Iraqi Journal of Veterinary Medicine Vol. 33, No. 2, 2009

Reproductive Hormonal Evaluation Of Female Mice After Treatment With Pentoxifylline

***Salema. L. Hassan ;* Rajiha, A. Al- Naimi ; ** Saad S. Al-Dujaily**

*** College of veterinary medicine / Baghdad University**

****Institute of Embryo Research and Infertility treatment Al-Nahrain University**

Summary

The aim of this study was the assessment of pentoxifylline (PTX) effects on female reproductive hormones as well the body and reproductive organs. Sixty white mice with approximately same ages (eight) weeks and body weights were randomly divided into (six.) equal groups 10 mice for each group. Group 1: Received tap water along the period of experiment and considered as a control group. Animals of group 2,3,4,5 and 6 were treated with 16 mg (PTX) / Kg B.W. daily for 2,4,6,8 and 10 weeks respectively. Before and after treatment all animals were weighed, and blood samples were taken for follicle stimulating hormone (FSH), lutenizing hormone (LH) and Estradiol (E2) hormones analysis.

After post – Mortem examination reproductive organs were excised and weighed. The experiment revealed that PTX administration caused increase in total body and reproductive organs weights. Concerning the biochemical tests results revealed elevation in concentration of FSH, LH and E_2 hormones especially significant ($P<0.001$) in treated groups of (8 and 10 wks duration). Compaired with another animal groups conclusion. Administration of PTX in low dose for 10 weeks has a significant effect on reproductive hormones. This will influence reproduction and litter size manifested after mating with untreated males.

التقييم الهرموني التكاثري لأناث الفئران بعد المعالجة بعقار البنتوكسفيلين

***سليمة لفتة حسن، * راجحة عبد الستار النعيمي، ** سعد صالح الدجيلي**

*** كلية الطب البيطري – جامعة بغداد**

**** معهد أبحاث الأجنة وعلاج العقم – جامعة النهرين**

تأريخ التسليم 11/1/2009

تأريخ القبول 1/6/2009

الخلاصة

هدفت الدراسة الى تقييم تأثيرعقار البنتوكسفيلين فيما يخص الهرمونات التكاثرية لأناث الفئران . أستخدمت في التجربة 60 فأرة بيضاء بعمر 8 أسابيع وذات أوزان متقاربة.قسمت الحيوانات الى ست زمر متساوية (10 / فأرات لكل زمرة) أعطيت الزمرة الأولى ماء شرب وعدت زمرة سيطرة للزمر الأخرى. الزمرة الثانية والثالثه والرابعه والخامسه والسادسه تناولت 16 ملغم/ كغم من وزن الجسم من عقار البنتوكسفيلين ولمدة 2، 4،6،8 و 10 اسابيع على التوالي.وزنت الحيوانات قبل بداية وبعد انتهاء التجريع الفموي للعقار وجمعت

عينات الدم بعد كل اسبوعين من التجريع لغرض إجراء التحاليل المختبرية الخاصة بالهرومونات التكاثرية الهرمون المنبه للجريب والهرمون الملوتن والاستراديول تم أجراء الصفة التشريحية للحيوانات وأستأصل الجهاز التناسلي لأغراض الوزن . أن تناول عقار البنتوكسفيلين ادى الى زيادة في كل من وزن الجسم الكلي ووزن الجهاز التناسلي . وكذلك زيادة انتاج الهرمون المنبه للجريب والهرمون الملوتن والاستراديول خصوصاً في الزمرٍ المعالجة لمدة8 ، 10 أسابيع . يمكن أن تعزى هذه النتائج الى التأثير الغير مباشر لعقار البنتوكسفيلين على الانظيمات الخاصة بتركيب الستيرويدات من خلال تأثيره على المستقبلات التي تؤدي إلى زيادة إنتاج الهرمونات التكاثريه مما تؤدي الى زيادة تحفيز الاعضاء التناسلية . نستنتج من هذه الدراسة أن للبنتوكسفيلين تأثيراً واضحاً على التكاثر وعلى أعداد الأجيال الجديدة من خلال زيادة انتاج الهرمونات التكاثريه فضلا عن زيادة اوزان الاعضاء المختلفه للجهاز التناسلي الانثوي .

