EMBRYOTOXICITY AND TERATOGENICITY OF LITHIUM CARBONATE IN WISTAR RAT* 

(Lithium; foetotoxicity; skeletal malformation)

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SUMMARY

Lithium carbonate was administered orally to pregnant Wistar rats from day 6–15 of gestation at doses of 50 and 100 mg/kg. Evidence of embryotoxic and teratogenic potential of lithium carbonate was noticed at the dose of 100 mg/kg. Reduction in number and weight of the litter, increase in the number of ruptured, wavy ribs, short and deformed bones of the limbs, or an increased incidence of incomplete formation of sternebrae and wide bone separation in the skull were the important findings suggesting the nature and extent of embryotoxicity and teratogenicity of lithium carbonate in Wistar rats.

INTRODUCTION

Lithium salt therapy is common in the treatment of manic depressive psychosis and other psychiatric disorders [1–3]. Women of child-bearing age suffering from psychiatric disorders are often required to be treated with lithium salts during mania; hence the knowledge of possible embryotoxic and dysmorphogenic effects of lithium salts is important. It has been reported that children of mothers on lithium treatment during pregnancy have shown malformations involving the heart and other organs [4]. Trautner et al. [5], Tuchmann-Duplessis and Mercier-Parot [6] and Gralla and McIlhenney [7] were unable to detect foetal malformations in rats treated with lithium salts, but Szabo [8] and Wright et al. [9] observed foetal malformations in mice and rats treated with lithium.

*81(Thalidomide) is being used in the treatment of manic depressive psychosis [1–3]. Women of child-bearing age suffering from psychiatric disorders are often required to be treated with lithium salts during mania; hence the knowledge of possible embryotoxic and dysmorphogenic effects of lithium salts is important. It has been reported that children of mothers on lithium treatment during pregnancy have shown malformations involving the heart and other organs [4]. Trautner et al. [5], Tuchmann-Duplessis and Mercier-Parot [6] and Gralla and McIlhenney [7] were unable to detect foetal malformations in rats treated with lithium salts, but Szabo [8] and Wright et al. [9] observed foetal malformations in mice and rats treated with lithium.

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Johansen and Ulrich [10] and Johansen [11], who examined the teratogenic effects of lithium in Wistar rats, did not observe any malformations. Since we were unable to locate any reference on foetal malformations caused by lithium in Wistar rats, the embryotoxic and teratogenic effects of lithium carbonate observed in our studies on Wistar rats are reported.

MATERIALS AND METHODS

The day of observation of spermatozoa in the vaginal smear under the microscope was termed day 0 of gestation. 44 conceived female Wistar rats weighing between 200-250 g bred and reared in our animal house were randomly assigned to 3 dose levels.

The dams were housed singly in polypropylene cages. Feed (M/s Hindustan Lever Ltd) and sterilized water were given ad lib. Rice husk was used as bedding material, which was changed frequently. Lithium carbonate was homogenized and suspended in 0.2% sterile agar at 50 mg/ml. Doses were given by gavaging from day 6 to day 15 of gestation, which is considered as the period of organogenesis [12]. The doses selected were comparable to the human therapeutic doses. Control rats received an equal volume of agar during the same period.

Body weights were taken on days 0, 6, 13 and 20. Doses were computed based on the body weights taken on day 6 and day 13. Observations were made every day for onset and duration of symptoms. On day 20 of gestation, the dams were killed by an overdose of ether anaesthesia. The foetuses were recovered by caesarian section and following observations were made: gross abnormalities; total number of implantations; number of live and dead pups; number of early resorptions; number of late resorptions, and body weight and sex of live pups.

The uteri of dams which did not appear to be pregnant were stained with a 10% aqueous solution of sodium sulfide and were examined for evidence of early resorption sites [13].

Live foetuses were killed by etherisation. One third of the total number of foetuses/sex/dam were fixed in Bouin's fluid and the remaining 2/3 in 95% ethanol.

The free-hand razor-blade technique of Wilson [14] was employed to study visceral malformations in the foetuses fixed in Bouin's fluid.

The foetuses fixed in ethanol were eviscerated, cleared in 2% KOH and stained with Alizarin Red S stain to study skeletal malformations [15, 16].

RESULTS

Lithium carbonate, when administered at 100 mg/kg from day 6 to day 15 of gestation, was found to be embryotoxic and teratogenic in Wistar rats. No foetal malformations were found in the dams treated with 50 mg/kg. The effect of lithium carbonate on the foetuses of rats treated during the period of organogenesis are
TABLE I

EFFECT OF LITHIUM CARBONATE ON WISTAR RAT FOETUS

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Implantations</th>
<th>Live pups</th>
<th>Resorptions</th>
<th>Average weight of pup (g)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.25</td>
<td>9.00</td>
<td>1.00</td>
<td>3.65</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>(n = 20)*</td>
<td>± 0.68</td>
<td>± 0.68</td>
<td>± 0.24</td>
<td>± 0.07</td>
<td>± 0.10</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>8.69</td>
<td>8.23</td>
<td>0.31</td>
<td>3.33</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td>(n = 13)</td>
<td>± 0.78</td>
<td>± 0.78</td>
<td>± 0.17</td>
<td>± 0.30</td>
<td>± 0.08</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>7.91**</td>
<td>4.73*</td>
<td>1.91</td>
<td>2.42*</td>
<td>2.24*</td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>± 0.71</td>
<td>± 1.29</td>
<td>± 0.97</td>
<td>± 0.40</td>
<td>± 0.37</td>
<td></td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± S.E. *P<0.01; **P<0.05.

