Pregabalin's effect on human genetic material: *in vitro* study.

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ABSTRACT: *Purpose of the study:* Pregabalin is a prescription drug approved for the treatment of generalized anxiety disorder; partial epilepsy; neuropathic pain and fibromyalgia. It is an alpha-2-delta ligand, structurally related to the neurotransmitter GABA that inhibits calcium currents via high-voltage-activated channels containing the a2d-1 subunit.
Aim of the present study was to investigate the *in vitro* effect of pregabalin on healthy human cultured lymphocytes, by estimating three sensitive cytogenetic indices: Sister Chromatid Exchanges (SCEs), Proliferation Rate Index (PRI) and Mitotic Index (MI).
Methods: SCEs are considered as one of the most sensitive markers of genotoxicity, whereas PRI is one of the most reliable markers of cytostatic activity and MI shows precisely the ability of the cell to proliferate. We prepared eight pregabalin solutions commonly used in clinical practice. The solutions were added to cultures of peripheral blood lymphocytes taken from two young healthy donors. After 72 hours of incubation with the appropriate technique the cultured lymphocytes were plated on glass slides, stained with the Fluorescence plus Giemsa method and the above indices were estimated with the optical microscope.
Results and Conclusions: Pregabalin at therapeutic doses exhibited dose-dependent cytogenetic activity *in vitro*, increasing SCE frequencies and diminishing PRI levels in normal human lymphocyte cultures. Interestingly, the variation of the magnitude of MI reduction seems to be directly related to the decrease of PRI values as well as to the increase of SCE frequencies. Considering that the use of pregabalin is rapidly increased, further studies in other cell lines and in *in vivo* experimental settings are needed in order to evaluate its effect on genetic material.

Key Words: Pregabalin, Cytogenetic activity, Sister chromatid exchanges, Proliferation rate index, Mitotic index.

INTRODUCTION

Pregabalin [(S)-3-(aminomethyl)-5-methylhexanoic acid] is an anticonvulsant drug that is used for the treatment of a variety of neurological and psychiatric disorders[1] such as partial epilepsy[2,3,4,5,6], neuropathic pain associated with diabetic peripheral neuropathy[7,8], post-herpetic neuralgia[8,9], fibromyalgia[10,11,12,13], generalized anxiety disorders[14,15,16,17] and cancer pain[2,18]. Pregabalin is structurally related to Gabapentin (Figure 1). Both drugs are derivatives of the neurotransmitter γ-aminobutyric acid (GABA)[1,2,3].

It is an alpha-2-delta ligand that inhibits calcium currents via high-voltage-activated channels containing the a2d-1 subunit and reduces neurotransmitter (noradrenaline serotonin, dopamine and substance P) release in hyperexcited neurons leading to attenuation of postsynaptic excitability[1,2]. Pregabalin unlike other anxiolitic compounds does not bind directly to $GABA_A$, $GABA_B$ and benzodiazepine receptors.

In addition, GABA metabolism is not affected either. Pregabalin also interacts with opioids, benzodiazepines, barbiturates and ethanol and so far it is thought that the potential for abuse of this drug is less than benzodiazepines potential. However, new evidence may challenge the prevailing view[19,20].

Despite its extended use, cytogenetic effect of pregabalin remained unknown. Previous studies suggest that other compounds modulating GABA-ergic

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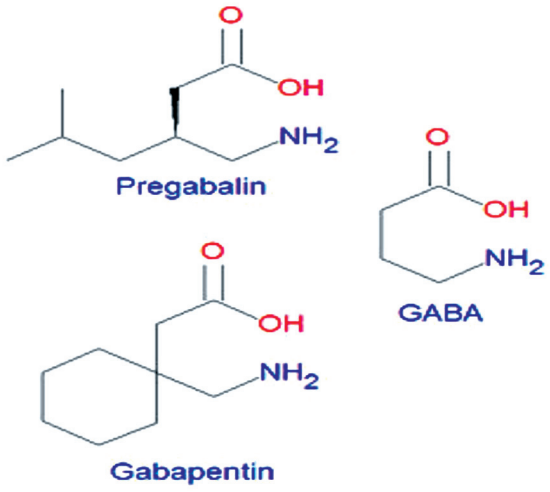

Figure 1.

neurotransmission including benzodiazepines exhibit statistically significant genotoxicity in human lymphocyte cultures[21].

