

Histological study on the ovaries of female mice treated by carbamazepine

دراسة نسجية على مبايض اناث الفئران المعاملة بالكاربامازيبين

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ABSTRACT

Carbamazepine is an antiepileptic drug used widely for the treatment of epileptic seizures and neuropathic pain. Several malformations in humans, mainly polycystic ovaries, have been reported as a consequence of its use during pregnancy. The association between maternal use of carbamazepine and pathology of the ovary has not been very well understood.

The purpose of this study was to examine the association of oral administration of Carbamazepine during pregnancy and the histological changes in the ovaries of mice.

Timed-pregnant mice were divided into experimental and control groups. 60 mice in the experimental group received daily oral of 15 mg/kg of carbamazepine via intragastric tube on gestational days 0 to 18. 20 mice were used as control group. They received normal saline via the same route. Dams underwent laparotomy on pregnancy days 13,15, and 18 and the ovaries were collected. Routine histological processing of the ovaries histology of paraffin sections stained with haemotoxylin and eosin , were conducted.

The ovary under the effect of the drug, there was signs of degeneration and necrosis in tissues of the cortex, medulla and the surface epithelium. Dramatic decrease in the number of ovarian follicles compared with the control group, with numerous small collections of fluid (follicles). There was noticeable decrease in the space of antrum and damage to the corona radiata in these follicles.

This study, to the best of our knowledge, showed that oral administration of carbamazepine at therapeutic comparable human doses during organogenesis can cause many disturbances in the process of oogenesis. Poly cystic ovary was ultimate results of carbamazepine .The consequences of these findings has to be noticed when carbamazepine is administered during human pregnancy.

Keywords: *Carbamazepine; ovary abnormalities; female mice ; pregnancy*

المستخلص :

الكاربامازيبين هو احد الادوية المستخدمة في علاج الصرع ونوبات التشنج والتهابات الاعصاب المصاحبة للصرع. بينت الدراسات التي تناولت هذا العقار حدوث حالات عديدة من التشوهات كعوارض جانبية منها اضطراب التركيب النسجي للمبيض .

ان الهدف من هذه الدراسة هو ايجاد العلاقة بين استخدام العقار خلال فترة الحمل والتشوهات التي تحدث في المبيض وخاصة خلال مرحلة اخذ العلاج اثناء فترة الحمل.

قسمت الفئران الي مجموعتين مجموعة السيطرة والتي تضم عشرون فأرا ومجموعة معاملة والتي تحوي ستين فأرا. تم تجريع حيوانات السيطرة عن طريق الفم بواسطة الانبوب المعدي بمحلول الملح الفزيولوجي , أما حيوانات التجربة فعند بداية الحمل ومن وقت ظهور السداة المهبلية , حيث اعتبر هذا اليوم يوم صفر من الحمل واليوم الذي يليه هو اليوم الاول من الحمل, فقد تم تجريعها عن نفس الطريق بمحلول مائي يحوي تركيز 15 ملغم ا كغم من وزن

الجسم . تم جمع المبايض من الفئران الحوامل لكلا المجموعتين عند ايام الحمل 13 و 15 و 18. خضعت كل المبايض التي جمعت للتثبيت والطرير في شمع البارافين والتقطيع بطرق التقنية النسيجية التقليدية للحصول على شرائح تم صبغها بواسطة الهيماتوكسلين والايوسين.

اظهرت المقاطع المستعرضة للمبايض للفئران في طور التكوين والتطور للفئران المعاملة عند يوم 13 من الحمل تجمعا اقل لجريبات المبيض الغير ناضجة مع تنكس كبير لمحتويات الجريبات الناضجة والتي بالرغم من ازدياد عددها بصورة غير طبيعية الا انها اظهرت صغر الغار وحدوث تحطم في طبقة التاج المشع .

لقد اظهرت النتائج ان هنالك مضار جانبية تحدث في عملية الاباضة بعد تناول الكاربامازيبين حتى ولو كان بجرعة مقارنة للجرعة الدوائية التي تعطى للنساء الحوامل وخاصة عند الفترة التي تستعد فيها الاناث لعملية الاباضة . ونتيجة تأثير الدواء هو حصول حالة تكيس المبايض . لذا ننصح النساء الحوامل بأخذ الحبيطة والحذر عند تناول عقار الكاربامازيبين خلال فترة الحمل.

