5-16-2006

Effects of Pre-natal Exposure to Arsenic on Gene Expression in Fetal Rat Kidney

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THESIS

BY

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2006

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THESIS

Presented in Partial Fulfillment of the Requirements for

the Degree Master of Science in the

Graduate School of Texas Southern University

By

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2006

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Texas Southern University, 2006

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Several types of cancer have been linked to arsenic in water. Recently, the EPA set the arsenic standard for drinking water at 10 parts per billion to protect consumers served by public water systems from the effects of long-term, chronic exposure to arsenic. Arsenic concentrations in ground water are generally higher in the Western United States, and appear to be lower in the Southeastern United States. Higher doses of arsenic are known to induce apoptosis whereas the chronic exposure of lower doses may lead to various adverse effects. Thus it is crucial to understand the genotoxicity and alteration in gene expression due to lower doses of arsenic. It has been reported that arsenic levels in drinking water are increased for populations living near arsenic-producing sites. It has also been reported that arsenic crosses the placenta and is also found in breast milk. Fetuses are therefore exposed in utero and postnatally. To date, there are no reports of the genotoxic effects of arsenic from these types of exposures.

In the literature, animal studies have indicated an increased incidence of chromosomal abnormalities when rats were given oral doses of sodium arsenate (4mg As/kg/day) for 2-3 weeks, suggesting ingested arsenic may cause chromosomal effects. In human fibroblast cells,

arsenite is known to induce oxidative damage, chromosomal aberrations, cell cycle arrest, and aneuploidy. The manifestation of these cellular responses is dependent on changes in gene expression, which can be analyzed.

The hypothesis behind this study is that prenatal exposure to arsenic alters gene expression during development to cause various tumor types. The purpose of this research is to explore the changes in gene expression after chronic exposure to the lower doses of the toxin arsenic, which may lead to possible adverse health outcomes. In this study, we have examined the effect of arsenic at low levels on rat fetuses to determine the effects on fetal gene expression.

Timed pregnant female rats were exposed to 200ppm sodium arsenate ad libitum via drinking water during the embryonic period gestational day 7 through 21. The pups were retrieved immediately after birth for dissection. Fetal kidney tissue was harvested and pooled from treated and control animals. Maternal kidney samples were also collected. RNA isolation was performed from the tissue samples and analysis for Bcl2, Bax, Cyclin D1, MnSOD and PCNA genes was done using real time PCR.

The results of this study revealed significant increases in the expression of PCNA, MnSOD, and Bax of the mother kidney. There were no significant changes observed in the expression of these genes in arsenic-exposed fetal kidney as compared to the age-matched untreated fetal kidney. The finding of this study indicates that lower dose arsenic exposure enhances cell proliferation, increases antioxidant defense as well as accelerates cell apoptosis by increasing the expression of Bax, a pro-apoptotic gene in the maternal kidney. The arsenic-induced increased cell proliferation, increased antioxidant defense, and acceleration of cell apoptosis may ultimately lead to cell transformation and tumor development. In summary, this study provides an insight into the mechanism of arsenic-induced carcinogenesis.

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ACKNOWLEDGEMENTS

This work is dedicated to my mom, Ruthie, and my husband, Ziyadah Holmes. Mom, thanks for believing in me and always encouraging me to pursue my dreams. Without you, I would not be the person I am today. Ziyadah, thanks for your unconditional love and support. You have made the last 6 years of my life incredible!

I would also like to thank Dr. Desirée Jackson, the chair of my committee, for her patience, wisdom and guidance throughout my graduate career. By her actions, she is a leader and a true inspiration. I also would like to thank the members of my Committee, Dr. James DuMond, Dr. Olufisayo Jejelowo, and Dr. Karen Williams for their time, interest and support.

A special thanks to Dr. Kamaleshwar Singh for his time and assistance in my research. I am grateful to Mrs. Helen Pittman-Cockrell for her exceptional organizational skills and motivation. I would also like to thank my family and friends for their support and encouragement. Last but not least, I would like to thank God who is the head of my life, without you none of this would be possible.

CHAPTER 1

INTRODUCTION

Arsenic is a known human carcinogen and induces a variety of human diseases. This element is ubiquitous in nature and is released into the environment via industrial processes, agricultural, and medical applications. Because of this natural distribution, drinking water is the most common source of arsenic exposure for the general population (23). Recently, the EPA set the arsenic standard for drinking water at 10 parts per billion to protect consumers served by public water systems from the effects of long-term, chronic exposure to arsenic. Water systems were required to comply with this standard by January 23, 2006 (10). Arsenic concentrations in ground water are generally higher in the Western United States and appear to be lower in the Southeastern United States (29).

