ORIGINAL ARTICLE

Variation in response to selenium folic acid and vitamin E in arsenic exposed aerobic flora in rats

ZK Choudhry[1], M Misbahuddin[2], UKFK Mojlish[3]

[1]*Department of Pharmacology, International Medical College, Gazipur*
[2]*Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka*
[3]*Department of Biochemistry, International Medical College, Gazipur*

ABSTRACT

Gut bacteria are considered to be body's first line of defense against ingested xenobiotics. Various nutritional and enviormental factors play a role in bacterial growth and multiplication. Bacteria exposed to arsenic in high concentration for a long period showed growth inhibition. Influence of nutrition on bacterial growth and multiplication was observed by giving selenium (0.4 μg/day), vitamin E (1 mg/day), folic acid (200 μg/day) supplementation. Selenium and vitamin E were able to overcome the inhibitory effect of arsenic on gut flora. Selenium not only increased gut bacterial count, it also increased arsenic excretion in stool. Folic acid could not overcome the inhibitory effect of arsenic on gut flora but there was significant decrease in liver arsenic level suggestive of hepatic mehylation of arsenic.

Introduction

Arsenic classified by World Health Organization (WHO) as group I carcinogen[1] is becoming a cause of major health concern, due to its ever increasing concentration in drinking water. In many areas of Bangladesh.[4] it is much above the safe standard level (0.01 mg/L) set by WHO. Use of arsenic contaminated water for the purpose of irrigation has also led to its entry into food chain. Increased arsenic consumption over a period of years leads to arsenicosis. It is not only a cause of carcinogenesis[2], but has also been related to cardiovascular[3], gastro intestinal[4], respiratory[5] and endocrine disease[6].

Ingested arsenic is removed from the body by a process of methylation in liver and also by bacteria in gastrointestinal tract[8]. Hepatic methylation of arsenic is considered to be toxic due to formation of DMA-III and MMA-III[7]. Bacterial excretion of arsenic though safe, studies suggest that bacteria exposed to arsenic for prolonged period in high concentration (1 mg/L), arsenic exerts an inhibitory effect on them[8]. Enviormental and nutritional factors play an important role in determining type and extent of gut bacterial colonisation[9,10]. In this study arsenic exposed bacteria have been treated with nutrients like selenium, vitamin E and folic acid to see whether they play any role in bacterial growth and multiplication in bacteria exposed to arsenic stressed enviorment

Materials and Methods

Healthy young adult male rats of Long Evans Norwegian strain, weighing 160-180 g and 3-4 months old were taken for the purpose of study. They were kept in animal house in stainless steel cages. Saw dust was used as bedding and changed every alternate day. A 12 hours light/12 hours dark cycle was maintained. They were fed standard pellet diet and allowed to drink ad libitum.

A total of 48 rats were used for the purpose of study and divided into two groups. One group received arsenic (1 mg/L) in drinking water ad libitum for a period of 4 weeks. Both the groups were then treated with vitamin E (1 mg/day),

selenium (0.4 μg/day) and folic acid (200 μg/day) orally through Ryles tube for a period of 2 weeks. Control group comprising 6 rats received normal diet and drinking water ad libitum. Microscopic examination of stool was done on day 0, day 7 and day 14. Rats were sacrificed on day 14 and liver was preserved for arsenic estimation.

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

Liver and stool arsenic estimation: remaining portion of stool was dried and arsenic estimated by SDDC method. 500 mg of liver tissue was taken and arsenic estimated by SDDC method.

Results

Gut bacterial count in control group ranged from 6.91 to 7.17×108 cfu/g dry weight of stool. (Table I). In rats treated with arsenic in drinking water a significant decrease in gut bacterial count was observed from day 0 to day 14. On day 0 count was 6.82×108 cfu/g dry weight of stool, whereas on day 14 it declined to 3.51×108 cfu/g dry weight of stool.

Rats treated with arsenic selenium and vitamin E showed a normal count as that of control group from day 0 to day 14 (Table I) rats that received arsenic and folic acid a significant decrease in gut bacterial count was observed on day 14, bacterial count declined to 2.57 × 108 cfu/g dry weight of stool.

Stool arsenic level in Control group of rats was 3.55 mg/g to 3.63 mg/g dry weight of stool, it increased significantly 4.58 mg/g dry weight of stool in rats that received arsenic with selinium (table II). It remained unchanged in rats that received arsenic and vitamin E and decreased significantly 2.67 mgm/g dry weight of stool in rats that received folic acid and arsenic.

Table I : Effects of arsenic, vitamin E, selenium and folic acid on gut bacterial count in rates

	Bacterial count /cfu/g dry weight of stool)		
	Day 0	Day 7	Day14
Control	$6.91 \times 10^8 \pm 0.45 \times 10^8$	$6.94 \times 10^8 \pm 0.52 \times 10^8$	$7.17 \times 10^8 \pm 0.34 \times 10^8$
Arsenic (1 mg/L)	$6.82 \times 10^8 \pm 0.50 \times 10^8$	$7.25 \times 10^8 \pm 0.83 \times 10^8$	$3.51 \times 10^8 \pm 1.77 \times 10^8$
Arsenic (1 mg/L) + Vitamin E (1 mg/day)	$6.96 \times 10^8 \pm 0.40 \times 10^8$	$7.22 \times 10^8 \pm 0.50 \times 10^8$	$6.74 \times 10^8 \pm 0.47 \times 10^8$
Arsenic (1 mg/L) + Folic acid (200 μg/day)	$7.22 \times 10^8 \pm 0.50 \times 10^8$	$4.35 \times 10^8 \pm 0.60 \times 10^8$	$2.57 \times 10^8 \pm 0.84 \times 10^8$
Arsenic (1 mg/L) + Selenium (0.4μg/day)	$6.73 \times 10^8 \pm 0.58 \times 10^8$	$7.52 \times 10^8 \pm 1.23 \times 10^8$	$7.25 \times 10^8 \pm 0.93 \times 10^8$
Vitamin E (1 mg/day)	$6.92 \times 10^8 \pm 0.48 \times 10^8$	$7.47 \times 10^8 \pm 0.62 \times 10^8$	$4.41 \times 10^8 \pm 1.87 \times 10^8$
Folic acid (200 μg/day)	$6.91 \times 10^8 \pm 0.46 \times 10^8$	$5.26 \times 10^8 \pm 0.97 \times 10^8$	$3.85 \times 10^8 \pm 1.46 \times 10^8$
Selenium (0.4 μg/day)	$6.79 \times 10^8 \pm 0.57 \times 10^8$	$6.06 \times 10^8 \pm 0.95 \times 10^8$	$5.85 \times 10^8 \pm 0.79 \times 10^8$