The aim of the present preliminary study was to investigate the *in vitro* effect of pregabalin on human cultured lymphocytes, by estimating sensitive cytogenetic indices as SCEs, PRI and MI. SCEs has been identified as one of the most sensitive indices among sensitive biomarkers of genotoxicity, such as chromosomal aberrations, comet assay and micronuclei. PRI and MI have been used as sensitive indicators for the evaluation of the cytostatic activity of various environmental hazards or therapeutic agents (Mourelatos, 1996).

MATERIALS AND METHODS

***In vitro* SCE and PRI assays.** In this study, blood samples were given by two healthy donors 18 and 19 years old, who were non-smokers, not receiving any drugs, not consuming considerable quantities of alcohol, or not having suffered any kind of infection for the last 15 days.

Human lymphocyte cultures were prepared by adding in 5 ml chromosome medium (RPMI-1640, Biochrome, supplemented with 20% fetal calf serum, 0,63% L-glutamine, 0,63% penicillin/streptomycin and 2% phytohaemagglutinin) at the beginning of the culture life, the following:

- 11-12 drops of normal human heparinized whole blood
- 5μg/ml 5-Bromodeoxyuridine (BrdU) and
- The pregabalin solutions (A=2,5μg/ml, B=5μg/ml, C=10μg/ml, D=15μg/ml, E=30μg/ml, F=60μg/ml, G=120μg/ml and H=150μg/ml final concentrations per culture)

The concentrations ranging from C to G were equivalent to the ones more commonly used in clinical practice (therapeutic doses per os: 150-600 mg/day). The cultures were incubated at 37° C for 72 hours in the dark to minimize photolysis of 5-Bromodeoxyuridine. Colchicine was added 2h before the collection of the cultures. The cells were then collected by centrifugation and exposed to 0,075M KCl for 10 minutes. The hypotonic solution spreads the chromosomes and hemolyses the red blood cells. The pellet was fixed three times with methanol:acetic acid (3:1). Drops of concentrated suspension of cells were placed on microslides that allow to air dry. For SCE, PRI and MI analysis, the slides were stained by a modification of the fluorescence plus Giemsa procedure to obtain harlequin chromosomes[21,,22].

Statistical Analysis. In order to estimate SCEs, 30 suitably spread second division cells from each culture were blindly scored. For PRI calculation, 100 cells in the first, second, third and higher divisions from each culture were blindly scored and the formula: $PRI=M_1+2M_2+3M_3/100$ was applied, where M_1, M_2 and M_3 are the percent values of cells in the first, second, third and higher divisions, respectively. For MI analysis, 1000 nuclei were scored. MI=number of metaphases/total number of cells. For the statistical evaluation of the experimental data, Student's t-test was performed to determine whether any SCE values differed significantly from the controls and the χ^2-test was used for PRI and MI comparisons. The Pearson product moment correlation coefficient r was applied for calculating the correlation between SCEs, PRI and MI. A criterion for testing whether r differs significantly from zero was used whose sampling distribution is Students test with n-2 degrees of freedom.

RESULTS

Table 1 illustrates the cytotoxicity of pregabalin, presented as dose- dependent increase of SCE frequency of the two donors' cultured lymphocytes. This increase shows statistically significance ($P < 0,001$) at pregabalin concentrations' D-H, while a small decrease (not statistically significant) in SCE rate has been found at concentrations A and B.

Table 1. Effect of Pregabalin on SCE frequency in human lymphocyte cultures.

Dosage (μg/ml)	1st donor	2nd donor
Control	8,21	8,33
A 2,5	8,19	8,31
B 5	8,13	8,27
C 10	9,56	9,67
D 15	10,45*	10,78*
E 30	11,21*	11,49*
F 60	12,01*	12,44*
G 120	13,19*	13,54*
H 150	15,46*	15,71*

**Statistically significant ($p < 0,001$) increase over the corresponding control (t-test).*

PRI of the lymphocytes, in Table 2, is decreased at concentrations A-H but this decrease becomes statistically significant ($P < 0,001$) only at concentrations F-H.

MI values, in Table 3, show a dose- depended deceased at concentrations B-F, and an insignificant increased at concentrations G and H. Furthermore, a correlation was observed ($P < 0,001$) between:

- the magnitude of the SCE induction and the MI alterations
- the MI alterations and PRI alterations and
- the magnitude of the SCE induction and the PRI alterations

Table 2. Effect of Pregabalin on PRI values in human lymphocyte cultures.

Dosage (μg/ml)	1st donor	2nd donor
Control	2,61	2,48
A 2,5	2,59	2,47
B 5	2,58	2,46
C 10	2,54	2,4
D 15	2,49	2,35
E 30	2,43	2,3
F 60	2,39	2,26
G 120	2,37	2,25
H 150	2,3**	2,21**

***Statistically significant ($p < 0.001$) decrease over the corresponding control (χ^2 test).*

Table 3. Effect of Pregabalin on MI values in human lymphocyte cultures.

