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Research article

GENDER AND SEGMENTAL STUDY OF ARSENATE UPTAKE BY THE EVERTED GUT SACS OF MICE

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ABSTRACT

Background: Humans are exposed to both inorganic and organic arsenic through environmental, medicinal and occupational situations. The main source of arsenic exposure is drinking water with high levels of arsenic. **Aim:** This study was undertaken to investigate the gender and segmental difference in arsenate (As V) uptake by everted gut sacs of mice. **Materials & methods:** By using the everted gut sac technique, the serosal and mucosal uptake of Arsenate (2 mM) in both sexes of mice was studied in the intestinal segments. The Arsenate in the samples of fluid present in serosal and mucosal compartments of everted gut sacs was estimated by Hydride-Generation Atomic Absorption Spectrophotometer. **Results:** There was a steady increase in both serosal and mucosal uptake of Arsenate in both duodenum and ileum with a rise in the initial Arsenate concentration of the incubation medium. The mucosal uptake of Arsenate was significantly higher in duodenum than ileum ($P<0.001$). Both serosal and mucosal uptakes were elevated in the duodenal segment of male mice when compared to female mice except mucosal uptake in ileum. **Conclusion:** These results indicate that there is a gender and segmental difference in the uptake of AS (V), which can be explored pharmaceutically to reduce the arsenic toxicity in population at risk.

Keywords: Arsenic transport, Everted gut sacs, Arsenic toxicity.

INTRODUCTION

Arsenic, a ubiquitous element present in various compounds, is mainly transported in the environment by water. Arsenic can exist in four valency states[1]. The predominant form of inorganic arsenic in aqueous and aerobic environments is arsenate (As V) whereas arsenite (As III) is more prevalent in anoxic environments. Humans are exposed to inorganic or organic arsenic through environmental, medicinal and occupational situations. The main source of arsenic exposure for the general population is drinking water with high levels of arsenic[2,3]. The food contains both organic and inorganic arsenic, whereas drinking water contains primarily inorganic forms of arsenic. A variety of adverse health effects e.g., skin and

internal cancers, cardiovascular and neurological effects have been attributed to arsenic exposure, primarily from drinking water.

Arsenic can be absorbed from the gastrointestinal tract after ingestion of arsenic containing food, water, beverages or medicines, or as a result of inhalation and subsequent mucociliary clearance. The concentration of arsenic in unpolluted ground water is in the range of 1-10 µg/ L. In many parts of the world - India [4], Bangladesh [4], Chile [5], North Mexico [6] , Argentina [7] and Taiwan [8] the arsenic concentrations (> 1 mg As/ L) in groundwater was found to be elevated. Though various chelators are available for acute arsenic toxicity, so far there is no known drug available to prevent the absorption of arsenic at the intestinal level. The preventive measures are mainly aimed at reducing the levels of arsenic in the drinking water [9]. Since the gut remains the main portal of entry of this metal into the human body and the inorganic arsenicals like pentavalent and trivalent forms are rapidly and extensively absorbed from the gastrointestinal tract [10,11], it was decided to study the effect of varying the Arsenate concentration in the incubation medium on Arsenate transport in all segments of intestine using the everted gut sacs of both the sexes of mice.

MATERIALS AND METHODS

Chemicals and Reagents: Sodium arsenate ($Na_2HAs_5O_4$ $7H_2O$; molecular weight 312.01) and other analytical laboratory chemicals and reagents were procured from Sigma –Aldrich Chemicals (St. Louis, MO.USA).

Animals: After the approval by Institutional Animal Ethical Committee (IAEC) of SVMCH&RC. Healthy adult male (40) and female (40) Swiss albino mice, weighing approximately 25-31 g were used for present study. Mice were acclimatized for 7 days to light from 06:00 to 18:00 h, alternating with 12 h darkness, maintained under controlled conditions of temperature (25 ± 2°C), humidity (50 ± 5%) and a 12-h light–dark cycle. Mice were allowed standard laboratory commercial feed obtained from Gold Mohur Animal Feeds (Bangalore, India) throughout the experiment and water *ad libitum*.

