Immunotoxicity of Chronic Phenytoin Administration in Epileptic Patients

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ABSTRACT

Objectives: To evaluate the probable cellular and humoral toxic immune response in epileptic patients on phenytoin treatment. Subjects and Methods: The total and differential leucocytic count, the peripheral blood T-lymphocyte subsets percentage (CD4⁺% and CD8⁺%) and serum immunoglobulins M and G were determined in 69 phenytoin treated epileptic patients from Suez Canal University Hospital and 23 healthy control subjects. Results: The level of total leucocytic count, neutrophils, monocytes, lymphocytes, CD4⁺%, CD4⁺/CD8⁺ ratio and immunoglobulins M and G were significantly lower in phenytoin treated patients especially those with long term treatment in comparison to healthy control subjects. The level of eosinophils and CD8⁺% were significantly higher in phenytoin treated patients. Conclusion: Toxic immuno-suppression effect of phenytoin can involve the cell mediated immunity in the form of lymphopenia, low percentage of circulating CD4⁺ and CD4⁺/CD8⁺ ratio and the humoral immunity in the form of low serum concentration of immunoglobulins M and G. (Egypt J. Neurol. Psychiat. Neurosurg., 2006, 43(1): 435-441)

INTRODUCTION

Epilepsy is a common neurological problem. Its prevalence is generally taken as between 5-10 cases per 1000 persons. As a sizeable population is affected by epilepsy, its effective management and avoidance of toxic side-effects are particularly the matter of concern. Phenytoin is the oldest non-sedative antiepileptic drug and is the most effective drug against partial seizures and the generalized tonic-clonic seizures. A marked amount of clinical observations indicated the existence of an association between the use of antiepileptic drugs and immunological disturbances and/or immunotoxic reactions. Little is known about the effect of phenytoin on both cellular and humoral immune system, as most studies in this field deal with alterations in immunoglobulins serum levels and a very rare studies were concerned with the distribution of T-cell subsets in peripheral blood. Thus, existing data in this field are difficult to interpret and give precise conclusions. Therefore, the present study was done to evaluate the probable toxic cellular and humoral immune response in epileptic patients on phenytoin treatment.

SUBJECTS AND METHODS

Sixty nine (69) phenytoin treated epileptic patients (group1) ranging in age from 12 to 60 years were selected from Neurology out patient clinic at Suez Canal University Hospital. The diagnosis of epilepsy was based on the presence of the characteristic convulsive seizures, documented with electroencephalogram (EEG). The duration of phenytoin treatment was > 6 months and the dose was 200-300 mg/day. According to the duration of treatment by phenytoin the patients were divided into 3 groups (each group 23 patients):
- Group 1-A: duration > 6-12 months.
- Group 1-B: duration > 12-24 months.
- Group 1-C: duration > 24 months.

Twenty three healthy subjects ranging in age from 18 to 45 years, taking no medications and with no history of malignancy, inflammatory or
infectious disease were recruited as controls (group 2).

Blood samples were collected from the patients and controls for:
- Evaluation of the natural immunity by counting the total leucocytic count on EDTA-anticoagulated blood sample by using automated blood cell counter apparatus (Automated blood cell counter System cx 21 Japan) and differential leucocytic count on blood films.5
- Evaluation of the cellular immunity by identification and counting T-lymphocytes subsets (CD4+ and CD8+) with monoclonal antibodies directed against relevant CD markers by using flow cytometry (FCM) after the separation of mononuclear cells from the EDTA anticoagulated peripheral blood on Ficoll-Hypaque density gradient (Biotest Co, Germany).7
- Evaluation of humoral mediated immunity by measurement of concentration of serum immunoglobulins G and M (IgG & IgM) by single radial immunodiffusion (Biocientifica S.A. Biochemist-Pharmacist) after centrifuging of 1 ml of peripheral blood.8

Data were expressed as mean ± SE, the difference between values was examined statistically by Students t-test and P value < 0.05 was considered significant.9

**RESULTS**

Table (1) shows the effect of duration of phenytoin administration on mean absolute total leucocytic count in epileptic patients. There is a significant decrease in the count in both groups 1-B and 1-C when compared to control group and group 1-A (P<0.05) although all the values were within the normal adult range (4000-11000 cell/mm³).

Table (2) shows the effect of duration of phenytoin administration on mean absolute differential leucocytic count in epileptic patients. There is a significant decrease of neutrophils in both groups 1-B and 1-C when compared to control group and group 1-A (P<0.05). There is also a significant decrease in lymphocytes by absolute number in groups 1-A, 1-B and 1-C when compared to control group (P<0.05) and in both groups 1-B and 1-C when compared to group 1-A (P <0.05). There is a decrease in absolute count of monocytes in groups 1-B and 1-C when compared to control group (P<0.05) and a significant increase in eosinophil count in groups 1-A, 1-B and 1-C when compared to control group (P<0.05).

