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ASSESSMENT OF DNA DAMAGE INDUCED BY CARBAMAZEPINE IN EPILEPTIC WOMEN

Ruşen DÜNDARÖZ[1], Halil İbrahim AYDIN[1], Tayfun GÜNGÖR[2], Faysal GÖK[1], Metin DENLİ[3], Volkan BALTACI[4],

[1]Department of Pediatrics, Gülhane Military Medical Academy, School of Medicine, Ankara
[2]Department of Gynecology and Obstetrics, Dr. Zekai Tahir Burak Women's Hospital, Ankara
[3]Department of Health Administration of the Turkish Army, Ankara
[4]Department of Genetics, Başkent University School of Medicine, Ankara

ABSTRACT: Pregnancy is one of the most difficult problems to be solved in epileptic female patients. The main concern with antiepileptic drugs (AEDs) during pregnancy is the Teratogenicity. Carbamazepine (CBZ) is one of the most frequently prescribed AEDs. Its potential toxic effects on DNA have been investigated by various tests, but the results were contradictory. To clarify this toxicity, comet assay was performed in peripheral lymphocytes of 32 epileptic women treated with CBZ monotherapy for at least one year. This was performed with a control group that included 16 and non-drug using healthy females. The damaged (limited and extensive, migrated) cells in patients' group were significantly higher than that of the control group ($p < 0,05$) indicating a detectable DNA damaging effect of CBZ monotherapy on human lymphocytes. The comet scores in patients who have a mean blood CBZ level of >8 mcg/ml were higher than those of the patients who have less than <8 mcg/ml; however, this difference was not statistically significant ($p > 0,05$). No significant correlation was noted between the duration of the therapy and the comet scores either ($p > 0,05$). We suggest that CBZ have mutagenic, carcinogenic and teratogenic effect and this effect may increase with high therapeutic levels and begins within the first year of the treatment.
[Keywords: Carbamazepine, epilepsy, comet assay, teratogenicity, DNA damage]

INTRODUCTION

Epilepsy is the most frequent neurological disorder during pregnancy (1). One in every 250 newborns is exposed to AEDs in utero (2). Meadow was the first who described an association between anticonvulsant drugs taken during pregnancy and congenital anomalies in 1968 (3). The risk of congenital malformations in newborns prenatally exposed to AEDs is around 5%, which is 2 to 2.5 times of that of the general population (4). There is no definite agreement that any one of the four major drugs used for the treatment of convulsive disorders (phenytoin, phenobarbital, valproic acid, and CBZ) is more teratogenic than others (5). Valproate and CBZ are associated predominantly with spina bifida aperta (1-2 and 0.5-1.0 % risk, respectively) and hypospadias (2). Samren, at al., reported an increased risk of major congenital anomalies in children of mothers with epilepsy treated with antiepileptic drugs during pregnancy as compared with children of healthy controls. The most significant increase in risk was in children exposed in utero to valproic acid or CBZ monotherapy (6).

CBZ reduces the spreading of abnormal impulses in the brain by blocking sodium channels, thereby inhibiting the generation of repetitive action potentials in the epileptic focus and highly effective against for all partial seizures and tonic-clonic seizures. It is also used in trigeminal neuralgia, occasionally in manic-depressive disorders and alcohol withdrawal symptoms (7, 8, 9, 10, 11, 12). It is one of the most frequently prescribed drugs to pregnant epileptic women (13). It was showed that CBZ readily can cross placenta (14, 15, 16). In utero exposure to CBZ may result in "carbamazepine syndrome", characterized by

facial dysmorphic features and mild mental retardation(17). Anophthalmus, bilateral severe microphthalmus and unilateral optic disc coloboma were also reported in infants exposed to CBZ in utero (18). Its effect on DNA has been evaluated both in vivo and in vitro by sister chromatid exchange (SCE) and chromosome aberration assays.

Comet assay (single-cell gel electrophoresis) has been recognized as a sensitive indicator of DNA strand which breaks at the single cell level. It is widely used not only as a vitro test to assess the mutagenic and carcinogenic effect of various environmental substances, but also presented as a valuable method of monitoring human population exposed to genotoxic agents (19, 20, 21, 22). The comet assay that is a rapid and easy method suitable for detecting DNA damage of human lymphocytes, has not been used in epileptic patients receiving carbamazepine therapy so far.

In the present study, we investigated the potential toxic effects of long term CBZ monotherapy on DNA using the comet assay in the peripheral blood lymphocytes of epileptic women.

