhaled aerosols. By a priori determining the laboratory conditions necessary for animals tests to accurately mimic human conditions, the use of interspecies models is very cost effective. We propose, therefore, that models be actively integrated into pulmonary pharmacology studies.

Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

134.2

A novel cysteinyl-leukotriene functional receptor in the human lung.

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Cysteinyl-leukotrienes (cys-LTs) are known to contract muscle preparations by activation of either $CysLT_1$ or $CysLT_2$ receptors. $CysLT_1$ antagonists (MK571 and ICI198615) do not block the cys-LT contractions in human pulmonary veins. There is little information on antagonism of cys-LT contractions in human pulmonary arterial preparations (HPA). The aim of this study was to characterize the CysLT receptors in HPA. HPA ring preparations, where the endothelium had been removed, were set up in organ baths (10ml) containing Tyrode's solution and indomethacin (INDO, 1.7μM). Concentration-effect curves were produced with either LTC_4, LTD_4 or LTE_4 in the absence (control) or presence of either MK571 (1μM; 30min), ICI198615 (1μM; 30min) or BAYu9773 (3μM; 30min). The pEC_{50} values were calculated (-log M of EC_{50} value) and pK_B values were determined for the antagonists.

LTC_4 and LTD_4 were equipotent in contracting HPA (pEC_{50}, 7.61±0.07 and 7.96±0.09, respectively, n=20-22 lung samples). LTE_4 did not contract HPA. LTC_4 contractions were antagonized by MK571 (pK_B=7.02±0.36) ICI198615 (pK_B=7.20±0.38) and BAYu9773 (pK_B=6.26±0.26). These antagonists did not modify the LTD_4-induced contractions. The findings suggest the presence of a $CysLT_1$ receptor with low affinity for $CysLT_1$ antagonists and a $CysLT_3$ receptor on HPA.

134.3

DIFFERENCES IN BIOELECTRIC RESPONSES TO HYPEROSMOLARITY IN EPITHELIUM OF FRESH TRACHEAL SEGMENTS (FE) AND AIR-LIQUID INTERFACE EPITHELIAL CELL CULTURES (CE) FROM GUINEA PIGS.

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In guinea-pig perfused trachea preparations, application of methacholine (MCh) to the serosal surface elicits contraction of the airway smooth muscle. Increasing the tonicity of the modified Krebs-Henseleit solution (MKHS) bathing the epithelium with D-mannitol (D-M) causes an epithelium-dependent relaxation. In this study, we compared bioelectric responses of FE and CE (30% ciliated cells) to these agents using Ussing chambers. When applied to the serosal surface of FE, MCh (3 10-7 M) elicited a smooth, monotonic increase in short circuit current (Isc). The subsequent addition of D-M (0.27 - 266.8 mosM added) to the mucosal MKHS of FE elicited D-M concentration-dependent decreases in Isc. In contrast to responses of FE, CE responded to the serosal application of MCh with a rapid, transient increase in Isc, which was followed by a sustained plateau. Also in contrast to responses of FE, cumulative increases in osmolarity with D-M (0.27-84.3 mosM) added to the mucosal compartment led to increases in Isc, but further increasing the osmolarity(84.3-266.8 mosM) decreased Isc and decreased transepithelial resistance concomitantly. The change in the responses of CE might reflect the absence of released or constitutive substances in the airway wall, or a phenotypic modification occurring under culture conditions. Source of research support: NIOSH

134.4

Mexiletine inhibits pharmacological actions of salbutamol through blockade of β_2-adrenoceptors in bovine tracheal smooth muscle

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Mexiletine, a class Ib antiarrhythmic drug, has been shown to prevent the irritant-induced bronchoconstriction, however, the effect of this drug on the airway smooth muscle has not yet been investigated intensively. In this study, we examined the effect of mexiletine on tension and salbutamol- and forskolin-induced relaxant responses of bovine tracheal smooth muscle contracted with methacholine (0.3 μM). Mexiletine (5-500 μM) concentration-dependently attenuated the salbutamol-induced relaxation and accumulation of adenosine 3',5'-cyclic monophosphate. On the other hand, mexiletine did not affect the forskolin-induced responses. In radioligand binding experiments, mexiletine concentration-dependently displaced the specific binding of [^{125}I]-cyanopindolol

