THE EFFECT OF ANTIEPILEPTIC DRUGS ADMINISTERED IN PREGNANCY ON MICRONUCLEUS FREQUENCY IN CORD BLOOD LYMPHOCYTES

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Abstract
Objectives: Epidemiological data indicate that the pregnancies of epileptic women constitute about 1% of all pregnancies. A large group of antiepileptic drugs (AEDs) applied in long-term monotherapy or polytherapy produce toxic metabolites as well as free radicals and reactive oxygen species. The aim of this study was to assess the potential genotoxic effect of AED therapy in pregnancy on DNA structure of umbilical cord blood lymphocytes.

Material and Methods: The study group were 30 newborns (14 males and 16 females) of mothers receiving long-term AED therapy during pregnancy. The AED considered were carbamazepine, valproic acid, phenyltriazine, benzodiazepine, gamma-aminobutyric acid and sulfonamide analogues. The controls were infants born to mothers not exposed to any medication in pregnancy (n = 20). Positive controls were the same infants, but in this case Nitrogranulogen (Sigma) was added to the collected cord blood samples (n = 11). Micronucleus (MN) assay was used as an indicator of chromosome damage. The frequency (%) of MN/1000 binucleated cells and the nuclear division index (NDI) were calculated.

Results: Mean MN frequency and NDI were respectively 0.110 (±0.152), 1.592 (±0.206) in the study group and 0.050 (±0.061), 1.628 (±0.178) in the controls (statistically non-significant difference, p > 0.1).

Conclusion: The findings did not reveal any genotoxic effect or inhibition of nuclear division in cord blood lymphocytes by AED metabolites. This was reflected by the absence of significant between-group differences in the mean MN frequency and NDI.

Key words: Antiepileptic drugs, Micronuclei, Mother, Newborn

INTRODUCTION

Many antiepileptic drugs (AEDs) have been found to be mutagenic and teratogenic in laboratory animals [1–3]. Pippenger [4] presumed that every major AED introduced into the market before 1990 was implicated in cognitive and/or physical defects of newborns and infants. The congenital developmental anomalies induced by the old-generation antiepileptic drugs such as carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB), and valproic acid (VPA) taken in pregnancy, are thought to be embryopathies associated with exposure to antiepileptic drugs [4–7].

It is widely postulated that a large group of antiepileptic drugs applied in long-term mono- or polytherapy account for the formation of toxic metabolites or free radicals. It is particularly the free radicals and reactive oxygen species that exhibit the genotoxic activity [4,8]. It has been shown that several antiepileptic drugs (CBZ, PHT, PB, VPA) significantly increased the frequency of chromosomal aberrations.
The culture medium was Eagle’s fluid 1959 (MEM) with added 10% (v/v) fetal calf serum (Biomed) and antibiotics: crystalline penicillin (100×IU ml⁻¹) and streptomycin (100 μg×ml⁻¹). Phytohaemagglutinin M (Gibco) at 0.1 ml/10 ml culture medium was used for stimulation of cell division. A final concentration of 6 μg×ml⁻¹ of cytochalasin B (Sigma) was added to the culture at 44 h to arrest

The micronucleus (MN) assay has become the preferred method used to evaluate chromosome damage, for it enables reliable assessment both of chromosome loss and chromosome breakage [11]. In fact, there are four recognized mechanisms by which micronuclei and micronucleus-like structures can arise: mitotic loss of acentric fragment, a variety of mechanical consequences of chromosomal breakage and exchange, mitotic loss of whole chromosomes, and apoptosis [12].

The aim of the present study was to evaluate the potential genotoxic effect of antiepileptic drugs on DNA structure of cord blood lymphocytes of the newborns whose mothers were treated for epilepsy during pregnancy. MN assay was used as an indicator of chromosome damage.

MATERIAL AND METHODS

The study group were 30 newborns (14 males and 16 females) of mothers receiving long-term AED therapy in pregnancy. The drugs administered are listed in Table 1. The controls were infants born to mothers not exposed to any medication in pregnancy (n = 20). Positive controls were the same infants, but in this case Nitrogranulogen (Sigma) was added to cord blood samples (n = 11). The deliveries took place at the Feto-Maternal and Gynaecology Department, Medical University of Łódź, Poland. Subject to analysis were cord blood lymphocytes of the infants from the study and control groups. 10 ml of umbilical cord blood was collected under sterile conditions immediately after separation of umbilical cord and placenta.