Arsenic contamination of drinking water is a serious environmental problem worldwide because of the large number of contaminated sites that have been identified and the large number of people at risk. Arsenic has been found in at least 1,014 of the 1,598 current or former National Priorities List (NPL) sites (1). It has been seen in populations living near arsenic-producing sites that arsenic levels in drinking water are increased near those sites (12). Human exposure near these hazardous sites could involve inhalation of arsenic dusts in air, ingestion of arsenic in water, food or soil, or dermal contact with contaminated soil or water. The likely effects of human health concerns

from ingestion of arsenic are gastrointestinal irritation, peripheral neuropathy, vascular lesions, anemia, skin diseases and cancers of the internal organs including the bladder, kidney, liver and lung (7).

Programmed cell death or apoptosis plays an essential role in the development of multicellular organisms and in pathological processes. Dysregulation of apoptosis is involved in the development of many diseases, cancer in particular. The genes that have been implicated as being involved in apoptotic pathways include Bcl2 which inhibits apoptosis. Bcl2 is a protein which exists in delicate balance with other related proteins whose function is to block cells from apoptosis death. Through several mechanisms Bcl2 proteins assist cancer cells in defying the killing effects of chemotherapy. Various types of cancer including leukemia have been shown to express high levels of Bcl2 proteins (5). Some functions of Bcl2 may be mediated through interaction with Bax, a Bcl2 homolog (24). Bax is a pro-apoptotic gene. When Bax predominates, programmed cell death is accelerated, and the death repressor activity of Bcl2 is countered.

Apoptosis induced by p53, a tumor suppressor gene, is blocked or delayed by overexpressed Bcl2, but cells continue to be growth inhibited. This implies that p53 functions are separate and that Bcl2 affects only the p53-induced apoptosis pathway (22). It has been shown that p53 down-regulates Bcl2 expression and up-regulates Bax expression in the murine leukemia cell line M1, thereby leading to apoptosis (27).

Cyclin D1, one of the G1 cyclins, is frequently overexpressed in several types of carcinoma and is thought to play an important role in tumorigensis and tumor progression including hepatocellular carcinoma (13). Recent genetic analysis has revealed abnormalities in cell-cycle regulators, which likely contribute to carcinogenesis via

aberrant cell-cycle progression. A restriction point has been identified late in the G1 phase, just before the G1-S-phase transition, in which cycling is stimulated by G1 phase cyclins. Cyclin D1 is a proto-oncogenic regulator of the G1-S-phase checkpoint and appears to phosporylate pRb^3 by binding to cyclin dependent kinase 4, a melanoma gene, or cyclin dependent kinase 6, a protein complex found elevated in several tumor types (20). Cyclin D1 is overexpressed in many cancers as a result of gene amplification or translocations targeting the D1 locus on human chromosome 11q13 (13).

PCNA is a processivity factor for DNA polymerase-δ and is expressed in a cell cycle-dependent manner (4). PCNA is expressed at high levels in the S phase of the cell cycle. It is required for cell cycle progression in vivo, because antisense targeting of PCNA mRNA inhibits growth factor-stimulated proliferation in cultured cells (18). In the developing kidney, PCNA expression is restricted to the nephrogenic zone. PCNA expression is down regulated rapidly as renal epithelial cells differentiate and acquire functional characteristics. Previous studies have shown that p53-mediated inhibition of cellular growth is accompanied by selective downregulation of PCNA gene expression (21). These findings suggest that elevated levels of cellular p53 may restrict PCNA expression through transcriptional repression.

Several recent studies have shown that tumor metastasis was suppressed by manganese-containing superoxide dismutase (MnSOD) (33). Moreover it has been noted that tumor cells generally have low levels of MnSOD. Among the antioxidant enzymes, MnSOD has been intensively studied as a potential tumor suppressor gene. Recent studies showed that transfection of the human MnSOD gene suppressed tumorigenicity of both human melanoma and breast cancer cell lines (33).

Gestation is often a period of high sensitivity to carcinogenesis. Studies have shown that after administration of inorganic arsenic during gestation, the metalloid can readily cross the placenta and enter the fetal system (6, 9, 19). This is an indicator that transplacental exposure to arsenic is a plausible exposure route and may carry risks typical of other types of exposure.

Pregnant women are exposed to arsenic through the water supply in amounts anywhere from 2 to 20 ug/L (28). In a World Health Organization study, arsenic was detected in human breast milk at concentrations of 0.00013 – 0.00082 ppm (25). In Northwest Argentina, where the drinking water contains approximately 200 ug/L the concentration of arsenic in cord blood (median,9ug/L) was almost as high as in maternal blood (median,11ug/L) (9). Fetuses are therefore exposed in utero and postnatally to arsenic. To date, there are no reports of the genotoxic effects of arsenic from these types of exposures.

