MECHANISMS OF ARSENIC TOXICITY IN MUMMICHOGS (*FUNDULUS HETEROCLITUS*)

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The goals for this project have been to better understand how arsenic elicits its toxicity. Arsenic is found in many Superfund sites and is considered to be a human carcinogen, although the exact mechanism of action is not well understood. Investigators have suggested that arsenic may work through an estrogenic mode of action (Chattopadhyay et al., J. Toxicol. Sci. 24:425-431,1999). This is in concordance with a new human epidemiological study that showed a reduction in live births in women who had drank arsenic-contaminated well water in Bangladesh, as well as a 2.6-fold increase in stillbirths and a 3.3-fold increase in spontaneous abortions (Ahmad et al., Environ. Health Perspect. 109:629-631, 2001). There is also recent evidence that arsenic inhibits glucocorticoid receptor-mediated transcription (Kaltreider et al., Environ. Health Perspect. 109:245-251, 2001). To this end, mummichogs are being used to examine the mechanism of action of arsenic and to correlate altered gene expression with physiological changes in the organisms, including altered reproduction, development, and growth.

Adult mummichogs (*Fundulus heteroclitus*) were collected, and half were exposed to 0.4 ppm sodium arsenate for eight days in 70% seawater. The eight days represents the approximate period between spawning on a seminlunar cycle, which the concentration is representative of wells in the U.S. that contain high levels of arsenic. Mating studies between control and treated mummichogs were performed to examine the number of eggs produced, their viability, hatchability, and hatchling survival. Total RNA was extracted from the adult livers and from 1 month-old hatchlings to prepare subtractive cDNA libraries to be used in differential gene expression studies.

The total number of eggs produced was not different between the two groups (control: 565$\pm$210; exposed: 737$\pm$393), nor was percentage egg viability (control: 59.9$\pm$19.2%; exposed: 67.5$\pm$15.6%), or percentage hatched eggs (control: 52.4$\pm$18.55%; exposed: 58.7$\pm$12.3%). Performing student's t-tests revealed a statistical difference in spinal morphology between the two groups, with an average of 1.45% of the hatchlings having stunted or curved tails in the arsenic-exposed group versus only 0.5% of the hatchlings in the control group. cDNA libraries were constructed from the adult livers and from 1-month old hatchlings, and the differentially-expressed cDNAs were spotted onto macroarrays. Several genes have been tentatively identified, based upon their homology with other fish sequences in GenBank, and include an ADP/ATP translocase, an ATP synthase, keratin, myosin light chain 2, a hemopexin-like protein, and a heart-type fatty acid-binding protein. Most of the genes were found to be upregulated in the fish that were exposed to arsenic, however these results need to be confirmed. We anticipate that these arrays will elucidate patterns of gene expression and thereby yield more insight into arsenic's mode of action and the resultant physiological changes in the organism.

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