All animals maintained compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Tissue preparation: This was assessed by usage of everted gut sacs prepared from the mice. Under anaesthesia the intestine was excised carefully and fat and mesenteric attachments were removed. Everted sacs of 6 cm length were prepared from the duodenum according to the method described by Wilson and Wiseman [12]. The distal end of the sac was tied with a ligature (000 Ethilon Black braided nylon). A ligature was placed loosely around the proximal end.

After weighing, the empty sac was filled with 0.5 ml of the desired incubation medium (serosal compartment) using a micro syringe (Gas tight syringe 1750, Hamilton Company, USA) fitted with a blunt needle. The filled sacs were slipped off the needle carefully and the loose ligature on the proximal end was tightened. After weighing, the distended sacs were placed in 5 mL of the same incubation medium contained in a 25 mL Erlenmeyer (siliconized) flask. After gassing for 1 min with 100% oxygen, the flasks were tightly stoppered and incubated at 37°C for 1 h in a metabolic shaker bath (Techno India Ltd, Pune, India) at a frequency of 90-110 shakes/ min. At the end of the 1 h incubation period, the sacs were removed from the flasks, blotted and weighed again. The incubation medium contained (in mM) NaCl (135), KCl (11) and $CaCl_2$ (0.04) dissolved in phosphate buffer of the desired concentration (KH_2PO_4 and Na_2HPO_4) at pH 7.4. Sodium arsenate was added to give a final concentration of 2 mM.

Estimation of Arsenate**:** After incubation in a water bath at 37°C with shaking for varying periods, the sacs were emptied and samples of fluid from the mucosal and serosal compartments

were collected. The final Arsenate concentrations of mucosal and serosal fluids were determined. The amounts of Arsenate removed from these fluids were calculated and characterized as 'mucosal uptake' and 'serosal uptake' respectively. These were expressed as µmol/ gm tissue wet weight/ hr. Arsenate was estimated by using Hydride Generation-Atomic Absorption Spectrophotometer (HG-AAS, GBC Instruments Pvt. Ltd., Australia, Model-916)[13.]

Enterocyte Viability Test: Enterocytes were isolated by mechanically vibrating the emptied gut sacs at the end of 90 min incubation. The cells were then incubated with 0.2% trypan blue solution at 37°C to check the viability[14]. The sample was taken into consideration only if 80% or more of mucosal cells excluded the dye showing their viable nature.

Statistics

Data were expressed as mean ± SEM. One-way ANOVA test and Student's unpaired t-test was performed. $P < 0.05$ was considered to be significant.

RESULTS

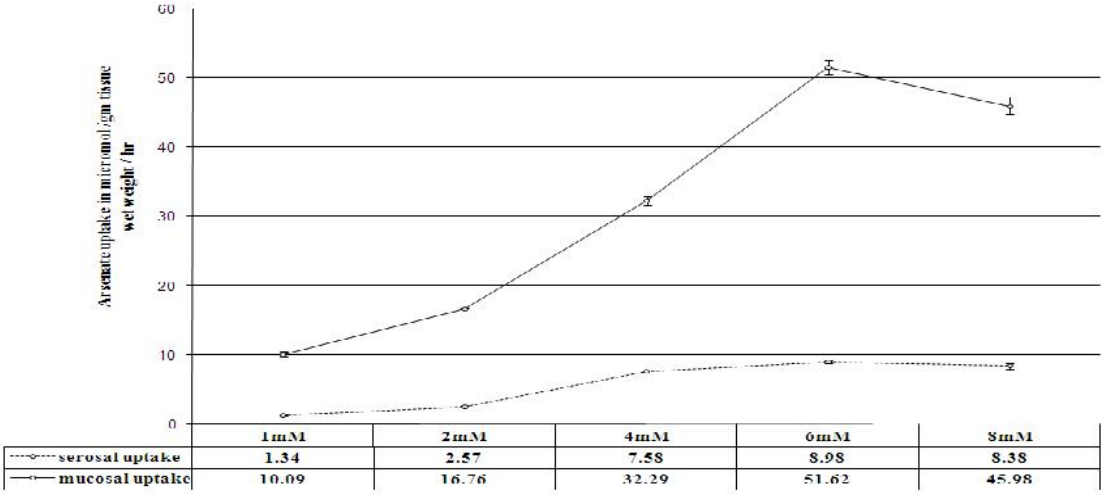
Effect of varying arsenate concentration in the incubation medium on arsenate transport of duodenum: From fig 1. it is seen that the mucosal uptake of Arsenate was increased with the rise in the initial Arsenate concentration of the incubation medium, reaching a maximum value at a medium concentration of 6 mM. From then on, the serosal uptake remained fairly steady whereas a slight decline is noted in the mucosal uptake. The mucosal uptake at this point is about thrice the value obtained with a concentration of 2 mM. The serosal uptake on the other hand reached a maximum value at 6 mM and showed a mild insignificant decline at higher initial Arsenate concentration in the medium.

