Mutagenic and Teratogenic Effects of Indomethacin and Cyclosporine-A on Dams and Fetuses of Mice

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Summary Eighty pregnant female mice were allotted among 16 groups. The animals were given intraperitoneal injection equalized to the therapeutic doses used for human. Indomethacin was given (25, 75 mg/kg b.wt.) and cyclosporine-A was given (5, 10, 15 mg/kg b.wt.). They induced significant increase in fetal resorption and significant decrease in fetal body weight. Also, reduction in the sizes of the skeletons of embryos was observed. Dosage of 150 mg/kg b.wt. of indomethacin was the lethal dose to dams. Various chromosomal aberrations in maternal bone marrow cells and embryos cells and mitotic activity were recorded, quantitated and statistically analyzed. Indomethacin (75 mg/kg) induced more chromosomal aberrations in both pregnant females as well as fetuses than the lower dose (25 mg/kg). Cyclosporine-A (15 mg/kg b.wt.) induced an increase in chromosomal aberrations in bone marrow cells of female mice than lower dose (5, 10 mg/kg b.wt.), while no significant differences were observed between the (5, 10 mg/kg) doses. Also, cyclosporine-A (15 mg/kg) showed highly significant increase in chromosomal aberrations in embryos than medium (10 mg/kg) or lower doses (5 mg/kg).

Key words Indomethacin, Cyclosporine-A, Teratogenic, Cytogenetic, Embryos, Mice.


Cyclosporine-A is a cyclic undecapeptide of fungal origin having potent immunosuppressive properties in man and different animal species. Cyclosporine-A used in the prevention of rejection organ transplantation and also in the treatment of rheumatoid arthritis (Cohen et al. 1984, Borel 1989, Charles et al. 1994). Cyclosporine-A produces some adverse effects in both experimental animals and humans. In animals cause maternal toxicity and in humans growth retardation have been noted. Also, cyclosporine-A appears to be related to the development of tumors in animals and humans (Olshan et al. 1994, Little 1997, Rezzaani et al. 1997, Armenti et al. 1998).

The aim of the present study is to investigate the teratogenicity and cytogeneicity of therapeutic dose of indomethacin and cyclosporine-A, that study the morphological changes and the types of chromosomal aberrations in pregnant mice and their fetuses.

Materials and methods

Eighty virgin females and 40 fertile males mice were used in this study. The animals were obtained from the Animal House Colony, National Research Center, Dokki, Giza, Egypt. Female mice were placed in the cage of adult fertile males by a ratio 2:1. The next day females exhibiting a
vaginal plug, the appearance of the vaginal plug was designated as 0 day of pregnancy (Gasser et al. 1992). The animals were divided into 16 experimental groups, each group consists of 5 pregnant females. The animals were given intraperitoneal injections equalized to the therapeutic doses used for human, calculated according to (Paget and Barnes 1964) from tested drugs on day 7, 9, 11 of gestation. The first 8 groups were sacrificed on day 14 of gestation for teratological and cytogenetical study as the following:

1) Control group: (distilled water), 2) Indomethacin group: (25 mg/kg b.wt.), 3) Indomethacin group: (75 mg/kg b.wt.), 4) Indomethacin group: (150 mg/kg b.wt.), 5) Control group: (5% dextrose), 6) Cyclosporine-A group: (5 mg/kg b/wt.), 7) Cyclosporine-A group: (10 mg/kg b.wt.), 8) Cyclosporine-A group: (15 mg/kg b.wt.).

The others 8 groups were injected with the same doses of the tested drugs and solvent but sacrificed on day 19 of gestation for skeletal examinations. For teratological effects, the number of living embryos, number of resorption embryos, number of haematomat and the embryos body weight were recorded. For skeletal examinations, fetures were preserved in 95% ethyl alcohol, cleared in 1% potassium hydroxide and stained in Alcian blue for cartilage and alizarin red-S for bone according to the method described by Webb and Byrd (1994). For cytogenetic effects, metaphase spreads were prepared according to Yosida and Amano (1965). Fifty metaphase spreads were prepared from each maternal bone marrow cells and their fetuses. The types and frequency of aberrations were recorded and photographed. Mitotic activity of the cells was calculated as the number of dividing cells including prophases and metaphases per 1000 cells. Statistical analysis was carried out using analysis of variance, one way by Walpoe and Myers (1972).