Arsenic concentrates in the kidney during urinary elimination, thus this organ cannot avoid exposure. Although the kidney represents a target for the accumulation and toxicity of arsenic, an understanding of the molecular targets of this metal in the kidney is limited. A study was designed to examine the molecular impact of arsenite and arsenate at low concentrations. The findings of this study were that enhanced DNA binding activity of activating protein-1 (AP-1) and Elk-1, transcription factors, correlated with increased gene expression of c-fos, a proto-oncogene involved in cellular proliferation . C-myc, a gene activated during hepatocarcinogenesis, was also induced by aresnite and arsenate. These results suggest that acute arsenic challenge is associated with discrete alterations in the activity of signaling pathways and gene expression in renal tissue (25).

The objective of this study is to explore the changes in gene expression after chronic exposure to the lower doses of the toxin arsenic which may lead to possible adverse health outcomes. This study examined the effect of arsenic at low levels on rat fetuses to determine the effects on fetal gene expression.

To test this hypothesis, timed pregnant Hsd female rats were exposed to 200 ppm of sodium arsenate ad libitum via drinking water during the embryonic period gestational day 7 through gestational day 21. Pups were sacrificed immediately after birth for dissection of the kidneys. Isolation of RNA was performed and analysis for Cyclin D1, PCNA, Bax, Bcl2 and MnSOD genes was performed. These genes are markers involved in cell cycle regulation, cell proliferation, a pro-apoptotic gene, an anti-apoptotic gene and an antioxidant defense gene, respectively.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Arsenic-related publications have greatly increased in recent years, partly as a result of the enormous disaster in the Bengal region of India and neighboring Bangladesh where millions have been exposed to high levels of arsenic in drinking water. In West Bengal alone, nine districts encompassing an area of 38,000 km² and with a population of about 42.7 million are affected (8). In the U.S. inorganic arsenic continues to be a major concern largely based on its carcinogenic potential after occupational or environmental exposure. Inorganic arsenic exposure in humans is associated with development of malignancies in various tissues. Epidemiological studies have repeatedly shown clear dose-response relationships between environmental arsenic levels and human cancer incidence.

It is becoming increasingly clear that high dose exposure to arsenic compounds differs from low dose exposure with regard to genotoxicity (32), types of reactive species formed (2), signal pathways activated (9) and gene expression (17). Many stress proteins seem to be induced only at high dosage.

In a recent study, pregnant C3H mice were exposed to sodium arsenate in drinking water for a brief period of gestation. Transplacental inorganic arsenic exposure produced a dose-dependent induction of tumors in the liver, adrenal, lung and ovary of

the offspring after they had become adults (31). These results are interesting because of the brief ten day duration of maternal inorganic arsenic exposure in this experiment. This exposure was carcinogenic to the offspring after they reached adulthood and well after the arsenic exposure had ended. Genomic analysis of liver tumors and tissues around the tumors revealed several patterns of aberrant gene expression associated with transplacental arsenic carcinogenesis. This clearly correlates with the idea that arsenic may and can be easily transferred across the placenta and induce unwanted gene expression.

In human fibroblast cells, arsenite is known to induce oxidative damage, chromosome aberrations, cell cycle arrest, and aneuploidy, and the manifestation of these cellular responses is dependent on changes in gene expression. Treatment of human fibroblast cells during G2 of the cell cycle with sodium arsenite resulted in arrest of cells in the G2 phase, interference with mitotic division, inhibition of spindle assembly, and induction of chromosome endoreduplication in the second mitosis (30).

Arsenic-induced oxidative damage is also evident in exposed populations. In a small study, researchers showed that exposure to inorganic arsenic in a contaminated area of Inner Mongolia, increased lipid peroxide serum levels and decreased nonprotein sulfhydryl serum levels, two indicators of oxidative stress. Similarly, residents of Northeastern Taiwan experienced elevated levels of reactive oxidants with a decrease in plasma antioxidants from exposure to arsenic-contaminated water sources (26).

There is evidence suggesting that arsenic may inhibit DNA repair systems. In general, disruption of DNA repair and other measures to ensure damaged cells do not proliferate has significant implications for carcinogenesis. Because inorganic arsenic can

directly interact with proteins by altering their structure and function, it has been hypothesized that arsenic interaction with critical enzymes inhibits DNA repair. In vitro studies have suggested that arsenic could inhibit the activity of DNA ligases (16).

