1833 INHIBITORY EFFECTS OF NICOTINE ON INFLAMMATION AND LEUKOCYTE MIGRATION.

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Cigarette smoking is a major cause of morbidity and mortality worldwide; additionally, smokers take longer to recover from injuries. Conversely, smokers have a lower risk for some inflammatory diseases and nicotine may have a therapeutic potential in some inflammatory diseases. We have reported that nicotine, a major component of cigarette smoke, suppresses the antibody response and T cell function. Using the turpentine-induced sterile abscess model, where inflammation is accompanied with increase in body temperature (fever), we show that chronic treatment of LEW rats with nicotine suppresses the fever response as well as the accumulation of leukocytes at the site of turpentine injection. Thus, in addition to adaptive immunity, chronic nicotine also suppresses the innate immune responses (i.e., inflammation and fever). To understand the mechanisms underlying the anti-inflammatory effects of nicotine, PBMCs from control and 3-wk nicotine-treated animals were analyzed for migratory responses to chemotactic stimuli in the transwell system. Our results show that, cells from nicotine-treated animals exhibit significantly lower migration in response to the neutrophil chemoattractant, formyl-methionyl-leucyl-phenylalanine (fMLP), or the lymphocyte chemoattractant, monocyte chemoattractant protein-1(MCP-1). Thus, chronic nicotine exposure inhibits the migration of leukocytes toward the site of inflammation. This effect might stem from the inability of leukocytes to respond to chemoattractants. Because inflammation is critical for wound healing process, the property of nicotine to moderate leukocyte migration might explain the suppressive effects of smoking in some inflammatory diseases and wound repair, and the therapeutic potential of nicotine in moderating some inflammatory diseases. Supported by a grant from NIDA (DA04208).

1834 ROLE OF P38 MAP KINASE IN REGULATING THE INHIBITORY EFFECTS OF UVB LIGHT ON CYCLOOXYGENASE-2 EXPRESSION IN MOUSE MACROPHAGES.

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Ultraviolet light of high energy and shorter wavelengths (UVB, 290-320 nm) is toxic to the skin. In normal skin, UVB light can induce an inflammatory response while in diseased tissue, it exhibits anti-inflammatory and immunosuppressive activity. It is well recognized that cells in the skin release lipid mediators, including prostaglandins (PGs) and leukotrienes that promote inflammation. The synthesis of PGs is dependent on the activity of cyclooxygenase (COX), an oxidoreductase that converts arachidonic acid into the common PG precursor, PGH_2. An inducible form of the enzyme known as COX-2 is expressed in the skin during inflammation. In earlier work we reported that UVB light is a potent inhibitor of lipopolysaccharide (LPS)-induced COX-2 expression in macrophages, a cell type known to mediate inflammation in diseased tissue. In the present studies, we examined the mechanism. Using RAW264.7 macrophages, we found that UVB light (2.5-25 J/cm^2) rapidly phosphorylates and activates the c-Jun-NH_2-terminal kinase (JNK), p44/42 mitogen-activated protein (MAP) kinase [extracellular signal-regulated kinase 1/2 (ERK1/2)], and p38 MAP kinase. PD-98059, an inhibitor the ERK1/2 kinase, and SP600125J, an inhibitor of the JNK kinases, were effective inhibitors of UVB light-induced ERK1/2 and JNK phosphorylation, respectively, in the macrophages. However, the inhibitors did not alter UVB light-induced inhibition of COX-2 expression. Unexpectedly, SB-203580 [(4-(4-Fluorophenyl)-2- (4-methylsulfinylphenyl)-5-(4-pyridil)imidazole], a p38 kinase antagonist, was found to block UVB light-induced p38 phosphorylation and inhibition of LPS-induced COX-2 in the macrophages. Taken together, these data indicate that activation of p38 MAP kinase plays an important role in mediating UVB light phototoxicity. Selective modulation of p38 MAP kinase may be an important target for treating epidermal inflammatory diseases. Support: NIH ES06897, ES03647 and ES05022

1835 COMPARISON OF THE ALLERGENIC POTENCY OF ALPHA-HEXYLCINNAMALDEHYDE (HCA) AND 2-MERCAPTOBENZOTHIAZOLE (MBT) IN SIX STRAINS MICE IN MURINE LOCAL LYMPH NODE ASSAY (LLNA).

