Arsenic toxicity in pancreas of diabetic rats

Neetu Singh, S.V.S. Rana

Toxicology Laboratory, Department of Zoology, Ch. Charan Singh University, Meerut, India–250 004

Neetu Singh, S.V.S. Rana receive International Toxicology Research Award-2014

Article history:
Received: 17 June, 2014
Accepted: 22 June, 2014
Available online: 08 December, 2014

Keywords:
Arsenic trioxide (As\textsubscript{2}O\textsubscript{3}), pancreas, diabetes mellitus, insulin, rat

Corresponding Author:
S.V.S. Rana*
Professor
Email: sureshvs_rana@yahoo.com, sureshvs.rana@gmail.com

Neetu Singh
Research Assistant

Abstract
Toxicity of arsenic trioxide (As\textsuperscript{III}) in exocrine and endocrine pancreas of rat has been addressed in this communication. Experimental diabetes was induced in rats through alloxen monohydrate (12.5 mg/100g). Arsenic trioxide was administered to diabetic rats (4 mg/100g) on each alternate day for 30 day. Thereafter, observations on accumulation of iAs in pancreas, insulin, liver, and histopathological studies were made. Results show that inorganic arsenic (iAs) is cumulative in pancreas. Further it damages \(\beta\)-cells and affect insulin secretion. In alloxan induced diabetic rats, accumulation of iAs in pancreas is diminished but no improvement in insulin concentration is noticed. Histopathological observations showed atrophy in the acinar tissue and insulitis in the islets. Further, pycnosis and apoptosis were recorded in arsenic treated diabetic rats. Ultrastructural observations reveal increased number of condensed vacuoles in the pancreas of iAs treated rats. Proliferation of zymogen granules was witnessed in arsenic treated diabetic rats. It is concluded that pancreas is also a suitable target organ for arsenic. Further, diabetes mellitus changes the toxico-kinetics of arsenic in rat.

Citation:

All Rights Reserved with Photon.

Photon Ignitor: ISJN22947439D720709122014

1. Introduction

Arsenic is an established environmental poison (Hughes et al., 2007; IARC, 2004; NRC, 1999). It affects numerous systemic functions and organs, viz. nervous system, hematopoietic system, liver, kidneys and skin (Blom et al., 1985).

Chronic arsenic exposure has also been reported as a potential risk factor for type II diabetes (Rahaman et al., 1998; Rahaman and Axelson, 1995). It is a metabolic disorder characterized by hyperglycaemia, insulin resistance in peripheral tissues, and altered insulin secretory capacity of pancreatic \(\beta\) cells (Kahn et al., 1993; Kahn, 2003). Several studies in different ethnic groups during different study periods have shown an association between arsenic exposure and the occurrence of diabetes mellitus (Chen et al., 2007; Navas-Acien, 2008).

Effects of arsenic on glucose metabolism have been attributed to its reactivity towards thiol (SH) groups (Aposhian et al., 1989; NRC, 1999). During acute poisoning, arsenite inhibits pyruvate and \(\alpha\)-ketoglutarate dehydrogenases, the essential enzymes for gluconeogenesis and glycolysis (Aposhian et al., 1989). A recent report from our laboratory showed that iAs when administered to alloxan diabetic rats improved their liver function (Singh and Rana, 2009). Further, convincing reports show that diabetes alters the pharmacodynamics and pharmaco-kinetics of drugs/xenobiotics in man and animals (Chawalit et al., 1982; Longhurst et al., 1986). Therefore, it could modulate its toxic manifestations. An earlier report from our laboratory also indicated such involvement in liver (Singh and Rana, 2009). The aim of the present study was to demonstrate the
involvement of pancreas, if any, in arsenic toxicity. Secondly, it is an attempt to investigate the effects of diabetes mellitus on arsenic toxicity in rat.

2. Experimental

Male Wistar rats (150 ± 20 g) were procured from the animal facility of Jamia Hamdard, New Delhi. They were acclimatized to laboratory conditions (room temperature 25 ± 5° C, relative humidity 50 ± 10%) for 2 weeks before starting the experiment. Each rat was housed separately in a polypropylene cage and offered pelleted food (Golden Feeds, New Delhi) and tap water ad libitum. All animal treatments and protocols received prior approval of Institutional Ethical Committee.

2.1 Treatments

Rats were separated at random in six groups, each containing five rats. Diabetes mellitus was introduced in rats of groups c, d, e and f by injecting alloxan monohydrate (12.5 mg/100 g/l body weight) intraperitoneally as described earlier (Singh et al., 2006). The induction of diabetes in these rats was confirmed by estimating blood glucose by Folin and Wu method using a commercial kit supplied by Span Diagnostics, Surat (India). Rats of groups b, d, and f were administered arsenic trioxide (4 mg/100 g/l body weight) on alternate day for 30 days as described earlier (Singh et al., 2006). Rats of groups e and f were injected insulin, and rats of group ‘A’ were injected with saline only to serve as controls. Alloxan monohydrate was procured from Loba Chemie (Mumbai, India). Arsenic trioxide was supplied by C.D.H., Mumbai whereas insulin (bovine) was procured from U.V.S. Limited Mumbai (India).