Dosage (μg/ml)	2nd donor	3rd donor
Control	43	39
A 2,5	42	38
B 5	41	36
C 10	37***	33***
D 15	32***	29***
E 30	28***	25***
F 60	27***	24***
G 120	28	25
H 150	30	27

****Statistically significant ($p < 0.001$) decrease over the corresponding control (χ^2 test).*

DISCUSSION

Pregabalin, along with similarly- structured Gabapentin, has a different mechanism of action in comparison to other known anticonvulsants. Multiple mechanisms of action have been proposed to describe the effect of this drug but so far these mechanisms have remained poorly understood[1]. It is also believed that pregabalin probably shares common neural mechanisms with SSRIs and benzodiazepines[23], which except for their therapeutic roles, affect the immune system as well. Further knowledge of the way pregabalin affects human DNA will provide us a better understanding of its mechanism of action. Besides, it concerns a considerable number of patients since it is approved for a variety of diseases.

The important therapeutic role of pregabalin and the lack of knowledge on its effect on human DNA motivated us to investigate another aspect of its action by estimating sensitive cytogenetic indices such as SCEs, PRI and MI, in order to study the cytotoxic and cytostatic activity of pregabalin *in vitro*.

The results show that pregabalin causes a dose-dependent, statistically significant increase of SCEs frequency from concentrations D-H, although it caused a small not statistically significant decrease of SCEs frequency at concentrations A and B. (Figure 2).The SCEs' increase followed by an equally significant decrease of MI (although it was observed a small increase of MI at concentrations G and H, Figure 3) and also a decrease of PRI values (Figure 3) which was statistically significant only at concentration H in both of the two experiments.

Living cells have the ability to excise and repair a large variety of damage on DNA. High SCE values could be due to a considerable number of DNA damages[24] that could not be repaired before T lymphocytes reach S phase *in vitro*. An inability of the repair mechanisms to restore DNA damage could increase SCEs too[21,22]. So the statistically significant increase of SCE values on cultured lymphocytes caused by pregabalin, shows that pregabalin has valuable cytotoxic action at least in the above mentioned doses.

Although, in this experimental work, the decrease of SCE values at concentrations A and B of pregabalin is not statistically significant, it should be further investigated because pregabalin in smaller doses may have a protective role on DNA molecule by the reinforcement of repair mechanisms or/and by the lack of pregabalin-induced DNA damage in small doses[24].

MI and PRI in small therapeutic doses show no statistically significant decrease (Figures 3, 4). These results suggest a noncytostatic behavior of pregabalin in these doses, at least *in vitro*. On the contrary, at concentrations D-F, the decrease of the MI index was statistically significant and the correlation between MI and PRI values is very strong, so pregabalin has a cytostatic behavior *in vitro* at concentrations D-F. Interestingly, though PRI (Figure 3) continues to decrease

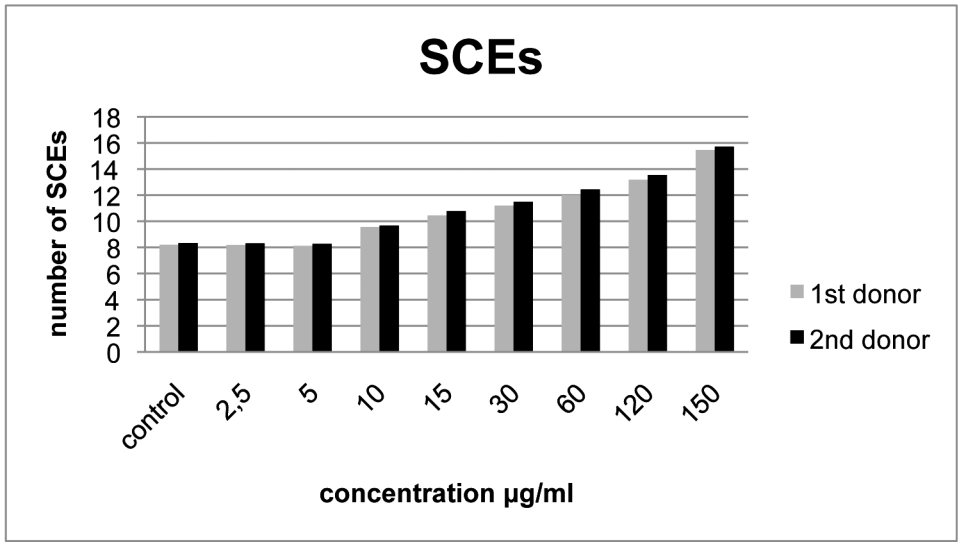

Figure 2.

