

Melatonin and Vitamin E Protects Against Sodium Arsenite-Induced Skeletal Malformations in Rats

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Abstract: There are many reports that show the teratogenic effects of drugs can be prevented by application of antioxidant drugs and stimulation of the maternal immune system. Therefore, in this study, the prophylactic effect of melatonin and vitamin E on teratogenic effects of arsenite sodium was studied. The study was performed on pregnant rats that were divided into five groups. The control group received normal saline and test groups received melatonin (10 mg/kg), sodium arsenite (11 mg/kg), sodium arsenite (11 mg/kg) plus melatonin (10mg/kg) and sodium arsenite (11 mg/kg) plus vitamin E (100 mg/kg), intraperitoneally at 10th day of gestation, respectively. Fetuses were collected at 20th day of gestation and after determination of weight and length; they were stained by Alizarin red - Alcian blue method. Cleft palate, spina bifida, sternal anomalies and fused ribs incidence were 50, 50, 55 and 25% in fetuses of rats that received only sodium arsenite. Cleft palate, spina bifida, sternal anomalies and fused ribs incidence were 8.33, 8.33, 8.33 and 20% in group which received sodium arsenite plus melatonin. However, Cleft palate, spina bifida and sternal anomalies incidence were 22.22%, 18.5%, 22.22% and 0% in group which received sodium arsenite plus vitamin E. The mean of weight and length of animals' fetuses that received melatonin and vitamin E were significantly greater than those received only sodium arsenite. Although melatonin had more prophylactic effect than vitamin E on incidence of sodium arsenite- induced cleft palate and spina bifida, but this difference was not significant.

Key words: Sodium Arsenite • Melatonin • Vitamin E • Cleft palate • Spina bifida • Teratogenicity • Rat

INTRODUCTION

Arsenic (As), being a potent environmental toxic agent, leads to development of various hazardous effects on human health [1]. It is a naturally occurring element that exists in several oxidative states but it is the pentavalent (arsenate, As⁵⁺) and trivalent (arsenite, As³⁺) forms that is almost prevalent in environment and has toxicological significance. Arsenite is considered a more potent developmental toxicant [2]. The U.S. Food and Drug Administration have calculated the mean daily intake of inorganic arsenic (arsenite and arsenate) to be approximately 0.5 g/kg body weight for adult including sources from air, water and food. Because of the extensive distribution and associated toxicity of as, contamination of drinking waters represents a worldwide problem [3].

Beyond its effects on adult organisms, arsenic is a well-established teratogen in chicken [4] and rodents [5, 6], arsenic products embryo and fetal lethality. At lower doses, it causes a variety of malformations, particularly cephalic axial dysraphic disorders (exencephaly), but also including genitourinary and skeletal defects. Inorganic arsenic has been shown to freely cross the placenta and accumulate in the embryonic neuroepithelium [3] and has been linked to various embryotoxic outcomes, including neural tube defects, reduced organ and body weight, malformation and fetal death [2, 7]. Cellular mechanism of arsenic toxicity involves generation of reactive oxygen species (ROS) [8]. Enhanced generation of ROS after arsenic exposure alters cells' intrinsic antioxidant defenses and results in oxidative stress [9].

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Non-specific stimulation of the maternal immune system in mice during the peri-conception period appears to have a broad spectrum efficacy for reducing teratogen induced birth defects from a variety of sources including chemical agents, x-rays and diabetes mellitus [10, 11]. Maternal immune stimulation reduced or blocked digit and limb defects [12], tail malformation, cleft palate [13] cranial defects [14] and neural tube defects [15, 11]. The operating mechanisms by which such immune stimulation reduces fetal dysmorphogenesis are unknown; however, the collective literature suggests that immunoregulatory cytokines of maternal or placental origin may be effector molecules that normalize dysregulated apoptosis or timing of cell proliferation in the fetus [11, 13]. Stimulation of maternal immune system or antioxidant drugs can decrease or prevent drug-induced embryonic abnormalities [12, 16]. For example, macrophage activation decreases incidence of cleft palate and digital and tail anomalies in fetuses of mice that received urethane and methyl nitrous urea [10]. Interferon gamma reduces urethane-induced cleft palate and granulocyte-colony stimulating factor decreases cyclophosphamide-induced distal limb abnormalities in mice [17].

Melatonin or N-Acetyl-5-methoxytryptamine, the main secretory product of pineal gland, participates in many physiological functions due to its efficacy as a free radical scavenger and indirect antioxidant [18, 19]. Because of its small size and lipophilicity, melatonin crosses biological membrane easily, thus, reaching all compartments of the cell. Melatonin has also been shown to be an efficient protector of DNA [20], protein and lipids in cellular membrane [21] as well as antagonist of a number of endogenous and exogenous free radicals attach or during cellular processes [22].

