

EFFECTS OF NEPHRITE JADE WATER BY OK-SAN GA® IN CHUNCHEON ON SPERM QUALITIES AND HISTOPATHOLOGICAL CHANGES IN BISPHENOL A(BPA) ADMINISTERED RATS

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Abstract

A wide ranges of chemicals released into the environment have potential to interfere with physiological and development process by disrupting endocrine pathways. The study was performed to examine the effects of nephrite jade water on sperm qualities and histopathological changes in bisphenol A (4,4'-isopropylidenediphenol, Sigma Co., USA) treated rats. Bisphenol A was administered in drinking water (tap water vs jade water) at 0, 0.05, 0.5, 5.0, 50.0 mg/L to pregnant Sprague-Dawley rats from mating period through postnatal day 21 (weaning). Estimated daily intake of BPA ranged from approximately 1.8ug to 2.1 mg/head/day. One-hundred fifty S.D. rats with 3-week old were randomly assigned to one of five different bisphenol A water treatment. A comprehensive assesment of physical development, organ weights, and functions were conducted. The concentration of serum testosterone in Jade water group was increased in low level of BPA group (0.05 & 0.5 mg/L), however, high level group (5.0 & 50.0 mg/L) was not different in testosterone level compared with those of tap water groups. Sperm motility showed a decreasing tendency in parallel with BPA dose level. Although no statistical difference among groups (water sources & BPA concentrations) was observed in sperm counts, Jade water groups was higher in sperm concentrations than those of tap water groups. Histopathological examination seemed that the testis showed normal in Jade water groups regardless of BPA dose level. On the other hand, there were similarly decreased spermatogenesis in tap water groups regardless of BPA dose level. In conclusion, these results revealed that the nephrite jade water might be useful in preventing or/and relieving the disrupting effect of BPA and in enhancing the spermatogenesis. Further studies are needed.

Introduction

The incidence of reproductive abnormalities in the male has been reported to have increased during the past 50 years. It has been suggested that these changes may be attributable to the presence of chemicals with oestrogenic activity in our environment (Saunder et al., 1997). Endocrine disruptors (EDs) are exogeneous chemicals which interfere several aspects of natural hormone profiles. EDs with estrogenic activity have been recently reported to cause animal reproductive problems. Bisphenol A (BPA) is a chemical used primarily as a monomer in the manufacture of numerous chemical products, such as epoxy resins and polycarbonate (Cagen et al., 1999). This study was performed to investigate the effect of nephrite jade water produced by Ok-SanGa of Chuncheon in Korea on the sperm qualities and histopathological changes in bisphenol A(BPA) administered S.D. rats.

ENDOCRINE DISRUPTORS

Material and Methods

Experiment (BPA exposure)

Animal

One-hundred fifty (75 male and 75 female) S.D. rats with 3-week old were assigned to one of five different BPA diluted-water treatment. Rats were caged individually with a 12-hour light:dark cycle.

BPA treatment and group

4,4'-isopropyl-idenediphenol (Sigma Co. Ltd) BPA was administered to induce the reproductive toxicity for three generations in drinking water. BPA was diluted with tap water in dose levels of no BPA (A), 0.05mg/L (B), 0.5mg/L (C), 5.0mg/L (D), 50.0mg/L (E). (Table 1)

Experiment (BPA detoxification)

Animal

Administered to investigate the detoxifying effects of two types of water on pups of the 3rd generation. Dams were supplied BPA diluted-water until weaning period. The pups were supplied BPA diluted-water after 30 days of birth including suckling period.

Measurements

- Weight of body & testes^o
- Development of reproductive organ & organ weight
- Histopathological examination of testes & liver
- Delivering & raising rates
- Serum hormone concentration
- Sperm count & motility

Histopathological examination (Light microscopy)

Male rats were anesthetized with ethyl ether. Then, the testes were surgically excised and immediately preincubated in mixed solution (2.5 % glutaraldehyde-1 %, paraformaldehyde) overnight. After, washed with 4 % sucrose (0.1M phosphate buffer) and postincubated in solution (1 % osmium tetroxide) for 2 hour, and rewashed with 4 % sucrose (0.1 M phosphate buffer) two times. Washed tissues were dehydrated with ethyl alcohol series (60 % ~ 100 %) and acetone (100 % 2 times). The samples were embedded in paraffin wax and sectioned at 1 μ (ultramicrotome, Sorvall MT-5000), stained with hematoxylin and eosin, and observed using light microscopy.

Results and Discussion

When several levels of BPA-diluted water were administered to male and female Sprague Dawley rats for three consecutive generations, sperm motility tended to decrease according to BPA dose level. Sperm concentrations were numerically higher in jade water treated group when detoxifying effects of the water were studied. Serum testosterone concentrations were higher in low BPA group, but this effect was not observed in the highest BPA group. On the other hand, Sertoli cells of testis were histologically normal in jade water treated group regardless of BPA dose level, but tap water treated groups had shown decreased spermatogenesis regardless of BPA dose level. However, there were no significant histopathological changes. In conclusion, administration of BPA-diluted water did affect the reproductive systems, but the degree of damage by BPA was improved by jade water treatment.

