Teratogenic Effect of Lithium Carbonate in Early Development of Balb/C Mouse

MOHSEN NOKHBATOLFOGHAHAI[1]* AND KAZEM PARIVAR[2]
[1]Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran
[2]Department of Biology, Science & Research Branch, Faculty of Basic Sciences, Islamic Azad University, Tehran, Iran

ABSTRACT

Lithium carbonate is used as a standard treatment for manic depression. While researchers have investigated the teratogenic effects of lithium carbonate on embryos of various animals in later stages of development, very limited work has been done on the probability of effects at early stages of development. In this study, the teratogenic effect of lithium carbonate was investigated at earlier preimplantation through implantation stages of development of Balb/C mouse embryos. A therapeutic dose (i.e., 300 mg/kg b.w.) was injected into mice intraperitoneally on days 3.5, 4.5, 5.5, and 6.5 of pregnancy. Then, on day 15.5 of gestation, embryos were collected from the pregnant animals. Among the embryos, 71.7% were healthy, 10.7% resorbed, 3.1% showed lordosis, 8.1% were underdeveloped and 8.4% had eye malformations. Significant increases ($P < 0.05$) in the number of hepatic megakaryocytes and nucleated red cells were also observed among experimental embryos. Analysis of maternal serum proteins prepared from dissected animals showed a significant increase or decrease ($P < 0.05$) in the levels of serum proteins albumin, α2 globulin, β globulin, and γ globulin. This research on early developmental stages suggests that pregnant mothers need to be aware of possible teratogenic effects at early stages of pregnancy, although it has been thought that the egg envelope can prevent teratogens from entering. In this case, mothers may need to stop lithium carbonate treatment before they make a decision to become pregnant. Anat Rec, 291:1088–1096, 2008.

Key words: lithium carbonate; teratogenic effect; early development; Balb/C mouse

Experimental studies have shown for several decades that lithium salts cause several teratogenic effects in animals. In recent years, it has also been shown that lithium is still teratogenically and toxicologically important in adults as well as embryos (Sharma and Iqbal, 2005; Zarnescu and Zamfirescu, 2006; Allagui et al., 2006; Tandon et al., 2006; Tsaltas et al., 2007). Extensive usage of lithium and its salts in pharmaceuticals, air conditioning, and in several biological and chemical laboratories increases the probability of this metal being in close contact to humans. Toxic effects of lithium salts may also result from medication. Clinical studies have recently shown that lithium carbonate causes several teratogenic effects when patients are treated by long-term administration of the medicine (Lewicki et al., 2006; Garcia et al., 2007; Fujii et al., 2007).

Studies on patients suffering from manic depression reveal that the majority of these patients are women and many are of reproductive age (Sharma and Rawat, 1986; Kao and Elinson, 1989; Yonkers et al., 1998). This raises concerns, because several reports suggest a high risk of fetal malformations when lithium carbonate is used during pregnancy (Yonkers et al., 1998). Lithium carbonate passes through the placenta easily (Smithberg et al., 1984), and after a short time, its concentration in fetal blood almost equals that in maternal blood serum (Weinstein and Goldfield, 1969).

*Correspondence to: Mohsen Nokhbatolfoghahai, Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran 71454. Fax: 0098-711-2280916. E-mail: nokhbeh@susc.ac.ir

