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The influence of arcenic contaminated water in insulin receptors in mice tissues

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ABSTRACT

Background: Arsenic is one of the most potent environmental carcinogens. Chronic exposure to the element has been associated with various non-cancerous diseases, including diabetes mellitus. Although mechanisms of arsenic-induced diabetes have not been identified, it seems that the element or its metabolites interfere with insulin-stimulated signal transduction pathway or with critical steps in glucose metabolism. The aim of this in vivo study was to correlate type 2 diabetes development with chronic arsenic exposure and to investigate the distribution of insulin receptors in kidneys and muscles.

Methods: Sixteen Balb/c mice were divided into two groups: the first group was chronically exposed to arsenic (50 ppm) in drinking water, whereas a control group consumed distilled water. After the end of a 6 week period, animals were sacrificed and the kidneys and sciatic muscles were prepared for immunohistocemical analysis.

Results: All animals in the arsenic group presented fasting hyperglycemia. The weight gain in both groups was not statistically significant different. The immunohistochemical staining revealed significant decrease in the number of insulin receptors in both kidneys and sciatic muscles of the arsenic exposed mice.

Conclusion: Our results have shown that prolonged drinking of arsenic-contaminated water is associated with decreased insulin Receptor (IR) concentration and increased glucose levels which may play a role in type 2 diabetes induction. Morphological and immunohistochemical changes caused by arsenic predominate in organs like kidneys and skeletal muscles.

Introduction

Arsenic is a heavy metal which name derived from the Greek word arsenikon (potent). Arsenic is a natural occurring toxic metalloid found all over the earth. In the environment, it is found in two forms, organic and inorganic. Inorganic forms are more toxic than the organic and they predominate in surface and groundwater reservoirs [1]. Arsenic can be easily solubilized in ground water. Natural arsenic is usually found at levels greater than 10 mg/l (0.01 ppm) in groundwater, which is higher of the standard for drinking water according to US Environmental Protection Agency [2]. Moreover, human derived arsenic pollution has driven to soil and drinking water contamination [1].

Diabetes mellitus (DM) has a growing prevalence worldwide and is becoming a serious threat to human health. It concerns a group of metabolic diseases characterized by hyperglycemia as the result of multiple defects in insulin secretion by pancreatic β-cells and/or insulin action on peripheral tissues. Type2 DM is a multiorgan disease without a specific etiology that involves both peripheral insulin resistance and insufficient insulin production due to pancreatic β-cell dysfunction [3,4].

There are many studies demonstrating that chronic exposure to arsenic in drinking water increases rates of various chronic diseases such as cancer, nervous system diseases, peripheral vascular disease, gangrene, hyperkeratosis (hardened skin) and endocrine dysfunction [5-8]. Arsenic associated diabetes mellitus is widely studied and well defined but there is not enough evidence on the pathophysiology of this association. It has been proved, in vivo as well as in vitro, that arsenic promotes β-cells apoptosis via oxidative stress [9]. Numerous studies of arsenic associated diabetes mellitus, as that of Makris et al on Cypriot population demonstrate a strong association between arsenic consumption through drinking water and DM [10].

Insulin Receptor (IR) plays a crucial role on glucose metabolism and DM, as it is necessary for insulin activity. We studied the Insulin Receptor existence on mice muscles and kidneys after their exposure to arsenic through contaminated drinking water. The aim of this study was to investigate in vivo the correlation between DM and arsenic consumption and its impact on IR expression on kidneys and muscles.

Materials and Methods

Sixteen adult Balb/c mice of the same age (6 weeks) and weight (21gr±1) were utilized in this study. They were housed in macrolon cages under standard laboratory conditions (21±2° C, food and water ad libitum). Mice were divided randomly in two groups consisted of four female and four male respectively. The first group (control group) consumed distilled water, whereas the second group (group A) consumed Arsenic trioxide (As2O3) (Sigma-Aldrich) contaminated water (50ppm). Both groups were exposed to the above specific conditions for a period of six weeks. Having completed the administration period, all mice were anaesthetized and sacrificed. Kidneys and sciatic muscles were removed and placed in 10% formalin for light microscopy examination.

Kidneys and sciatic muscles samples designated for light microscopy study were then dehydrated through a series of increasing ethanol concentrations (25%, 50%, 70%, 80%, 96%

and absolute) and finally cleared with xylene. Then, the samples were embedded in parafin wax and sections of 5 μm were taken and stained with hematoxylin – eosin and immunohistochemical staining using e2f3 (Lifespan Biosciences) monoclonal antibody for IR.

The intensity of the staining of IRs on kidneys and sciatic muscle cells was evaluated in a scale of four grades: negative (-), low (+), moderate (++) and strong (+++).