Introduction

Pentoxifylline (PTX) is a dimethyl xanthine derivative belongs to a group of vasoactive drugs used in humans for the treatment of peripheral and cerebral vascular diseases caused by impairment of the microcirculation (1) . PTX is also used in the treatment of male infertility in human by enhancing sperm motility both *in vivo* (2) and *in vitro* (3) , in cases of normozoospermia and asthenozoospermia (4). It has an inhibitory effect on phosphodiesterase that enhances sperm motility by increasing intracellular cAMP (5) .

Studies on reproductive performance in rats , mice , and rabbits revealed no evidence of impaired fertility or harm to the fetus due to PTX (6) .Also , females with endometriosis associated infertility may get benefit from the use of PTX without significantly affecting embryo development (7).

The aim of this investigation was to evaluate the hormonal changes after treatment with PTX in female mice , body and reproductive organs weight.

Materials and methods

1. Experimental design:

Sixty mice were divided into 6 equal groups after labeling them with ear or tail marking and weighing them using mechanical balance as follows: control G1: treated orally with tap water. G2-G6: treated orally with 16 mg ptx/ daily) for 2,4,6,8 and 10 weeks respectively. After 2 weeks, the body weights of G2 recorded then mice were anesthetized by ether under glass beaker in order to facilitate blood collection .Blood samples were drawn directly by cardiac puncture (8). Serum was separated from coagulated blood samples after 15 minutes by centrifuge at 2500 rpm for 5 minutes. The serum was separated and stored at -20 ◌ْC until hormonal assay FSH, LH, and E_2.

Method of assay

The method of performing radioimmunoassay is as follows:-

A-An antibody that is highly specific for the hormone to be measured is produced.

B- A small quantity of this antibody is mixed with a quantity of fluid from the animal containing the hormone to be measured and a known amount of radio active iodinated hormone.

C- After binding has reached equilibrium, the antibody- hormone complex is separated from the unbound iodinate hormone by a variety of the physicochemical means.

D- The amount of hormone present in the plasma can be inferred by comparing with "standard curve" (9).

Mice reproductive system was sampling quickly excised and immersed in few drops of normal saline to be cleared from surrounding adipose tissue. The genital organs were weighed with electrical balance (Sartourius/ Germany), examined grossly for any abnormality .G3, G4, G5, G6, and control could were sampling in the same way..

2. Treatment:

Pentoxifylline is presented in the form of coated tablets containing 400mg (Aventis USA).One hundred and sixty (160) mg of the coated tablets were dissolved in 100 ml of tap water to obtain a stock solution from which 0.1 ml was given orally to each 10 gm of living body weight of the experimental mice. This amount of the solution will provide a dose of 16 mg/kg. B.W/ day of the drug. This dose was individually adjusted according to body weight of each animal and given via a fine plastic stomach tube given to G2, G3, G4, G5 and G6. While the control group (G1) given tap water only.

Statistical analysis was calculated by students t-test differences between values were considered significant at $p<0.05$ and highly significant at $p<0.01$(10).

Results

A-Weight changes:

The results showed that there was a highly significant increase ($p<0.01$) in the body weight of mice of G4 and G5 (25.348 ± 0.40, 27.008 ± 0.347) gm respectively, as well as, there was a significant increase ($p<0.01$) in the G2, G3 and G4 (22.085± 0.258, 23.535±0.553, 22.674 ± 0.609) gm respectively compared with that of the control 20.421±0.137 gm (Table 1).

Also the changes in the body weight between G4 and G5 were significant ($P<0.05$) and highly significant ($P< 0.01$) between G2, G3 and G4, comparing with G5 and G6 (Table 2).