Effect of varying Arsenate concentration in the medium on Arsenate transport of ileum: From Fig 2, it is seen that the mucosal uptake of Arsenate increased with a rise in the initial Arsenate concentration of the incubation medium but reaching a maximum value at a medium concentration of 4mM. From then a decline in Arsenate uptake was noted at higher initial concentration in the medium. The mucosal uptake at this point is about thrice the value obtained with a concentration of 2mM. The serosal uptake reached a maximum value at 6mM followed by a mild insignificant decline. The serosal and mucosal uptake at different Arsenate concentrations between duodenum and ileum are significant except the serosal uptake at 6mM and 8mM Arsenate concentration.

Time study: Fig 3. The serosal and mucosal Arsenate uptake steadily increases with time. The serosal uptake when expressed as a percentage over mucosal uptake tends to remain at 37% and decreases to about 22% at the end of 60 minutes. At the end of 90 min of incubation, the serosal uptake becomes 31% .The drained sacs at the end of 90 minutes were tested for viability using trypan blue test ; 80% - 90% of the mucosal cells showed an exclusion of the stain showing their viable nature.

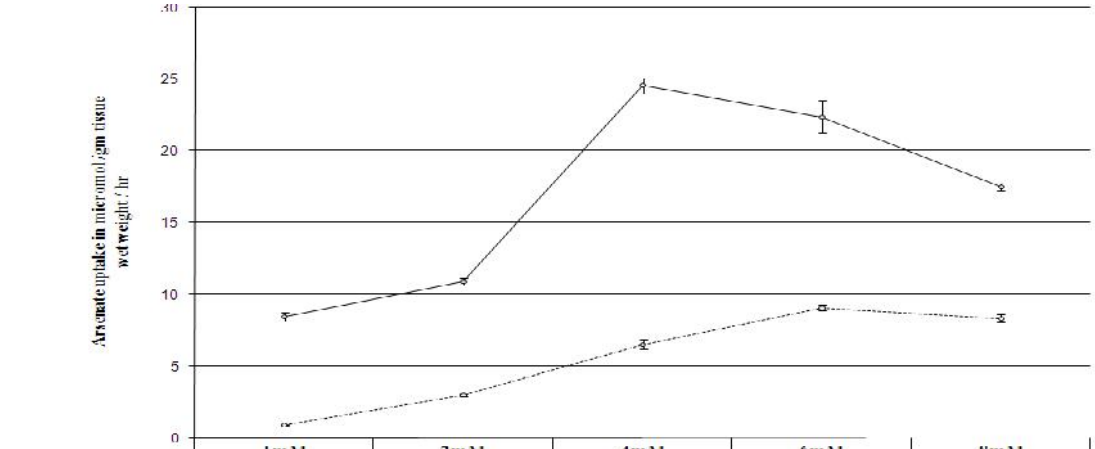
Gender and Segmental Study

The Arsenate transport in various segments of intestine namely duodenum, jejunum and ileum was studied successively in male and female mice and are represented by Fig 4 and 5. It is apparent that Arsenate transport is maximal in the proximal segment for males whereas the terminal segment of the female mice gave the maximal Arsenate transport. It tended to plunge to a low value in the second segment of both the groups. From there a steady increase was noted in successive segments of the intestine. Both serosal and mucosal uptakes were elevated in male when compared to female mice except mucosal uptake in ileum.