Statistical analysis

The quantitative evaluation was made from photomicrographs of the tissue samples from each group. The Mann-Whitney U-test was used for comparisons between the 2 groups. Data was analyzed using PASW Statistics 18, Release Version 18.0.0, 2009 (SPSS, Inc., Chicago, IL). A value of $p<0.05$ was considered statistically significant.

Results

Glucose level

All mice showed normal glucose levels at the beginning of the study (Table 1). Mice of control group didn't show significantly altered glucose levels after the experimental period (glucose levels were below 124 mg/dl). On the contrary, glucose levels of group A mice after exposure to arsenic (50ppm) for six weeks were significantly higher than before. Glucose levels of five out of eight mice exceed 200 mg/dl and the highest is 214 mg/dl. The lowest glucose levels account for 181 mg/dl, which are much more than those of control group mice after exposure (Table 1).

Glucose levels (mg/dl)					
Control Group	Group A				
	Before exposure	After exposure		Before exposure	After exposure
Mouse 1	120	122	Mouse 1	118	189
Mouse 2	118	123	Mouse 2	121	201
Mouse 3	122	121	Mouse 3	123	202
Mouse 4	120	124	Mouse 4	119	195
Mouse 5	119	119	Mouse 5	122	211
Mouse 6	117	120	Mouse 6	121	213
Mouse 7	118	124	Mouse 7	117	214
Mouse 8	124	122	Mouse 8	122	181

Table 1. Glucose levels of mice peripheral blood at the beginning and after completion of the six week period of arsenic consumption.

Weight

Mice of both groups gained weight which was not statistically significant ($p>0.05$) compared to their initial weight. Group A mice gained more weight comparatively to those of control group but the difference is slight and not statistically significant ($p>0.05$) (Table 2). Control group mice seem to gain weight during the experimental period as well as those of group A. Although after treatment, the average body weight of control group mice are almost 3 gr less than that of group A mice. This difference is not statistically significant ($p>0.05$) (Table 2).

Weight (grammars)				
Control Group	Group A			
	Before treatment	After treatment	Before treatment	After treatment
Mouse 1	20.6	22.5	Mouse 1	20.9	25.7
Mouse 2	21.6	23.0	Mouse 2	21.3	25.9
Mouse 3	21.2	22.0	Mouse 3	21.7	22.0
Mouse 4	21.5	22.5	Mouse 4	21.2	22.9
Mouse 5	21.7	24.0	Mouse 5	21.8	28.7
Mouse 6	21.8	21.0	Mouse 6	21.6	24.2
Mouse 7	20.7	20.0	Mouse 7	21.5	27.0
Mouse 8	21.8	24.9	Mouse 8	20.6	24.9
Average	21.37	22.48	Average	21.33	25.17

Table 2. Mice weight of control group and group A before and after the six week period of exposure to arsenic.

Insulin Receptor concentration on kidney and sciatic muscle tissue

IR was measured by immunohistochemical essay on kidney and muscle tissues. Control group mice showed adequate IR concentration in kidney as in muscle tissues. IRs in group A mice are very rare and their concentration is much lower compared to control group.

Seven out of the eight control group mice kidney tissues showed a strong existence of Insulin Receptor and only one showed a moderate existence of the receptor (Table 3). On the other hand group A mice showed decreased insulin receptor concentration on kidney tissue. Insulin receptor was moderately present to kidney tissue of five mice and its existence was low to the other three mice of the group (Table 3).

Measurement of IR concentration on sciatic muscle tissue showed high concentration of insulin receptor in five control group mice as well as a moderate concentration in the remaining three mice (Table 4). Two group A mice sciatic muscle tissues were found to show moderate existence of IR, three other of the same group showed low IR concentration and the remaining three of the group were negative for the IR.

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IR on kidney			
Control Group	Group A		
Mouse 1	+++	Mouse 1	++
Mouse 2	++	Mouse 2	++
Mouse 3	+++	Mouse 3	+
Mouse 4	+++	Mouse 4	++
Mouse 5	+++	Mouse 5	++
Mouse 6	+++	Mouse 6	+
Mouse 7	+++	Mouse 7	+
Mouse 8	+++	Mouse 8	++

Table 3. Insulin Receptor concentration measured by immunohistochemistry (staining) on kidney in mice of control

group and group A at the end of the experimental period.

