REPRODUCTIVE BIOLOGY

SHORT COMMUNICATION

The effect of carbamazepine on sperm counts in Wistar rats - reflecting upon its mitogenic potential[1]

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Received: 28 February 2007; accepted: 30 June 2007

SUMMARY

The present study was aimed to investigate the effects of carbamazepine, an antiepileptic drug, on sperm count in rats. Male Wistar rats were treated with carbamazepine at doses of 9, 18, and 36 mg/kg for five consecutive days. Following the last exposure, on days 14 and 35, spermatozoa were collected from epididymis and counted. On day 14, carbamazepine treatment decreased the sperm number in a dose dependent pattern. On day 35, 9 mg/kg and 36 mg/kg of carbamazepine increased the sperm number in comparison with untreated rats. The results of the study suggest that carbamazepine is a germ cell mitogen. *Reproductive Biology 2007 7 2:177–181.*

[1]This research project was part of a presentation that was awarded at the Indian Pharmacological Society sponsored Southern Regional Conference 27-28.05. 2006, JNMedical College, Belgaum, India

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Key words: carbamazepine, mitogenic factor, germ cells

INTRODUCTION

Carbamazepine (cbz; 5H-dibenz(b, f)azepine-5-carboxamide; $C_{15}H_{12}N_{10}$) is commonly used to treat epilepsy and trigeminal neuralgia [7]. It is reported to have psychotropic activity in depressed epileptic patients [1]. Carbamazepine and its active metabolite, 10,11-epoxycarbamazepine, limit repetitive firing of action potentials evoked by sustained depolarization [7]. The drug is absorbed slowly and erratically after oral administration and spreads rapidly into all tissues. It is metabolized into active and inactive metabolites and excreted in urine [7]. Carbamazepine increases serum sex hormone-binding globulin (SHBG) concentration in both men and women with epilepsy. Over time, the increase in serum SHBG level leads to diminished bioactivity of testosterone and estradiol, which may result in diminished potency in men and menstrual disorders in some women, and thus to reduced fertility [4].

Neural tube defects are associated with exposure to carbamazepine, and the risk of spinabifida has been calculated to be about 1% [6]. Carbamazepine was found to be teratogenic inducing craniofacial defects, nail hypoplasia and developmental delay in human offspring [5]. The drug is classified by the FDA as pregnancy category D [3]. Dietary carbamazepine (25, 75 and 250 mg/kg/day) when administered to Sprague – Dawley rats for two years resulted in a dose-dependent increase in the incidence of hepatocellular tumors in female and benign interstitial cell adenomas in the testes of males [3]. Hence the knowledge concerning mitogenic effects of this drug due to long term human exposure is essential from an iatrogenic point of view. The present study was aimed to investigate the effects of carbamazepine on sperm counts in the rat.

MATERIALS AND METHODS

Male albino rats of Wistar strain (9-13 weeks old, 135-200 g) were maintained in the facility of Kasturba Medical College, Mangalore, Karnataka, India under standard laboratory conditions. The rodents had *ad*

libitum access to food and water. The five-day LD 50 for carbamazepine was found to be 72 mg/kg. The dose selected for assessment of cytotoxic/ cytogenic potential of cbz was 1/2, 1/4, and 1/8, of the LD50 i.e. 36 mg/kg, 18 mg/kg and 9 mg/kg. Sixty male rats were segregated into 12 groups of five in each group.

One control group was treated with 0.1 ml of sterile water (normal control), the second control group was given 2% gumacasia (suspender of carbamazepine; 0.25 ml/100 g body weight). Cyclophosphamide (20 mg/ kg; positive control; Endoxan - Asta , Germany Remedies Ltd, Batch No. 4178-4179) and carbamazepine (9, 18 and 36 mg/kg; Tegrital™, Novartis India Ltd., Batch No OX 125V) were dissolved in 2% gumacasia, and administrated intraperitoneally for five consecutive days at intervals of 24 hours. On days 14 and 35 following the last treatment, the animals were anaesthetized with 30 mg/kg pentobarbital sodium and then sacrificed by cervical dislocation. Laparotomy was conducted and reproductive organs exposed. The right epididymis was removed and minced in 1 ml of phosphate buffered saline (PBS, pH 7.2) and the suspension was filtered through 80 µm nylon mesh. One drop of 1% aqueous eosin Y was added to the filtrate and kept for 30 minutes, then an aliquot of the suspension was diluted in PBS. The sperm count was conducted according to the standard procedure [8, 9] using Neubaur chamber. Data were presented as a mean±standard error. Data for 14 day were fitted using linear regression. Data for 35 day were analyzed by Mann-Whitney test [8].

