The Teratogenicity of Cyclophosphamide on Skeletal System and Neural Tube of Fetal Mice

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Abstract: Cyclophosphamide (CP) as an alkylating agent which is used for treatment of cancer and to prevent rejection of tissue transplantation. Therefore, in this study, the teratogenic effects of CP were compared. This study was performed on 11 pregnant mice that were divided into two groups. Control group received normal saline and test group received CP (20 mg/kg) intraperitoneal at 10th day of gestation, respectively. Fetuses were collected at 19th day of gestation and after determination of weight and length; they were stained by Alizarin red - Alcian blue method. Cleft palate, spina bifida and exencephaly incidence were 62.79%, 62.79% and 30.23% in fetuses of mice that received only CP, respectively. In addition, skeletal anomalies incidence including limbs, vertebrae and sternum defects were observed by CP. The mean of weight and length of animals' fetuses that received normal saline were significantly greater than those received CP.

Key words: Cyclophosphamide • Embryo Malformations • Teratogenicity • Mice

INTRODUCTION

Cyclophosphamide (CP) as an alkylating agent is used for treatment of cancer and to prevent rejection of tissue transplantation. CP has several toxic effects including hemorrhagic cystitis. Metabolites of cyclophosphamide, especially acroleine modulates its toxic effects [1, 2]. Studies on the biologic alteration of CP have results in the suggestion that nor-nitrogen muscard is the active alkylating agent [3]. CP has also been reported to be teratogenic in rats. Single intraperitoneal injection of CP, 20 to 40 mg/kg, on gestational Days 10 through 15 were found to induce exencephaly, microcephaly, syndactyly, hexadactyly and missing ribs in the offspring. The critical period was found to be Days 12 and 13 [4]. In the rabbit the subcutaneous administration of 50 mg of CP per rabbit on Days 10 through 13 consecutively induced primarily cleft palate with associated hypognathus, resorptions and decreased fetal size [5, 6]. Injection of 300 mg and 100 mg of CP per rabbit were lethal to the dam after the second injection and induced 100% fetal resorptions, respectively [5].

The purpose of the present investigation is to report the teratogenic action of CP on neural tube and skeletal system in another mammalian species; like mouse especially.

MATERIALS AND METHODS

Male and female healthy mice of NMRI strain, 6-8 weeks of age, weighing 28-30g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Mice were fed ad libitum by standard laboratory pellet (Pars khurakdam, Shushtar, Iran.) and tap water. A 12h light-12h dark cycle was maintained. Room temperature was at 23±2°C with a relative humidity of 45-55%.

Females were mated overnight with males. Pregnancy was ascertained the next morning by presence of a vaginal plug and this time was designated as gestational day (GD) 1. Pregnant mice (n=11) were randomly divided into two groups (5 pregnant mice in treatment group, 6 pregnant mice in control group) and treated as follow:

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Control group received normal saline (10 ml/l g); test group received CP (20 mg/kg) [7] intraperitoneally, respectively.

The animals were sacrificed by cervical dislocation at 19th day of gestation. Following laparotomy, the uterus was exteriorized and the number and location of fetuses and resorption were noted, then their weight and length (crown-rump length) were measured. Individual fetuses were examined carefully for external anomalies then fetuses were stained by Alizarin red-Alcian blue method [8] and investigated by stereomicroscope (Nikon, SMZ200, Japan) for skeletal malformations. The incidence of skeletal malformations was determined and was compared in the groups.

Statistical significance between groups was determined using SPSS program and compared by one way analysis of variance (ANOVA). Binomial data were examined using the Chi-square test. The minimum level of significance was p< 0.05.

RESULTS

Fifty fetuses were obtained from six mice of control group. There were no macroscopic anomalies in the control animals. In the control group palatal closures of fetuses were normal at gestational day 20 (i.e., palatal shelves had grown vertically on the sides of the tongue, then horizontally to meet and fuse). CP induced cleft palate, spina bifida and exencephaly at 62.79%, 62.79% and 30.23% incidence, respectively (Table 2).

Open eye and polydactyly, syndactyly, synpolydactyly and several anomalies in sternum were observed (Fig. 1, 2, 3, 4) and their incidence is shown in Table 2. Mean weight and length (P<0.0001) were significantly decreased in the group which received CP (Table 1).

