U.S. Wheat and Barley Scab Initiative
Annual Progress Report
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Cover Page

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Year:	**FY2000**
Grant Number:	**59-0790-9-060**
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$59,594.00**

Project

Program Area	Objective	Requested Amount
Food Safety, Toxicology, Utilization	Study Human Susceptibility To Trichothecene Mycotoxins Associated With Fusarium Head Scab.	$59,594.00
	Requested Total	$59,594.00[1]

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Principal Investigator Date

[1] Note: The Requested Total and the Amount Granted are not equal.

Project 1: Study Human Susceptibility To Trichothecene Mycotoxins Associated With Fusarium Head Scab.

1. What major problem or issue is being resolved and how are you resolving it?

Vomitoxin (VT or deoxynivalenol) and other trichothecenes are elaborated during head blight and thus pose a potential threat to human health. There have been several European studies that have suggested that a lower action level for VT be considered (120 ppb) rather than the 1-2 ppm being employed by most countries. Currently, the Joint Expert Committee on Food Additives (JEFCA) will be meeting in 2001 to review safety concerns for VT and other mycotoxins. I am working with this group and the International Life Sciences Research Institute to provide a state-of-the-art review on toxicological issues related to VT.

Based on studies in the mouse immune system, we believe that the most critical step for VT toxicity induction is its action on cell signaling in leukocytes (white blood cells). We propose to determine if human leukocyte cytokine dysregulation and/or apoptosis induction are indeed targeted by the same levels of VT and related 8-ketotrichothecenes as are their mouse equivalents. If this is true, then the risk of low ppm levels of VT to humans will be extremely small when one considers the diversity of the human diet and the actual potential level of VT exposure in human tissues. Such evidence is critical because it would support the argument against establishing lower action levels than those currently set for VT.

2. Please provide a comparison of the actual accomplishments with the objectives established.

For Objective 1, we have proposed to examine the cytotoxic and immunologic effects of 8-ketotrichothecenes in cloned human leukocyte lines. We have utilized the human Jurkat cell line as a model for human T cells. Acridine orange-ethidium bromide and MTT assays have been used to evaluate the capacity of the 5 8-ketotrichothecenes to induce apoptosis (programmed cell death) in this line. It was determined that the concentrations of VT, 15-acetyl deoxynivalenol, nivalenol, fusarenone-x and 3-acetyl nivalenol to induce apoptosis in 50% of the cells were 280,320,400,1400 and 5600 ng/ml, respectively. Using a sensitive ELISA, we have determined that at lower doses members of the deoxynivalenol family can superinduce secretion of the cytokine IL-2 by as much as 11-fold. Doses of VT, 15-acetyl deoxynivalenol and 3-acetyl deoxynivalenol as low as 35, 40 and 700 ng/ml were capable of this superinductive effect. We have determined that these compounds readily activate MAP kinase signaling cascades We have preliminary data to suggest that similar effects are apparent for nivalenol and fusarenone-x. In a second aspect of this work, we are examining the human U-937 cell line as a model for macrophage cells. We have determined in preliminary experiments that the 8-ketotrichothecenes can induce apoptosis in this cell line but may preferentially suppress proinflammatory cytokine production. These experiments are being replicated over a wider range of toxin concentrations and different co-stimuli.

For Objective 2, we have proposed to evaluate the cytotoxic and immunologic effects of 8-ketotrichothecenes on leukocytes isolated from human blood. We have begun setting up for this phase of the project by arranging to obtain cells from two sources: blood collected from volunteers at the Michigan State Clinical Center and blood cells collected as byproducts of blood processing from the Red Cross Blood Center. Initially we will optimize culture conditions for measuring T cell, macrophage and B cell function.

For Objective 3, we have proposed to use the above models to develop quantitative structure-activity relationships (QSARs) for 8-ketotrichothecenes. We have evaluated the QSAR approach using existing data on induction of apoptosis and cytotoxicity by twenty four trichothecenes was evaluated in four murine B lymphoma cell lines. The effect of trichothecene physical and chemical characteristics was assessed. Three dimensional structures were generated using Allinger's MM2 molecular mechanics force field analysis (Chem 3-D Software, Cambridge Soft, Cambridge MA). Physico-chemical properties were calculated using

Molecular Modeling Pro (WindowChem Software, Fairfield, CA). The software generated 34 variables, although many were highly correlated (collinear). To reduce problems associated with collinearity, Pearson correlation, Gittins condition number, and consideration of variable calculation were used to select each variable to be used in further analysis: Hansch Log P (Log P), Crippen's Log P (CRIP), CNDO Log P (CNDO), Kier's molecular connectivity index (CON), Hansen's solubility parameter (SOL), hydrophilicity-lipophilicity balance (HLB), lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO). Eight data sets were analyzed using principal components analysis (PCA). These data suggest that electrostatic interactions are an important indicator of trichothecene apoptosis and cytotoxicity. This study will provide the basis for conducting similar comparisons in human leukocytes using the data generated in Objectives 1 and 2.

3. What were the reasons established objectives were not met? If applicable.

It has taken us longer to establish the optimal culture conditions for the cloned human cell lines then originally anticipated because of their fastidious nature and changes in personnel in our lab. We have overcome these problems. We have also chosen to focus on T cells and macrophage clonal models in objective 1 and use these data in an the expanded leukocyte panel from human blood for Objective 2 that includes B cells.