Table 1. The antiepileptic drugs administered in pregnancy

<table>
<thead>
<tr>
<th>Monotherapy (n = 21)</th>
<th>Polytherapy (n = 9)</th>
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<tbody>
<tr>
<td>CBZ (n = 10)</td>
<td>CBZ+VPA (n = 3)</td>
</tr>
<tr>
<td>VPA (n = 6)</td>
<td>CBZ+VPA+bezodiazepine (n = 1)</td>
</tr>
<tr>
<td>Phenytoin (n = 5)</td>
<td>CBZ+phenyltriazine (n = 2)</td>
</tr>
<tr>
<td></td>
<td>VPA+phenyltriazine (n = 1)</td>
</tr>
<tr>
<td></td>
<td>sulfonamide analogues+GABA (n = 1)</td>
</tr>
<tr>
<td></td>
<td>sulfonamide+CBZ (n = 1)</td>
</tr>
</tbody>
</table>

CBZ — carbamazepine, VPA — valproic acid, GABA — gamma-aminobutyric acid analogues.

The culture medium was Eagle’s fluid 1959 (MEM) with added 10% (v/v) fetal calf serum (Biomed) and antibiotics: crystalline penicillin (100×IU ml⁻¹) and streptomycin (100 μg×ml⁻¹). Phytohaemagglutinin M (Gibco) at 0.1 ml/10 ml culture medium was used for stimulation of cell division. A final concentration of 6 μg×ml⁻¹ of cytochalasin B (Sigma) was added to the culture at 44 h to arrest

![Fig. 1. Bi-, tri- and quadrinuclear cells with single micronuclei.](image)
cytokinesis. At 72 h of incubation, the cultures were harvested by centrifugation at 1000 rpm for 10 min. Hypotonic shock was performed using 0.075 M KCl (Serva) at 20°C for 5 min. The cells were centrifuged, and Cornoy’s fixative (methanol: acetic acid, 3:1, v/v) solution was freshly added and dropped on slides. The slides were stained with 2% Giemsa (pH 6.8) (Sigma). Nitrogranulogen (Sigma) at the concentration of 0.25 μg×ml⁻¹ was used for positive control.

The number of MN per 1000 binucleated (BN) cells for each sample was scored, and the frequency (%) of MN per 1000 BN cells was calculated (Fig. 1).

A minimum of 500 viable cells were scored to determine the frequency of cells with 1, 2, 3, or 4 nuclei and to calculate the nuclear division index (NDI) using the formula [11]: NDI = (M1+2(M2)+3(M3)+4(M4))/N, where M1-M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored. Basic statistics: arithmetic mean and standard deviation, were calculated. The obtained results were subjected to statistical analysis with Mann-Whitney U test. P < 0.05 was considered the level of significance.

RESULTS

Detailed results are displayed in Table 2. The mean MN frequency in binucleated cells for the study group, control group, and positive control were 0.110 (±0.152), 0.050 (±0.061), and 2.658 (±1.731), respectively. The respective mean NDI values were 1.592 (±0.206), 1.628 (±0.178), and 1.718 (±0.249).

In the study group, the mean MN and NDI values did not differ significantly from the findings for the control group (p > 0.1). When the study group and positive control were compared, a statistically significant difference (p < 0.001) was noted only for the mean MN frequency. A similar difference (p < 0.001) was observed between the control group and positive control. However, in view of the small size of the investigated groups (Tab. 1), the statistical analysis of the findings could not be performed.

DISCUSSION

Studies on genotoxicity of various groups of antiepileptic drugs, that would employ the methods of cytogenetic tests in vitro (structural chromosome aberration test, SCE test, MN test), have been widely reported [1,9,10,12,13]. Among these authors, Celik [12] noted a statistically significant increase in MN frequency in cultured peripheral blood lymphocytes exposed to carbamazepine at the doses of 8, 10 and 12 μg×ml⁻¹, as compared to the findings for controls (p < 0.001). However, to our knowledge, no authors have thus far discussed the results of cytogenetic tests on cultured lymphocytes from umbilical cord blood of the newborns whose mothers received antiepileptic therapy in pregnancy.