MATERIALS AND METHODS

Thirty-two female patients between the ages of 20-35 who are on a long-term treatment of CBZ mono-therapy (for 1-6 years, mean 2,7 years) were studied. The Epileptic female patients who had normal menstrual cycles, and who were in, otherwise, good health were accepted. They were also nonsmokers. The patients who have not had normal therapeutic levels of CBZ (4-10 mcg/ml) for the last one year were not included into the study. Control group consisted of 16 healthy, nonsmoker female patients, who had normal menstrual cycles and did not use any long-term drugs. Both, the patient group and control group were informed about the study. The informed consent of the patients and the necessary permissions from the ethical committee were obtained. The blood samples were taken from the control and patient groups within 20^{th} and 27^{th} days following the beginning of their menstrual bleeding. To our knowledge, neither the patients receiving CBZ nor the control group were exposed to any other mutagenic agents (e.g., radiation, chemicals, lifestyle, smoking, drugs, or viruses) during the at least one year before the study. All subjects were healthy at the time of sampling. Five ml of blood was carefully layered over eigh ml Lymphocyte Separation Medium and centrifuged at 2000 x g for 15 min. After the plasma layer was removed and saved, the buff coat was carefully removed and the cells were washed with TC-199 medium. Then they were collected by a 10 min centrifugation at 1000 x g. Lymphocytes were re-suspended at approximately 10^{7}/ ml in TC-199 medium with 20% v/v plasma and 10% v/v plasma and v/v DMSO. Lymphocytes were transferred to microfuge tubes, and they were stored at -20°C. The comet assay was performed as described previously (23). Comets from as broken ends of the negatively charged DNA molecule become free to migrate in the electric field towards the anode. The assay provides direct determination of the extent of DNA. Damage in individual cells and the extent of DNA damage can be assessed from the length of DNA migration. And, this is derived by subtracting the diameter of the nucleus from the total length of the image. The degree of damage was determined by grading the cells as: Normal (undamaged - no migration), Limited migration (at low damage levels, stretching of attached strands of DNA, rather than migration of individual pieces is likely to occur), and Extensive migration (with increasing numbers of breaks, DNA pieces migrate freely into the tail forming comet images). Minimum of 100 cells were analyzed for each study subject. Slides were scored blindly by the independent investigator. Statistical comparisons between the grade of DNA damages in control/patient groups were analyzed by using Mann-Whitney-U test.

RESULTS

The clinical data including ages, duration of treatments and blood levels of the drug and the comet scores of the 32 patients and 16 controls are shown in table 1 and 2. The mean age of the patients was 26.1 ± 6.7 years and of the control group was 26.5 ± 6.3 years. The comparison of the ages of the patients and the controls showed no statistically significant difference ($p > 0,05$). However, limited and extensive migrated cells in epileptic patients receiving CBZ were statistically higher than those of the controls (respectively $p=0.002$ and $p=0.0001$). The damaged (limited and extensive migrated) cells in patients who have a mean blood CBZ level of >8 mcg/ml were higher than those of the patients who have less than <8 mcg/ml; but, this difference was not statistically significant ($p > 0,05$). No relationship was observed between the comet scores in the patient group and the duration of treatment, either ($p > 0,05$).

DISCUSSION

Several trials evaluated the toxic effects of CBZ on DNA, but the results were contradictory. In a study about the effect of CBZ on human chromosomes in vivo and in vitro, no significant increase of chromosomal aberrations has been found. The SCE in vivo, however, significant increase of dose-dependency in chromosome aberrations were showed in vitro analysis (24). In an other investigation, it has been found that the total chromosome aberrations and SCE were significantly increased in patients undergoing treatment with CBZ for different periods starting from 6 months up to 15 years (25). On the other hand, Flejter et al. reported no detectable chromosome damaging effects of phenytoin alone, carbamazepine alone, or a combination of these two antiepileptic drugs. And, therefore, no correlation between the rate of either the chromosome damage or SCEs and the age, sex, drug blood level, or daily dose (26)

The single-cell gel-electrophoresis-assay has already been used in both in vitro and in vivo studies to assess DNA damage and repair induced by various agents in a variety of mammalian cells. The alkaline single-cell gel-electrophoresis-assay is now widely used for measuring DNA damage in somatic cells and has been successfully applied to detect lymphocyte samples from human populations. The single-cell gel-electrophoresis-assay is a potentially sensitive system to assess induced genotoxic damage in vivo and in vitro (20).

Several studies evaluated the effects of CBZ on DNA, but none of them has used the comet assay until now. For the first time, we used the comet assay to investigate the toxic effects of CBZ on DNA in the peripheral lymphocytes of female epileptic patients treated with only CBZ. We found a significant difference in the comet scores between the patients and the healthy women. The factors that may have influence on the comet scores (age, sex, race, nutrition, environ etc.) were similar in both groups. Physiological factors that may have effects on DNA are reproductive hormones; evaluation of SCE frequencies during a normal menstrual cycle demonstrated a higher rate of ovulation, and in the luteal phase as compared to the early follicular phase (27). In our study, all the subjects (patients and the control group) were at the same phase of the menstrual cycle (within 20^{th} and 27^{th} days following the beginning of menstrual bleeding) at the time of sampling. Therefore, we think that the difference in the comet scores was induced by the exposure to the CBZ. Samren et al. reported an increased risk of major congenital abnormalities in the offspring exposed in utero to CBZ, however they did not find a relationship between the occurrence of major congenital abnormalities and the dose of CBZ (6). We compared the comet scores of the patients considering blood drug levels. We found a higher excessive and limited damaged cells in patients who have a mean blood CBZ level of >8 mcg/ml compared with patients who have less than <8 mcg/ml. But, this increase is not statistically significant. Our results support the finding of Samren et al. in regard to the dose-effect relationship