الكلمات الدالة: كاربامازيبين, تشوهات المبيض, اناث الفئران, الحمل.

Introduction

As a neurological disorder , epilepsy is widely distributed among populations in the world [1,2]. Carbamazepine is one of the antiepileptic drugs which are used to treat the symptoms of epilepsy. Carbamazepine is indicated for focal seizures and generalized tonic-clonic seizures [3] . It is considered as a drug of choice in partial epilepsy .Carbamazepine can cause a range of idiosyncratic reactions, the most common of which is a skin rash.

The goal in treating the person with epilepsy is to suppress the seizure of the patient. The reproductive endocrine effect of antiepileptic drugs (AEDS) should be taken in consideration when prescribed to the women, especially, if the anticipated duration of the treatment is long.

Reproductive endocrine disorders are more common among women with epilepsy than among normal women. These disorders have been attributed to epilepsy itself, but could be related to antiepileptic-drug therapy[4,5] .

In order to distinguishing the side effects of antiepileptic drugs (AEDs) from the many other factors that influence the patients. The present experiment was planned using carbamazepine as monothearypy.

The effect of carbamazepine on the histology of the ovary has not been widely studied, thus the present experiment was planned to elucidate the histological picture of the mouse ovary and relate that to the human being.

MATERIAL AND METHODS

The present study was carried out on 100 albino mice (80 pregnant female and 20 male).The average weight of each mice was 25-30 gm .They were kept in clean properly ventilated cages and fed a commercial laboratory diet. After mixing the female mice and male mice the timing of pregnancy was calculated at the time of appearance of vaginal plug. The antiepileptic drug carbamazepine was supplied as a tablet containing 200 mg of the drug by Novartis AG Company. The mice were divided into two groups, control and treated. The treated group includes 60 pregnant female, which were given carbamazepine drug via intragastric tubing, once daily, at a concentration of 15 mg/kg body weight, which is the maximum allowable human therapeutic dose [6] . Carbamazepine suspended in distilled water. This doses represent minimal and maximal therapeutic doses in humans, and were calculated for rats according to [7]The administration of the drug started at the time of appearance of vaginal plug which is usually mark the day zero of pregnancy and the next day was considered as the day one of pregnancy [8] .The rest of the pregnant mice (20 mice) were considered as duration of administration of the drug continued throughout the pregnancy till the allocated day for each subgroup. Maternal weights were measured daily

throughout the experiment. Dams were observed for any signs of clinical toxicity. Females were sacrificed on days 13, 15, and 18 of pregnancy. The ovaries collected were fixed in neutral buffered formalin for 3 days, to be processed for routine histological paraffin embedding technique. Serial 5-8 μm thick transverse sections of the fetuses were cut and stained with haematoxylin and eosin [9].

Results

Effect of carbamazepine (15 mg/kg) on the mouse ovary:

The cross sections of the ovaries in control group were surrounded by free surface epithelium of simple cuboidal cells. The cortex was containing several ovarian follicles in different stages of development, viz: primordial follicles, primary follicles, secondary follicles and mature follicles. The follicle was characterized by a large follicular antrum that makes up most of the follicle. The secondary oocyte was located eccentrically. It was surrounded by the zona pellucida and a layer of several cells known as the corona radiata. The ovarian stroma was consisting of network of connective tissue and fibroblasts. The medulla was merely a loose fibroelastic connective tissue containing a number of blood vessels (Figs. 1 and 2).