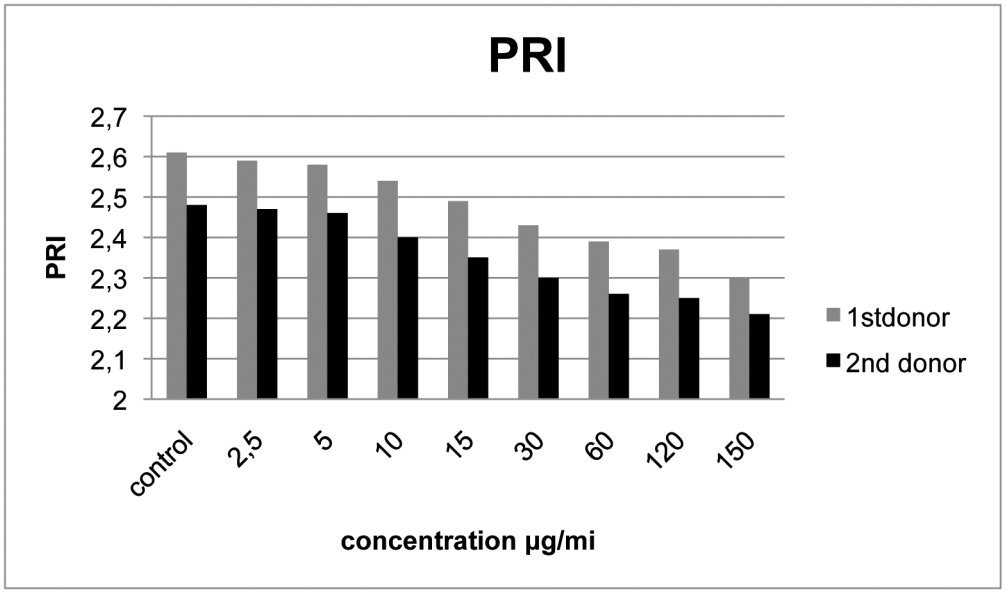

Figure 3.

at concentrations G and H, MI presents an unexpected increase (Figure 4) at these concentrations, which is not statistically significant, but the correlation of these alterations between MI and PRI is very strong. At the same concentrations the correlation between MI-SCEs and PRI-SCEs is equally strong. This observation should be subject to further investigation as it is an unexpected result and it can provide us with further information about the way pregabalin affects DNA at the largest therapeutic doses approved.

This preliminary study shows that pregabalin, a new drug, has a very interesting cytogenetic behavior that should be tested in more detail. Our next step then would be the examination of cytogenetic behavior of pregabalin in a larger amount of donors and in *in vivo* experiments.

Disclosure Statement

No competing financial interests exist.

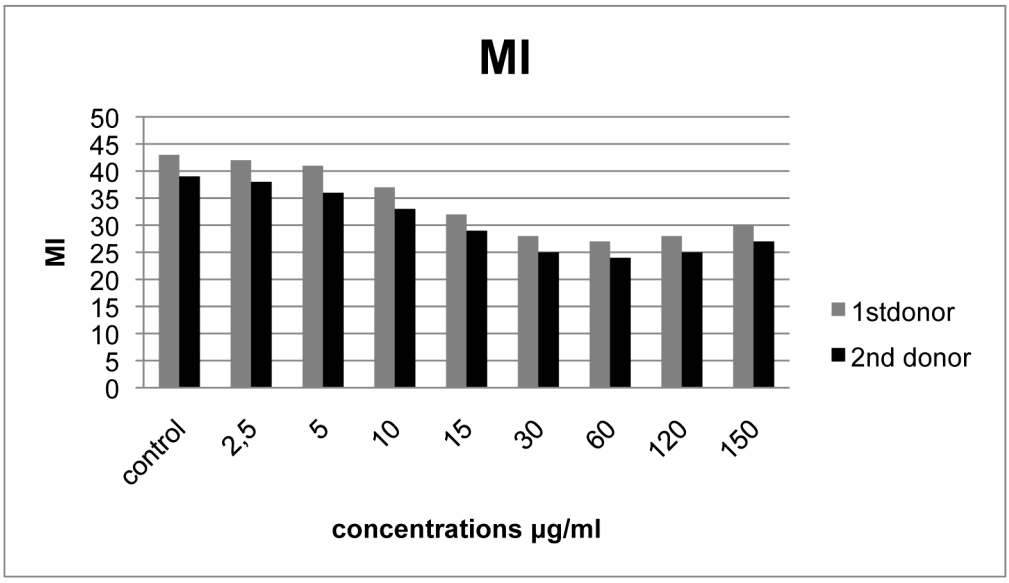

Figure 4.

