APPENDIX V

ADD-ON STUDY ON mRNA

PROTOCOL FOR HEAT STRESS INDUCED mRNA RESPONSE

The purpose of this project is to determine the feasibility of measuring mRNA response to heat stress in a working population under exertion. Uro-epithelial cells would be harvested from routine urine collections and the mRNA activity analyzed in the Kavanagh lab at the University of Washington. Subjects would be workers at the Hanford Nuclear Reservation's Tank Farms, with urine collected for a NIOSH/DOE study on heat stress. This summer's pilot would involve approximately 20 workers.

BACKGROUND

Cellular heat response can be characterized by either direct protein damage or by the up regulation of protein synthesis including aberrant proteins. Families of heat shock proteins (HSPs) have been described, which are now generally categorized as a subset of stress proteins(1). New techniques in molecular biology have made study of gene regulation of HSPs possible, opening up an exciting area of cellular toxicology. mRNA induction can be used as a fingerprint of both physical (heat) and chemical exposure with characteristic gene responses for each class of exposure. This "fingerprinting" is best characterized for chemical exposure(2).

Previous techniques to quantify mRNA induction have relied upon somewhat tedious and expensive techniques (Northern hybridization, slot blot or reporter gene expression) which do not allow many simultaneous mRNA species to be analyzed. Our technique utilizes restriction landmark cDNA scanning (RLCS) on a two-dimensional gel similar to that described by Suzuki, et al (3).

STUDY POPULATION & METHODS

The study population has been organized by Dr. Ken Rosenman at Michigan State University with cooperation from the United Brotherhood of Carpenters. The workers are from the United States Department of Energy's Hanford Reservation tank farm. This desert steppe along the Columbia River often has summer temperatures above 90 degrees Fahrenheit. Because of the extremely hazardous contents of these tanks, and their poor characterization, fully self-contained occlusive suits are often worn during work in this area.

Voided urine samples will be collected before and after the work shift. Following measurement of specific gravity and pH the sample will be centrifuged and the cell pellet collected and resuspended in a solution containing RNAse inhibitors. The resuspended cells would then be frozen on dry ice.

Ideally this preparation step will occur immediately following the void in the field. Cells can remain viable in fresh urine for a short period (30-60 min.) so that transport under refrigeration to a near-by laboratory may be possible.

Frozen cells then will be transported by express mail to the Kavanagh Lab in Seattle for sorting and analysis. Commercially available and locally produced cDNA stress response gene probes will be used to characterize induction of the stress response in uro-epithelial cells. The probes will include genes coding for the heat stress proteins of the Hsc70 family and more general stress response genes such as thiol glutathione, Gadd43, Gadd153 and p21(Waf1). Because there is wide variation in the species and quantity of mRNA induction depending upon cell type, we hope to identify the stress genes which are most responsive in the transitional uro-epithelial cells harvested in a routinely voided urine sample.

While the small number of samples in this pilot may preclude obtaining statistically significant comparisons, we will analyze any intra-individual differences before and after the workshift. We will also look for a relationship between the extent of exposure and the degree of mRNA induction. Because many oxidizing and alkylating chemicals also cause stress response induction, we will attempt to establish whether chemical exposures confound the heat response in this population. We are hopeful that this pilot project will lead to a larger study of mRNA induction of HSPs next year allowing for more meaningful analyses.

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REFERENCES

1. Hightower, LE, Heat shock, stress proteins, chaperones and proteotoxicity. Cell 66: 191-197, 1991.

2. Todd MD, Lee MJ, Williams JL, Nalezny JM, Gee P, Benjamin MB and Farr SB, The CAT-Tox(L) assay: a sensitive and specific measure of stress-induced transcription in transformed human liver cells. Fundamnetals of Applied Tox.. 28: 118-28, 1995.

3. Suzuki H, Yaoi T, Kawai J, Hara A, Kuwajima G and Wantanabe S, REstriction landmark cDNA scanning (RLCS): a novel cDNA display system using two-dimentional gel electrophorysis. Nucleic Acids Res. 24: 289-296, 1996.

Appendix V

HEAT STRESS
CONSENT FORM

I volunteer to be tested for the effects of heat. The purpose of this investigation is to learn more about the effects of heat. I will benefit from the activity because I will be informed of the results which may identify medical problems I need to address.

I understand that I will complete a medical history questionnaire, my physical fitness level and body density will be measured, neurological testing will be performed, my urine will be measured for signs of dehydration and I will complete a questionnaire on how I am feeling. This testing will be done at the beginning, throughout, and at the end of the day. Testing will be done on 3-4 working days and perhaps on one non-working day. There is minimal risk for the tests. There is no risk for the questionnaire, neurological, urine, pulse, ear, oral, skin temperature or body density testing. The neuralgic testing will consist of me sitting in front of a computer and performing activities such as tapping my finger, or remembering numbers. I will also be asked to squeeze a gauge to measure hand strength and to stand on a platform to measure balance. The urine testing will measure my hydration before, during and after work. If I agree to a supplementary urine test by signing a separate consent form, my urine will also be tested for the activity of proteins that are altered by heat. Urine samples will not be used for any other purpose. Body density will be measured with small calipers that measure skin fold thickness. The skin will be pinched between the calipers on my arm, back, and chest for males, and the arm, hip and abdomen for females. There is no pain or risk from this measurement. To test my physical fitness, I will be asked to ride an exercise bicycle. This test, which is similar to tests conducted at the YMCA, will be done to a submaximal level , and will be stopped if I develop chest pain or other symptoms. As with any exercise, there is the risk of precipitating a heart attack.

I agree to participate with the understanding that:

1. I will be paid my contractual rate of pay for all work time that I spend on this study, including extra time before or after my normal work shift. If I participate on non-working days I will receive an amount equal to one and one-half times my regular hourly rate for each hour that I participate.

2. I will receive a copy of my results in a timely manner.

3. My results will be kept confidential.

4. My participation is voluntary and I am free to discontinue my participation at any time with no negative consequence to me.

5. The information from my test and those of others will be summarized and issued as reports. These reports will be written to keep all information about me and others confidential.

6. This testing is limited and does not replace a general medical exam.

7. I can contact Buck Cameron at (206) 935-7748 at any time to ask questions about this project and its current status.

8. If I am injured while participating in the study, I need to seek emergency care as I normally would with a job-related injury. The study has no funds to provide emergency or long-term care.

My Name is (Print)	**Witness Name (Print)**
My Signature	**Witness Signature**
Date	**Date**