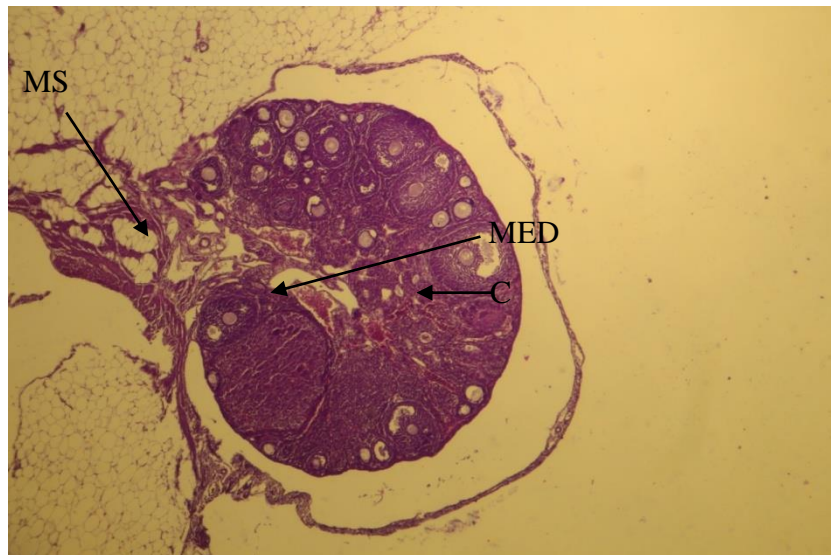


Fig. 1: Micrograph of Cross section of the mouse ovary of control group showing the cortex (C),the medulla (MED) and mesoovarium (MS).H &E .X4.

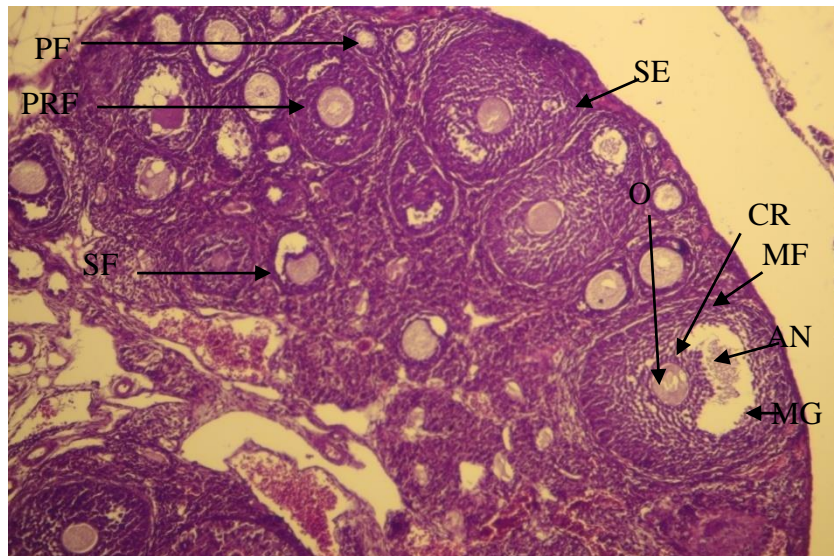


Fig. 2: Micrograph of normal structure of the ovary in the control group. The surface epithelium(SE), follicles in different stages of development also shown; primary follicles (PF),primordial follicle (PRF), secondary follicle (SF),and mature follicle (MF) with an oocyte (O) ,antrum (AN),corona radiata (CR) and granulosa membrane (MG). H&E stain; X10.

In treated group, the ovary under the effect of the drug, there was signs of degeneration and necrosis in tissues of the cortex, medulla and the surface epithelium. Dramatic decrease in the number of ovarian follicles compared with the control group, with numerous small collections of fluid (follicles). There was noticeable decrease in the space of antrum and damage to the corona radiata in these follicles. Numerous mature follicles were present at the same time which tend to be pushed down towards the medulla. There was hemorrhagic spots in between the follicles. (Figs. 3 and 4).