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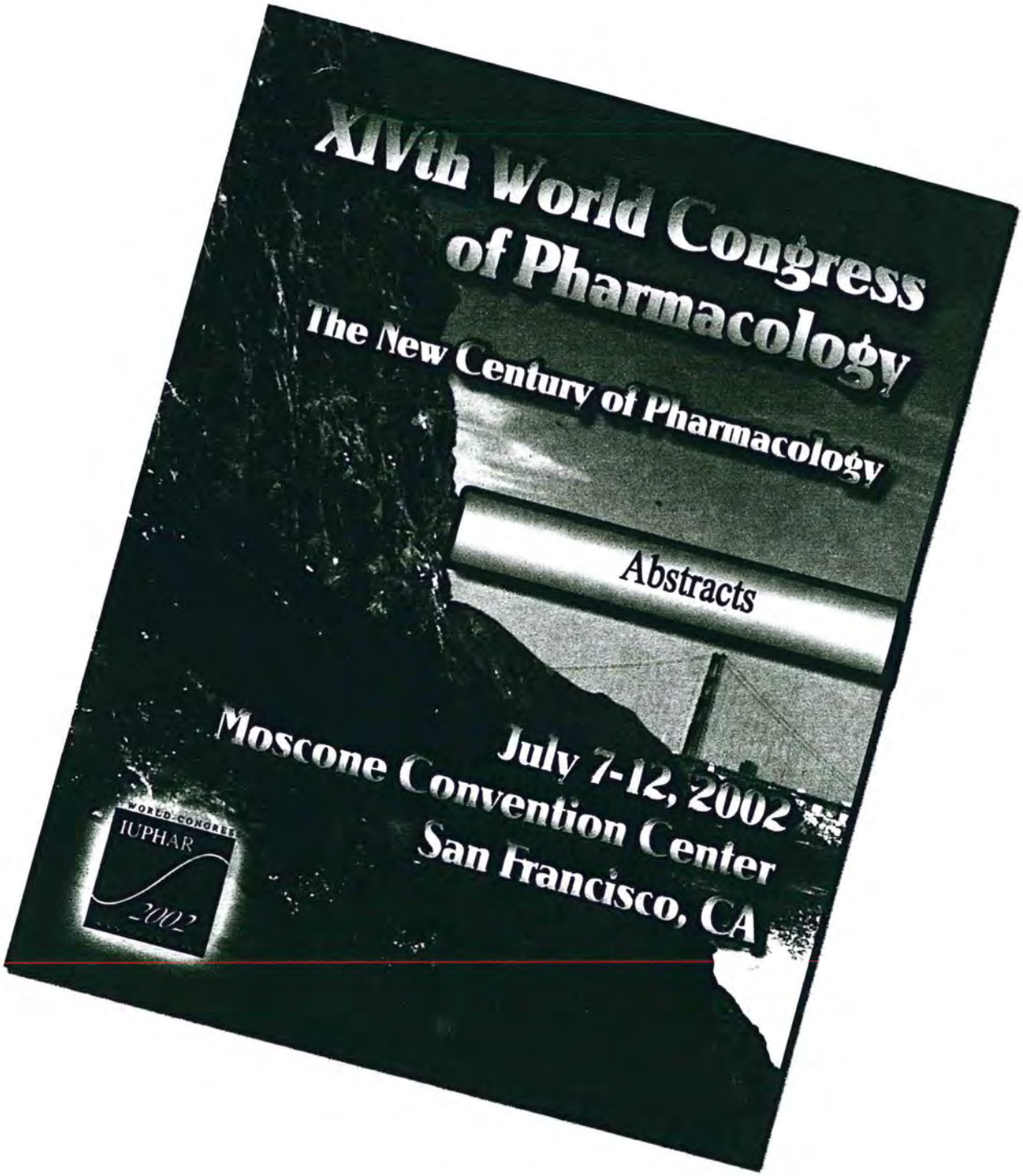

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