2.2 Collection, preparation, and analysis of samples

Collection, preparation, and analysis of samples. After 30 days, rats were starved overnight and killed by light ether anesthesia the next morning. The pancreas was carefully removed and processed. Blood was collected through cardiac puncture and processed suitably for further investigations.

2.3 Histopathology of pancreas

Small pieces of pancreas collected from each group were fixed in 10% neutral formaldehyde and embedded in paraffin. Six-micron thick sections were stained with hematoxylin and eosin and examined under research microscope (Olympus, Japan).

2.4 Electron microscopic studies

Very small cubes (1 mm³) of pancreas were immersed in 2.5% glutaraldehyde, post fixed in 1.0% osmium tetroxide, dehydrated through a graded series of ethanol and embedded in Epon 812 after several changes of propylene oxide. Ultra thin sections stained with uranyl acetate and lead citrate were examined under a Phillips, CM10 transmission electron microscope at Electronic Microscope Facility, All India Institute of Medical Sciences, New Delhi.

2.5 Estimation of arsenic

Samples of pancreas were digested in concentrated nitric acid and diluted with double distilled water. One gram of the sample was digested in 10 ml of concentrated nitric acid at 80° C for 16 h. The digests were stored at 4°C till analysis. A 2 ml aliquot of the digest was analyzed for arsenic by hydride generation at pH 6 using sodium borohydride as the reducing agent. Sodium borohydride generation assembly compatible with atomic absorption spectrophotometer (ECIL, India) was used. Arsenic concentration was measured at 193 nm using a hollow cathode lamp.

2.6 Determination of insulin

Insulin was determined in serum samples using ELISA method with the help of a commercial kit procured from Monobind Inc. Lake Forest, CA, USA.

2.7 Statistical inferences

Students’ t test was employed for statistical conclusions.

3. Results & Discussion

Present observations show that weight of pancreas decreased in arsenic treated rats. However, it increased in arsenic treated diabetic rats. Insulin treatment to diabetic and arsenic fed rats could not restore the weight of pancreas. Result on pancreas body weight ratio showed a decrease in arsenic treated rats but an increase in arsenic treated diabetic rats (Table1).

Arsenic was found to be cumulative in pancreas too. Accumulation of arsenic was higher in arsenic treated rats in comparison to arsenic treated diabetic rats suggesting that diabetes mellitus does not favor the accumulation of arsenic in pancreas (Table 1).

Insulin concentration showed a non-significant increase when arsenic was administered to
diabetic rats whereas it decreased in arsenic treated rats. Insulin therapy given to diabetic and arsenic treated failed to restore insulin concentration (Table 1).

### 3.1 Histopathological observations

#### 3.1.1 Light microscopy
Histopathological observations further suggest those exocrine and endocrine pancreases are suitable targets of arsenic toxicity. Pancreas of control rats showed normal acinar cells and islets morphology (Figure 1). Acinar cell atrophy was recorded in arsenic treated rats. The endocrine pancreas showed damage to beta cells (Figure 2). Pancreas of alloxan and arsenic treated rats showed tissue damage. Pycnotic and apoptotic nuclei were also observed in the islets (Figure 3).

**Figure 1:** T.S. of the pancreas of control rat shows normal acinar cells (AC) and islets of Langerhans (IL). (H/E×400)

**Figure 2:** T.S. of the pancreas of arsenic-treated rat shows acinar cell (AC) atrophy and damage of beta cells in islets (IL). (H/E×400)

**Figure 3:** T.S. of pancreas of alloxan and arsenic-treated rat shows pycnotic nuclei in islets (IL) and acinar cell damage (AC). (H/E×400)

#### 3.1.2 Electron microscopic observations
The exocrine pancreas of normal rats shows several zymogen granules, Golgi bodies and condensed vacuoles (Figure 4). The exocrine pancreas of arsenic treated rats showed increase in the number of condensed vacuoles.
and decrease in the number of Golgi bodies (Figure 5). The pancreas of diabetic rats showed extensive proliferation and condensation of endoplasmic reticulum and increased number of condensed vacuoles. Exocrine pancreas of arsenic treated diabetic rats showed several condensed vacuoles and Golgi bodies (Figure 6).

Figure 4: Electron micro-photographs of pancreas of control rat shows several zymogen granules (z) and nucleus (NU). (2200X)

Figure 5: Electron micro-photographs of the pancreas of arsenic-treated rat shows increased number of condensed vacuoles (V), zymogen granules (z), mitochondria (M) and nucleus (NU). (4600X)

Inorganic arsenic causes physiological and pathological changes in several human and animal tissues. Acute as well as chronic effects have been reported in skin, gastrointestinal system, nervous system, liver, kidney, respiratory and cardiovascular systems. (ATSDR, 2005; Chen et al., 1997 a, b; IARC, 2004; 2006; NRC, 1999; WHO, 1981; 2001). Further ingested or inhaled arsenic through occupational or environmental exposure is involved in the development of cancer in skin, liver, kidney and urinary bladder (IARC, 2004; WHO, 2001). Although several reports on the association between iAs and diabetes mellitus are available, effects of arsenic trioxide on pancreas were hardly addressed.