Reproductive system weight:

The reproductive system weight showed a highly significant increase ($p<0.01$) in G5 and G6 (0.171±0.004, 0.245 ± 0.008) mg respectively. Also, there were a significant increase in G2, G3 and G4 (0.093± 0.0021 , 0.138 ± 0.005 , 0.117 ± 0.003) mg respectively compared with that of the control group 0.140 ± 0.0022 mg , (Table 1). The changes in reproductive system weight between groups were highly significant ($P< 0.01$) between all treated groups and significant between G3with G4 (Table 2).

B-Hormonal Changes

Table 3 showed that serum levels LH µlu/ml were highly significant increase ($p<0.01$) in G6 1.137 ± 0.256, while in G3, G4 and G5 showed significant changes ($p<0.05$) 0.293 ± 0.023, 0.283 ± 0.006 , 0.330 ± 0.057 respectively . Group 2 showed no significant changes 0.170 ± 0.005 compared with that of control 0.160 ± 0.005. While the FSH levels µlu/ml showed a significant increase in G2, G3, G4 ($p<0.05$) (4.730 ± 0.015, 5.21 ± 0.008, 5.69 ± 1.10, 5.730 ± 0.682) respectively. With highly significant increase ($p<0.01$) in G5 and G6 (5.730 ± 0.682; 6.213 ± 0.287) µlu/ml respectively. Estradiol pg/ml levels become highly significant ($p< 0.01$) in G6 300.8 ± 11.5, with significant

elevation ($p<0.05$) in G2, G3, G4 and G5 267.4 ± 43.9, 264 ± 32.0, 264/4 ± 38.0, 293 .7 ± 6. 75 pg/ ml respectively (table 3).

In general the treatment showed that there were hormonal (FSH, LH and E2) differences between G2 with G3 while the difference was significantly increased between G2, G6 with G3, G4, and G5. While E2 was significant between group 2, 3 with groups 5, 6 and highly significant between group 4, 5 (Table 4).

Table (1): Changes in the body weight gm and reproductive system weight mg after oral administration of pentoxifylline to mature female mice x = S.E. n= (n =10)

weight	Control group	2weeks treatment	4weeks treatment	6weeks treatment	8weeks treatment	10weeks treatment
	G1	G2	G3	G4	G5	G6
B.Wt. (g)	20.421± 0.137	*22.085 ± 0.258	*23.535 ± 0.553	*22.674 ± 0.609	**25.348 ± 0.40	**27.008 ± 0.347
Rep.sys.wt. (mg)	0.104 ± 0.0022	*0.093 ± 0.0021	*0.138 ± 0.005	*0.117 ± 0.003	**0.171 ± 0.004	**0.245 ± 0.008

*P<0.05 Significant changes
**P<0.01 High significant changes

Table (2): Changes in the body weight and reproductive system weight after oral administration of 16 mg/ daily of Pentoxifylline to mature female mice. (Between groups) x = S.E. n= (n =10)

Weight	2weeks treatment	4weeks treatment	6weeks treatment	8weeks treatment	10weeks treatment
	G2	G3	G4	G5	G6
B.Wt.(g)	22.085±0.258[b]	23.535±0.553[b]	22.674±0.609[a]	25.348±0.40	27.008±0.347
Rep.sys.wt. (mg)	0.093±0.0021[d]	0.138±0.005[c]	0.117±0.003[d]	0.171±0.004[d]	0.245±0.008[d]

B.Wt.(g): a= significant between group4 with group5
b=high significant between group2, 3, 4 with group 5, 6.
Rep.sys.wt. (mg): c=significant between group3 with group4
d=high significant between all groups
B.Wt.: Body weight.
Rep.sys.wt.: Reproductive system weight.