While p53 does not actively repair DNA, it is critical to the DNA repair process because its expression regulates a critical cell cycle checkpoint that can suspend cell cycle progression and allow for repair of damaged DNA. Studies have shown that arsenic can both induce and inhibit p53 expression, and its regulation of other genes (11).

Furthermore, epidemiological studies in highly exposed populations demonstrate that arsenic exposure results in increased micronuclei incidence and sister chromatid exchanges in bladder cells (3). In a 1999 evaluation of arsenic toxicity, the National Research Council concluded that the most accepted explanation for arsenic carcinogenicity is that arsenic induces chromosomal abnormalities without interacting directly with DNA (23).

Changes in DNA methylation patterns may contribute to alterations in gene transcription. For example, it was shown that low-level arsenic exposure induced cell proliferation in human keratinocytes (14). Proliferative effects were associated with increased transcription of cyclin G1 and protein kinase C delta. In addition to the upregulation of proliferative genes, increased transcription of the DNA repair-related genes as well as genes known to respond to oxidative stress was noted.

A study on the standardized mortality ratios with arsenic exposure from ground water in Argentina revealed dose-related association between arsenic exposure and risk of kidney and lung cancers (15). Although toxicological and metabolic interactions of arsenic have been suggested by epidemiological literature, the past experimental studies

have mostly focused on acute, high-dose interaction, leaving long-term, low-dose interaction unexplored. This study explored the effect of exposure at low levels to see if there is an effect on gene expression in the tissues of exposed fetuses.

CHAPTER 3

DESIGN OF THE STUDY

Animal Exposure to Arsenic

Timed pregnant Hsd female rats were used as the subjects. The arsenic group [N=3] was exposed to 200ppm sodium arsenate ad libitum via drinking water. The control group was given water ad libitum. Each rat was given 200mL of water or water with arsenic every other day. On these days the weight of each animal, the amount of water consumed and remaining was recorded. This was done during the embryonic period gestational day 7 through gestational day 21.

Pups were sacrificed immediately after birth for dissection. The animals were given isofluorine via inhalation and then dissected. The dissected tissues were fast frozen in liquid nitrogen and stored at -40°C until the mRNA isolation was performed.

mRNA Isolation

Homogenization. Kidney tissues were homogenized in 1mL of Trizol reagent (Invitrogen Life Technologies) using a power homogenizer.

Phase Separation. The homogenized samples were incubated at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes. Chloroform was added in the amount of 0.2mL. The sample tubes were shaken vigorously by hand

for 30 seconds and incubated at room temperature for 10 minutes. The samples were centrifuged at 10,750 rpm for 15 minutes.

RNA Precipitation. RNA was precipitated by mixing the aqueous phase with 0.5mL of isopropyl alcohol, incubated at room temperature for 10 minutes and centrifuged at 10,750 rpm for 15 minutes.

RNA Wash. The RNA pellet was washed with 1mL of 75% ethanol.

Redissolving the RNA. RNA was resuspended in 40uL of DEPC water and incubated on ice.

Quantification. 7.5uL of RNA was quantified in a final volume of 3mL. Readings were taken at 260, 280 and 320 nanometers respectively. The RNA was frozen at -40°C until Real Time PCR analysis was done.

Real Time PCR

Gene analysis was done using Real Time PCR (RT-PCR). One-step RT-PCR kit with SYBR Green was used for amplification of total RNA (200ng) and by following the manufacturer's protocol (BioRad). Single-step RT-PCR amplification was performed in 96-well optical reaction plates using iCycler (Bio-Rad Laboratories, Hercules, CA) programmed for reverse transcription at 50°C for 15 minutes, denaturation and RT enzyme inactivation at 95°C for 5 min, followed by 40 cycles of 10s denaturation at 95°C and 30s annealing and extension at 60°C. Melting curves were verified between 55 and 95°C with 0.5° temperature increments. The threshold cycle number (Ct value) was analyzed using iCycler IQ optical system software (Bio-Rad, version 3.0a). Quantitative PCR was normalized to the Ct value of beta-actin RNA from same sample and the level

of expression of each gene was given as a relative expression. Amplification reactions for each sample were performed in triplicate. A non-template control was also included in each experiment.

Primers for the MnSOD gene which is involved in antioxidant defense, cyclin D1 gene which functions in cell cycle regulation, PCNA gene which is an indicator of cell growth, Bcl2 gene, an anti-apoptotic gene and a housekeeping gene β-actin-GAPDH were used for Real Time PCR. The primer sequences used are given in Table 1.

Data Analysis

Statistical analysis of the data comparing the controls and each treated group was determined by a Student's t-test, using two-tailed tests. For all the experiments, the significance level was set at $P < 0.05$.

