STUDY ON LIVER ARSENIC LEVEL IN ANTIBIOTIC TREATED RATS

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Abstract

Rats treated with arsenic (1mg/L) in drinking ad libitum *an increase in liver tissue arsenic was observed when orally pretreated with streptomycin (500mg twice daily). Inhibition of gut flora was confirmed by microscopic examination of stool. Control group showed a mean gut-bacterial count of 7.13-7.26×10^{8}cfu/g dry weight of stool, when administered with streptomycin orally (500mg twice daily) gut-bacterial count was not countable on day 7. Liver tissue arsenic level increased to 5.78 mg/g of liver tissue compared to that of the control group of 3.33mg/g of liver tissue. A decrease in gut-bacterial count of 2.83×10^8 cfu/g dry weight of stool was observed on day 14 in rats that were not pretreated with streptomycin but received only arsenic (1mg/L) in drinking water* ad libitum *throughout the study period. Increase in liver arsenic level in this group was almost similar to that of streptomycin pretreated rats.*

Key words: *Arsenicosis, Liver arsenic level, Antibiotic, Streptomycin, Long Norwegian rat.*

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Introduction

Arsenicosis is a major health hazard in an underdeveloped country like Bangladesh. Environmental pollution of arsenic is from industrial sources, use of wood preservatives, metallurgical, mining and use of insecticides.[1] Significantly high level of arsenic in ground water has been observed in some areas of Bangladesh. Maximum safe permissible limit of arsenic in water by WHO is 0.01mg/L. In many areas of Bangladesh, particularly along the Ganges, Brahmaputra, Delta it is above the safe drinking standard.[5]

Water contaminated with arsenic is used for purpose of irrigation has led to its redistribution in food stuff.[2] Use of organic arsenicals like roxersone in poultry feed, as an intestinal palliative and to increase growth has also led to its distribution in food stuff.[2] Antibiotics now a days are being used by practitioners at all levels most of the time without proper indication. Irrational and improper use of such antibiotics might have an adverse effect, leading to inhibition of gut flora.[3,4]

Gut flora are considered to be bodies first line of defence mechanism against ingested xenobiotics. So indiscriminate and improper use of antibiotics in areas where people are chronically exposed to arsenic through drinking water can result in permanent and severe damage to human health, including lesions of skin[1], mucous membrane, digestive tract[2] and damage to respiratory[3], circulatory[4], endocrine[5] and nervous system.[6] Arsenic is a documented human carcinogen associated with skin, liver and lung cancers and has been classified by WHO's International Agency for Research on Cancer into Group-I. Epidemiological studies suggest that persons with impaired arsenic metabolism are at increased risk of arsenicosis. Detoxification of arsenic takes place both in liver and by gut flora.[9] Hepatic methylation of arsenic is being challenged as growing number of reports of studies in experimental model system indicate that hepatic methylation of arsenic is more toxic than methylation by gut flora.[18] This study was carried out with a view to see the inhibitory effect of antibiotics and gut flora and its effect on hepatic methylation of arsenic.

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Materials and Methods

Animals: Healthy young adult male rats of Long Norwegian Strains, weighing 160-180g and 3-4 months old were taken for the purpose of study. They were kept in stainless steel cages in animal house. Saw dust was used as beding and changed every alnernate day, a 12 hours light/ 12 hours dark cycle was maintained. They were fed standared pellet diets and allowed to drink *ad libitum*.

A total of 24 rats were used in this study and divided into four groups. Group A was control, Group B received only streptomycin (500mgm twice daily) orally, group C received only arsenic (1mg/L) and group D received arsenic with streptomycin orally for 14 days. Stool was cultured in MacConkey's agar and complete inhibition of gut flora was ensured by microscopic examination of stool.

Stool specimen collection, dilution and culture: Fecal pellets were collected in clean, sterile glass container as soon as they were passed by the animal. A portion of fresh stool specimen was taken in sterile labelled test tubes containing 1ml of normal saline, vortexed and centrifuged at 1600g. The supernatant was decanted and serial dilution (1-5) was carried out in sterile test tubes containing1ml of normal saline. From the fifth test tube 10µl of specimen was taken and cultured in MacConkey's agar at 37°c for 24 hours, for colony count. Stool cultures were performed on day 0, day 7 and day 14 of administering drugs.

Liver and stool arsenic estimation:
Remaining portion of stool was estimated for arsenic. Animals were sacrificed on day fourteen under light chloroform anaesthesia. Liver taken out and arsenic estimation done. Both stool and liver arsenic were estimated by SDDC method (silver diethyldithiocarbamate method).

