1162 PERINATAL EXPOSURE TO Δ-9-TETRAHYDROCANNABINOL (THC) INDUCES APOPTOSIS IN THE FETAL THYMUS

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Marijuana, or Cannabis sativa, is one of the most widely used drugs of abuse worldwide. Previous studies from our laboratory have shown that THC, the major psychoactive component of marijuana, induces immunosuppression by triggering apoptosis in the thymus and the spleen. However, very little is known about the effects of THC on the fetal immune system development and homeostasis. In this study, we treated pregnant mice with THC on day 16 of gestation, which corresponded to the beginning of fetal T cell development, and found that the treatment resulted in a dose- and time-dependent decrease in fetal thymic cellularity. This correlated with an increase in the level of apoptosis, as demonstrated by TUNEL staining and cleavage of caspase-3 and 7. In addition, we found that THC induced apoptosis through both the cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), as its effects could be blocked by both CB1- and CB2-inhibitors in vivo. Finally, we found that perinatal exposure to THC resulted in a transient decrease in the percentage of double-positive T cells found in the fetal thymus. Together, these data suggest that perinatal exposure to THC can have a profound effect on the development of T cells in the fetus leading to immunosuppression, which bears tremendous implications for mothers abusing marijuana during pregnancy (Supported by NIH grants R01 ES009098, R01 DA016545, R01 HL058641, R01 AI058300 and R01 AI053703).

1163 NITROSATIVE STRESS INDUCES PHOSPHATIDYLSERINE EXTERNALIZATION: SIGNALING ROLE IN PHAGOCYTOSIS

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Aminophospholipid translocase (APT) is responsible for asymmetric distribution of phosphatidylserine (PS) across plasma membrane. The enzyme contains catalytically competent cysteines whose oxidation/alkylation results in loss of its activity. Because protein S-nitrosylation can act as a regulatory redox-sensitive mechanism, we hypothesized that nitrosative stress enhances PS externalization in cells by inhibiting APT activity. This pathway should be particularly important during inflammation whereby oxidative/nitrosative burst generated by macrophages may cause direct nitrosylation or trans-nitrosylation of APT in target cells. To experimentally address this hypothesis we utilized HL-60 cells that express high activity of APT. S-nitroso-L-cysteine-ethyl ester (SNCEE) and S-nitroso-glutathione (GSNO) were used as prototypical cell-permeable and cell impermeable trans-nitrosylating reagents. HL-60 cells externalized PS in response to SNCEE or GSNO treatment (50-100 μM, 0.5h at 37oC) as evidenced by annexin V binding assay and fluorescence microscopy. No cytotoxic effects were induced by either of the trans-nitrosating agents. RAW 264.7 macrophages elicited enhanced phagocytizing activity towards "nitrosylated" HL-60 cells. We speculate that our proposed mechanism of macrophage induced nitrosative stress contributes to effective clearance of apoptotic cells and regulates switching of acute inflammatory response to anti-inflammatory phase as has been observed in the lung and in the brain in in vivo experiments with inhalation of single-walled carbon nanotubes and cortical trauma, respectively. Supported by NIH HL70755, NIOSH OH008282, ES09648, AHA0535365N, Human Frontier Science Program.

1164 ACTIVATION OF INTRACELLULAR TRPV1 INDUCES ER STRESS RESPONSE AND CELL DEATH

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The endoplasmic reticulum (ER) is the major site of protein folding in cells. Depletion of ER calcium can cause unfolded or misfolded proteins to accumulate, inducing ER stress, cell cycle arrest and possibly cell death. Activation of Eukaryotic Translation Initiation Factor 2α Kinase 3 (EIF2AK3) during the unfolded protein response, induces transcription of activating transcription factor 4 (ATF4), which subsequently upregulates transcription of DNA damage-inducible transcript 3 (DDIT3). These events drastically alter transcription in the cell. Preliminary data suggests that treatment of human lung bronchial epithelial (BEAS-2B) cells with capsaicin induces ER stress through activation of the vanilloid receptor 1 (TRPV1) which is present in the ER membranes of these cells. Altered transcription of several ER stress markers was identified using one-way SAM analysis of cDNA microarrays after treatment with capsaicin. Transcriptional modulation of DDIT3, DDIT1, ATF3, Stanniocalcin 2 (STC2), Cyclin G2 and Cyclin D1 were assessed. DDIT3, DDIT1, ATF3, Cyclin G2 and STC2 were significantly increased in cells treated with capsaicin while Cyclin D1 was reduced. Selective knockdown of EIF2AK3 via pretreatment with a gene-specific siRNA significantly attenuated these responses. ER stress responses and cell death were inhibited by LJO-328, a cell permeable TRPV1 antagonist, but not by two inhibitors of calcium flux originating from extracellular sources, EGTA and Ruthenium Red. These findings support our hypothesis that capsaicin is cytotoxic through the induction of ER stress via activation of TRPV1 receptors present in the ER of these cells. This work was supported by HL069813.