Results

Table 1 shows the teratogenic effects of indomethacin (25, 75 mg/kg b.wt.) on mice embryos on day 14 of gestation. The results indicated that there were significant difference between control and different doses in total implantation, number of living embryos, number of resorbed embryos, embryos body weight and number of haematomat. Dosage (150 mg/kg b.wt.) of indomethacin was the lethal dose to dams. Table 2 represents the teratogenic effects of cyclosporine-A (5, 10,
Effects of Indomethacin and Cyclosporine-A on Dams and Fetuses of Mice

Table 3. Teratogenic effects of intraperitoneal injection of indomethacin on embryos on day 19 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.wt.)</th>
<th>Total implantation</th>
<th>No. of living embryos</th>
<th>Resorbed embryos</th>
<th>Mean body weight of embryos</th>
<th>No. of haematoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.80±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.80±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>7.60±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.80±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>7.00±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.40±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.40±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.42±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.80±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error.
Means of different letters (a, b, c) in the same column are significantly different (P<0.05).

Table 4. Teratogenic effects of intraperitoneal injection of cyclosporine-A on embryos on day 19 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.wt.)</th>
<th>Total implantation</th>
<th>No. of living embryos</th>
<th>Resorbed embryos</th>
<th>Mean body weight of embryos</th>
<th>No. of haematoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.20±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.20±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>7.80±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.80±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.60±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>7.80±0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.80±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.45±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.20±0.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>7.40±0.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.20±0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.20±0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.27±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.20±0.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error.
Means of different letters (a, b, c, d) in the same column are significantly different (P<0.05).

15 mg/kg b.w.t.) on mice embryos on day 14 of gestation. The data recorded that there were significant differences between treated groups and control group in total implantation, number of living embryos and embryos body weight but in the number of resorbed embryos showed significant difference between 15 mg/kg b.w.t. dosage and control group. Also, in the number of hematoma showed significant difference between 10 mg/kg b.w.t. dosage and control group. Table 3 represents teratogenic effects of indomethacin (25, 75 mg/kg b.w.t.) on pregnant mice on day 19 of gestation. The data showed that, there were significant differences between treated groups and control group in the number of living embryos, number of resorbed embryos and embryos body weight but in the total implantation and the number of hematoma showed significant differences between 75 mg/kg b.w.t. and control group. Table 4 shows teratogenic effects of cyclosporine-A (5, 10, 15 mg/kg b.w.t.) on mice embryos on day 19 of gestation. The results recorded that there were significant differences between different doses and control group in total implantation, number of living embryos and embryos body weight but in number of resorbed embryos showed significant differences between the control and the treated groups of 10 and 15 mg/kg b.w.t. Also, in the number of haematoma showed significant difference between control and 15 mg/kg b.w.t.

Skeletal examinations of the fetuses that obtained from dams that injected with indomethacin and dams that injected with cyclosporine-A which sacrificed on day 19 of gestation showed reduction in size of the skeletons as compared to those of control groups (Fig. 1).

In this study various chromosomal aberrations were recorded in bone marrow cells of pregnant mice and their fetuses. The numerical aberrations were peridiploidy (including hypoploidy and hyperploidy) and polyploidy.

Structural chromosomal aberrations were chromatid and chromosomal gaps, chromatid breaks (including deletions and fragments), centromeric attenuation and others (including endomitosis and ring-shaped). Mitotic activity was recorded in all treated groups. Tables 5 and 6 show highly significant differences in total aberrant cells and mitotic activity between control and treated groups in maternal bone marrow cells and embryo cells treated with indomethacin (25, 75 mg/kg b.w.t.). Tables 7 and 8 show the effect of cyclosporine-A (5, 10, 15 mg/kg b.w.t.) on maternal bone marrow cells and embryo cells. The data showed highly significant differences in total aberrant cells and mi-
totic activity between control and treated groups, but there were no significant difference in chromosomal gaps between control and treated groups in both maternal bone marrow cells and embryos cells (Fig. 2).

Discussion

The present study has clearly demonstrated the teratogenic and cytogenetic effects of indomethacin and cyclosporine-A on pregnant mice and their fetuses.

In the present study, indomethacin (25, 75 mg/kg b.wt.) induced highly significant decreased in the number of living embryos and increased in fetal resorption. These findings might be attributed to the interference of the administered drug to transfer of some essential nutrients responsible for the development of embryos as leucin and magnesium (Tuchmann-Duplessis 1975). These results were in agreement with some authors, as Persaud (1974), El-Banna et al. (1976) and Abou-Tarboush and Massoud (1993) in pregnant rats, rabbit and mice. Also, these results were recorded with others non-steroidal anti-inflammatory drugs as in phenylebutazon in mice by Yoshida et al. (1980). In addition indomethacin induced highly significant reduction in fetal body weight. This reduction may be due to the inhibitory effect of the drug on cell proliferation. Also, in this study Alcian blue and Alizarin red-S stained fetuses that their mothers treated with indomethacin showed growth reduction in the skeletons. These results were in accordance with those reported by Koga et al. (1981) and Abou-Tarboush et al. (1993). Furthermore 150 mg/kg b.wt. dosage of indomethacin was toxic to dams. This result was in agreement with Abou-Tarboush and Massoud (1993).