Table (3) shows the effects of duration of phenytoin administration on T-lymphocytes subsets percentage in epileptic patients. The mean of CD4+% shows a decrease in groups 1-B and 1-C in comparison to control group and group 1-A (P<0.05). While, CD8+% shows a significant increase in groups 1-A, 1-B and 1-C when compared to control group (P<0.05) and groups 1-B and 1-C when compared to group 1-A (P<0.05). CD4+/CD8+ ratio decreased significantly in groups1-A, 1-B and 1-C when compared to control group (P<0.05).

Table (4) shows the effect of duration of phenytoin administration on mean serum IgM and IgG in epileptic patients. Serum IgM titer shows a significant decrease in groups 1-A, 1-B and 1-C when compared to control group (P<0.05). Group 1-C shows a significant decrease when compared to groups 1-A and 1-B (P<0.05). Serum IgG titer in group 1-C shows a marked and significant decrease when compared to control group and groups 1-A and 1-B (P<0.05).
Table 1. Effect of duration of phenytoin administration on mean absolute total leucocytic count (Cell/mm$^3$) in epileptic patients.

<table>
<thead>
<tr>
<th>Total WBCS count</th>
<th>Control group</th>
<th>Group (1-A)</th>
<th>Group (1-B)</th>
<th>Group (1-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SE</td>
<td>9000±279</td>
<td>7900±494</td>
<td>6188±312$^{a,b}$</td>
<td>5695±315$^{a,b}$</td>
</tr>
</tbody>
</table>

Groups 1-A: Patients treated by phenytoin for a period >6-12 months, 1-B: Patients treated by phenytoin for a period >12-24 months, 1-C: Patients treated by phenytoin for a period >24 months.

Paired t test: (a) P < 0.05 compared to control group, (b) P < 0.05 compared to group 1-A.

Table 2. Effect of duration of phenytoin administration on mean absolute differential leucocytic count (cell/mm$^3$) in epileptic patients.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group (1-A)</th>
<th>Group (1-B)</th>
<th>Group (1-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>4219±259</td>
<td>3968±138</td>
<td>3451±216$^{a,b}$</td>
<td>3074±309$^{a,b}$</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>4274±165</td>
<td>3130±275$^a$</td>
<td>2305±170$^{a,b}$</td>
<td>2105±110$^{a,b}$</td>
</tr>
<tr>
<td>Monocytes</td>
<td>436±32</td>
<td>418±31</td>
<td>259±12$^a$</td>
<td>249±16$^a$</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>110±3</td>
<td>135±4$^a$</td>
<td>157±5$^a$</td>
<td>162±3.9$^a$</td>
</tr>
</tbody>
</table>

Groups 1-A: Patients treated by phenytoin for a period >6-12 months, 1-B: Patients treated by phenytoin for a period >12-24 months, 1-C: Patients treated by phenytoin for a period >24 months.

Paired t test: (a) P < 0.05 compared to control group, (b) P < 0.05 compared to group 1-A.

Table 3. The effect of duration of phenytoin administration on mean of T-lymphocyte subsets percentage (CD4$^+$% and CD8$^+$%) in epileptic patients.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group (1-A)</th>
<th>Group (1-B)</th>
<th>Group (1-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4$^+$%</td>
<td>49.35±1</td>
<td>43.32±1.1</td>
<td>38.76±1.19$^{a,b}$</td>
<td>36.86±1.17$^{a,b}$</td>
</tr>
<tr>
<td>CD8$^+$%</td>
<td>27.86±1.1</td>
<td>32.6±0.89$^a$</td>
<td>36.14±1.24$^{a,b}$</td>
<td>37.35±1.16$^{a,b}$</td>
</tr>
<tr>
<td>CD4$^+$/CD8$^+$ ratio</td>
<td>1.77±0.08</td>
<td>1.33±0.03$^a$</td>
<td>1.07±0.05$^a$</td>
<td>0.99±0.04$^a$</td>
</tr>
</tbody>
</table>

Groups 1-A: Patients treated by phenytoin for a period >6-12 months, 1-B: Patients treated by phenytoin for a period >12-24 months, 1-C: Patients treated by phenytoin for a period >24 months.

Paired t test: (a) P < 0.05 compared to control group, (b) P < 0.05 compared to group 1-A.