E: early resorption; L: late resorption.

From Table I. Reduction in the number of implantations, number of live foetuses and their body weights and a higher number of resorptions were noticed in the 100 mg/kg treated group. Foetuses of 50 mg/kg lithium carbonate-treated dams were comparable to controls.

Reduction in the size of foetuses, shortening of the limbs and various skeletal abnormalities (Fig. 1) as listed in Table II, were observed in the foetuses of 100 mg/kg treated dams. 

Reduction in the size of foetuses, wavy ribs and shortening of limb bones produced by lithium carbonate on the left.
TABLE II
SKELETAL MALFORMATIONS OBSERVED IN FOETUSES FROM WISTAR RATS TREATED WITH LITHIUM CARBONATE

<table>
<thead>
<tr>
<th>Findings</th>
<th>Dose (mg/kg body wt/day)</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control* (n = 95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Wide bone separation in skull</td>
<td>5 (5.26)</td>
<td>4 (3.74)</td>
<td>10 (18.52)</td>
</tr>
<tr>
<td>(2) Incomplete ossification of sternaebrae</td>
<td>10 (10.53)</td>
<td>8 (7.48)</td>
<td>21 (38.89)</td>
</tr>
<tr>
<td>(3) Wavy ribs</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(4) Shortening of bones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Radius and ulna</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(b) Humerus</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(c) Tibia and fibula</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(d) Femur</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(5) Deformity in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Scapula</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(b) Pelvic bones</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(6) 14th rib</td>
<td>11 (11.58)</td>
<td>16 (14.95)</td>
<td>7 (12.96)</td>
</tr>
</tbody>
</table>

Control*: the number of foetuses examined; values in parentheses are the percentages.

No visceral malformations were seen in the foetuses of 50 and 100 mg/kg treated dams which were examined by free-hand razor-blade sectioning.

DISCUSSION

The Scandinavian Health Service collected 40 cases of pregnant women on lithium therapy. Among the 40 neonates 38 were normal, 2 had various malformations, e.g., club feet, spina bifida and meningocoele [17], whereas a case of a manic woman treated with lithium salts throughout pregnancy, delivering a normal child, also was reported [18].

Although various authors, as mentioned in the introduction, have observed the teratogenic effects of lithium carbonate in man and various animals, there has been no previous report of its teratogenicity in Wistar rats. Experiments have been conducted in the past with doses ranging from 50 to 200 mg/kg in rats for the possible teratogenic effects of lithium salts [6, 7, 11]. With daily doses of 100 mg/kg, Tuchmann-Duplessis and Mercier-Parot [6] did not observe any malformations in rat. In higher doses they found maternal mortality. Similarly in our study toxic effects and maternal mortality were noticed at the dose of 200 mg/kg. Hence the doses were restricted to 50 and 100 mg/kg only.

In this study lithium carbonate at a dose of 100 mg/kg showed significant embryotoxic and teratogenic effects in Wistar rats. The embryotoxic potential of...
lithium exhibited in other animals [17] was confirmed in Wistar rats through this study. Reduction in the number and body weight of live foetuses and increase in the number of resorptions in the dams treated with 100 mg/kg lithium carbonate were observed. However, no such embryotoxic effects were noticed in rats treated with 50 mg/kg.

The skeletal abnormalities observed in foetuses of rats treated with 100 mg/kg/day confirm the teratogenic effects of lithium carbonate in Wistar rats. Shortening of the limbs, found in the gross observations, was attributed to the shortening of the bones of the respective limbs. Wavy ribs were found in 52% of the foetuses observed for skeletal abnormalities. Mankes et al. [19] have reported 1% wavy ribs in control Long-Evans rats, but in our study wavy ribs were not found in the foetuses of control Wistar rats. Various skeletal abnormalities observed ranged from 18-52%. These abnormalities were absent in control and 50 mg/kg-treated rat foetuses. Wide bone separation in the skull and incomplete ossification of sternebrae were found in control and 50 mg/kg-treated rat foetuses, but their incidence was significantly higher in the 100 mg/kg-treated rat foetuses. Therefore it can be concluded from this study that lithium carbonate can produce embryotoxicity and teratogenicity in the Wistar rat. Considering similar observations in various species including man, lithium therapy in pregnant women calls for a judicious use, appropriately weighing the risk-and-benefit factor.

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REFERENCES