Results

Table I

Inhibilion of gut flora

Groups	Bacterial Count (cfu/g dry weight of stool)		
	Day 0	Day 7	Day 14
Control	$7.13 \times 10^3 \pm 0.49 \times 10^3$	$7.19 \times 10^3 \pm 1.06 \times 10^3$	$7.26 \times 10^3 \pm 0.06 \times 10^3$
Only streptomycin (1g/Day)	$7.08 \times 10^3 \pm 0.54 \times 10^3$	Not Countable	Not Countable
Only Arsenic (1mg/L)	$7.01 \times 10^3 \pm 0.56 \times 10^3$	$7.74 \times 10^3 \pm 1.33 \times 10^3$	$2.83 \times 10^3 \pm 3.33 \times 10^3$
Arsenic (1mg/L) + streptomycin (1g/Day)	$7.01 \times 10^3 \pm 0.56 \times 10^3$	Not Countable	Not Countable

All valuable result are presented as mean ±SD and Statistically significant P value is >0.05

Table II

Stool & Liver arsenic concentration

Groups	Stool mg/g dry weight		Liver tissue mg/g	
	Day 0	Day 7	Day 14	Day 14
Control	3.38±0.63	3.74±0.67	3.63±0.79	3.33±0.92
Only streptomycin (1g/Day)	3.56±0.34	3.31±0.52	3.72±0.67	3.63±0.21
Only Arsenic (1mg/L)	3.33±0.68	3.31±0.72	2.39±0.27	5.40±0.71
Arsenic (1mg/L) + streptomycin (1g/Day)	3.43±0.36	2.58±0.24	2.52±0.27	5.78±0.46

All valuable result are presented as mean ±SD and Statistically significant P value is >0.05

The mean gut bacterial count in control group was 7.13 to 7.26×10^8 cfu/g dry weight of stool (Table I). Oral administration of streptomycin caused a reduction in gut bacterial count to not countable level on day 7 and day 14. Rats treated only with arsenic also showed an inhibition of gut flora from 7.01×10^8 cfu/g dry weight stool to 2.83×10^8 cfu/g dry weight of stool, here gut flora reduced significantly but did not go to uncountable levels. So it was observed that both streptomycin and arsenic inhibit gut flora but rate of inhibition of streptomycin was more than that of arsenic, being almost 100% incase of streptomycin treated rats. Mean stool arsenic level in control group ranged from 3.38-3.63 mg/g dry weight of stool (Table II). It decreased significantly on day14 to 2.52 mg/g dry weight of stool in rats that received streptomycin with arsenic and 2.39 mg/g dry weight of stool in rats that received only arsenic.Liver arsenic level in control groups of rats was 3.33 mg/g of liver tissue (Table II). An increase in liver arsenic level was observed in groups that received arsenic with streptomycin 5.78mg/g of liver tissue and only arsenic 5.90 mg/g of liver tissue. In streptomycin treated group liver arsenic level increased to 5.78 mg/g of liver tissue an increase of 62.16% compared to control group.

Discussion

Inhibition of gut bacteria by streptomycin and subsequent administration of arsenic, caused a significant increase in liver tissue arsenic level. Liver tissue arsenic level also increased in rats that received only arsenic. Increase in streptomycin treated group was more than in untreated group. Inhibitation of gut bacteria was also observed and was more marked in strepto-mycin treated rats, where it decreased to uncountable levels uggesting an important role of gut bacteria in arsenic metabolism.

Gut bacteria play an important role in metabolic, tropic and protective functions and play a vital role in bodies immunity.[10] Several studies carried out in this aspect show that bacteria play an important role in arsenic detoxification.[11,13] Liver is an important organ of arsenic detoxification, but this concept has been challenged now as growing number of reports of studies in experimental model system indicate that hepatic methylation of arsenic is more toxic than methylation by gut flora.[18]More stress is being given to detoxification by gut flora.Arsenic detoxification in liver results in formation of MMA III and DMA III which are more toxic than any inorganic arsenic or any other pentavalent intermediates.[12] Gut flora produce MMMV and DMV which are non toxic and finally TMA III in gaseous form.[9] Besides arsenic microbes have been found to reduce a wide range of toxic metals through detoxification and elimination.[14] Which suggests an important role of gut bacteria in bodies defence mechanism.

In this study inhibition of gut bacteria in rats by oral streptomycin and subsequent administration of arsenic showed a significant increase in liver arsenic level, it was also observed that chronic exposure to arsenic also had an inhibitory effect on gut bacteria andincrease in liver arsenic level. Increase in liver arsenic level streptomycin treated group was more,it could be concluded that inhibition of gut flora results in increase entry of arsenic inside the body. Exposure to arsenic results in formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic.[15] It plays a role in blackfoot disease, Diabetes mellitus,[16] gastrointestinal, neurological and cardiovascular diseases.[6]

People in Bangladesh are exposed to arsenic throughfood[2] and drinking water,[5]inhibitory effect of arsenic in gut flora and increased liver concentration of arsenic requires proper and rational use of antibiotics. Irrational use of antibiotics might lead to inhibition of gut flora and increase the risk of arsenacosis, particularly in arsenic exposed areas leading to increased incidence of systemic diseases, which not only increases morbidity and mortality but also puts a heavy pressure on the countries economy.

Maintenance of healthy gut bacteria is very important to maintain good health. It has been

observed that environmental and nutritional factors play a major role in determining the extent and type of colonization by gut bacteria.[18] Use of probiotics i.e foods which contain live bacteria beneficial to health and prebiotics i.e foods which contain certain non digestable oligosaccharides which selectively stimulate the growth of bifidobacteria in the colon[19] along with judicious use of antibiotics is advocated to prevent or cure infections, disease and perhaps immunopathologic disorders.

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