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While findings on embryotoxicity and teratogenicity among invertebrates show that lithium has detrimental effects, the data for vertebrates are dependent on the animal and strain treated as well as on the dosage and duration of exposure (Smithberg and Dixit, 1982). The specific problems also associated with lithium exposure vary during different stages of gestation (Yonkers et al., 1998). Among vertebrates, numerous reports (Wright et al., 1971; Allakhverdiev and Smol'nikova, 1983; Manhas and Kumar, 1988; Jurand, 1988; Simard et al., 1989; Hansen et al., 1990; Giles and Bannigan, 1997; Scheinfeld and Faad, 2004) are available about teratogenic effects of lithium carbonate, few on humans; most on laboratory animals. For example, fertilized chick eggs were injected with 0.05 ml of various dilutions of lithium carbonate ranging from 0.5 percent to 1 percent (Manhas and Kumar, 1988). The embryos were observed at various intervals on 6th, 8th, 11th, and 13th day after injection. The results showed an increased rate of mortality and malformations in the chicks injected with lithium carbonate. In another investigation, injection of lithium carbonate at the rate of 300 mg/kg body weight to mice on their gestation day 9 caused death in some embryos, growth retardation and central nervous system malformation in others (Jurand, 1980). These results were later confirmed by other researchers (Jurand, 1988; Giles and Bannigan, 1997). There are also reports of skeletal malformation as a result of lithium carbonate treatment (Marathe and Thomas, 1986; Sharma and Rawat, 1986). In another study, lithium carbonate at the rate of 300 mg/kg body weight was injected to Balb/C mice on days 7.5, 8.5, and 9.5 of pregnancy. These treatments caused embryo malformations such as anophthalmia, microphthalmia, exohepaty, exencephaly, skeletal malformation, and resorption (Smithberg and Dixit, 1982; Parivar and Zeynali, 1995). Smithberg and Dixit (1982) tested the teratogenicity of lithium carbonate in two inbred strains of mice (Strains 129 and A/J mice). Their results suggested that six times the therapeutic amount of lithium in human serum level is teratogenic in mice. In the human embryo, these teratogenic effects are targeted at the most vital parts such as heart and blood vessels (Weinstein and Goldfield, 1975). However, in the case of the human embryo, determination of time intervals (critical periods) during which the application of this drug is most detrimental awaits further investigation. Due to deleterious teratogenic effects of this medicine on the human embryo, the aim of the present study was to use the Balb/C mouse as an animal model for studying the effects of lithium on mouse embryos not as usual on late development, but on earlier preimplantation through implantation stages of development. Research on early developmental stages is important, because pregnant mothers need to be aware of possible teratogenic effects resulting from treatment. In this case, mothers may need to stop lithium carbonate treatment before they make a decision to become pregnant.

MATERIALS AND METHODS

Animals and Treatments

The study was approved by the animal welfare committee of Tehran Training University. Balb/C mice (10–12 weeks) were used in our experiments. The mice were kept in polypropylene cages at 21 ± 2°C under natural photoperiod (12 hr light and 12 hr dark). Appearance of a vaginal plug was considered as day zero of pregnancy. The LD50 value was selected based on a previous study conducted with the same mouse strain and same chemical (Parivar and Zeynali, 1995). To measure the LD50, increasing values of lithium carbonate solution were injected in a series of six pregnant mice. The experiment was terminated when approximately 50% of the animals die after 48 hr of treatment. The LD50 value was approximately 450 mg/kg b.w. and the teratogenic dose was 300 mg/kg b.w. For studying the effects of lithium on mouse embryos at early stages of development, 85 pregnant mice were taken and divided into three groups—experiment, control, and untreated. Mice were taken on days 3.5, 4.5, 5.5, and 6.5 of pregnancy in each group. The animals of the experimental group, were injected with 300 mg/kg body weight of lithium carbonate solution in water intraperitoneally. The control group was injected with the same volumes of normal saline solution (0.9% w/v of NaCl) only, and the third group remained untreated. Gestation was terminated on day 15.5, and the uterine contents were removed. After collection of embryos from the uterus, their amniotic membranes were removed carefully and the weight of embryos and their placentas were determined. Measurements also were made of the crown-rump (CR) length by calipers. Embryos were fixed in Bouin's solution for at least 3 hr for morphological and histological studies. Each series of experiments was repeated three times.

Blood Sampling

Mice were anesthetized with ether and maternal blood samples from untreated, control, and lithium carbonate-treated animals were taken by cardiac puncture and was used for serum protein measurements. Serum was prepared from collected blood and stored at −20°C until required for later studies.

Sectioning and Tissue Staining

Embryos were prepared for sectioning and staining according to the following procedures: specimens were rinsed in several changes of normal saline and then fixed in Bouin's solution for at least three hours. The embryos were washed in tap water several times and then dehydrated in an ascending ethanol series (30%, 50%, 70%, 90%, and 100%). The specimens were prepared for wax embedding using a paraffin bath (57–60°C), and were sectioned at 7 μm then stained with Hematoxylin-Eosin (conventional method). Sections were examined over a range of magnifications and photographed using a Zeiss photomicroscope, M3. Lithium is reported to have a direct effect on vascular development in chick embryo (Giles and Bannigan, 1999). To find out if lithium affects blood vessel development in mouse embryos, the vessels in histology sections were chosen randomly for RBC counting in both experimental and control samples. In each field, the ratio (number) of nucleated RBCs to nonnucleated RBCs were counted. The same procedure was carried out for the counting of megakaryocytes. The slide section of liver at stage 15.5 of development was designed for histopathological investigation, specifically to count the number of hepatic megakaryocytes as the activity of hematopoiesis is marked