IR concentration on sciatic muscle			
Control Group		Group A	
Mouse 1	++	Mouse 1	++
Mouse 2	+++	Mouse 2	-
Mouse 3	+++	Mouse 3	+
Mouse 4	++	Mouse 4	+
Mouse 5	+++	Mouse 5	-
Mouse 6	+++	Mouse 6	-
Mouse 7	++	Mouse 7	+
Mouse 8	+++	Mouse 8	++

Table 4. Insulin Receptor concentration measured by immunohistochemistry (staining) on sciatic muscle tissue in mice of control group and group A at the end of the experimental period.

Location of insulin receptor on kidney and sciatic muscle tissue

In control group, insulin receptors of kidney are located in the interstitial tissue, in vessels, in glomerular and lightly in convoluted tubules (images 1,2). Insulin receptors of sciatic muscles are mainly located in connective tissue and vessels (image 10)

In group A, with diabetes mellitus, insulin receptors are not found in the largest part of kidney but only in the internal medulla and interstitial connective tissue (images 3,4,5,6). Insulin receptors of sciatic muscles in control mice are located in the same structures as in control group (images 7,8,9).

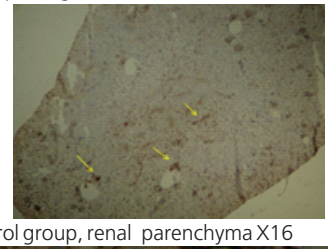


Image 1: control group, renal parenchyma X16

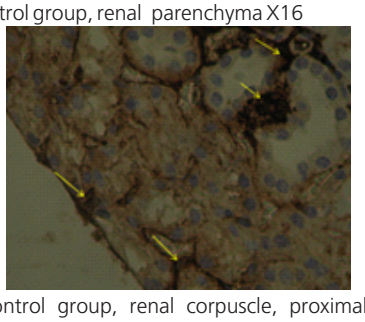


Image 2: control group, renal corpuscle, proximal and distal convoluted tubules X284

