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Endocrine Reproductive Effects of Antiepileptic Drugsin Male Rats

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Abstract: Both epilepsy and antiepileptic drugs induced endocrine reproductive disorders. To exclude the epilepsy as a cause of reproductive disturbances, this study was carried out to investigate the reproductive side effects of antiepileptic drugs in healthy rats. Effects of antiepileptic drugs on LH, FSH, testosterone and weights of sexual and secondary sex organs, semen quality and the progeny of treated male rats were investigated. All antiepileptic drugs used in this study induced significant decline in the level of LH and testosterone. However carbamazepine, phenobarbital and phenytion induced significant reduction in FSH level, while, the decline induced by clonazepam and sodium valproate was insignificant. All antiepileptic drugs induced significant decline in the testis weight except carbamazepine and phenobarbital. On the other hand, all antiepileptics caused significant reduction in the weight of seminal vesicle, while; only sodium valproate and phenytion caused significant reduction in the weight of epididymis. However, no significant change was recorded in the weight of prostate in all groups. All antiepileptic drugs caused significant reduction in sperm count; however the reduction with carbamazepine was not significant. Viable sperm percent was significantly declined and malformed sperm percent was significantly increased with all antiepileptic drugs used in this study. These results were discussed according to central and peripheral effects previously recorded for antiepileptics.

Key words: Endocrine · Reproduction · Hormones · Antiepileptics · Rats · Carbamazepine · Phenobarbital · Phenytoin · Clonazepam · Sodium Valproate

INTRODUCTION

Reproductive disorders are usually common among women and men with epilepsy. They are generally associated with many consequences of reproductive endocrine disorders [1, 2]. Sexual dysfunction including decreased libido, erectile dysfunction and an orgasmia were recorded in 20-50 % of epileptic men [3], while polycystic ovary syndrome, hypothalamic amenorrhea, hypoprolactinemia and premature menopause were recorded in epileptic women [1]. However, most authors mentioned that both epilepsy itself and antiepileptic drugs have been implicated in reproductive endocrine pathophysiology [1, 2, 4]. However, in addition to the effects of antiepileptic drugs on hypothalamic-pituitary-gonads axis [4, 5]; some of antiepileptic drugs also act as liver enzyme inducers. Phenobarbital, phenytion and carbamazepine decreased serum sex hormone binding globulin concentration in both men and women. This effect diminished bioactivity of testosterone and estradiol resulted in reducing fertility by diminishing potency in men and menstrual disorders in women [6]. Accordingly reproductive disturbances could be attributed to the use of antiepileptic drugs or to epilepsy itself and to exclude the epilepsy, this study was designed to investigate the endocrine reproductive effect of antiepileptic drugs in healthy rats.

MATERIAL AND METHODS

One hundred forty four rats (72 males and 72 female) were used in this experiments, they were taken from lab animal house in the college of science, university of Thi qar. They were 8-12 wks old and weighing 200-250 gm. They were housed for 2 wks before the experiment to be adapted on the experiment environment. Water and diet were offered *Ad. libitum*. The temperature of the housing room was adapted to 23±3°C, with 14 hr day / 10 hr

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darkness. Males were divided into 6 groups. The 2nd, 3rd, 4th, 5th and 6th groups were given carbamazepine 25 mg/kg, clonazepam 0.05 mg/kg, sodium valproate 300 mg/kg, phenobarbital 10 mg/kg and phenytoin 50 mg/kg respectively as a single oral daily dose suspended in dimethylsulfoxide (DMSO) for 60 days . The first group was given DMSO by the same method and for the same period to serve as control. The volume of DMSO is adjusted to 0.2 ml /rats. After the treatment periods, half of males in each group were anesthetized with ether, blood samples were collected by cardiac puncture, scrotum was open, the right testis epididymis as well as seminal vesicle and prostate were isolated from each animal. After weighting of these organs, the number of sperms / mg in the left epididymal head were counted according to the method of Sakamoto and Hashimoto [7]. Viable sperm count and sperm malformation ratio were determine by routine lab tests [8]. Serum was isolated from blood sample and used for determination of LH, FSH and testosterone levels by ELIS[11]. To determine the effect of antiepileptic drugs on the progeny of treated males, the rest 6 males in each group were mated with females (2 females / 1 male) during proestrus period and for 24 hours. The presence of the sperms in the vaginal smears was considered as day 1 of the pregnancy. Half of the females were killed in the day 14 of the pregnancy to determine litter size, weight and fetal resorption ratio, while, the other half were left till parturition to determine the previously mentioned parameter as well as the gross teratogenic effects . Statistical package of social sciences (SPSS) was used to determine the significancy among groups.

