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Effect of a New Phenanthroline Derivative on Testicular Tissue and Serum Testosterone Concentration and its Contraceptive Affects on Adult Balb/C Mouse Strain

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Abstract: In this research, the effect of 2, 6-diaminopyridinum as a new phenanthroline derivative was studied on the hypophysis-gonad axis, testicular tissue and sperm production in male Balb/C mice. Fifty adult male Balb/C mice were divided in five groups. First group was considered as untreated control. Saline was injected to second group and the remaining three groups received intraperitoneal injection of 15, 20 and 25 mg kg⁻¹ of 2, 6- diaminopyridinum every other day for 20 days. The LD₅₀ was determined to be 35 mg kg⁻¹ body weight. The testicular tissues were studied morphologically and the serum concentration of FSH, LH and testosterone were measured. The results showed that 25 mg kg⁻¹ diaminopyridinum decreased the number of germ cells significantly and serum testosterone level with no change on FSH and LH levels. This study indicates that 25 mg kg⁻¹ of phenanthroline may directly affect testicular tissue causing a lower testosterone level and spermatogenesis in mice.

Key words: Phenanthroline, hypophysis-gonad axis, testicular tissue, spermatogenesis

INTRODUCTION

Scientific changes and developments in technology lead to control many diseases, increasing lifetime decreasing mortality and overpopulation in developing countries. Nowadays most of contraceptive methods are used on women. Due to the side effects of some drugs on women (Wu, 1981), men should actively participate on this problem. This contribution is limited to use of condoms and vasectomy (Stephan, 1994). These methods are difficult and sometimes impossible to use by some people. Discovery of the antifertility activity of gossypol as a male contraceptive drug was considered after reducing fertility in one of the Chinese villages where the seed and root of the cotton plant was used traditionally. Preceding these researches lead to discovery of some new drugs such as sulfonamides, nitrofurans and quinine derivatives that had antifertility activity in men (Costenito *et al.*, 1990; Karol, 1960; Natalie, 1982).

Phenanthroline and its ferrous compounds, which are used in chemistry (Skoog *et al.*, 1988), are biologically effective on sperm maturation (Gottlieb and Meizel, 1987). Also some of its derivatives consisting cobalt have been studied previously (Shokravi *et al.*, 2003). The effects of new derivatives of phenanthroline containing copper have been studied on spermatogenesis. So far, the effects

of many derivatives of phenanthroline on life of sperm have been investigated and showed weakness of sperm maturation (Sadeghipour Roodsarie *et al.*, 1999).

In this study, the effects of new derivatives of phenanthroline containing copper, 2, 6-diaminopyridinium-1, 10-phenanthroline-2, 9-decarboxylate on spermatogenesis and hypophysis-gonad axis have been investigated in male adult mice to use it as a new suggestive male contraceptive drug with more specific activity.

MATERIALS AND METHODS

Primarily new derivative of phenanthroline containing copper (2, 6-diaminopyridinum-1, 10-phenanthroline-2, 9-decarboxylate) as a green and odorless powder in organic chemistry laboratory of Teacher Training University of Tehran was prepared and purified with recrystallization method (Moghimi *et al.*, 2003). The normal saline in 37°C was used as solvent for dissolving and injection of the drug. Adult male Balb/C mice strain weighing between 25-30 g which was obtained from the Animal Production Department of Shiraz Medical University were used. The animals were kept in standard conditions (14 h lighting, 10 h darkness and 21±2°C) and had access to sufficient amounts of water and food. Fifty male mice were randomly

divided into five groups (N = 10). First group as control group was used as untreated, while the second group as sham group received only normal saline. The experimental groups (1, 2 and 3) received intraperitoneal injection of 15, 20 and 25 mg kg⁻¹ BW of drug every other day for 20 days. The injection was done after determination of lethal dose 50 (LD₅₀) of the drug which was 35 mg kg⁻¹ BW.

Injections were done every other day for 20 days time because the period of sperm maturation continued for 35 days and considerable differentiation changes can happen in first 20 days (Gilbert, 1997). The animals weighted and killed after day 20th of first injection and their testes fixed in Bouin's fixative for histological studies. The blood of animals was collected separately in sterilized tubes which contained no anti-coagulant substance for measurement of testosterone, LH and FSH. The collected blood samples were centrifuged at 2000 RPM for separation of serum and were kept at the temperature of -2°C till the time of hormone assay.

Both testes of animals were removed after opening the abdomens. The volume and weights of testes were measured and after fixation, embedding and staining with hematoxyline and eosin method the number of sertoli, leydig, spermatogonia, primary spermatocyte, spermatid and spermatozoid cells were determined.

Primarily the long and short diameters of testes were measured and testes volumes were determined with the following formula (Courtade *et al.*, 1998):

$$V = \left(\pi * \frac{D^2}{4} \right) . L . K$$

V = Volume, L = Long diameter of testes,
D = Short diameter of testes, K = 0.9

The hormone measurement was carried out using Radio Immuno Assay (RIA) method by kits with precision of 0.01 made in Radim Company and results were studied by gamma counter Kentron model made in Switzerland. Statistical analysis of data was done using one-sided variance and the average and standard deviation were determined and the difference between the experimental and control groups were evaluated using t-test.