Also, in this investigation, cyclosporine-A (5, 10, 15 mg/kg b.wt.) induced highly significant decrease in the number of living embryos, increase in fetal resorption and cyclosporine-A induced gross malformations induced by growth retardation, which lead to highly significant reduction in fetal body weight. In addition in this study fetus that stained with Alcian blue and Alizarin red-S

Fig. 1. Effect of indomethacin. A) and cyclosporine-A, B) on embryos body weight on day 19 of gestation, showing differences between control and treated groups. Photograph of indomethacin, C) and cyclosporine-A, D) maternally treated fetuses on day 19 of gestation, showing the different decreases in the size of the skeletons between control and treated groups.
Table 5. Mean values of different chromosomal aberrations induced by indomethacin in bone marrow cells of pregnant female mice (n=5) on day 14 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.w.)</th>
<th>Numerical variations</th>
<th>Structural aberrations</th>
<th>Total aberrant cells</th>
<th>Cells with more than one type</th>
<th>Mitotic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peridiploidy</td>
<td>Polyplidoy</td>
<td>Total</td>
<td>Chromatid gap</td>
<td>Chromosomal gap</td>
</tr>
<tr>
<td>Control</td>
<td>1.40±0.29⁹</td>
<td>0.00±0.00⁹</td>
<td>1.60±0.35⁹</td>
<td>1.40±0.44⁹</td>
<td>0.00±0.00⁹</td>
</tr>
<tr>
<td>25</td>
<td>2.60±0.29⁷</td>
<td>0.20±0.18⁷</td>
<td>2.80±0.35⁷</td>
<td>2.40±0.44⁷</td>
<td>0.00±0.00⁷</td>
</tr>
<tr>
<td>75</td>
<td>3.80±0.29⁶</td>
<td>2.60±0.18⁶</td>
<td>6.40±0.35⁶</td>
<td>5.40±0.44⁶</td>
<td>0.80±0.22⁶</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error. Means of different letters (a, b, c, d) in the same column are significantly different (P<0.05). ¹ including deletion and fragment, ² including endomitosis and ring.

Table 6. Mean values of different chromosomal aberrations induced by indomethacin in embryos (n=25) on day 14 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.w.)</th>
<th>Numerical variations</th>
<th>Structural aberrations</th>
<th>Total aberrant cells</th>
<th>Cells with more than one type</th>
<th>Mitotic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peridiploidy</td>
<td>Polyplidoy</td>
<td>Total</td>
<td>Chromatid gap</td>
<td>Chromosomal gap</td>
</tr>
<tr>
<td>Control</td>
<td>1.28±0.18⁹</td>
<td>0.00±0.00⁹</td>
<td>1.28±0.24⁹</td>
<td>0.96±0.20⁹</td>
<td>0.00±0.00⁹</td>
</tr>
<tr>
<td>25</td>
<td>3.88±0.18⁷</td>
<td>0.60±0.14⁷</td>
<td>4.48±0.24⁷</td>
<td>3.44±0.20⁷</td>
<td>0.76±0.14⁷</td>
</tr>
<tr>
<td>75</td>
<td>4.40±0.18⁶</td>
<td>2.92±0.14⁶</td>
<td>7.32±0.24⁶</td>
<td>6.20±0.20⁶</td>
<td>1.52±0.14⁶</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error. Means of different letters (a, b, c, d) in the same column are significantly different (P<0.05). ¹ including deletion and fragment, ² including endomitosis and ring.
Table 7. Mean values of different chromosomal aberrations induced by cyclosporine-A in bone marrow cells of pregnant female mice (n=5) on day 14 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.w.)</th>
<th>Numerical variations</th>
<th>Structural aberrations</th>
<th>Total aberrant cells</th>
<th>Cells with more than one type</th>
<th>Mitotic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peridiploidy</td>
<td>Polyplploidy</td>
<td>Total</td>
<td>Chromatid gap</td>
<td>Chromosomal gap</td>
</tr>
<tr>
<td>Control</td>
<td>1.40±0.31a</td>
<td>0.00±0.00a</td>
<td>1.40±0.35a</td>
<td>1.40±0.32a</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td>5</td>
<td>2.40±0.31b</td>
<td>0.80±0.26b</td>
<td>3.20±0.35b</td>
<td>2.40±0.32b</td>
<td>0.20±0.20b</td>
</tr>
<tr>
<td>10</td>
<td>3.00±0.31bc</td>
<td>0.80±0.26bc</td>
<td>3.80±0.35bc</td>
<td>2.80±0.32bc</td>
<td>0.40±0.20bc</td>
</tr>
<tr>
<td>15</td>
<td>3.40±0.31c</td>
<td>3.00±0.26bc</td>
<td>6.40±0.35c</td>
<td>4.40±0.32c</td>
<td>0.40±0.20c</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error.
Means of different letters (a, b, c, d) in the same column are significantly different (P<0.05).
1 including deletion and fragment, 2 including endomitosis and ring.

