Genotoxic Evaluation of Duloxetine II. The Effect on the Number of Sister Chromatid Exchanges, the Mitotic Index, and the Proliferation Kinetics in Mouse Bone Marrow

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Duloxetine is an antidepressant which has showed valuable results, particularly in patients with major depression. This type of drugs is known to require genotoxic studies as part of their preclinical safety evaluation. In the case of duloxetine, however, there have been controversial results. Therefore, we considered it worthwhile to extend studies on the matter in an attempt to reach a conclusion. The present assay was made in mouse bone marrow to evaluate the capacity of the drug to induce sister chromatid exchanges (SCE), as well as to modify the proliferation kinetics and the mitotic index. Three doses of the antidepressant were tested (2, 20, and 200 mg/kg), besides the control mice were administered with purified water, and the positive treated animals administered with 1 mg/kg of doxorubicin. The results indicated a moderate but significant increase of SCE with the three tested doses, no effect regarding the mitotic index and a small reduction in the proliferation kinetics. Although in our assay the drug showed a lower effect, the present study agreed with a previous report that analyzed the amount of micronuclei in mouse peripheral blood, and it confirmed the relevance of evaluating the genotoxic effect of antidepressants, specifically duloxetine by applying diverse tests.

Key words antidepressant; duloxetine; sister chromatid exchange; mouse

Depression is a complex disease related with the activity of genetic, neurochemical, social, and environmental factors, and it is expressed in different clinical types and intensities. The disease has become a significant public health problem in most countries. Besides the clinical impact, other human aspects are also significantly perturbed, such as the economic, social, and labor areas.1–3) A few data may be useful to show the relevance of the disease and the need to counteract its expansion: a) it is estimated that about 350 million people suffer from depression in the world, b) depression is the main cause of lost years by disability, c) it is the most significant risk factor for suicide and a leading cause of death worldwide, and d) in Mexico, a national survey of psychiatric epidemiology estimated that about 8.4% of the population has suffered from an episode of major depression in their life span with a median age of initiation at 24 years.1,4,5)

Its therapeutic management is mainly based on the use of pharmacologic drugs and psychological treatments. In the first case, numerous medications have been developed and applied to control the disease along the years, with variable results. However, research is constantly in progress to discover new drugs with better effects and lower collateral damage. Presently, the mechanism of action disclosed by antidepressants is varied, including bi-tri or tetracyclic drugs, inhibitors of monoaminoxidase, and inhibitors of the recapture of different neurotransmitters, such as dopamine, serotonin, and noradrenaline.6)

Duloxetine or (2S)-N-methyl-3-(1-naftioxy)-3-(2-tyenil) propan-1-amine (Fig. 1), is an antidepressant which acts as an inhibitor of the recapture of both noradrenaline and serotonin, with a low inhibitory effect in the recapture of dopamine.6,7) It is prescribed for the treatment of major depression, generalized anxiety, and diabetic neuropathic pain.6,7)

Studies in the genotoxic field have been reported with heterogeneous results, for example, in a revision made by Barambilla et al.,8) earlier studies have reported negative results with the Ames test, and in the evaluation of mouse bone marrow chromosomal aberrations, sister chromatid exchanges (SCE), and in the DNA damage (observed with the comet assay) in Chinese hamster; however the same authors mention the development of hepatic tumors in female mice treated with 140 mg/kg of the drug for 120d. In a more recent assay, authors reported a significant increase in the number of micronuclei in an acute and a subchronic assay made in mouse.9) Besides, DNA damage by xenobiotics is known to originate in distinct forms, knowledge that support the pertinence of evaluating the genotoxic potential through different
tests, moreover in drugs that are commonly prescribed, such as duloxetine. The study of SCE in mouse bone marrow has proved to be a sensitive measure to determine DNA/chromosome damage, among other reasons because bone marrow is a highly proliferative tissue where the chromosome morphology has been well studied, the appropriate number of second-division metaphases can be found, and the length of its cellular division is also well established. Therefore, based on the indicated antecedents and advantages of the selected mouse model, we decided that it was worthwhile to extend the research about the possible genotoxic potential of the drug. As part of this research in progress, in the present report we examined its effect on the amount of sister chromatid exchanges, as well as on the mitotic index and cellular proliferation kinetics in mice.

MATERIALS AND METHODS

Chemicals and Animals Duloxetine (Cymbalta® Elly Lilly & Co., Mexico City, Mexico) was obtained as the usually prescribed pharmaceutical medication. Höechst 33258, 5-bromo-2′-deoxyuridine (BrdU), colchicine, and doxorubicin were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). The Giemsa stain was purchased from Merck (Mexico City, Mexico), and sodium citrate, sodium chloride, potassium phosphate, and sodium phosphate, the salts to prepare the buffers, were obtained from J.T. Baker S.A. (Mexico City, Mexico).

Thirty Swiss Webster male mice of 25 ± 2g of weight were used for the experiment. Animals were obtained from the Breeding and Care Unit of the Autonomous University of Hidalgo State. Groups of six mice each were placed in polypropylene cages at a mean temperature of 23°C, 12h light/dark cycles, and 40% of relative humidity. They were given free access to food and water. Twenty-two hours after this step, animals were cervicaly dislocated, the two femurs dissected, their bone marrow extracted and placed in a hypotonic solution (KCl 0.075M at 37°C). Each sample was then centrifuged at 1500 rpm for 10 min, the supernatant eliminated and three fixative steps with methanol–acetic acid (3 : 1) were carried out. Each fixation was made for 10 min followed by a centrifugation at 1500 rpm for 10 min. After that, a few drops of the cell suspension were placed on clean slides and flamed for a few seconds. In our assay, the low dose of the antidepressant corresponded to 120 mg/d of duloxetine in humans, which is in the range of the high therapeutic dose level prescribed in an adult, while the highest tested dose corresponded to about 70% of the LD₅₀ (282 mg/kg) obtained in the laboratory with the method of Lorke. The intermediate dose maintained a logarithmic scale.