Table (3): Shows the effects of daily 16 mg of PTX on hormonal serum level in female mice x = S.E. n= (n =6)

	Control group	2weeks treatment	4weeks treatment	6weeks treatment	8weeks treatment	10weeks treatment
	G1	G2	G3	G4	G5	G6
LH mlu/m	0.160± 0.005	0.170± 0.005	*0.293± 0.023	*0.283± 0.006	*0.330± 0.057	**1.137± 0.256
FSH mlu/m	4.433± 0.403	*4.730± 0.015	*5.21± 0.008	*5.69± 1.10	**5.730± 0.682	**6.213± 0.287
E2 pg/ml	150.57± 0.15	*267.4± 43.9	*264± 32.0	264.4± 38.0	*293.7± 6.75	**300.8± 11.5

*P<0.05 Significant changes
**P<0.01 High significant change

Table (4): Hormonal changes after oral administration of 16 mg / daily of Pentoxifylline to mature female mice.

	2weeks treatment	4weeks treatment	6weeks treatment	8weeks treatment	10weeks treatment
	G2	G3	G4	G5	G6
LH mlu/m	0.170± 0.005[a]	0.293± 0.023[b]	0.283± 0.006	0.330± 0.057	1.137± 0.256
FSH mlu/m	4.730± 0.015[c]	5.21± 0.008	5.69± 1.10	5.730± 0.682	6.213± 0.287[c]
E2 pg/ml	267.4+ 43.9[d]	264± 32.0	264.4± 38.0	293.7± 6.75	300.8± 11.5

a= significant between group2 with group3 and 5
b=significant between group3 with group 4 and 6
c=significant group2, 6 with group3, 4 and 5
d= significant between group2 and 3 with groups 4, 5 and 6
e=high significant between group 4 and 5.

Discussion

The biochemical results (Tables 3 and 4) showed a significant increase in serum E2 level from the first 2 wks of experiment with a highly significant increase in G6 (10 wks treatment) this elevation may be explained to the effect of PTX on enzymes involved in steroid hormone synthesis like aromatase (cyp19) which catalyzes the conversion of androsterndione to estrone and conversion of testesterone to E2 . Ovary theca interna cells have many LH receptors , and LH acts via cAMP to increase conversion of cholesterol to androstenedione some of the androstenedione is converted to E2 which enters the circulation , the theca interna cells also supply androstenedione to the granulosa cells the granulosa cells which have FSH and LH receptors and make estradiol when provided with androgens , and FSH facilitates their secretion of E2 by action via cAMP to increase their aromatase activity , mature granulosa cells also acquire LH receptors, and LH also stimulates E2 production (11) . As PTX causes highly significant increase in E2, this may cause positive feedback action on gonadotropin secretion.

The increase in the body weight may be correlated to the elevation of E2 level since it had been known that estrogens increase the whole-body metabolic rate in addition to promoting the deposition of subcutaneous fat and increase hepatic synthesis of steroid-and thyroid hormone-binding proteins (12, 13).It has been known that estrogen exert an important protein anabolic effect in chickens and cattles, possibly by stimulating the secretion of androgens from the adrenal, and estrogen treatment has been used commercially to increase the weight of domestic animals (14).Furthermore estrogens can cause an increase in osteoblastic activity and inhibit osteoclastic activity in the bones and also promoting the linear. skeleton growth (15,16) which might be participating in the rising of body weight..

The increase in the reproductive system weight can be attributed to the direct effects of estrogens on genitalia, estrogens had a dramatic effects on the uterus, it lead to increased blood flow and accumulation of extracellular fluid. In addition to, an increased water content of the tissues (17, 18). The alteration in weight is also due to an increase in the number of cells present (hyperplasia) (19).

The result showed that there were significant changes in body, reproductive system weights and hormonal changes between treated groups. We thought that this may be attributed to different duration of treatment by PTX, and the anabolic effect of accumulated PTX through the period of treatment.