Table 8. Mean values of different chromosomal aberrations induced by cyclosporine-A in embryos (n=25) on day 14 of gestation

<table>
<thead>
<tr>
<th>Dose (mg/kg b.w.)</th>
<th>Numerical variations</th>
<th>Structural aberrations</th>
<th>Total aberrant cells</th>
<th>Cells with more than one type</th>
<th>Mitotic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peridiploidy</td>
<td>Polyplploidy</td>
<td>Total</td>
<td>Chromatid gap</td>
<td>Chromosomal gap</td>
</tr>
<tr>
<td>Control</td>
<td>0.92±0.13a</td>
<td>0.00±0.00a</td>
<td>0.92±0.18a</td>
<td>0.92±0.18a</td>
<td>0.00±0.00a</td>
</tr>
<tr>
<td>5</td>
<td>2.48±0.13b</td>
<td>1.00±0.16b</td>
<td>3.48±0.18b</td>
<td>1.28±0.18b</td>
<td>0.36±0.15b</td>
</tr>
<tr>
<td>10</td>
<td>2.88±0.13bc</td>
<td>2.24±0.16bc</td>
<td>5.12±0.18bc</td>
<td>3.84±0.18bc</td>
<td>0.48±0.15bc</td>
</tr>
<tr>
<td>15</td>
<td>3.56±0.13c</td>
<td>2.80±0.16bc</td>
<td>6.36±0.35c</td>
<td>4.36±0.18c</td>
<td>0.44±0.15c</td>
</tr>
</tbody>
</table>

Above values represent mean±standard error.
Means of different letters (a, b, c, d) in the same column are significantly different (P<0.05).
1 including deletion and fragment, 2 including endomitosis and ring.
and their mothers treated by cyclosporine-A showed growth reduction in the skeletons. These results were in accordance with many authors as Wagih et al. (1987), Gasser et al. (1992) and Olshan et al. (1994) who reported that, cyclosporine-A produces some adverse effects in both experimental animals and humans. Also, Rezzani et al. (1997) who found that, cyclosporine-A induced sever intrauterine growth retardation in pregnant rats and Armenti et al. (1998) reported that, treatment of pregnant women with cyclosporine-A induced growth retardation which lead to a reduction in fetal body weight. This finding indicated that, cyclosporine-A had crossed the placenta. Also, Klintman et al. (1984) showed significant levels of cyclosporine-A in the placenta and breast milk of a renal transplant patient, and recommended that, mother treated with cyclosporine-A avoid breast feeding.

The present study demonstrated that indomethacin and cyclosporine-A induced chromosome abnormalities in bone marrow cells of pregnant mice and their fetuses. In this study, indomethacin induced highly significant differences in structural chromosomal aberrations in treated groups than control, such as chromatid and chromosomal gaps, chromatid breaks (including deletions and fragments), centromeric attenuation and others (including endomitosis and ring). Also, highly significant decrease in the mitotic index was observed in all stages of the experiment.

These results may be attributed to the clastogenic effect of the used drug. These results were in agreement with many authors, such as De Hondt et al. (1985), El-Ghor et al. (1988) following administration of diclofenace sodium and phenylebutazone in mice and Abd-El-Aziz et al. (1989) with piprofin in pregnant mice. In addition, these results were recorded Mattar et al. (1992), Mahrous and Kamal (1995) and Giri et al. (1996).

Also, in the present investigation cyclosporine-A induced highly significant structural chromosomal aberrations in maternal bone marrow cells and their fetuses. Furthermore significant decrease in mitotic index were recorded. These findings suggest that cyclosporine-A has a mutagenic effect
and this effect may be related to the ability of the drug to interfere with the cell-mediator antigen presentation and also may be due to cyclosporine-A interference in the maternal immune response to specific embryonic antigens. These results were in accordance with some authors as Reneltova et al. (1978), Kucerova et al. (1982), Fukuda et al. (1987, 1988) and Anderson et al. (1995).

From the previous results, it could be concluded that both indomethacin as anti-inflammatory drug and cyclosporine-A as immunosuppressive drug induced teratogenic and mutagenic effects when given to pregnant mice. This may give more light on their hazards to pregnant women and their fetuses.

Generally the long-term treatment and the uncontrolled use of these drugs lead to many hazard effects to the individual and to his progeny. This might give more support to coming investigations concerning the dose, drug group, duration and the time of administration of these drugs to determine the minimal adverse effects and the maximal therapeutic efficacy.

References


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