Control group received standard diet and drinking water ad libitum. Arsenic was administered to different group in drinking water ad libitum. Ryles tube feeding was given for vitamin E, folic acid and selenium. All the groups were treated for 14 days. Each group had six rats. Stool culture was done in Mac Conkey's agar. Values are mean ± SD.

Table II : Stool and liver arsenic concentration following administration of arsenic, vitamin E, selenium and folic acid

	Amount of arsenic			
	Stool mg/g dry weight			Liver issue mg/g
	Day 0	Day 7	Day14	
Control	3.55 ± 0.34	3.25 ± 0.52	3.30 ± 0.32	2.84 ± 0.22
Arsenic (1 mg/L)	3.88 ± 0.48	3.55 ± 0.80	2.79 ± 0.54	3.57 ± 0.46
Arsenic (1 mg/L) + Vitamin E (1 mg/day)	3.80 ± 0.73	3.59 ± 0.86	2.84 ± 0.57	2.66 ± 0.51
Arsenic (1 mg/L) + Folic acid (200 µg/day)	3.55 ± 0.37	3.54 ± 0.35	2.67 ± 0.29	2.20 ± 0.33
Arsenic (1 mg/L) + Selenium (0.4 µg/day)	3.86 ± 0.65	4.49 ± 1.29	4.58 ± 1.29	2.24 ± 0.34
Vitamin E (1 mg/day)	3.40 ± 0.81	3.96 ± 0.91	3.40 ± 0.41	2.52 ± 0.43
Folic acid (200 µg/day)	3.42 ± 0.39	2.93 ± 0.47	2.19 ± 0.17	2.08 ± 0.47
Selenium (0.4 µg/day)	3.53 ± 0.37	3.48 ± 0.34	4.22 ± 0.46	2.54 ± 0.27

Control group received standard diet and drinking water ad libitum. Arsenic was administered with drinking water ad libitum. Vitamin E, folic acid and selenium was given orally through ryles tube. Arsenic was adminstered to different group in drinking water. All the groups were treated for 14 days. Each group had six rats. Stool and liver arsenic estimation was done by SDDC method. Values are mean ± SD.

Significant increase in mean liver arsenic level to 3.57 mg/g of liver tissue was observed in rats that received only arsenic compared to control group 2.84 mg/g of liver tissue. A significant decrease in mean liver arsenic level was observed in rats that received arsenic with selenium and arsemic with folic acid (Table II), 2.2 and 2.24 mg/g of liver tissue respectively rats receiving vitamin E showed no significant change in liver arsenic level.

Discussion

Studies have shown bacterial role in arsenic detoxification11[1,12]. When exposed to high concentrations of arsenic for a considerable period of time arsenic might exert inhibitory effect on gut flora as is observed by a decrease in gut floral count[16,8]. In this study rats administered with only arsenic a significant decrease in gut bacterial count and stool arsenic level and increase in liver arsenic was observed. Decreased gut bacterial count is suggestive of inhibitory effect of arsenic on gut bacteria an elevation of liver arsenic might be correlated to decreased bacterial count as studies suggest role of[17,5] bacteria in arsenic detoxification.

Gut bacteria play an important role in bodies metabolic, tropic and protective functions and also play a vital role in bodies immunity[9]. Nutritional and environmental factors influence the extent and type of colonisation by gut bacteria[13]. When selenium, vitamin E and folic acid were administered along with arsenic a variation in response was observed in gut flora. Gut bacterial count returned to normal in rats that received arsenic with seleneium and vitamin E, from which it can be assumed that vitamin E and selenium are able to overcome the inhibitory effect of arsenic on gut flora and that nutrition plays a role in bacterial growth and multiplication[13]. Observation in laboratory animals deprived of one or more dietary elements have confirmed the crucial role of vitamin E and selenium in arsenic metabolism[15]. An increase in stool arsenic level and decreased liver arsenic level in rats that received selenium is suggestive of bacterial role in arsenic detoxification. Arsenic and selenium being chemically similar ions, compete for sites of reduction in bacteria leading to increase arsenic in stool1[4,11], or alternately increased arsenic metabolism by selenium and hepatobiliary excretion[11].

This study suggested that inhibition of gut flora by arsenic can be overcome by use of selenium and vitamin E and also that increased bacterial

multiplication is associated with increased bacterial activity. Intestinal bacteria contain high amount of glutathione which can effectively reduce toxic substances[10].

However, future further extensive studies as regards role of selenium and vitamin E as nutrients to promote gut bacterial growth is needed. Moreover selenium might prove toxic and therefore dose adjustments and its related adverse effects also have to be evaluated.

Acknowledgement

Thanks to Department of Microbiology, Bangabandhu Sheik Mujib Medical University and especially to Prof. Dr. Ahmed Abu Saleh for his help and cooperation.

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