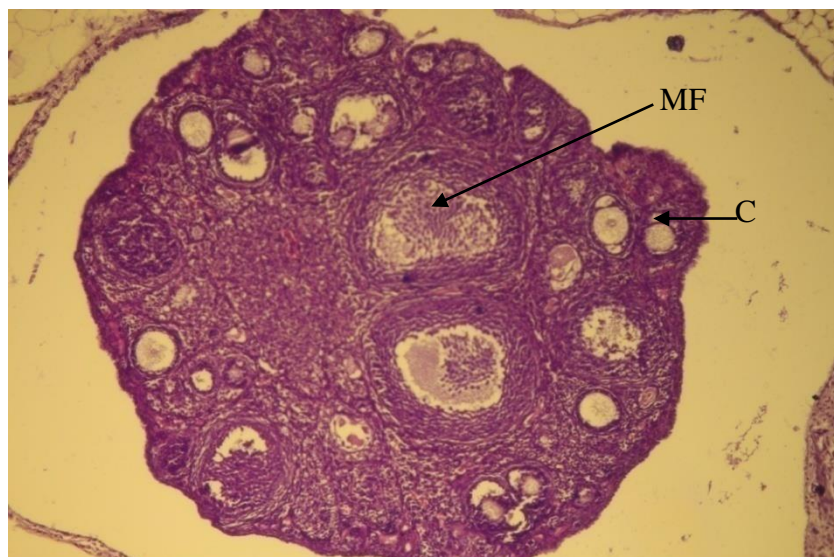


Fig. 3:Micrograph of Cross section in ovarian tissue of the carbamazepine treated group.Signs of degeneration in the cortical follicles (C),sinking down of the mature follicle (MF) towards the medulla was noticed .H&E stain. X4.

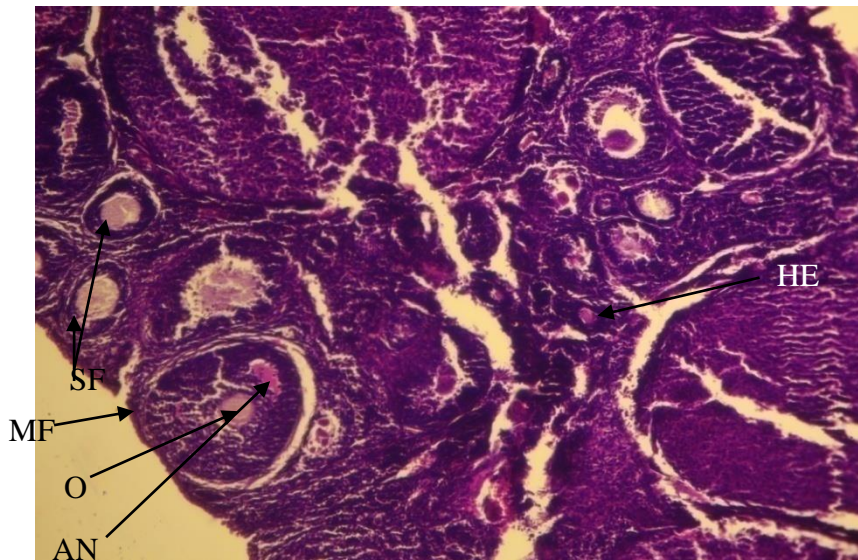


Fig. 4: Micrograph of Ovary from treated group express spots of congestion in the medulla (HE). secondary follicle (SF) , oocyte (O) ,antrum (AN), The disturbance in the mature follicle (MF) which has reached to the surface of the ovary is represented by decrease in the space of the antrum . H&E stain . X10.

Discussion

The present study has highlighted the degree of damage to the ovaries of carbamazepine treated female mice. The reproductive endocrine dysfunction in women with epilepsy is an important issue, and in recent years there is growing evidence to support the effect on sex hormones of both epilepsy and various antiepileptic drugs (AEDs) [10, 11]. Focal epileptic discharges from the temporal lobe may have a direct influence on the function of the hypothalamic-pituitary axis, thereby altering the release of sex steroid hormones [10]. The most noticeable changes in the treated group were the occurrence of several mature follicles in the ovary at the same time. Even with approaching the mature follicles to the surface of the ovary, these follicles were showing decrease in the antrum space with subsequent decrease in the antrum fluid, and damage to the corona radiata. All these signs are in fact signs seen in the polycystic ovary [12 , 13] .The current results are in agreement with [14] who pointed out that the exposure of the rats to carbamazepine was resulting in degeneration to the follicular cells .The former authors also noticed apoptosis to the follicular cells. But in contrast to their results which stated decrease in the number of follicles in the rat we have noticed increase in the number of mature follicles but with inability to rupture due to lack of follicular fluid and decrease in the antrum size. In fact the results published by [10] gave an explanation to our finding which link these results to the influence of the epilepsy and the drugs on the hypothalamic-pituitary like where the hypothalamic releasing hormones are normally affect the release of FSH and LH hormones [15] .The present work was in disagreement with the observations reached by [16] who stated that histology of the ovary in both the control and female treated by carbamazepine were the same.

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