Table 1. Individual data [age, duration of treatment (years), drug blood level (mcg/ml), grade of DNA damage in 100 cells by comet assay] from patients treated with carbamazepine.

Subject number	Age (years)	Duration of treatment	Blood drug level mcg/ml	Grade of damage in 100 cells		
				Undamaged (nomigration)	Limited migration	Extensive migration
1	25	2	9.50	81	11	8
2	24	3.5	4.05	80	11	9
3	21	4	6.09	82	10	8
4	27	5	4.72	88	3	9
5	31	1	5.75	82	9	9
6	29	1	7.48	90	4	6
7	35	3	8.05	91	3	6
8	27	2	4.74	92	3	5
9	25	1.5	9.30	85	7	8
10	35	5	4.35	83	8	9
11	32	1.5	8.98	77	9	14
12	23	1	7.00	80	11	9
13	23	4	10.8	82	10	8
14	21	3	5.02	88	4	8
15	20	1	8.43	86	7	7
16	27	1	7.50	89	4	7
17	31	2.5	4.90	89	5	6
18	20	1.5	5.88	91	3	6
19	33	2	9.00	86	7	7
20	20	2	6.15	86	5	9
21	25	3	6.90	84	6	10
22	27	2.5	4.83	91	5	4
23	21	6	6.87	87	6	7
24	29	5	11.9	84	9	7
25	33	2.5	4.53	89	5	6
26	22	4	7.73	90	10	-
27	21	3.5	5.50	83	10	7
28	29	2	7.05	90	4	6
29	33	2	5.45	95	3	2
30	20	2	4.76	92	4	4
31	22	7	5.93	80	9	11
32	23	1.5	4.29	94	5	1
Mean	26.1621	2.7656	6.6697	86.4688	6.5625	6.9687
±SD	6.7123	1.5605	2.0185	4.6070	2.7933	2.7880

There was not any significant difference in comet scores between patients receiving the drug during the last one year and the patients receiving more than one year. This finding suggest that this DNA damaging effect begins within the first year of the treatment.

In conclusion, we suggest that CBZ have mutagenic, carcinogenic and teratogenic effect and this effect may increase with high therapeutic levels and begins within first year of the treatment. We also suggest that in order to minimize the toxic effects of CBZ on DNA in epileptic patients, the least possible dose of carbamazepine must be prescribed to control seizures, especially during pregnancy.

Table 2. Individual data [age (years), grade of DNA damage in 100 cells by comet assay] of control group.

Subject number	Age (years)	Grade of damage in 100 cells Undamaged (no migration)	Limited migration	Extensive migration
1	21	91	5	4
2	20	91	6	3
3	35	96	4	-
4	28	97	2	1
5	29	93	5	2
6	34	96	3	1
7	27	98	2	-
8	24	92	7	1
9	36	95	4	1
10	24	92	7	1
11	23	93	3	4
12	27	94	5	1
13	25	97	3	-
14	26	100	-	-
15	24	97	2	1
16	25	98	2	-
Mean	26.5735	95.0000	3.7500	1.3125
±SD	6.3631	2.7809	1.9833	1.3125

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AUTHORS:

R. DÜNDARÖZ: (MD) Assistant Professor of Pediatrics Department of Pediatrics, Gülhane Military Medical Academy, School of Medicine, Ankara
H. İ. AYDIN: (MD) Assistant Professor of Pediatrics, Department of Pediatrics, Gülhane Military Medical Academy, School of Medicine, Ankara
T. GÜNGÖR: (MD),Department of Gynecology and Obstetrics,Dr. Zekai Tahir Burak Women's Hospital, Ankara
F. GÖK, (MD): Assistant Professor of Pediatrics, Department of Pediatrics, Gülhane Military Medical Academy, School of Medicine, Ankara
M. DENLİ, (MD): Associate Professor and Chairman, Department of Health Administration of the Turkish Army, Ankara, Turkey.

V. BALTACI: (MD), Associate Professor of Genetics, Department of Genetics, Başkent University School of Medicine, Ankara

ADDRESS FOR CORRESPONDENCE:
Dr. Ruşen DÜNDARÖZ
Bağ-Kur blk. 4. Blk. No: 69/14
06010 Etlik / Ankara