1165 GONIOTHALAMIN INDUCES APOPTOSIS IN VASCULAR SMOOTH MUSCLE CELLS

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Restenosis represents a major impediment to the success of coronary angioplasty. Abnormal proliferation of vascular smooth muscle cells (VSMCs) has been shown to be an important process in the pathogenesis of restenosis. A number of agents, particularly rapamycin and paclitaxel, have been shown to impact on this process. This study was to determine the mechanism of cytotoxicity of goniothalamin (GN) on VSMCs. Results from MTT cytotoxicity assay showed that the IC50 for GN was 4.4μg/ml (22μM) which was lower compared to the clinically used rapamycin (IC50 of 25μg/ml [27.346μM]). This was achieved primarily via apoptosis where up to 25.83 ± 0.44% of apoptotic cells were detected after 72 hr treatment with GN. In addition, GN demonstrated similar effects as rapamycin in inhibiting VSMCs proliferation using BrdU cell proliferation assay after 72 hour treatment at IC50 concentration ($p>0.05$). In order to understand the mechanisms of GN, DNA damage detection using comet assay was determined at 2 hour post-treatment with GN. Our results showed that there was a concentration-dependent increase in DNA damage in VSMCs prior to cytotoxicity. Moreover, GN effects were comparable to rapamycin. In conclusion, our data show that GN initially induces DNA damage which subsequently leads to cytotoxicity primarily via apoptosis in VSMCs. (Supported by IRPA Grant 06-02-04-0374-PR0014/06-05 &SAGA Grant NN-001-2005)

1166 ALTERATION OF THE HYPOTHALAMIC-PITUITARY GONADAL(HPG) AXIS IN WISTAR MALE RATS FOLLOWING A PREPUBERTAL EXPOSURE TO THE CHLOROTRIAZINE HERBICIDE SIMAZINE

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Chlorotriazine herbicides, such as atrazine and simazine (SIM), are used extensively in the U.S. each year and both parent compounds and metabolites are detected in ground water in areas of major usage. We found previously that atrazine suppresses serum luteinizing hormone and prolactin in the female rat by altering hypothalamic regulation. We have also demonstrated that puberty was delayed in the male and female rat when exposed to atrazine during the juvenile period. In addition, the chlorotriazine metabolites were equally potent in delaying puberty, which led to concern about cumulative effects of the metabolites and other parent compounds on pubertal development. For these reasons, we hypothesized that SIM, like atrazine, would delay pubertal progression in the male rat. In two separate experiments, males were administered SIM (0, 3.13,6.25, 12.5, 25, 50, 75, 100, 150 and 300 mg/kg) by oral gavage from postnatal day (PND)23 to 53. Males were monitored for preputial separation (PPS) and necropsied on PND 53. In both experiments, puberty was significantly advanced at 25 mg/kg. Consistent with the lower dose advancement of PPS, there was a non-monotonic dose response, with an elevation in LH, testosterone and androstenedione at the lower doses (6.25 to 75 mg/kg). There was a corresponding delay in PPS at 300 mg/kg and a decrease in seminal vesicle and prostate weights at the highest doses (150 to 300 mg/kg), with LH concentrations returning to normal. In conclusion, SIM appears to have a bimodal effect on puberty, with an increase in androgens and corresponding advancement in puberty at the lower doses and a delay in reproductive tract development in the higher doses. The fact that the two lower doses of SIM increased serum androgens and LH on PND53 and advanced PPS in the developing male would indicate that SIM activates the HPG axis. Further studies are planned to elucidate this activation and the specific mechanisms of these effects on male development. This abstract does not necessarily reflect EPA policy.

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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