We observed that arsenic is cumulative in pancreas. In diabetic rats, the concentration of arsenic is reduced whereas pancreas / body weight ratio is increased in arsenic treated diabetic rats. It has been postulated earlier that hyperglycemia restricts the accumulation of arsenic in liver (Singh and Rana, 2009). Increased excretion of arsenic in diabetic rats has also been suggested (Singh and Rana, 2009).

Several studies have documented the role of trace elements in islet function and development of diabetes mellitus (Chen et al., 2009). Tseng (2004) reviewed the potential mechanism of arsenic induced diabetes mellitus. Effect of inorganic arsenic on insulin has also been debated. Earlier studies have shown that exposure to inorganic arsenic (iAs) disrupts insulin production. Paul et al. (2008) showed that arsenite and its methylated metabolites inhibit insulin stimulated glucose uptake in cultured adipocytes by disrupting insulin activated signal transduction pathway. Further, arsenite has high affinity for sulphydryl groups and forms covalent bonds with the disulfide bridges in the molecule of insulin,
insulin receptors, glucose transporters (GLUTs) and enzymes involved in glucose metabolism (pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase). Insulin status declined in iAs treated rats. It has been suggested that iAs increases oxidative stress that upregulates the expression of tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) (Tseng., 2004). Both these cytokines might affect insulin and insulin dependent reactions in diabetic rats. In liver arsenic disturbs glucose production, whereas in beta cells, arsenic decreases insulin synthesis and reduces the expression of antioxidant enzymes (Diay-Villansenor et al., 2007). In diabetic and arsenic treated rats oxidative stress is diminished and expression of antioxidant enzymes is increased (Singh and Rana, 2009). This may down regulate the expression of cytokines (TNF α and IL-6) leading to moderate increase in insulin status.

Consequences of above mentioned changes i.e. iAs accumulation, insulin secretion and oxidative stress on pancreas were finally witnessed through histopathological changes in pancreas. Significant injury to exocrine and endocrine pancreas was expressed by iAs. Acinar cells were injured and so were the beta cells (Figure 2). A comparison with arsenic treated diabetic rats showed mild inflammation in acinar cells whereas pycnosis was observed in endocrine pancreas (Figure 3). No similar reports are seemingly available on arsenic toxicity with special reference to pancreas. However, exocrine pancreas may play minor role in the metabolism, detoxification or excretion of xenobiotics. Overall incidence of recognized xenobiotic toxicity in exocrine pancreas is low. Nevertheless, present observations provide additional information on arsenic toxicity with special reference to pancreas. We noted that inorganic arsenic was found to cause degenerative changes in the acinar cells and islet hyperplasia (inflammation, atrophy and fibrosis of the exocrine tissue). We noted that inorganic arsenic was found to cause degenerative changes in the acinar cells and islet hyperplasia. Whereas in arsenic treated diabetic rats, insulitis was observed in the endocrine pancreas. Damaged beta cells exhibited vacuolated cytoplasm and pycnosis of the nuclei.

Electron microscopical studies support light microscopical observations. Acinar cells secrete zymogen by exocytosis of zymogen granules into the acinar lumen in response to toxic stimulus. We observed increased number of zymogen granules and condensed vacuoles in the pancreas of inorganic arsenic treated rats. Whereas similar were the observations in arsenic treated diabetic rats.

It is concluded that acinar cells contain phase I and phase II drug metabolizing enzymes. Induction of these enzymes by inorganic arsenic like other xenobiotics may contribute to its toxicity in pancreas as suggested by Lawson and Kolar (1997).

These observations become important as a number of reports have indicated that exposure to iAs causes diabetes mellitus (Tseng, 2004). It has been suggested that arsenic may indirectly cause functional impairment in insulin secretion through generation of free radicals and oxidative stress that destroy the insulin secreting β-cells. Alternatively, it has been suggested that toxicokinetics of arsenic are altered in diabetic rats due to microsomal interference. A unique form of CYP450 appears in rat liver microsomes during diabetes (Past and Cook, 1982).

**Flow chart:** Showing the effect of diabetes on arsenic toxicity in pancreas

**Conclusion**

Present results demonstrated that arsenic is cumulative in pancreas. Further, it damages β-cells and impairs insulin homeostasis. Diabetes mellitus modulates its toxic manifestations in pancreas, like liver and kidney.

**Acknowledgements**

The authors are thankful to Dr. Y. Verma and Mr. Nitin Sharma for their excellent technical support.
Authors Contribution and Competing Interest

The authors have not conflict of interest. The experiments were carried out as a team work.

References


Chawalit K., Stret Argusa P., Thitapanda A., 1981. Comparative effects of diabetogenic agents on hepatic drug metabolism. Drug Metabolism and Disposition, 10, 81–86