The next step was to perform the differential chromatid staining process. For this purpose we put 1 mL of bisbenzimide 0.001M made in distilled water on the slides (Höechst solution), expanded it with a coverslip, and kept the slides in the dark for 30 min, then, we added the buffer of differentiation constituted by trisodium citrate plus monobasic, monohydrated sodium phosphate, at pH 7, and we exposed the slides to black light for 90 min. They were then immersed in distilled water, the coverslips detached, and slides were dried at room temperature. In a subsequent step, slides were placed

![Image](image-url)
in a Coplin vessel with citrate–saline solution at 60°C for 15 min, they were briefly washed in water at 60°C followed by cold water, and finally they were air dried and stained with 5% Giemsa made in phosphate buffered saline (PBS), at pH 6.8 (Fig. 2).

Three measurements per mouse were made at 1000× magnification by means of a Nikon Eclipse 80i microscope: 1—the number of SCE in 30-second-division metaphases, 2—the mitotic index in 1000 metaphases, and 3—the classification of first (M1), second (M2), and third (M3) division metaphases in 100 cells. With the obtained data we calculated the average generation time (AGT) according to the formula: \((22/1M1+2M2+3M3) \times 100\).

The statistical analysis of the obtained data was made by applying a one-way ANOVA and the post-hoc Student–Newman–Keuls test using the software Sigma plot, version 12.1.

RESULTS

Figure 3 shows the results obtained regarding the number of SCE induced by duloxetine. In this study, a mean of 3.07 SCE/cell was found in the control group, while mice treated with doxorubicin (a well-known mutagen) triplicated such value. As regards the effect of the antidepressant, our results showed a significant increase in the number of SCE with each of the three tested doses although no dose–response was observed. A mean increase of 58% over the control level was observed. Moreover, the cumulative frequency of cells vs. SCE number shows a displacement of the obtained curve with duloxetine respect to the control data curve. For example, about 4 SCE/cell were present in 40% of cells treated with duloxetine, while about 2, and 8 SCE/cell were observed with this percentage in the control, and doxorubicin treated cells, respectively (Fig. 4).

The results obtained in regard to the proliferation kinetics determined for duloxetine are shown in Table 1. We can observe that a usual cell generation kinetics for mouse (with slight variations) was found for the tested agents, that is, about a triple increase of the M2 values, and about a 50% reduction of the M3 values in comparison with the obtained M1 level. With these data the mean time required to reach two cell generations (AGT) was determined to fluctuate around 12h. However, the fluctuation of duloxetine was observed to be always lower than 12h, suggesting a shorter cell turnover related with a small arrest of the first cellular division.

Finally, the mitotic index values are presented in Fig. 5. The results presented in the Figure shows that the values obtained with duloxetine are similar to those observed in the control animals; although the high dose of the drug presented certain decrease, this was not statistically significant, and therefore, the obtained data indicated no cytotoxic effect of the drug in the present stated conditions. Doxorubicin (an antineoplastic agent) as expected, was cytotoxic, inducing approximately a threefold reduction of the index with respect to the control value.

Table 1. Cell Proliferation Kinetics Determined in Mice Treated with Duloxetine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>M1 μM</th>
<th>M2 μM</th>
<th>M3 μM</th>
<th>AGT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.50±1.38</td>
<td>61.00±0.77</td>
<td>9.50±1.57</td>
<td>12.27±0.21</td>
</tr>
<tr>
<td>DXO 1 mg/kg</td>
<td>27.67±0.55</td>
<td>63.00±0.59</td>
<td>3.93±3.66</td>
<td>12.11±0.08</td>
</tr>
<tr>
<td>DLE 2 mg/kg</td>
<td>20.17±1.000</td>
<td>68.00±0.79</td>
<td>11.83±0.64</td>
<td>11.48±0.04</td>
</tr>
<tr>
<td>DLE 20 mg/kg</td>
<td>20.50±0.60</td>
<td>68.17±0.65</td>
<td>11.33±1.29</td>
<td>11.53±0.08</td>
</tr>
<tr>
<td>DLE 200 mg/kg</td>
<td>19.67±1.410</td>
<td>70.33±0.78</td>
<td>10.00±0.63</td>
<td>11.57±0.12</td>
</tr>
</tbody>
</table>

DXO=doxorubicin, DLE=duloxetine. Each bar correspond to the mean±S.E.M. obtained in 30 second-division mitosis per mouse. Six mice per group. *Statistically significant difference with respect to the control value, and ** with respect to DXO. One-way ANOVA and post-hoc Student–Newman–Keuls test \((p<0.001) \left( F_{(4, 25)}=49.792 \right)\).

Fig. 3. Number of Sister Chromatid Exchanges (SCE) Determined in Mouse Bone Marrow Exposed to Duloxetine

Fig. 4. Cumulative Frequency of Cells with Respect to SCE per Cell in Mouse Bone Marrow

DXO=doxorubicin, DLE=duloxetine. Each line correspond to cumulative frequency in 30 second-division mitosis per mouse/six mice per group.
REFERENCES


