Immunotoxicity of 2,3,7,8-Tetrachlorodibenzo-\textit{p}-dioxin (TCDD) and Diethylstilbestrol (DES) in the Fetal Mouse Thymus and Liver

By

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Abstract

Diethylstilbestrol (DES) and 2,3,7,8-tetrachlorodibenzo-\emph{p}-dioxin (TCDD) have been identified as immunotoxicants causing thymic atrophy, thymocyte hypocellularity, phenotypic changes detected by CD4 and CD8 surface antigens, and progenitor T-cell targeting in the fetal mouse. We hypothesized that gestational exposure to these two compounds may lead to comparable histologic and gene expression alterations in the fetal mouse thymus and liver. Treatment of pregnant C57Bl/6 mice with doses of 5 or 10 $\mu$g/kg TCDD or 48 $\mu$g/kg DES by oral gavage on gestation days (gd) 14 and 16 severely depressed day 18 thymic cellularity. Histologic evaluation of day 18 fetal thymuses showed disruption of normal cortico-medullary architecture after TCDD or DES. Decreased thymocytes density was noted primarily in cortical zones where pyknotic cells were increased by either TCDD or DES treatment. Using day 18 thymocyte suspensions and flow cytometry, 7-AAD showed decreases in viable thymocytes from TCDD- or DES-treated fetal mice, and concomitant increases in thymocytes in early apoptosis. When thymocytes were do-identified with CD4 and CD8 cell surface antigen expression, enhanced apoptosis occurred in CD4$^+$CD8$^+$ phenotype after TCDD treatment. After DES exposure, increased apoptosis occurred in CD4$^-$CD8$^-$ and CD4$^-$CD8$^+$ thymocytes. Both TCDD and DES increased liver to body weight ratios and decreased ratios of hematopoietic cells to hepatic cells present. Cytomegaly was seen in hepatocytes of TCDD and DES treated animals, and these cells had more variable features, such as increased cytoplasmic basophilia and more prominent nucleoli. Real time quantitative PCR demonstrated that DES decreased c-jun, bcl-2, and PKC$\alpha$ mRNA expression. These results suggest a shift away from proliferative activity and may reflect alterations noted predominantly in the hematopoietic population. TCDD increased c-jun mRNA expression with modest decreases in PKC$\alpha$, and marked decreases in p53 also noted. Decreases in p53 suggest a pro-proliferative
status of hepatic cells, while decreases in PKCα may indicated decreases in phosphorylation of substrates required for normal cell cycle progression. The increased c-Jun suggests that this gene may play a role in the hepatocyte hyperplasia, as well as the diminution of hematopoiesis.
DEDICATION

I dedicate this work to my clinical pathology residency advisor, Dr. Bernard F. Feldman, who was a constant source of reassurance and motivation; to my son, Jake, whose presence brought balance to my life; to my mother, Esther, for her words that strengthened my resolve; to Renee and Kurt, Barbara and Margaret, and to the rest of my family and friends for their support and encouragement during this final stage of my academic training.
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In addition, I would like to extend a special thanks to Joan Kalnitsky for assisting with the flow cytometry experiments; to the Histopathology Laboratory at the Veterinary Teaching Hospital, VMRCVM, for preparing all tissue samples for histologic evaluation; and to Susanne Aref, Statistical Consulting Center, for her assistance with the gene expression analysis.
DECLARATION OF WORK PERFORMED

I declare that I, Elizabeth Gayle Besteman, performed all of the work performed in this dissertation except that which is identified below.

Joan Kalnitsky operated the flow cytometer. Steve Holladay and Bonnie Smith assisted in harvesting thymus and liver tissues respectively. The Histopathology Laboratory at the Veterinary Teaching Hospital at Virginia Tech prepared the tissue samples for microscopic evaluation after formalin fixation. Susanne Aref of the Statistical Counseling Center at Virginia Tech performed the analysis of the gene expression data.
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ABBREVIATIONS

7-AAD  7-aminoactinomycinD
AhR  aryl hydrocarbon receptor
APC  antigen presenting cells
ARNT  aryl hydrocarbon nuclear translocator protein
BERKO  estrogen receptor beta knockout
cDNA  complementary deoxyribonucleic acid
CFU-GM  colony forming unit-granulocyte macrophage
CT  comparative threshold
DC  dendritic cell
DERKO  double estrogen receptor knockout
DES  diethylstilbestrol
DEX  dexamethasone
DN  double negative
DNA  deoxyribonucleic acid
DP  double positive
E2  estradiol
ER  estrogen receptor
ERKO  estrogen receptor alpha knockout
FITC  fluorescein isothiocyanate
FTOC  fetal thymus organ culture
GD  gestation day
HAH  halogenated aromatic hydrocarbon
HBSS  Hanks buffered salt solution
IL  interleukin
mRNA  messenger ribonucleic acid
NK  natural killer cell
NOAEL  no observable adverse effect
OVA  ovalbumin
PCDD  polychlorinated dibenzodioxin
PCDF  polychlorinated dibenzofuran
PCB  polychlorinated biphenyl
PCR  polymerase chain reaction
PE  phycoerythrin
PFC  plaque forming cell
RTPCR  reverse transcription polymerase chain reaction
SP  single positive
SRBC  sheep red blood cell
TCD  3, 3’, 4, 4’-tetrachlorobiphenyl
TCDD  2,3,7,8-tetrachlorodibenzo-p-dioxin
TcR  T cell receptor
Tdt  terminal deoxynucleotidyl transferase
TEQ  toxic equivalency factors
TG  transgenic
TUNEL  terminal dUTP nick end labeling
|Abbreviation| Unit\nkg| kilogram\ng| gram\nmg| milligram\nµg| microgram\nmL| milliliter\nµL| microliter