RESULTS

As shown in Table 1, all antiepileptic drugs used in this study induced significant decline in the level of LH and testosterone. However carbamazepine, phenobarbital and phenytion induced significant reduction in FSH level, while, the decline induced by clonazepam and sodium valproate was insignificant.

All antiepileptic drugs induced significant decline in the testis weight except carbamazepine and phenobarbital. On the other hand, all antiepileptics caused significant reduction in the weight of seminal vesicle, while; only sodium valproate and phenytion caused significant reduction in the weight of epididymis. However, no significant change was recorded in the weight of prostate in all groups (Table 2).

Table 3 showed that all antiepileptic drugs caused significant reduction in sperm count; however the reduction with carbamazepine was not significant. Viable sperm percent was significantly declined and malformed sperm percent was significantly increased with all antiepileptic drugs used in this study.

Table 1: Effects of antiepileptic drugs on LH, FSH and testosterone level when administered for 60 days in male rats.

	Hormones level					
	LH IU/L		FSH IU/L		Testosterone IU/L	
Drugs	Mean±SD	Change %	Mean ±SD	Change %	Mean ±SD	Change %
Control	1.57±0.3[a]		2.20±0.14[a]		3.17±0.95[a]	
Carbamazepine	1.09±0.09[b]	-3.57b	-18.18	2.03±06[b]	-35.96	
Clonazepam	1.24±0.38[b]	-21.01	2.02±0.16[ab]	-8.18	1.88±0.75[b]	40.69-
Sod. Valproate	1.13±0.14[b]	-28.02	1.96±0.37[ab]	-10.90	1.55±0.44[b]	-51.10
Phenobarbital	1.25±0.60[b]	-20.38	1.72±0.51[b]	-21.81	1.64±0.46[b]	-48.26
Phenytoin	1.17±0.17[b]	-25.47	1.58±0.40[b]	-28.18	1.60±0.53[b]	-49.52

Similar letter means not significant

Table 2: Effects of antiepileptic drugs on testis, epididymis, prostate and seminal vesicle weight when administered for 60 days in male rats

	Testis weightGm		Epididymis weightGm		Prostate weightGm		seminal vesicle weight gm	
Drugs	Mean ±SD	%Change	Mean ±SD	Change %	Mean ±SD	Change %	Mean ±SD	Change %
Control	1.34±0.03 a		0.41±0.02 a		0.40±0.03 ab		0.98±0.09 a	
Carbamazepine	1.30±0.05 a	-2.98	0.41±0.03 a	0.0	0.40±0.03 ab	0.0	0.77±0.13d	-21.42
Clonazepam	1.20±0.08 b	-10.44	0.37±0.05 b	-9.75	0.41±0.04 a	+2.50	0.89±0.12 bc	-9.18
Sod. Valproate	1.11±0.05c	-17.16	0.29±0.03 b	-29.26	0.33±0.04 b	-17.50	0.79±0.12 d	-19.38
Phenobarbital	1.33±0.04 a	-0.74	0.42±0.02 a	+2.43	0.37±0.04 ab	-7.50	0.85±0.12 c	-13.26
Phenytoin	1.14±0.04bc	-14.92	0.30±0.05 b	-26.82	0.34±0.03 b	-15.0	0.93±0.18 b	-5.10

Similar letter means not significant

Table 3: Effects of antiepileptic drugs on semen parameters when administered for 60 days in male rats.

	Semen analysis (mean±SD)					
	Sperm count		Viable sperm %		Sperm malformation ratio	
Drugs	Sperm/mg of epididymal head	Change %	Viable sperm %	Change %	Malformation %	Change %
Control	1443800±207662 a		40.66±5.789 a		15.83±4.53c	
Carbamazepine	1392400±111865 a	-3.56	28.16±6.91 b	-30.74	46.33±8.66 a	-192.67
Clonazepam	1126300±105027 b	-21.99	33.83±4.02 ab	-16.79	31.00±3.46 b	-95.83
Sod. Valproate	1086000±214601 b	-24.78	28.16±8.13 b	-30.74	38.00±10.91 ab	-140.05
Phenobarbital	1081800±1357180 b	-25.07	26.33±4.32 b	-35.24	25.16±4.57bc	-58.93
Phenytoin	979867.66±198966 b	-23.13	32.00±5.62 ab	-21.29	32.33±6.25 b	-104.23