Table 4. The effect of duration of phenytoin administration on mean serum immunoglobulins M and G (mg/dl) in epileptic patients.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Group (1-A)</th>
<th>Group (1-B)</th>
<th>Group (1-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin M</td>
<td>15.1±11.5</td>
<td>64.1±5.4$^a$</td>
<td>55.5±5.9$^a$</td>
<td>35.1±5.8$^{a,b,c}$</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>945±107</td>
<td>795.6±90</td>
<td>745±35.1</td>
<td>403.4±105$^{a,b,c}$</td>
</tr>
</tbody>
</table>

Groups 1-A: Patients treated by phenytoin for a period >6-12 months, 1-B: Patients treated by phenytoin for a period >12-24 months, 1-C: Patients treated by phenytoin for a period >24 months.

Paired t test: (a) P < 0.05 compared to control group, (b) P < 0.05 compared to group 1-A, (c) P < 0.05 compared to group 1-B.
DISCUSSION

Evaluating the immunotoxic potential of any drug is a standard component of safety assessment. Effects evaluated include the potential for drug to induce hypersensitivity and/or autoimmune reactions or to produce immunosuppression. Although humoral and cellular immune function abnormalities have been described in epileptic patients during antiepileptic drugs therapy, the incidence and the type of immunological aberrations and the contribution of each drug are still controversial. Many of the existing reports were difficult to compare, owing to differences in experimental design and patients populations.

Assays commonly used to assess immunotoxicity in clinical studies include: total and differential leucocytic count, serum immunoglobulins levels, cytokines analysis and lymphocyte subpopulations. In the present study we selected the measurement of total and differential leucocytic count to study non-specific immunity, percentages of T-lymphocytes subsets CD4+ and CD8+ to study cell-mediated immunity, measurement of serum IgG and IgM to study humoral immunity.

In the present study, with increasing the period of administration of phenytoin there is a significant decrease in mean total leucocytic count. These results denoting that the decrease in total leucocytic count is time dependent in epileptic patients under phenytoin treatment. Chronic phenytoin treatment causes also a significant decrease in the mean count of neutrophils, monocytes and lymphocytes. While it causes eosinophilia in all groups of patients. Kaur et al., reported that phenytoin can produce hematological abnormalities such as atypical lymphocytes and eosinophilia along with hypersensitivity syndrome in epileptic patients on phenytoin treatment. In an animal study evaluating the toxic immune effect of phenytoin on female mice, Tucker et al., noticed that the committed granulocyte macrophage progenitor cells, colony-forming unit-granulocyte macrophage were inhibited by phenytoin. Bone marrow cells were folate deficient, as determined by the inability of these cells to convert deoxyuridine to thymidine. They suggested that phenytoin inhibits folate utilization or metabolism at the cellular level, selectively affecting bone marrow stem cells and resulting in altered stem cell kinetics resulting in alteration of immune response. In their study Kaul et al., proved that the chronic administration of phenytoin on peripheral lymphocytes brought mitotic delay and it decreases the mitotic index of the first, second and the third metaphysic with reduction of the proliferation index with proliferation delay per cycle. Phenytoin also is considered as one of the drug-induced interferon gamma release with direct effect on the B-lymphocytes after incubation of peripheral blood lymphocytes with phenytoin which was considered as a first step in its immunotoxic effect.

Little is known about the effects of phenytoin therapy on cellular immunity and studies designed in the past to measure both cellular immune parameters were very conflicting. In the present study the results revealed that there is a significant decrease in CD4+ in all groups of patients in comparison to control group especially with long term treatment. The CD8+ show a significant increase in all groups of patients. The CD4+/CD8+ ratio is significantly decreased with increase duration of exposure. The present results are in agreement with the results of Basaran et al., who stated that the phenytoin has a suppressive effects on the total number of CD4+ and CD4+/CD8+ ratio. However, Eeg-Olofsson et al., in their study on epileptic patients under treatment of phenytoin found a significant increase in CD8+ percentage with low CD4+/CD8+ ratio. In a study in mononuclear cells from peripheral blood of 10 healthy blood donors were incubated with phenytoin 20 mg, the results revealed that the percentage of active E-rosette forming T-lymphocytes was decreased compared with control. Phenytoin reduced the percentage of active T-lymphocytes in 9 of the 10 suspensions.
The present results can be explained according to a huge amount of clinical observations indicated the existence of an association between phenytoin and immunological disturbances. In vitro phenytoin inhibits DNA synthesis in human lymphocytes and reduces the percentage of active T-helper lymphocytes (Th cells). In an experimental study on rat T-lymphocytes, Winn et al. postulated that phenytoin is bio-activated to a reactive intermediate leading to increase formation of reactive oxygen species (ROS) which can damage essential macromolecules including DNA molecules and induce DNA double strands breaks (DSBs) in a concentration and time dependent manner.