RESULTS AND DISCUSSION

The epididymal sperm counts in the control animals was $29.10\pm0.97\times10^6$. Treatment with carbamazepine decreased, in a dose dependent manner, the sperm number counted on day 14 (fig. 1); a 33% reduction in sperm counts was observed at the highest dose (36 mg/kg). Treatment with 9 mg/kg and 36 mg/kg carbamazepine on day 35 significantly increased the sperm counts in comparison to control values ($p<0.01$). Cyclophosphamide (20 mg/kg) caused a higher reduction in sperm counts (day 14: to $8.66\pm0.43\times10^6$; day

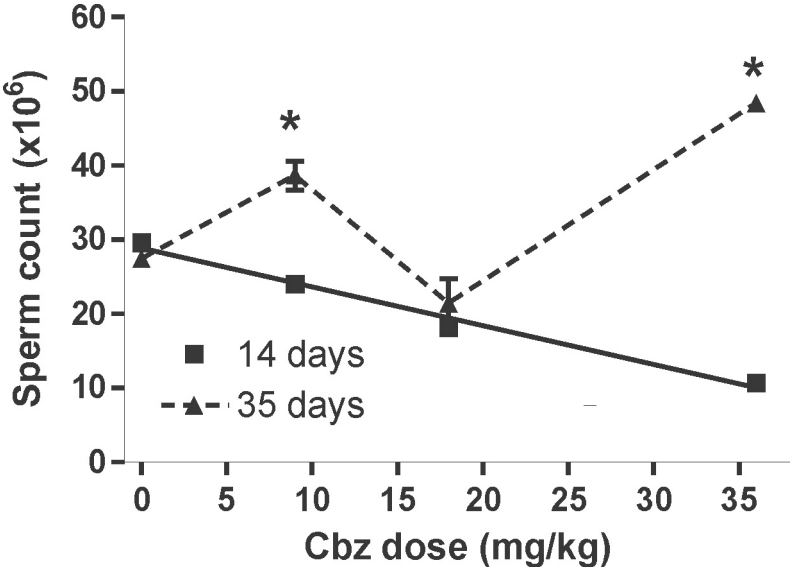

Fig. 1. Effect of carbamazepine administered for five consecutive days on rat epididymal sperm counts (mean±SEM) measured 14 and 35 days post-treatment. Data for day 14 were fitted using linear regression ($y = -1.19x + 55.2$; $r^2 = 0.98$, $p<0.01$). For day 35 sampling Mann-Whitney test was used for calculations, *significant difference from control ($p<0.01$)

35: to $9.14\pm0.15\times10^6$) than that of carbamazepine, independently on the cbz dose and time of counting.

This study was undertaken to evaluate the effects of cbz on germ cell counts in Wistar rats which would reflect on its mitogenic potential. Spermatozoa obtained after days 14 and 35 post treatment were, during the time of exposure to the drug, at the stage of spermatids and spermatogonia, respectively [2, 8]. Chemicals that affect Sertoli cells are known to reduce sperm counts, since these cells regulate and orchestrate spermatogenesis [8]. Drugs depress the spermatogenesis in mammals due to their cytotoxicity [8] which results in the death of immature germ cells present in the seminiferous epithelium [7, 8]. Due to cell removal, total cell number available for spermatogenesis also diminishes, reducing the daily sperm production [8]. The results of this study suggest that carbamazepine is absorbed from the peritoneal cavity and reaches the

germ cells. This drug is toxic to spermatids because it decreased the sperm counts on day 14 after treatment. However, after 35 days, sperm counts returned to the normal level with 18 mg/kg and much higher than normal level of sperm number were observed with 9 mg/kg and 36 mg/kg of carbamazepine. These results suggest that carbamazepine may act as a mitogen in spermatogonial cells. It is generally thought that increased sperm count implies that a compound has mitogenic potential [10]. In view of this study's result, carbamazepine could be considered as a mitogen in rats as well as a potential mitogen in humans, therefore there is a need for vibrant pharmacovigilance to scrutinize its carcinogenic potential and further studies with BrdU incorporation.

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