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Keyword LLNA The Murine Local Lymph Node Assay (LLNA), which has been accepted by the OECD since 2000 as a stand alone alternative to the Guinea Pig Maximization Test (GPMT), has been largely used for assessing the allergic contact dermatitis potential of chemicals. According to OECD Draft Guideline No. 429 [November 2000] the animal species of choice are CBA/Ca or CBA/J mice. To test and compare the suitability of these and other mouse strains for assessing the allergenic potency of a chemical, two positive-control chemicals (HCA and MBT) were tested at three doses in the following six mouse strains at RCC: CBA/CaOlaHsd, CBA/Ca (CruBR), CBA/Jlbm (SPF), CBA/JNCrj, Balb/c and NMRI. For each strain, test groups of four mice were topically treated with HCA or MBT at 5%, 10% or 25% (w/w) in acetone:olive oil, 4:1 (v/v) on three consecutive days. A control group for each strain of mouse was treated with vehicle only. Five days after the first topical application the proliferation capacity of the lymph node cells was determined by their incorporation of 3HTdR, which was measured in a beta-scintillation counter, compared with that recorded in negative control groups. The experimental results are briefly summarized in Table 1. The test results indicate that CBA/CaOlaHsd mice should be the first choice for assessing allergenic potency. Balb/c mice are also a potential test strain for LLNA tests. CBA/Ca (CruBR) and NMRI strains of mice are not suitable for assessing the allergenic potency of chemicals.

Strain	HCA			MBT		
	5% (w/v)	10% (w/v)	25% (w/v)	5% (w/v)	10% (w/v)	25% (w/v)
CBA/CaOlaHsd	5.0	10.5	44.7	5.3	8.6	6.6
CBA/Ca (CruBR)	1.4	1.3	2.6	1.2	2.3	4.1
CBA/Jlbm (SPF)	1	1.8	8.5	1.3	8	5.4
CBA/JNCrj	2.3	4.5	8.2	1.7	3.6	0.6
Balb/c	6	3.8	9.6	6.1	9.6	7.5
NMRI	0.8	1.9	3.5	1.2	1	1.2

1836 INFLAMMATORY RESPONSE AND FREE RADICAL FORMATION IN SKIN OF B63CF1 MICE WITH DIMINISHED LEVELS OF GLUTATHIONE AFTER PHENOL EXPOSURE.

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A number of phenolic compounds that are utilized in industry (e.g., for production of resins, paints, lacquers, cosmetics and pharmaceuticals) are toxic to skin (i.e., can cause rash, dermal inflammation, contact dermatitis, leucoderma, and/or cancer promotion). The biochemical mechanisms of dermal toxicity of phenolic compounds are not well understood. We observed alpha-phenyl-N-*tert*-butylnitrone (PBN) spin-trapped free radicals generated in the skin of female B6C3F1 mice after topical exposure to phenol (3.5 mmol/kg, 100 µL, 1 h). The exposure also resulted in oxidation of GSH and protein thiols, and decreased levels of total antioxidant reserves and vitamin E in the skin. We also compared the effects of phenol in mice with normal or diminished levels of GSH. The magnitude of phenol-induced PBN-spin-trapped radical adducts in skin of mice with diminished levels of GSH (either pre-treated with DL-buthionine sulfoximine, BSO, or 1, 3-bis(2-chloroethyl)-1-nitrosourea, BCNU) was remarkably higher as compared to those in mice treated with phenol alone. Topical exposure to phenol also resulted in increased inflammatory cell infiltration in the skin of mice pre-treated with BSO or BCNU. To identify mediators involved in skin inflammation after phenol and phenol plus BSO exposure, we used JB-6 mouse epidermal cells. Using ELISA, we found that phenol and BSO plus phenol induced increases in IL-1β and prostaglandin E_2 production in the cells as early as 1 h post-treatment. Real time PCR of ICAM-1 revealed mRNA expression starting at 3 h after exposure of the cells to phenol and BSO plus phenol. Additionally, western blot analysis showed higher expression of COX-2 in cells exposed to BSO plus phenol. In the skin, we also observed mRNA expression of COX-2 and IL-1β in mice treated for 2 h with phenol or BSO plus phenol. These data indicate that mediators, such as IL-1β, ICAM-1 and prostaglandin E_2, are involved in early stages of skin inflammation in response to phenol and phenol plus BSO.

1837 EVALUATION OF THE PHOTOTOXIC AND PHOTOALLERGIC POTENTIAL OF METHYL N-METHYL ANTHRANILATE.

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Methyl N-methylanthranilate, which occurs naturally in many citrus oils, is used in both fragrances and flavors. Earlier studies reported that methyl N-methylanthranilate was phototoxic in hairless mice at a concentration of 50% in methanol and in humans at a concentration of 5% in hydrophilic ointment. Further studies were conducted to determine if a no-effect level for phototoxic effects in humans could be established. Phototoxicity was evaluated using a 24-hour occluded application of