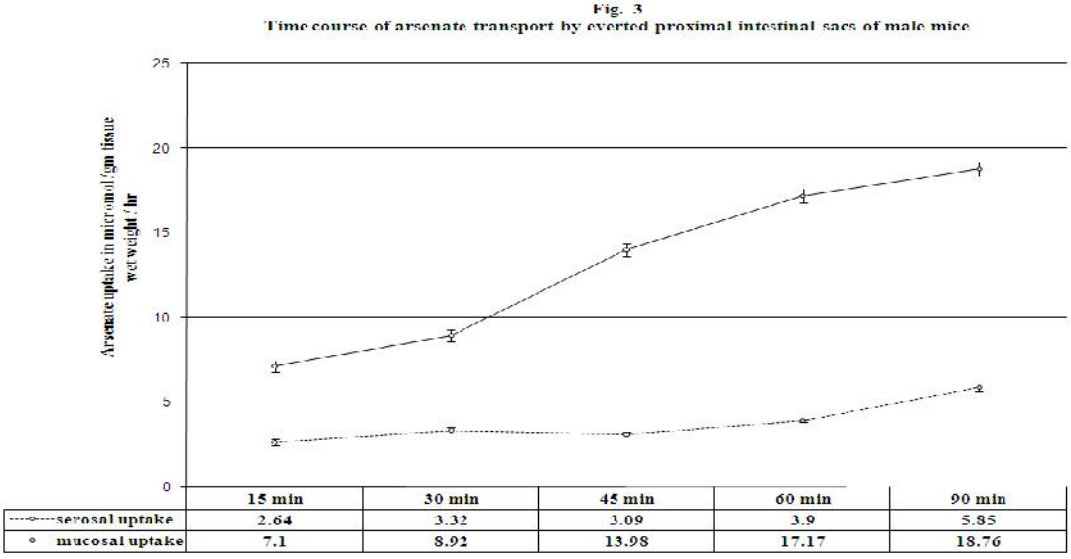
	1mM	2mM	4mM	6mM	8mM
serosal uptake	1.34	2.57	7.58	8.98	8.38
mucosal uptake	10.09	16.76	32.29	51.62	45.98

Fig.1: Effect of varying arsenate concentration in the medium on arsenate transport by proximal intestinal sac



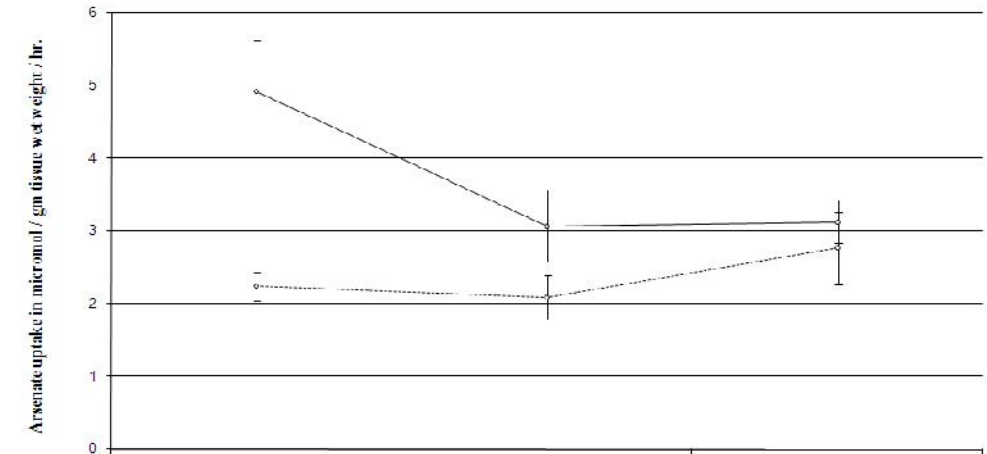
	1mM	2mM	4mM	6mM	8mM
serosal uptake	0.91	2.99	6.52	9.1	8.38
mucosal uptake	8.46	10.91	24.56	22.37	17.49

Fig.2: Effect of varying arsenate concentration in the medium on arsenate transport by distal intestinal sac