TABLE 1

Primer Sequences

Sequence Name	Sequence 5' to 3'
Bcl2 - F (RODENT)	ATGGGGTGAACTGGGGGATTG
Bcl2 - R (RODENT)	TTCCGAATTTGTTTGGGGAGGTC
Bax – F (RODENT)	GGGTGGTTGCCCTTTTCTACT
Bax – R (RODENT)	CCCGGAGGAAGTCCAGTGTC
CyclinD1 - F (RODENT)	GCACAACGCACTTTCTTTCTTTCCA
CyclinD1 - R (RODENT)	CGCAGGCTTGACTCCAGAAG
PCNA - F (RODENT)	GCCCTCAAAGACCTCATCAA
PCNA - R (RODENT)	TCTGGGATTCCAAGTTGCTC
MnSOD - F (RODENT)	ATTAACGCGCAGATCATGCA
MnSOD - R (RODENT)	TGTCCCCCACCATTGAACTT
β - Actin - F (RODENT)	ACACTGTGCCCATCTACGAGG
β - Actin - R (RODENT)	AGGGGCCGGACTCGTCATACT

CHAPTER 4

RESULTS AND DISCUSSION

Five gene primers were used to study the effects on fetal gene expression due to arsenic exposure at low levels in rat fetal kidney. The kidneys of the dams were also studied. Four out of the five sets of gene primers, Bcl2, Bax, PCNA and MnSOD, showed an increase in gene expression as compared to the untreated controls, while Cyclin D1 exhibited a decrease in expression in the fetal kidney as compared to the untreated controls.

The Bcl2 gene showed a higher increase in gene expression in the fetal kidney when compared to gene expression in the maternal kidney (Figure 1). Though an increase in expression was seen, it was not enough to meet the minimum significance level of 0.05 (Table 2).

Gene expression of Bax was higher in the fetal kidney than seen in the maternal kidney (Figure 2). The Bax gene showed no significant difference in expression, but the level of expression was very close to the set minimum significance level of 0.05 (Table 3). A significant increase was observed in the kidney tissue of the dam (Table 3). This implies that arsenic is crossing the placental membrane, inducing apoptosis through the Bax gene.

Expression of PCNA showed a very subtle increase in both the maternal and fetal kidney tissues (Figure 3). The minimum significance level was not observed in the fetal kidney tissue (Table 4). A significant level of gene expression was observed in the dam tissue (Table 4), which indicates that cell proliferation was being enhanced.

Cyclin D1 showed a decrease in expression in the fetal tissue with not much increase in expression observed in the dam tissue (Figure 4). The minimum significance level was not observed for gene expression in either of these tissue samples (Table 5).

Gene expression of MnSOD was higher in the maternal kidney tissue than in the fetal kidney tissue (Figure 5). A significant change in expression was not observed in the fetal kidney tissue, but was observed in MnSOD expression in the dam (Table 6). This increased expression in maternal tissue may be an indicator that tumor suppression via up-regulation of MnSOD may be activated in the dam in response to Arsenic treatment.

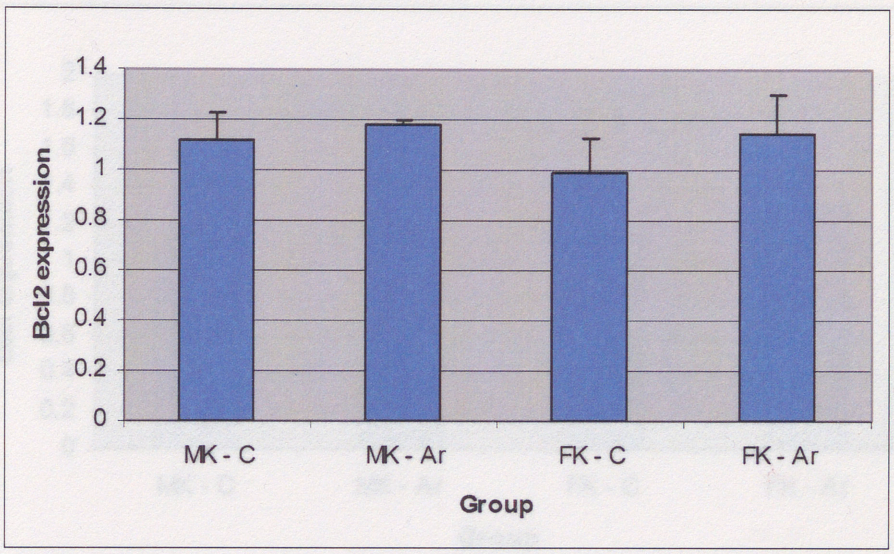
While increased expression of Bcl2, Bax, PCNA and MnSOD were seen in the arsenic-treated fetal kidney, these increases were not significant according to the statistical standard used to generate this data. However, it may still be implied that arsenic does have an effect on the expression of these genes after crossing the placental membrane as seen in the subtle changes in expression of these genes relative to the controls.