Vitamin E, is a natural antioxidant, believed to prevent diseases associated with oxidative stress [23]. Vitamin E is considered safe in pregnancy, although experiments that evaluate the safety of high-doses of vitamin E in pregnancy have not been reported [24].

In the present study, the preventive effect of melatonin and vitamin E on sodium arsenite-induced spina bifida and skeletal malformations in rats was compared.

MATERIALS AND METHODS

Sodium arsenite (Sigma, USA), melatonin (Rouz daru, Iran) and vitamin E (Darupakhsh, Iran) were purchased from commercial sources.

Male and female healthy rat of Wistar strain, 3-4 month old, weighing 200-250g were purchased (Razi Institute, Karadje, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimatization period. Rats were fed *ad libitum* standard laboratory pellet (Pars khurakdam, Shushtar, Iran.) and tap water. A 12-h light: 12-h dark cycle was maintained. Room temperature was at $23\pm 2^{\circ}\text{C}$ with a relative humidity of 45-55%.

Female rats were mated overnight with males. The vaginal plug was assumed as first day of gestation (GD1). Pregnant animals were divided into five groups (n=5) and treated as follow:

Control group received normal saline; the test groups received melatonin (10 mg/kg), sodium arsenite (11 mg/kg) [25], sodium arsenite (11mg/kg) plus melatonin (10 mg/kg) [26] and sodium arsenite (11 mg/kg) plus vitamin E (100 mg/kg) [27] intraperitoneally, respectively.

The animals were sacrificed by cervical dislocation 20th day of gestation and fetuses were collected and numbered, then their weight and length (crown-rump length) were measured. Fetuses were stained by Alizarin red-Alcian blue method [28] and examined by stereomicroscope for teratogenicity. The incidence of cleft palate, spina bifida and skeletal malformations were determined.

Statistical significance between groups was determined using SASS program and comparisons were made by one way analysis of variance (ANOVA) and Chi-square test. The minimum level of significance was $p<0.05$.

RESULTS

Forty-seven fetuses were obtained from six rats of the control group. In this group, palatal closures of fetuses were normal on gestational day 20 (i.e. palatal shelves had grown vertically on the sides of the tongue, then horizontally to meet and fuse) (Fig. 1,2 A) and no macroscopic anomalies were observed in them. Sodium arsenite induced cleft palate (Fig. 1,2 B), spina bifida (Fig. 3B), sternal anomalies (Fig. 5) and fused ribs (Fig. 4B) incidence were 50, 50, 55 and 25%, respectively (Table 1). Melatonin reduced incidence of sodium arsenite-induced cleft palate, spina bifida, sternal anomalies and fused ribs incidence were 8.33, 8.33, 8.33 and 20% respectively. Vitamin E reduced incidence of sodium arsenite-induced cleft palate, spina bifida, sternal anomalies and fused ribs incidence were 22.22, 18.5, 22.22 and 0%, respectively. Mean weight and length (CRL) were significantly

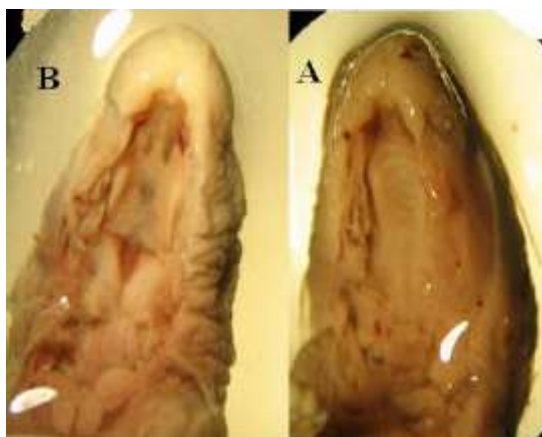


Fig. 1: A, B: Razor blade sections of rat fetuses of GD 20. A: Control skeleton. Note the cleft palate due to palatal shelf hypoplasia(B) in the treated case (11 mg/kg of sodium arsenite, treated on GD 10)

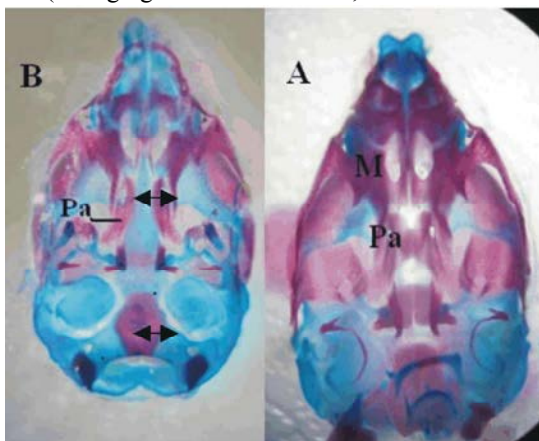


Fig. 2: Ventral view of skull of rat fetuses of GD 20, stained with alizarin red-S- alcian blue. A) Normal palatine bone B) Cleft palate induced by sodium arsenite (arrow). M: maxilla; Pa: palatine