Επίδραση της πρεγκαμπαλίνης στο ανθρώπινο γενετικό υλικό.

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ΠΕΡΙΛΗΨΗ: *Σκοπός:* Η πρεγκαμπαλινη είναι ανάλογο του γ-αμινοβουτιρικού οξέως. Συνδέεται σε μια επικουρική υποομάδα των ενεργοποιημένων από διαφορά δυναμικού διαύλων ασβεστίου στο κεντρικό νευρικό σύστημα, εκτοπίζοντας δραστικά την [3Η]-γκαμπαπεντίνη. Ενδείκνυται για την θεραπευτική αντιμετώπιση του νευροπαθητικού άλγους, της επιληψίας καθώς και του γενικευμένου άγχους.
Υλικό και Μέθοδος: Μελετήθηκε η επίδραση της πρεγκαμπανίλης *in vitro* στο ανθρώπινο DNA με τον υπολογισμό ευαίσθητων κυτταρογενετικών δεικτών. Οι χρωματιδιακές ανταλλαγές (Sister Chromatid Exchanges, SCEs) θεωρούνται ο πιο ευαίσθητος δείκτης γονοτοξικότητας, ο δείκτης ρυθμού πολλαπλασιασμού (Proliferation Rate Index, PRI) ένας από τους πιο αξιόπιστους δείκτες κυτταροστατικότητας, ενώ ο μιτωτικός δείκτης (Mitotic Index, MI) δείχνει με ακρίβεια την κατάσταση του κυτταρικού πολλαπλασιασμού. Αρχικά παρασκευάστηκαν διαλύματα πρεγκαμπαλίνης 8 διαφορετικών συγκεντρώσεων (Α=2,5μg/ml,Β=5μg/ml,Γ=10μg/ml,Δ=15μg/ml,,Ε=30μg/ml, ΣΤ=60 μg/ml, Ζ=120 μg/ml, Η=150 μg/ml), μεταξύ των οποίων οι συγκεντρώσεις από Γ εως Ζ είναι οι πιο συχνά χρησιμοποιούμενες στην κλινική πράξη. Τα διαλύματα προστέθηκαν σε καλλιέργειες λεμφοκυττάρων από περιφερικό αίμα δύο νεαρών υγειών αιμοδοτών. Μετά από 72 ώρες επώασης, με την κατάλληλη τεχνική τα καλλιεργημένα λεμφοκύτταρα επιστρώθηκαν σε αντικειμενοφόρους πλάκες, χρωματίστηκαν με την μέθοδο Fluorescence plus Giemsa και οι προαναφερθέντες δείκτες υπολογίστηκαν με το οπτικό μικροσκόπιο.
Αποτελέσματα: Μετά την στατιστική επεξεργασία των αποτελεσμάτων διαπιστώθηκε ότι οι SCEs αυξάνονται ανάλογα με την συγκέντρωση της πρεγκαμπαλίνης στις πιο συχνά χρησιμοποιούμενες συγκεντρώσεις ενώ δεν φαίνεται να επηρεάζονται σημαντικά στις συγκεντρώσεις Α και Β. Οι MI και PRI παρουσιάζονται σημαντικά μειωμένοι στις συγκεντρώσεις Γ,Δ,Ε ενώ στην Ζ,Η ο MI έχει αυξητική τάση ενώ ο PRI το αντίθετο. Τα παραπάνω αποτελέσματα κρίθηκαν στατιστικά σημαντικά με πιθανότητα σφάλματος p < 5%.
Συμπεράσματα: Οι συγκεντρώσεις της πρεγκαμπαλίνης που χρησιμοποιούνται στην καθημερινή κλινική πράξη φαίνεται να έχουν σημαντική επίδραση στο ανθρώπινο γενετικό υλικό *in vitro* γεγονός που χρήζει περαιτέρω διερεύνησης καθώς δεν υπάρχουν αντίστοιχα βιβλιογραφικά δεδομένα και αφορούν έναν μεγάλο αριθμό ασθενών.

Λέξεις Κλειδιά: *Πρεγκαμπαλίνη, Γονοτοξικότητα, Χρωματιδιακές ανταλλαγές, Δείκτης ρυθμού πολλαπλασιασμού, Μιτωτικός δείκτης.*

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