Similar letter means not significant

Table 4: Effects of antiepileptic drugs treatment to male rats on fetal and pup characteristics

	Fetal characteristics (examined in the day of parturition				Pup characteristics (examined in the1 4th day of gestation.					
Drugs	Litter size	Change %	Fetal weight gm	Change %	Litter size	Change %	Pup weight gm	Change %	Resorption ratio	Change %
Control	10.32±2.58 a		5.46±0.71 b		8.33±4.50 a		1.74±0.05 ab		0.33±0.81	
Carbamazepine	5.50±4.54 b	-46.75	5.40±0.42 b	-1.09	5.83±3.76 b	-30.01	1.64±0.03 b	-5.79	0.83±0.98	+151.51
Clonazepam	5.66±3.55 b	-45.20	5.76±0.28 a	+5.49	6.00±3.63 b	-27.79	1.67±0.06 b	-4.02	0.66±0.81	+100
Sod. Valproate	5.83±4.62 b	-43.56	5.50±0.10 b	+0.73	3.83±4.83 bc	-54.02	1.65±0.03 b	-5.17	0.66±0.81	+100
Phenobarbital	7.00±4.67 b	-32.23	5.60±0.34 ab	+2.56	5.16±4.21 bc	-38.05	1.70±0.06 b	-2.29	0.50±0.54	+51.51
Phenytoin	5.50±4.67 b	-46.75	5.79±0.26 a	-6.04	3.33±3.72 c	-60.02	1.79±0.02 a	-2.87	0.66±0.81	+100

Similar letter means not significant

Litter size estimated either in the 14th day of gestation or in the day of parturition was significantly decreased in all groups. However, there were no significant variations in the pup and the fetal weight among groups except in the progeny of clonazepam and phenytion treated males, which showed inverse correlation with litter size. Fetal resorption ratio was significantly increased in all groups (Table 4).

DISCUSSION

The effects of antiepileptic drugs on the reproductive function could be attributed to their central effects on hypothalamic-pituitary-gonad axis. Most of antiepileptic drugs acting as GABA-agonists. The GABA –ergic system is the predominant inhibitory system in the mammalian central nervous system. GABA nerve fibers showed high density in the hypothalamus, median eminence, posterior and intermediate lobes of pituitary [9]. GABA mediates its effects by activation of two types of receptors, the GABAA receptor (GABAAR) and the GABAB receptor (GABABR). GABAAR á1, á2, á3 and á5 subunits are found in hypothalamic nuclei [10] These results indicate that GABA-ergic system participate in the endocrine function. Many GABA – agonists antiepileptic drugs affected the endocrine – reproductive physiology. Benzodiazepines [11], barbiturates [11-13] and valproate [14, 15]. All these drugs decreased FSH, LH and testosterone levels.

On other hand, antiepileptic drugs which cause enzyme induction such as phenobarbital, carbamazepine and phenytion increased sex hormones – binding globulin (SHBG) as a result of stimulation of aromatase and cytochrom p 450 enzyme, thus they decreased the level of biological active testosterone. Carbamazepine also decreased LH and FSH levels [1]. Therefore, the decline in LH, FSH and testosterone levels in this study could be attributed to all the previously mentioned mechanisms.

The decline in the weight of sexual and secondary sex organs and the decline in the sperm count with an increase of dead and malformed sperm percent could be related to the effects of antiepileptic drugs on hypothalamic-pituitary-gonads axis. So, these findings are normal sequallae for the decline in the levels of LH and testosterone. Decreasing of litter size could be occurred as a result of deterioration of many reproductive parameters including low LH and testosterone levels, low sperm count and an increase in dead and malformed sperm percent [16].

Increase of fetal resorption ratio could be related to an increase in the sperm malformation ratio. Sperm malformation subsequent affected fetal development and

cause dominant fetal mutation according to the equation of Bader and Bader [Dominant lethal mutation index = $\frac{litter\ size\ in\ treated\ group}{litter\ size\ in\ control\ group}$ [17].

According to this equation it appeared that antiepileptic drugs have high dominant lethal mutation index. This dominant mutation usually killed the embryo in the early embryonic stage and subsequently resorped, which didn't give a chance to see the teratogenic effects.

CONCLUSION

According to these results, it appeared that antiepileptic drugs induced endocrine reproductive toxicity . These drugs should be used with minimum effective dose and the reproductive activities should be evaluated periodically during therapy.

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