References

1. Saunderson PT, Majdic G, Parte P, Millar MR, Fisher JS, Turner KJ, Sharpe RM., 1997. Fetal and perinatal influence of xenoestrogens on testis gene expression. *Adv. Exp. Med. Biol.*, 1997(424) : 99-110.
2. Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, VN, Veenstra GE, Harris LR., 1999. Normal reproductive organ development in wistar rats exposed to bisphenol A in the drinking water. *Regul. Toxicol. Pharmacol.*, 30(2) : 130-139.

ENDOCRINE DISRUPTORS

able 1. Effect of Feeding BPA-diluted Water for three generations on Parturition, Physical Development, and Sperm Parameters in Rats.

Parameters	BPA Level(mg/L)				
	0	0.05	0.5	5.0	50.0
no. dams(n=9)	6	7	5	6	4
pups	13.0±	12.4±	13.4±	12.0±	12.3±
per dam	1.4	1.1	1.7	3.6	1.0
total no. pups	78	87	67	72	49
birth wt.	6.27±	5.88±	5.87±	6.10±	6.10±
(g)	0.89	0.27	0.37	0.37	0.38
weaning wt.(g)	41.5±	38.2±	39.8±	36.2±	35.4±
ear*	6.3	1.9	4.4	1.9	4.4
teeth*	64.3	84.6	58.3	50.9	69.2
eye*	64.3	60.2	70.3	61.6	69.7
eye*	25.0	20.0	18.0	18.0	18.0
Anogenital					
hair*	71.4	35.0	34.2	49.4	22.5
BPA intake (?/day/?)	-	1.84±	28.3±	278.4±	2,077.4±
testis wt.(g)	0.17	8.7	155.3	658.7	
Sperm	2.29±	2.23±	2.41±	2.28±	2.19±
motility(%)	0.06	0.16	0.08	0.09	0.06
Count	68.7±	48.3±	57.5±	50.0±	45.0±
(10 ⁸ /?)	14.7	2.9	3.5	4.1	0.0
BPA intake (?/day/?)	3.4±	3.1±	2.5±	2.4±	1.8±
no. of follicle(n=3)	0.38 ^a	0.61 ^a	0.58 ^{ab}	0.48 ^{ab}	0.32 ^b
no. of CA**	-	2.56±	20.5±	203.2±	1,649.7±
color***	1.23	3.9	82.1	627.9	
Remarks****	210±	245±	156±	159±	169±
	37.5	16.1	14.0	2.6	11.5
	24±	28±	66±	35±	85±
	7.4	7.5	16.6	9.3	4.2
	0	2	3	1	1
	1	3	1	.	1

represents physical development of pups on day 14 of birth(%).

**CA, *corpus albicans*;

*** Uterus color(0~3) : 0, white ; 3, red ;

**** Number of individual represents fluid in the uterus.

Means with the same superscripts in the line are not significantly different (p<0.05).

ENDOCRINE DISRUPTORS

Table 2. Effect of Nephrite Jade Water of Ok San Ga® on Testis Weight, Sperm Parameters, Testosterone Level in rats fed BPA-diluted Water for three generations.

Parameters	BPA level(mg/L)									
	0 Control	0.05		0.5		5.0		50.0		
		Detoxification drinking water								
		Jade	Tap water	Jade	Tap water	Jade	Tap water	Jade	Tap water	
Liver wt. (g)	12.23 ±0.78	9.53 ±1.65	11.24 ±0.87	9.47 ±1.46	9.22 ±1.72	11.12± ±1.39	11.22 ±0.99	0.85 ±1.20	1.43 ±1.14	
Testis. wt. (g)	1.54 ±0.10	1.50 ±0.07	1.40 ±0.36	1.54 ±0.05	1.56 ±0.08	1.58 ±0.09	1.66 ±0.10	1.58 ±0.12	1.55 ±0.07	
Sperm count (×10 ⁸)	5.50 ±3.89	6.00 ±4.68	4.87 ±2.90	5.33 ±3.06	5.11 ±3.42	3.86 ±2.95	3.19 ±1.96	2.47 ±0.25	1.78 ±0.92	
Sperm motility(%)	65.6 ±18.0	74.7 ±7.3	61.8 ±16.4	65.9 ±16.7	63.9 ±19.7	60.3 ±14.8	59.8 ±13.0	60.6 ±8.9	43.3 ±2.9	
Hematocrit (%)	39.1 ±3.5	38.7 ±3.6	37.7 ±1.4	39.3 ±3.1	37.2 ±2.1	37.2 ±2.1	38.1 ±2.5	35.8 ±2.5	35.9 ±2.2	
Testosterone (ng/ml)	2.01 ±1.39	2.11 ±1.26	2.39 ±0.96	3.15 ±2.30	2.53 ±2.02	170 ±1.67	1.64 ±1.13	1.72 ±0.73	1.29 ±0.74	