Table 1: Comparison of weight and length in mice fetuses between control and treatment group

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of litters</th>
<th>Implantations</th>
<th>Resorbed fetuses</th>
<th>Live fetuses</th>
<th>Fetal length (mean± SEM)</th>
<th>Fetal weight (g): (mean± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>50</td>
<td>2(4)</td>
<td>48</td>
<td>24.80±0.18</td>
<td>1.36±0.01</td>
</tr>
<tr>
<td>CP</td>
<td>5</td>
<td>50</td>
<td>7(14)</td>
<td>43</td>
<td>15.65±0.03*</td>
<td>0.77±0.02*</td>
</tr>
</tbody>
</table>

Numerals in parantheses are percentages
* : Significant difference when compared with control group (P<0.05)

Table 2: Incidence of anomalies in mice fetuses of groups

<table>
<thead>
<tr>
<th>No. of fetuses with malformations</th>
</tr>
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<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>CP</td>
</tr>
</tbody>
</table>

Incidence of anomalies was significantly difference at groups which received CP with control (P<0.05)

DISCUSSION

These experiments have shown that the administration of single intraperitoneal injection of CP into the pregnant mouse on 10 day of gestation induced fetal anomalies. Sloth and Hales (1986) evaluated effect of mesna on cyclophosphamide-induced teratogenicity. They used CP at dose 10 and 15 mg/kg in rats in 13th day of gestation. They observed the CP can produce teratogenicity in 50 and 100% of fetuses with 10 and 15mg/kg, respectively [9]. They determined fetal defects similar with our study including cleft palate, exencephaly, open eye and limb defects.

Gibson and Becker (1968) reported cyclophosphamide-induced teratogenicity in mice. They used CP at dose 5 to 20 mg/kg in mice in one of 9-14th day of gestation. They observed the CP can produce teratogenicity in 67.3% of fetuses with 20 mg/kg [10]. They determined fetal defects similar with our study including cleft palate, exencephaly, polydactyly, syndactyly ectrodactyly and kinky tail. They also determined fetal weights and crown rump lengths similar with our study reduced significantly by cyclophosphamide. In the rabbit, CP induced cleft palate when given consecutively on Days 10 through 13 of gestation [6].

Of interest to the present finding are the results obtained in CP teratology experiments conducted in another strain of mice: strain DM/MK strain. Late in the course of present investigations a paper published in the Japanese literature [11] came to the authors’ attention which demonstrated the teratogenic response of the DM/MK mouse to CP. The anomalies reported to be induced by CP in the DM/MK strain were cleft palate, syndactyly and a statistically insignificant level of kinky tail occurrence. The dosages required to induced these
Fig. 1: Some skeletal defects in fetuses in the treated case (20 mg/kg of CP, treated on GD 10). Exencephaly, open eye, kinky tail and digital defects (up); synpolydactyly (left-down); polydactyly (right-down) in experimental group treated with 20 mg/kg of CP on GD 10

Fig. 2: Ventral view of skull of mice fetuses of GD 19, stained with alizarin red-S- alcian blue. A) Normal palate bone B) Cleft palate (arrow) in the treated case (20 mg/kg of CP, treated on GD 10). Pa: palate

Fig. 3: Dorsal view of vertebral column of mice fetuses of GD 19, stained with alizarin red-S- alcian blue. A) Normal B) Spina bifida (arrow) in the treated case (20 mg/kg of CP, treated on GD 10)., SP: spinous process

Fig. 4: Sterna of fetuses of GD 19, stained with alizarin red-S- alcian blue. A: Control. The chest wall together with the sternum and costal elements are dissected out and shown here. B and C: Experimental group treated with 20 mg/kg of CP on GD 10. Observe fusion of sternebra (B,C), hemisternebra (C) and unusual shape of xiphoid process

anomalies in the DM/MK strain were considerably higher than the dosages required in the Swiss Webster strain. In the DM/MK strain 25 mg/kg of CP was an ineffective teratogen, whereas 50 and 100 mg/kg subcutaneously produced the teratogenic effects when given daily during the period of the 11th to 14th days of gestation. Thus, another strain of mouse appears to be more resistant to the teratogenic effects of CP than the Swiss Webster mouse.

In chick embryo high dose of CP (6 micrograms) on day 3 induced facial clefts and limb malformations and strong clastogenic effects associated with mitotic inhibition were observed in all tissues investigated [12]. In another study, experimental eggs received varying amounts of the cyclophosphamide in 0.04 ml of normal saline on 3rd to 6th days of incubation and gross malformations of eyes besides other malformations were observed. Maximum number of malformations of eye were found in the 3rd day injected group which conforms to the fact that most organs pass through their period of greatest susceptibility, relatively soon after cellular differentiation begins [13].

Sherderer et al., (2008) reported a new anxiolytic afobazole (1-100 mg/kg perorally, Russia) dose-dependently abolished the embryotoxic and teratogenic effects of cyclophosphamide and reduced the range of induced malformations in outbred albino rats [14].

Fetuses of mothers who had been given cyclophosphamide presented a significant reduction in body weight, considered to reflect fetal changes and embryotoxicity of the drug. Similar weight reduction had been observed [15].
CP demonstrates species differences as well as strain differences. Several differences between the teratogenic effects of CP in the present mouse studies and in rats [4] suggest that CP may be handled differently by mice and rats and/or the conceptuses of these two species.

In conclusion, the present study shows spina bifida for the first time in teratogenicity cyclophosphamide in mice. The results show that CP to produce anomalies in mouse similar to skeletal anomalies in rats.

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