4. What were the most significant accomplishments this past year?

The most significant accomplishments this past year have been:

a. The observation that human cloned cell lines are affected differently then comparable murine lines was unexpected. For T cells, the human cultures appear more sensitive than murine cultures, whereas human macrophage cell cultures seem less sensitive than do those of murine origin. With these parameters defined, we can validate these findings in primary cultures using human blood leukocytes.
b. The human and murine cell lines appear to be centrally affected at the level of cell signaling by a common pathways – MAP kinase cascades.
c. The QSAR analysis offers a strategy for predicting the toxicity of other trichothecenes that may be simultaneously present wheat and barley infected by *Fusarium.*

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Related Peer Reviewed Articles

Bondy GS, Pestka JJ. 2000. Immunomodulation by fungal toxins. J Toxicol Environ Health B Crit Rev. 2000 3(2):109-143

Zhou, H-R., J.R. Harkema, J.A. Hotchkiss, D. Yan, R.A. Roth and J.J. Pestka. 2000. Lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol) synergistically induce apoptosis in murine lymphoid organs. Toxicol. Sciences 53(2):253-263.

Yang, G.H., B.B. Jarvis, Y.J. Chung, and J.J. Pestka. 2000. Apoptosis induction by the satratoxins and other trichothecene mycotoxins: relationship to ERK, p38 MAPK and SAPK/JNK activation. Toxicol. Appl. Pharmacol.164(2):149-160.

Li, S.G., Y.L. Ouyang, G.H. Yang, and J.J. Pestka. 2000. Modulation of transcription factor AP-1 in murine EL-4 thymoma cells by vomitoxin (deoxynivalenol). Toxicol. Appl. Pharmacol. 163(1):17-25.

Yang, G.H., S. Li, and J.J. Pestka. 2000. Down regulation of 78 kDa glucose-regulated protein by vomitoxin (deoxynivalenol) in murine EL-4 thymoma cells. Toxicol. Appl. Pharmacol. 162(3):207-217.

Pestka, J.J. and H-R. Zhou. 2000. Interleukin-6-deficient mice refractory to IgA dysregulation but not anorexia induced by vomitoxin (deoxynivalenol) ingestion. Food Chem. Toxicol. 38: 565-575

Yuan, Q.Y., J.J. Pestka, B.M. Hespenheide, L.A. Kuhn, J.E. Linz, and L.P. Hart. 1999. identification of mimotype peptides which bind to the mycotoxin deoxynivalenol-specific monoclonal antibody. Appl. & Environ. Micro. 65:3279-3286.

Banotai, C., J.I. Azcona-Olivera, D.M. Greene-McDowelle, and J.J. Pestka. 1999. Effects of vomitoxin ingestion on murine models for systemic lupus erythematosus. Food Chem. Toxicol. 37:533-543.

Zhou, H-R., J.R. Harkema, D. Yan, and J.J. Pestka. 1999. Amplified proinflammatory cytokine expression and toxicity in mice coexposed to lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol). J. Toxicol. Environ.. Health 56:115-136.

Related Presentations

Pestka, J.J. 2000. Deoxynivalenol. Symposium on Significance of Mycotoxins to the Global Food Supply. Sponsored by the International Life Sciences Institute in conjunction with the International Association for Food Protection 87th Annual Meeting (Atlanta, GA).

Uzarski, R., Clarke, J., Uzarski, D. and Pestka, J.J. 2000. Trichothecene-induced apoptosis in B cell lymphomas: Quantitative structure activity relationships. Cancer and Molecular Genetics in the 21st Century. Van Andel Research Institute. (Grand Rapids, MI)

Chung, Y., M. Lee, S. Li, B.B. Jarvis, and J.J. Pestka. 1999. Dose-related superinduction and suppression of cytokines by macrocyclic trichothecenes in murine clonal T-cell and macrophage models. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Pestka, J.J. 1999. Amplification of endotoxin-mediated cytokine expression and lymphocyte apoptosis by trichothecenes: A paradigm for microbe-toxicant interactions. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Riley, R. J.J. Pestka, and R.A. Roth. 1999. Chemical modifiers of response to food-borne microbial pathogens. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Uzarski, R.L. and J.J. Pestka. 1999. Deoxynivalenol (vomitoxin) and TNFα act synergistically to induce thymocyte apoptosis through mechanisms involving intracellular Ca2+, reactive oxygen species, MAPKs, and CASPASE-3. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Zhou, H.R. and J.J. Pestka. 1999. Potentiation and attenuation of transcription factor activity in murine spleen following oral exposure to the trichothecene vomitoxin (deoxynivalenol). 1999. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Yang G.-H., S. Li, and J.J. Pestka. 1999. Down-regulation of an endoplasmic reticulum chaperone, GRP78/BiP, by vomitoxin (Deoxynivalenol) in murine EL-4 thymoma cells. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Clarke, J.R., R.L. Uzarski, D.G. Uzarski, and J.J. Pestka. 1999. Qualitative structure activity relationship analysis of trichothecene mycotoxins. Society of Toxicology 38th Annual Meeting (New Orleans, LA).

Pestka, J.J. and G.-H. Yang. 1999. Effect of vomitoxin (deoxynivalenol) on the binding activity of a negative transcription factor for IL-2 by in murine EL-4 thymoma cells. Society of Toxicology 38th Annual Meeting (New Orleans, LA).