RESULTS

Volume and weight of testes: The results showed that there was no significant difference in volume and weight of testes between the experimentals, control and sham

Table 1: The average and standard deviation of sertoli cells after Intraperitoneally injections of different doses of 2, 6-diaminopyridinium in comparison with control and sham groups in adult male mice

Groups	Phenanthroline dose (mg kg ⁻¹)	Sertoli cells
Control	-	21.1±0.8
Sham	-	19.3±1.1
Experimental 1	15	20.5±0.9
Experimental 2	20	17.5±1.1
Experimental 3	25	16.0±0.9

P: NS

Table 2: The average and standard deviation of leydig cells after Intraperitoneally injections of different doses of 2, 6-diaminopyridinium in comparison with control and sham groups in adult male mice.

Groups	Phenanthroline dose (mg kg ⁻¹)	Leydig cells
Control	-	70.0±2.9
Sham	-	71.6±2.7
Experimental 1	15	71.5±1.3
Experimental 2	20	57.9±1.4
Experimental 3	25	44.0±1.2*

p≤0.05

groups. Also there was no significant of difference in the body weight of mice in different groups before and after injections.

Histological studies of testes: Histological studies showed that there was no significant difference in average number of Sertoli cells in different groups (Table 1). The results showed that the number of Leydig cells significantly decreased (p≤0.05) in the experimental group after receiving 25 mg kg⁻¹ of drug in comparison with control and sham groups but in the groups which received 15 and 20 mg kg⁻¹ no significant difference was observed (Table 2).

The average number of spermatogonia, primary spermatocyte, spermatid and spermatozoid cells significantly decreased (p ≤ 0.05) as in experimental group which received 25 mg kg⁻¹ BW of drug. The most significant difference (p≤0.01) in average number of sperm cells was observed in comparison with other groups (Table 3).

Measurements of hormone concentrations: The study of level of serum testosterone, FSH and LH showed that the average concentration of testosterone decreased significantly (p≤0.05) in experimental group after receiving 25 mg kg⁻¹ BW of drug in comparison with control and sham groups and was observed to be 1.03±0.04. There was no significant difference in the concentration of FSH and LH between the experimental, control and sham groups (Table 4).

Table 3: The average and standard deviation of spermatogenic cells after Intra-peritoneally injections of different doses of 2,6-diaminopyridinium in comparison with control and sham groups in adult male mice

Groups	Phenanthroline dose (mg kg ⁻¹)	Spermatogeny	Spermatocytes	Spermatid	Spermatozoid
Control	-	48±1.6	82±2.5	148±4.8	675±9.9
Sham	-	49±2.1	81±3.2	149±6.3	660±31.4
Experimental 1	15	41±1.4*	65±2.6*	108±3.8*	539±13.2*
Experimental 2	20	33±1.1*	42±1.9*	88±4.1*	324±21.6*
Experimental 3	25	22±1.4*	17.5±0.6*	54±3.8*	33±3**

*p≤0.05, **p≤0.01

Table 4: The average and standard deviation of Testosterone, LH and FSH serum concentration after injections of different doses of 2,6-diaminopyridinium in comparison with control and sham groups in adult male mice

Groups	Phenanthroline dose (mg kg ⁻¹)	Testosterone concentration (ng mL ⁻¹)	LH concentration (mu mL ⁻¹)	FSH concentration (mu mL ⁻¹)
Control	-	3.3±0.1	0.78±0.03	2.1±1
Sham	-	3.4±0.1	0.77±0.03	2.1±0.1
Experimental 1	15	3.2±0.2	0.74±0.03	2.1±0.1
Experimental 2	20	3±0.1	0.72±0.04	2.1±0.1
Experimental 3	25	1±0.04*	0.71±0.04	2.1±0.2

*p≤0.05

DISCUSSION

In the present study, the effect of 2, 6-diaminopyridinium on histological changes in the testes and concentration of LH, FSH and testosterone has been investigated. The results of this investigation showed application of this drug has no effects on the volume and weight of testes but can decrease the number of Leydig and sperm cells as well as decrease of the concentration of serum testosterone. Some drug compounds have a leading role. Changing on their chemical structure can produce new derivatives with ideal effects, as study on side effects of sulfonamides led to introduce some anhydrase carbonate enzymes inhibitors such as acetazolamide and reducing blood sugar drugs such as chlorpropamide. These results were obtained two decades after discovery of sulfonamides. The results of some investigations have shown that different compounds such as anticancer substances and cadmium can abort production of sperm cells although their irreversible effects and toxicity prevent clinical application of them (Wilson, 1996). Also it was shown that trimethoprim can reduce fertility without any effects on concentration of testosterone (Sadeghipour Roodsari, 1998).

In this study, the contraceptive effects of phenanthroline with copper on adult male mice have been investigated as a new male antifertility drug. Survey on average change in body and testes weight in different groups indicated that growth and development of animals were completely normal. This finding indicates one of the most advantages of this new compound.

Concerning these results, it is suggested that this drug may have direct effect on interstitial cells and leads

to decrease the number and activity of these cells although it has no effects on hypophysis gland and production of gonadotropins (Grizard and Artonne, 1997).

Also it seems that the drug probably causes a decrease in the number of spermatogenic cells regarding to direct effects on seminiferous tubules. It has been shown that the maximum dose of the drug had the most decrease in the number of cells. The significant reduction on spermatogenesis (p≤0.05) in experimental groups in comparison with sham and control groups showed that the most effective dose of the drugs is 25 mg kg⁻¹ BW.

Consequently, investigation of testes, study on sexual behaviors, hormone changes on hypothalamus level and changes on development and growth of embryo in further researches can contribute to find the positive effects of this new male contraceptive drug.

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