References

1. Muller, R., and Lehrach, F. (1981). Haemorheology and cerebrovascular disease: multi-functional approach with Pentoxifylline. Curr. Med. Res. and Opin. 7: 253-263.
2. Aparirio, NJ. Schuarzstein, L. de Turm=ner, E.A. (1980). Pentoxifylline (BL 191) by oral administration in the treatment of asthenozoospermia. Andrologia, 12: 228-231.
3. Yovich, J. M., Edirisinghe, W. R., Cummins, J. M. and Yovich, J. L. (1988). Preliminary results using Pentoxifylline pronuclear stage tubal transfer (PROST) program for sever male factor infertility. Fertil. Steril., 50: 179-181.
4. McKinney, K. A., Lewis, S. E. M., Thompson, W. (1994). Persistent effects of Pentoxifylline on human sperm motility, after drug removal, in normozoospermic and asthenozoospermic individuals. Andrologia.26:235-240.
5. Terriou, P., Hans, E., Giorgetti, C., Spach, J. L, Salzmann, J., Urrutia, U., and Roulier, R. (2000). Pentoxifylline initiates motility in spontaneously immotile epididymal and testicular spermatozoa and allow normal fertilization pregnancy and birth after intracytoplasmic sperm injection. J. Assist. Reprod. Genet.17:194-199
6. De oliveria, M.M., Torrer, A.A., da costa, E.P., decarvalano, R. (2000): Effect of Pentoxifylline on the vitro viability of equine spermatozoids. Sociedade Brasileira de Zootecnia, 33 (1): 449-457.
7. Xiaoyan, Z., Hany, Rakesh, K., Sharma, (2005). A shock Agarwal, and Tommaso Falcone. J. Assist. Reprod. Genetics.22:11-12.
8. Grasso, P., Rozhavskaya, M. and Reichert, L. (1997). A synthetic peptide corresponding to amino acid residues 24 to 37 of human follicle-stimulating hormone B-subunit accelerates the onset of puberty in male and female mice, Endocrinology. 183 (10): 4215-4219.
9. Guyton, A. C. , and Hall, J. E., (2006): Text Book of Medical Physiology , 10th ed., W.B. Saunders Company , India, Pp: 929-942.
10. Daniel, W. W. (1988). Multiple regression and correlation, In: Biostatics, A foundation for Analysis in the health science, Daniel, W.W. ed.
11. Ganong, W. F. (2005). Review of Medical Physiology, 21st ed., Lange Medical Book/ McGraw-Hill., Pp: 415-457.
12. Goldman, M.D. and Bennet, C. (2000). Menstrual cycle and infertility, In: Cecil text book of medicine, 21st ed., W.B. Saunders Company, Pp: 1327-1341.
13. Broukhin and Mrtin, G.B., (1997). Administration of fatty acids and gonadotrophin in the mature ram, Anim. Reprod. Sci. 49: 143-159.
14. Bownan, W.C. and Rand, M.J. (2000). Text Book of Pharmacology, 2nd ed., Bowman and William Cameron, Great Britain by University press, Cambridge.
15. Yen, S.S.C., Jaffe, R.B. and Barbieri, R.L. (1999)**.** The ovarian life cycle and practical evaluation of hormonal status and chronic an ovulation caused by peripheral endocrine disorder In: Reproductive Endocrinology, Physiology, Pathophysiology, and clinical management, 4th ed., W.B. Saunders company, USA Pp:533-539.
16. Gyton, A.C., and Hall, J.E., (2003): Text Book of Medical Physiology, 10th ed., W.B. Saunders Company, India, Pp: 929-942.
17. Raloff, J. (2008): Estrogen's Emerging Manly Alter Ego." Science News Online .
18. Lipsett, M. (1986). Steroid hormones. In: Reproductive Endocrinology, Yes, S.S.C.and jaffe, R.B., 2nd ., Philadelphia, Pa., W.B.Jaunders, Pp. 140-153.
19. James, E. (1971). Text Book of Vet. Physioslogy, Breazile, Beames, C.G. and Cardielhac, P.T., Philadelphia, Pp: 531