The findings of this study indicate that chronic exposure to lower doses of arsenic may enhance cell proliferation, increases antioxidant defense and accelerates cell apoptosis by increasing the expression of Bax, a pro-apoptotic gene in the dams. The arsenic-induced increases in cell proliferation, antioxidant defense and acceleration of cell apoptosis, may ultimately lead to cell transformation and tumor development. In

summary, our study provides an insight into the mechanism of arsenic-induced carcinogenesis. Further studies of the subject should be done using other genes that are involved in carcinogenicity.

FIGURE 1

Relative Expression of Bc12 (normalized by β–actin)



Bcl2 activity in mother kidney (MK) and fetal kidney (FK), comparing control (C) and arsenic (Ar) tissues.

TABLE 2

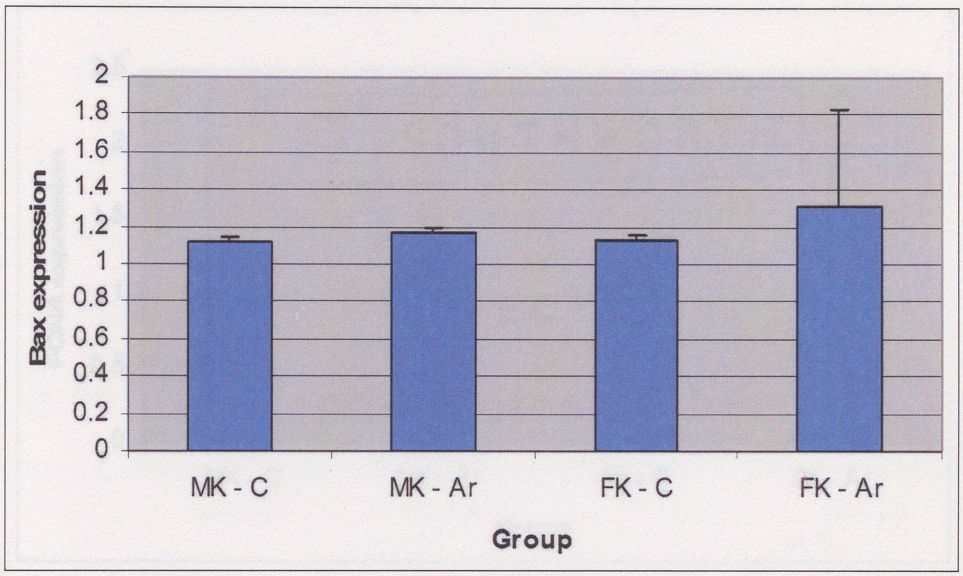
Bc12-Dam and Bc12-Fetal t-Test Paired for Means

t-Test: Paired Two Sample for Means

	Bcl2-Dam		Bcl2-Fetal	
	Variable 1	Variable 2	Variable 1	Variable 2
Mean	1.117637	1.184783	0.98921	1.147952
Observations	3	3	3	3
P(T<=t) two-tail	0.432026		0.205696	
T Critical two-tail, df 2	4.302653		4.302653	

FIGURE 2

Relative Expression of Bax (normalized by β-actin)



Bax activity in mother kidney (MK) and fetal kidney (FK), comparing control (C) and arsenic (Ar) tissues.

TABLE 3

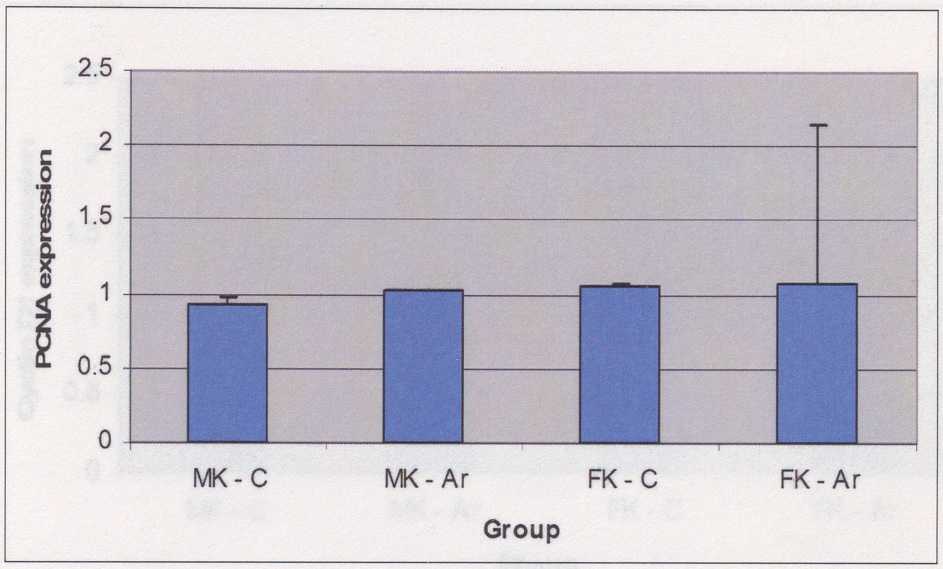
Bax-Dam and Bax-Fetal t-Test Paired for Means