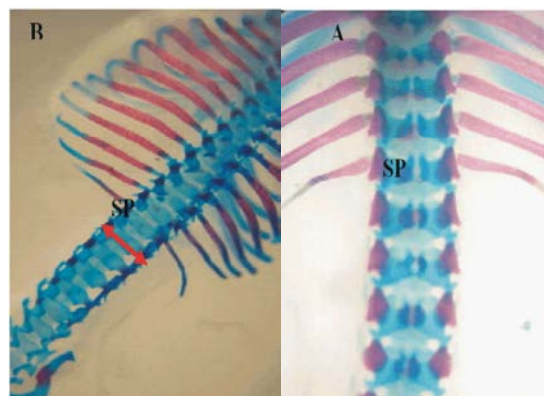


Fig. 3: Dorsal view of vertebral column of rat fetuses of GD 20, stained with alizarin red-S- alcian blue. A) Normal B) Spina bifida (arrow) induced by sodium arsenite. SP; spinous process

($P < 0.001$) decreased in group received only sodium arsenite. The means weight and length in groups that received melatonin and vitamin E were greater than the group received only sodium arsenite. There were not any aborted fetuses from total groups but percentage of resorbed fetuses were 4.08, 4.65, 50, 40 and 27.02% in groups that receives normal saline, melatonin, sodium arsenite, sodium arsenite plus melatonin and sodium arsenite plus vitamin E, respectively (Table 1).

DISCUSSION

Several studies have reported that the maternal immune stimulation can reduce teratogenic anomalies. Mechanisms of this effect have remained unclear, but it is thought the fetal gene expression has been modulated [16].

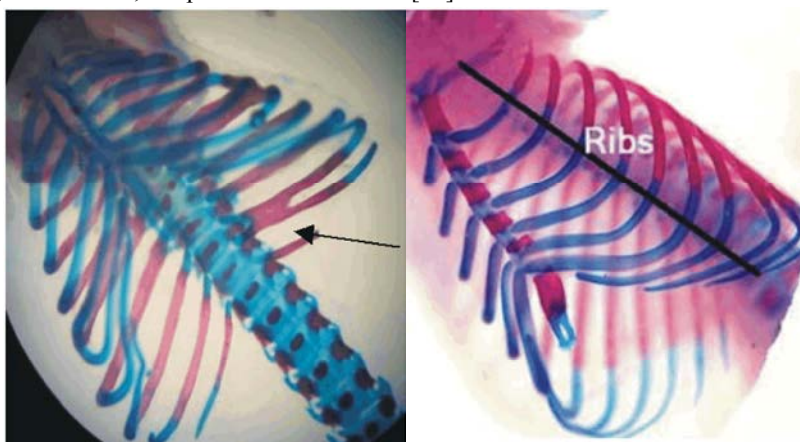


Fig. 4: Ventral view of vertebral column and ribs of rat fetuses of GD 20. A) Normal, B) Rib adhesion (arrow) induced by sodium arsenite which stained with Alizarin red- Alcian blue

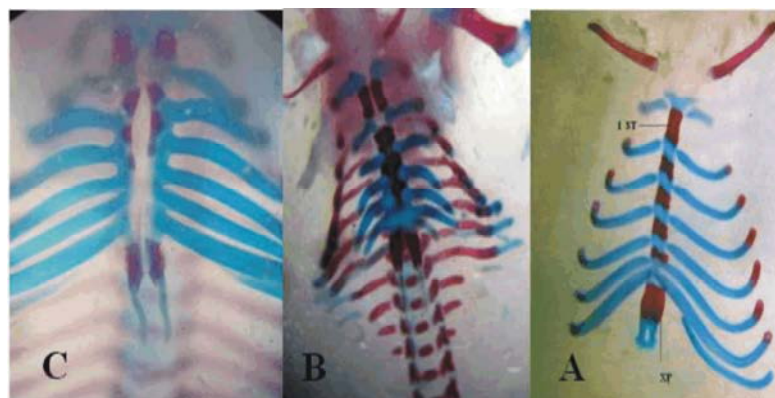


Fig. 5: Sterna of fetuses of GD 20, stained with alizarin res- S- alcian blue. A: Control. The chest wall together with the sternum and costal elements are dissected out and shown here. B and C: Experimental group treated with 11 mg/kg of sodium arsenite on GD 10. Observe fusion of sternebra (B,C), hemisternebra (C) and unusual shape of xiphoid process