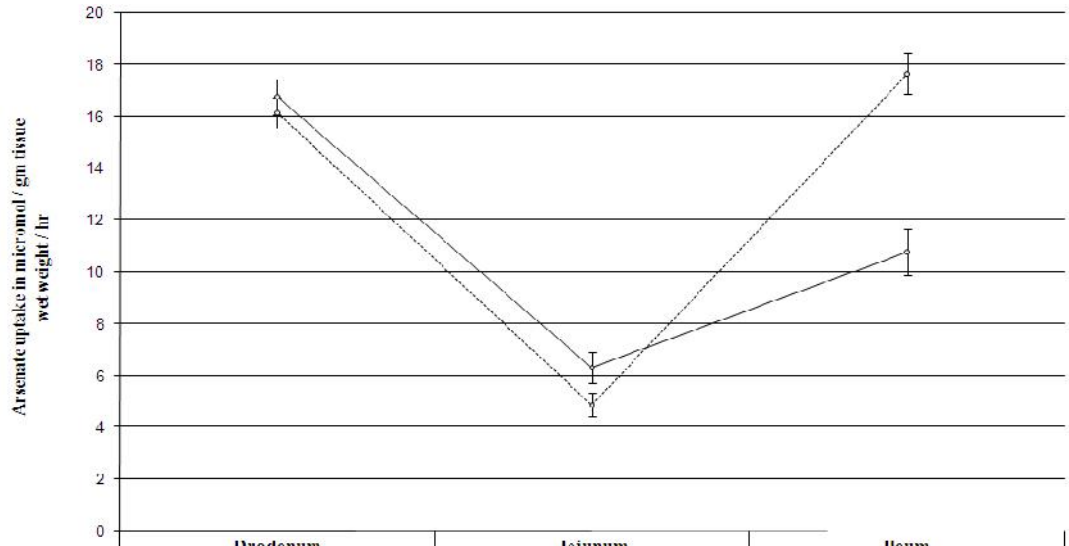
	15 min	30 min	45 min	60 min	90 min
serosal uptake	2.64	3.32	3.09	3.9	5.85
mucosal uptake	7.1	8.92	13.98	17.17	18.76

Fig.3: Time course of arsenate transport by everted proximal intestinal sacs of male mice



	Duodenum	Jejunum	Ileum
Male	4.92	3.07	3.13
Female	2.24	2.09	2.76

Fig.4: gender and segmental study of serosal arsenate uptake in evereted mice intestinal sac



	Duodenum	Jejunum	Ileum
Male	16.75	6.3	10.76
Female	16.14	4.88	17.64

Fig.5: Gender and segmental study of mucosal arsenate uptake in the everted mouse intestinal sacs

DISCUSSION

Our results clearly showed that both the serosal and mucosal uptakes of Arsenate in both duodenum and ileum were found to be linearly increased at varying Arsenate concentrations in the incubation medium. The observations of this study goes in agreement with the previous studies where the intestinal absorption of Arsenate in chick by means of in-situ ligated duodenal loop technique showed that, it is rapidly and completely absorbed (80-95%) from the lumen at the Arsenate concentration up to 5 mM, declining to about 50% absorption at 50mM. The total mucosal accumulation of Arsenate increases in a linear logarithmic fashion from 0.05 to 5 mM [14,15].

In KB oral epidermoid carcinoma cells, the cytotoxicity and intracellular accumulation of Arsenate were dramatically enhanced, equaling those of As III when cells were grown in phosphate -free medium and Arsenate uptake was dose-dependently inhibited by phosphate [16]. The previous studies have shown that the cellular

uptake of arsenite is four times higher than for Arsenate[17]. In KB (cell line) oral epidermal carcinoma cells, the uptake of arsenite were linearly correlated with extracellular concentrations suggesting that arsenite uptake is accomplished through simple diffusion through aquaglyceroporins [10].

The absorption of Arsenate by the rat small intestine was investigated, where intestinal absorption of Arsenate appeared to be carried out by a saturable transport process. The inorganic phosphate (Pi) produces a pronounced decrease in the intestinal absorption of As V. These results suggest that Arsenate shares a common transport system with phosphate which is an active secondary carrier mediated system depending on Na^+ and H^+ gradients [18].

Studies by Borowitz and Granrud [19] showed that Pi absorption appeared to decrease with age, as the 12 week old rabbits contained Na-Pi co-transporters only in duodenum and proximal jejunum compared with young animals. The significant increase in Arsenate uptake in duodenum could be attributed to the increase in the number of Na-Pi co-transporters in the duodenum.

CONCLUSION

The difference in the As V uptake by both sexes of mice observed in our study correlates well with the previous studies [20] indicating a clear sex difference in phosphate transport of duodenum in mice. Since As V is transported by the same transport protein, it is natural to see such difference in As V transport also. Hence, Na-Pi co-transporters in the duodenum may be used for further pharmaceutical exploration with a view to reduce the arsenic toxicity in population at risk.

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