t-Test: Paired Two Sample for Means

	Bax-Dam		Bax-Fetal	
	Variable 1	Variable 2	Variable 1	Variable 2
Mean	1.120552	1.164566	1.130767	1.308271
Observations	3	3	3	3
P(T<=t) two-tail	0.014488		0.061835	
T Critical two-tail, df 2	4.302653		4.302653	

FIGURE 3

Relative Expression of PCNA (normalized by β-actin)



PCNA activity in mother kidney (MK) and fetal kidney (FK), comparing control (C) and arsenic (Ar) tissues.

TABLE 4

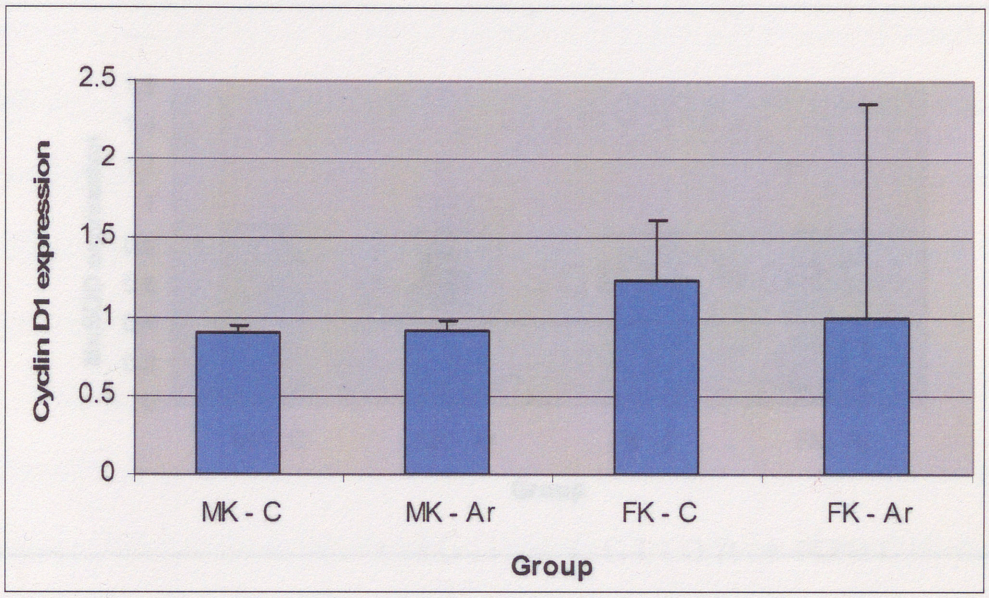
PCNA-Dam and PCNA-Fetal t-Test Paired for Means

t-Test: Paired Two Sample for Means

	PCNA-Dam		PCNA-Fetal	
	Variable 1	Variable 2	Variable 1	Variable 2
Mean	0.933875	1.362337	1.05148	1.07057
Observations	3	3	3	3
P(T<=t) two-tail	0.044135		0.752257	
T Critical two-tail, df 2	4.302653		4.302653	

FIGURE 4

Relative Expression of Cyclin D1 (normalized by β-actin)



Cyclin D1 activity in mother kidney (MK) and fetal kidney (FK), comparing control (C) and arsenic (Ar) tissues.