Table 1: Incidence of anomalies in rat fetuses of groups

Groups	No. of litters	Implantations	Resorbed fetuses	Live fetuses	Fetal length (mea n± SEM)	Fetal weight (g): (mea n± SEM)	No. of fetuses with malformations			
							Rib adhesion	Cleft palate	Spina bifida	Sternal malformations
Control	6	49	2(4.08)	47	37.48±0.38	4.73±0.09	0	0	0	0
Melatonin	6	43	2(4.65)	41	35.59±0.85	4.39±0.16	0	0	0	0
Sodium arsenite	7	40	20(50)	20	26.15±1.60*	2.33±0.26*	4(20)	10(50)	10(50)	11(55)
Sodium arsenite + melatonin	6	20	8(40)	12	33.33±1.10**	4.06±0.23**	3(25)	1(8.33)	1(8.33)	1(8.33)
Sodium arsenite + Vitamin E	6	37	10(27.02)	27	30.05±1.69#	3.13±0.30#	0	6(22.22)	5(18.51)	6(22.22)

Numerals in parantheses are percentages

*: Significant difference when compared with other groups (P<0.05)

** : Significant difference when compared with control and sodium arsenite groups (P<0.05)

#: Significant difference when compared with control, melatonin and sodium arsenite groups (P<0.05)

Incidence of anomalies was significantly difference at groups which received sodium arsenite with control and melatonin group (p=0.0001). Also this incidence was difference between groups received sodium arsenite (P<0.05)

Enhancing antioxidative effects can protect fetuses against teratogenicity [29]. Sharova *et al.* [13] showed that interferon - gamma and Freund's complete adjuvant reduced severity of the urethane - induced cleft palate in mice [13]. Torkinsky *et al.* [15] reported that immune stimulation in diabetic mice, which show a high spontaneous rate of cleft palate, decreased in malformed fetuses, significantly [15].

The types of arsenite-induced malformations noted in the present study are essentially in agreement with those reported by Hood [6]. The results of the present study demonstrated that skeletal malformation-induces sodium arsenite was ameliorated by melatonin and vitamin E. In the present study, both vitamin E and melatonin reduced the frequency of incidence of clefting. Melatonin decreased incidence of cleft palate more than vitamin E, but the difference was not significant.

Hood *et al.* [25] evaluated the teratogenic activity of sodium arsenite in mice. They observed that sodium arsenite can produce teratogenicity in fetuses of mice.

They observed fetal defects similar to our study including skeletal malformations. These anomalies were decreased by melatonin (10 mg/kg) and vitamin E (100 mg/kg).

Melatonin or N-Acetyl-5-methoxytryptamine, the main secretory product of pineal gland, is an antioxidant [19], scavenges the hydroxyl radical [18]. The studies in laboratory rodents and other domestic species suggested that melatonin did not affect prenatal growth, survival, or morphology of the conceptus once pregnancy had been established [30]. Jahnke *et al* [31] reported that melatonin with doses of 50 and 100 mg/kg/day from gd 6 through 19 had no effect on prenatal survival, fetal body weight, or incidence of fetal malformations [31]. The authors reported no significant differences in dam body weight or total numbers of fetuses, live fetuses, or abortions through Gd 18 [32]. In one study, melatonin with dose 5 or 10 mg/kg in mice protects against lipopolysaccharide-induced intra-uterine fetal death and growth retardation via counteracting lipopolysaccharide-induced oxidative stress [8]. Omurtag *et al.* [33] reported melatonin with

dose 10 mg/kg/day for 5 days protects against endosulfan-induced oxidative tissue damages in rats. In present study, the effect of melatonin is probably related to antioxidant activity.

Administration of vitamin E to pregnant diabetic animals decreases the rate of embryonic malformations and increases their body weight and enhances their maturation [27]. Boskovic *et al.* [34] reported that consumption of high doses of vitamin E during the first trimester of pregnancy was not associated with an increased risk for major malformations, but may be associated with a decrease in birth weight [34]. On the other hand, vitamin E supplementation of the ewe resulted in a significant increase in lamb birth weight [35]. In one study, Mittal and Floral reported that vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice [36]. Konar *et al.* [26] reported that vitamin E and melatonin had protective effects against high dose cadmium-induced oxidative damage.

In conclusion, probably sodium arsenite influences antioxidant system that produces teratogenic effects including skeletal malformations and spina bifida. Effects of sodium arsenite immunosuppression are mediated indirectly by inducing oxidative stress. On the other hand, melatonin is more effective than vitamin E in decreasing incidence sodium arsenite -induced skeletal malformations and spina bifida in fetuses of rat, but it was not significant. This would indicate that maternal immune stimulation blocks or prevents rather than reverses the deleterious effect of teratogenicity.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the research council of Shahid Chamran University for their financial supports.

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