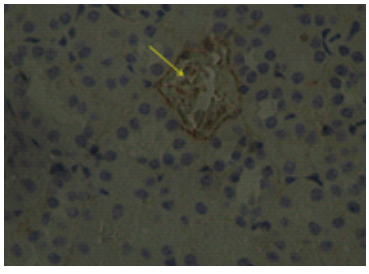


Image 3: group A, kidney medulla and renal corpuscle X284

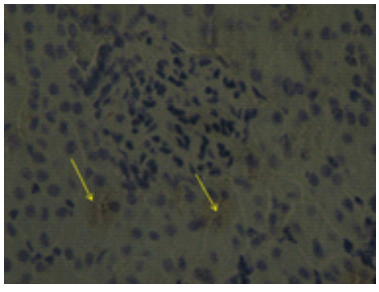


Image 4: group A, kidney cortex X160

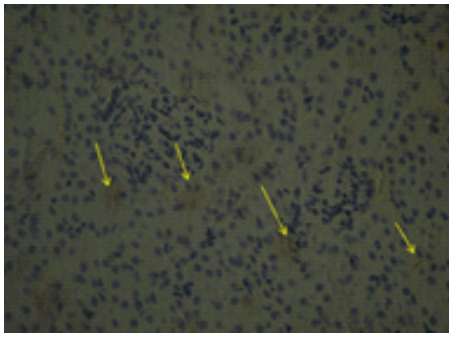


Image 5: group A, kidney cortex X40

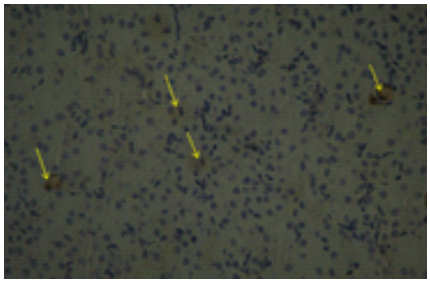


Image 6: group A, kidney medulla X160

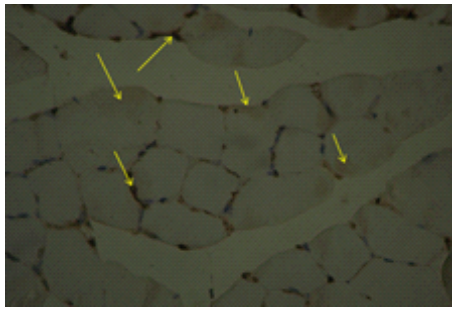


Image 7: group A, sciatic muscles X40

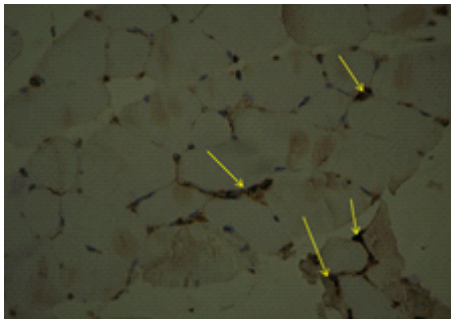


Image 8: group A, sciatic muscles X160

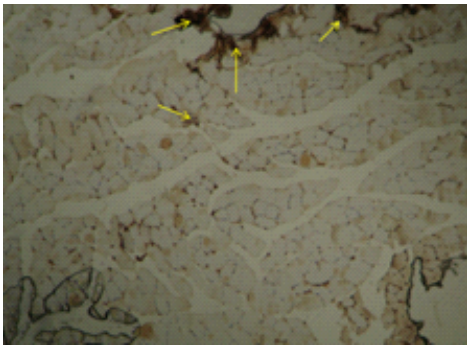
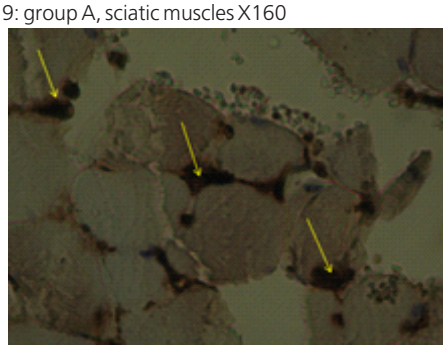


Image 9: group A, sciatic muscles X160



Discussion

Arsenic is a recognized toxicant and carcinogen and has been reported to have many adverse health effects including skin cancer and skin lesions, hypertension and chronic bronchitis [11]. Chronic exposure to arsenic is a potential risk factor for diabetes mellitus types 2 (DM2). Arsenic diabetogenesis affects millions of humans and needs millions of dollars for treatment. There is a dose dependent relationship between arsenic in drinking water and prevalence of diabetes [12-15]. The potential mechanisms of arsenic-induced diabetes, mainly on a biochemical basis, have been demonstrated in many studies. However, to our knowledge, little research has addressed the morphological effect of arsenic in tissues.

Normal glucose range in mice after a four hour fast is 60-130mg/dl. In case glucose value exceeds 200mg/dl then mice can be considered as diabetic [16,17]. Arsenic via drinking water increased glucose levels in mice of experimental group (50ppm). In fact, mean glucose values in the experimental group were significantly higher when compared with those of control group. Moreover in 5 out of 8 mice with increased glucose rates exceeded the benchmark of 200mg/dl and therefore those 5 mice can be considered as diabetic after exposure to arsenic. On the contrary, control group mice showed blood glucose levels lower than 200mg/dl (Table 1). Our results demonstrated increased glucose levels in male compared to female mice. A study of Palacios et al showed that male rats exposed to arsenic and lead for 3 months became insulin resistant and showed higher glucose levels whereas female rats remained sensitive to insulin [18]. Moreover, two of the female mice of group A cannot be considered as diabetic, even though they show elevated serum glucose levels.

Weight was changed throughout the experimental period in mice of both groups. However, this trend did not reach statistical significance. Weight differences were slight in both groups, but what can be easily seen is that all mice gained weight during the six week period (Table 2). It must noticed that most of the mice which consumed arsenic contaminated water became heavier than those of control group, even slightly, at the end of the experimental period.

The most interesting finding of the present study is the immunohistochemical analysis of insulin receptor using the e2f3 monoclonal antibody. Control group mice showed normal concentration of IR in kidney as well as in muscle tissue whereas experimental group mice showed significantly lower IR concentration in both kidneys and sciatic muscle. This finding combined with that of increased glucose levels in mice exposed to arsenic can lead us to the conclusion that arsenic reduces IR expression which may play part in DM2 prevalence. (Tables 3 and 4). The association between exposition to arsenic and DM has been is already known but the majority of studies focuses on oxidative stress. Arsenic promotes pancreatic -cells apoptosis by inducing oxidative stress and that must play a crucial role in DM2 pathophysiology [9]. Kim et al reported increased urinary glucose excretion in Korean general population after exposition to arsenic through drinking water. Urinary glucose excretion is associated with increased peripheral blood glucose levels and DM [19,20]. US adults exposed to arsenic through drinking water have a higher prevalence of DM2 than those who are not exposed to the compound. These findings can be combined with those of our study in order to understand arsenic associated DM.

Arsenic has a crucial impact on IR reduction in kidney and muscle tissue. Until now there is not a reference on arsenic impact on IR concentration on any kind of tissue after exposition to it and the impact of such a reduction on DM2 prevalence. It is needed to examine more tissue types and to further investigate the role of this reduction in IR number in DM2 in order to fully understand the pathophysiology of arsenic induced Dm2.

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