TABLE 5

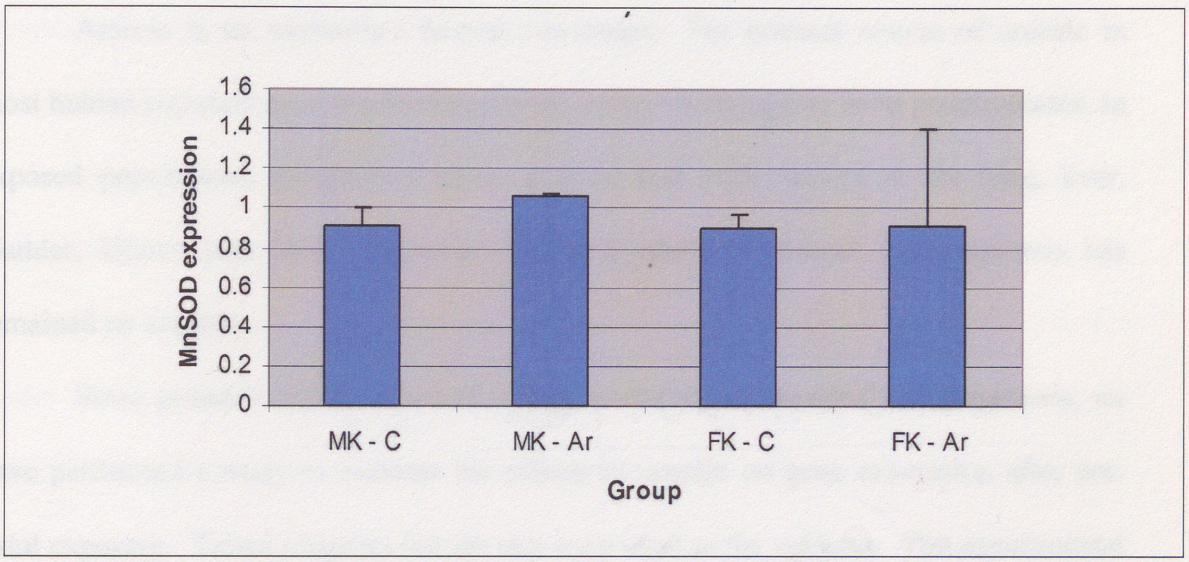
Cyclin D1-Dam and Cyclin D1-Fetal t-Test Paired for Means

t-Test: Paired Two Sample for Means

	Cyclin D1-Dam		Cyclin D1-Fetal	
	Variable 1	Variable 2	Variable 1	Variable 2
Mean	0.905233	0.913067	1.228216	0.998489
Observations	3	3	3	3
P(T<=t) two-tail	0.912366		0.437932	
T Critical two-tail, df 2	4.302653		4.302653	

FIGURE 5

Relative Expression of MnSOD (normalized by β-actin)



MnSOD activity in mother kidney (MK) and fetal kidney (FK), comparing control (C) and arsenic (Ar) tissues.

TABLE 6

MnSOD-Dam and MnSOD-Fetal t-Test Paired for Means

t-Test: Paired Two Sample for Means

	MnSOD-Dam		MnSOD-Fetal	
	Variable 1	Variable 2	Variable 1	Variable 2
Mean	0.839825	1.067642	0.893985	0.906245
Observations	3	3	3	3
P(T<=t) two-tail	0.003599		0.782204	
T Critical two-tail, df 2	4.302653		4.302653	

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Arsenic is an established human carcinogen. The primary source of arsenic in most human populations is the drinking water, where the inorganic form predominates. In exposed populations, inorganic arsenic is associated with tumors of the lung, liver, bladder, kidney and skin. However, the mechanism of arsenic carcinogenesis has remained an enigma.

Since gestation can be a period of high sensitivity to chemical carcinogenesis, we have performed a study to evaluate the effects of arsenic on gene expression after pre-natal exposure. Timed pregnant female rats were used as the subjects. The experimental group was given 200ppm sodium arsenate in distilled water while the control group was given distilled water from gestational day 7 to 21.

Expression of genes associated with cell proliferation, cell cycle control and apoptosis were analyzed in kidney samples from the fetuses of these two groups. This was done using Real Time PCR to determine the level of expression of these genes. A Student's t-test analysis was done to determine the significance of the data points gathered from Real Time PCR for each gene in the fetal tissue. Real Time PCR was also performed for the kidney tissue of the dams.

Using the parameters set for the Student's t-test, expression of these genes was not significantly different in the kidney tissues of the arsenic-treated fetuses when compared to the controls. However, there was a subtle increase in expression of MnSOD, PCNA, Bcl2 and Cyclin D1 seen in the fetal tissues. The expression level for Bax gene was very close to the level of significance set for this study. This is proof that arsenic is crossing the placental membrane causing the cells to go through apoptosis.

The increased gene expression levels for MnSOD, PCNA and Bax in the kidney tissues of the dam indicate that chronic exposure to lower doses of arsenic may enhance cell proliferation, increase antioxidant defense and accelerate cell apoptosis. In conclusion, the arsenic-induced increase may ultimately lead to cell transformation and tumor development.

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