In the present study, there is a significant reduction in serum concentration of the IgM compared to control especially with increasing the period of administration. The present results are coincided with the results reported by Badawy et al., which revealed that serum IgM was significantly decreased in the phenytoin treated patients in comparison to control. In a cohort study on epileptic patients on phenytoin treatment Ranua et al., reported that serum IgM and IgA concentrations were decreased and the low IgM levels were detected with long duration of epilepsy. Hence, the depressive effect of phenytoin on IgM was obvious. However in contrast to the present results, Basaran et al found no significant alteration of serum IgM titer in phenytoin treated patients with respect to healthy subjects in a comparative study on epileptic patients and healthy controls.

The present study showed that serum IgG titer is decreased in all groups of patients in comparison to control group especially with increasing the period of administration. The present findings are consistent with a study conducted to compare IgG levels in serum and saliva obtained from phenytoin-treated epileptic patients and control group. Serum IgG levels were significantly lower in phenytoin treated epileptic patients and no difference was found in salivary IgG.

In a study conducted for studying the mechanisms for suppression of immunoglobulin production by lymphocytes induced by phenytoin using the peripheral blood mononuclear cells (PBMCs) on patients, immunoglobulin production in PBMCs was suppressed by phenytoin and the suppressive effect on IgM and IgA tended to be greater than the effect on IgG production. Phenytoin can influence the immune system by modifying interleukin and chemokine concentrations; along with immunoglobulins concentrations.

The effect of chronic administration of phenytoin in mice was studied by Okada et al. They concluded that phenytoin modulates the immune response by suppression of cell-mediated immunity directly by reducing interleukins (IL-1 alpha) level (its major effects are activation of T and B cells and macrophages) and reducing interferon gamma level (its major effects are stimulation of macrophages and suppress Th2 cells) while it increases the IL4 level which has a suppressor effect on Th1 cells. Thus, phenytoin collectively suppresses T and B cells. Phenytoin, also can modulates the immune response indirectly by increasing ACTH and corticosteroid level with a net result of Th1/Th2 imbalance and initiating immunotoxicity.

REFERENCES

الخلايا المناعية الناتجة عن الاستعمال المزمن لعقار الفينيتوين في مرضى الصرع

استهدفت الدراسة تقييم التأثير السمي المحتمل لوجود عقار الفينيتوين كأحد العوامل المضادة لمرض الصرع على بعض المعايير الممثلة للجهاز المناعي للجسم، وقد أجربت الدراسة على 69 مريضاً يعانون من مرض الصرع بالإضافة إلى 23 شخصاً سليماً كمجموعة ضابطة. وقد تم تقسيم المرضى إلى ثلاث مجموعات طبقاً لفترة علاجهم بالعقار كالتالي:
- مجموعة (1-1) المريض الذين يخضعون للعلاج بالفينيتوين لمدة 6 - 12 شهراً.
- مجموعة (1-ب) المرضى الذين يخضعون للعلاج بالفينيتوين لمدة 12 - 24 شهراً.
- مجموعة (1-ج) المرضى الذين يخضعون للعلاج بالفينيتوين لمدة >24 شهراً.

قد تم دراسة التأثير على الجهاز المناعي للجسم من خلال ثلاثة معايير هي:
- تقييم المناعة الطبيعية عن طريق عد كرات الدم البيضاء الكلي والمناعي.
- تقييم المناعة_twitter محضرة الخلية عن طريق تحديد نسبة عدد خلايا الدم البيضاء الليفاوية (ت) والأدوات الثانوية لها (المحمولة والمصابة بالخلايا).
- تقييم المناعة Twitter محضرة الخلية عن طريق قياس تركيز الأجسام المناعية للمادة الغريبة (إيبوليرينات المناعة ج ، م) في المصل.

وقد أظهرت النتائج وجود تأثيرات دالة إحصائية في مرضى الذين يخضعون للعلاج بعقار الفينيتوين مقارنة بالمجموعة الضابطة خاصة المريض الذين يتقلدون العقار لفترات طويلة وذلك في النتائج التالية:
- القيمة المئوية لكل خلايا الدم البيضاء وخضرة وخلايا الدم البيضاء وحيدة النواة.
- النسبة المئوية من خلايا الدم البيضاء والخلايا المناعية (ت) المحمولة.
- نسبة بين خلايا الدم البيضاء (ت) المحمولة والخلايا المناعية (ت) المحمولة.
- تركيز الأجسام المناعية للمادة الغريبة (إيبوليرينات المناعة ج ، م) في المصل.

بينما كان هناك زيادة في النسبة المئوية للخلايا المناعية (ت) المحمولة خلايا الدم البيضاء ذو دالة إحصائية في المرضى الذين يخضعون للعلاج بعقار الفينيتوين مقارنة بالمجموعة الضابطة.