Our study concerned the possible genotoxic effect of long-term mono- or polytherapy with antiepileptic drugs in pregnancy on cord blood lymphocytes. As regards the monotherapy, the largest group (n = 10) were mothers receiving carbamazepine, followed by those who took valproic acid (n = 6) and phenyltriazine (n = 5). Carbamazepine and valproic acid belong to the old-generation antiepileptic drugs. However, the examined groups were rather too small to undertake statistical evaluation.

Table 2. MN frequency and NDI values in the groups examined

<table>
<thead>
<tr>
<th></th>
<th>Study group (n = 30)</th>
<th>Control group (n = 20)</th>
<th>Positive control (n = 11)</th>
</tr>
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<tbody>
<tr>
<td>MN (%) (mean±SD)</td>
<td>0.110 (±0.152)</td>
<td>0.050 (±0.061)</td>
<td>2.658 (±1.731)</td>
</tr>
<tr>
<td>NDI (±SD)</td>
<td>1.592 (±0.206)</td>
<td>1.628 (±0.178)</td>
<td>1.718 (±0.249)</td>
</tr>
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</table>

* p > 0.1 compared with control group; a p < 0.001 compared with positive control; b p > 0.05 compared with positive control; c p < 0.001 compared with positive control; d p > 0.1 compared with positive control.
It should be emphasized that the genotype of both the mother and the fetus may have influence on the transport of a given harmful agent through the placenta, as well as its absorption, distribution and binding to relevant receptors, thus affecting its teratogenicity. A great protective role is attributed to the efficiency of the detoxifying systems e.g. antioxidative enzymes (superoxide dismutase, glutathione-S-transferase, catalase, glutathione reductase) [4,7,8]. In the present study, the absence of significant differences in MN frequency in cord blood lymphocytes between the newborns of mothers exposed to antiepileptic drugs and not exposed to any medication in pregnancy, may point to the placental barrier as responsible for the observed finding. Many antiepileptic drugs exert influence on the enzymatic metabolic system with cytochrome P450, which leads to a decrease in maternal folate level in serum, resulting in its deficiency. The antiepileptic drugs that do not activate the cytochrome P450 system do not decrease folate concentration [7]. The main task of the metabolic pathways of folate is to supply 1-carbon units such as methyl (-CH₃), methylene (-CH₂), formyl (-CHO), for numerous enzymatic processes: purine and pyrimidine synthesis, protein synthesis, remethylation of homocysteine to methionine, and DNA methylation [14]. Fenech et al. [15] reported that MN frequency significantly and positively correlated with age in males and females, and was affected by dietary factors such as folate deficiency and plasma levels of vitamin B12 and homocysteine.

Two groups of fragile sites can be found in the human genome: heritable (or rare) and constitutive (or common). Both the types of fragile sites are characterized by a specific and precise localization in the chromosome [16]. The common fragile sites are now defined as the site-specific gaps or breaks seen on the metaphase chromosome after partial inhibition of DNA synthesis [17]. The common fragile sites are normally stable in somatic cells. However, when the cultured cells are treated with replication inhibitors, the fragile sites display gaps, breaks, rearrangements, and other features of unstable DNA [18]. A great number of fragile sites in chromosomes are the specific target of mutagens and carcinogens, and this selective localization of lesions is called "hot spots" [17,19]. Hodges et al. [20] reported that the patients with epilepsy seizures who were treated with diphenylhydantoin had a higher incidence of induced fragile sites (p < 0.001) than did the patients treated with anti-seizure medication other than diphenylhydantoin. Morel et al. [21] studied the fragile site at 10q23.3 in an amniocyte culture of a fetus exposed to phenytoin during pregnancy. The authors postulate that the fragile site at 10q23.3 in this fetus may have arisen secondary to a combination of polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and exposure to phenytoin, and is indeed FRA10A [21].

The results of the present study did not reveal any genotoxic effect or inhibition of nuclear division in cord blood lymphocytes by AED metabolites. This was reflected by the absence of significant differences in the mean MN frequency and NDI between the study and control group.

REFERENCES