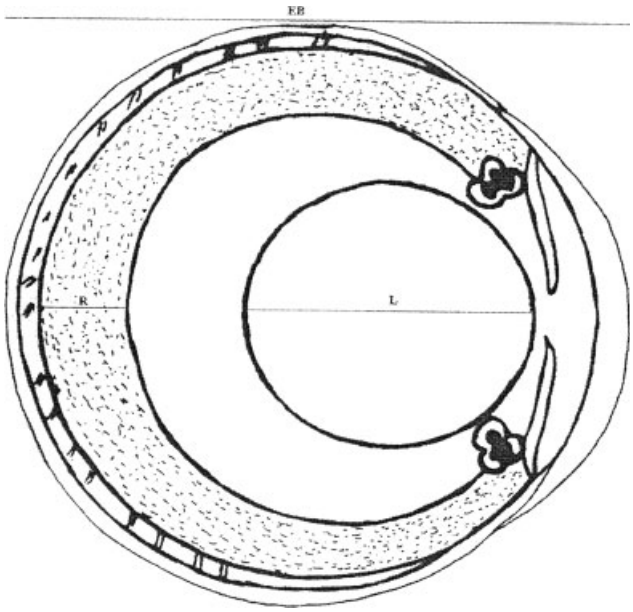

Fig. 1. Schematic image showing general organization of an eye in sagittal section (lateral view). (____) applied for measuring the length of eye ball (EB) and lens (L) diameters, also retina (R) thickness.

by presence of these cells in this stage of development. Three measurements of the eye (eye ball, lens diameter, and retina thickness) were recorded in experimental, control, and untreated embryos. The histological serial sections of the head through eye regions were prepared to measure the eye parameters, using light microscope with graticule and in relevant magnification (Fig. 1).

Analysis of the Main Blood-serum Proteins

Electrophoretic properties of blood serum proteins were studied according to the method described by the manufacturer (Helena Laboratories, Beaumont, TX). In this method, blood serum samples were placed on acetate plates, and Desaga electrophoresis equipment was used to separate the protein bands. The separated bands were then stained in Coomassie blue. The acetate plates were scanned, using a densitometer at 525 nm, and graphs and relative percentages of the five main serum proteins were prepared for statistical analysis at a later stage.

Statistical Analysis

The data obtained from the numbers and percentages of the abnormalities in different treatments (Table 1) were analyzed by using Z-test and Bonferroni Correction. Growth parameters such as embryonic weight, placenta weight and CR length obtained from different treatments (Table 2) were analyzed through multiple comparison and Tukey's procedure. The data obtained from the relative percentages of the serum proteins (Table 3) were also analyzed using Tukey's procedure. All tests were done at 5% level significance.

RESULTS

Macroscopic Observations

Experimental data in comparison to controls and untreated groups are presented in Table 1. Among 344 lithium-treated embryos from 37 litters examined for abnormalities, 247 embryos were healthy; 37 resorbed; 11 showed lordosis; 28 were undeveloped; and 21 had eye malformations. Among 275 control embryos, only 4 were resorbed embryos and no other malformations were seen. No malformation was seen among 293 embryos from the untreated mice. The resorbed embryos were found among all experimental treatments, but were significantly increased in number in 3.5 and 4.5 day injected embryos. Underdeveloped embryos were found to be significantly increased on days 3.5 and 6.5 of experimental treatments. A pronounced body curvature (lordosis) was also observed in some experimental embryos of days 3.5, 4.5, and 6.5. Figure 2A,B shows morphological differences between normal and lordosis embryos respectively, in sagittal section. The lithium carbonate also significantly decreased the weight of placenta and CR length of experimental embryos compared with control embryos except in day 4.5 of injection (Table 2). Experimental embryos of all days showed a significant decrease in their weight compared with control and untreated embryos.

Microscopic Studies

Microscopic examination of sections through the eye revealed a significant decrease in eyeball, and lens diameters and retinal thickness (microphtalmia) in the experimental mice embryos compared with controls in days 3.5 and 5.5 treatments. In the day 6.5 treatment, a significant decrease was only shown in eyeball diameter. No significant differences in eye malformations were seen in the day 4.5 treatment. Figure 3 shows the differences in eye ball diameter generally between normal (A) and malformed (B) embryos. Figure 4 (histogram) shows a comparison between eyeball diameter on different days of injection. Other malformations observed included: cleft palate in day 3.5, 5.5, and 6.5 treatments; exohepaty (Fig. 5A,B) in the day 5.5 treatment. Both exohepaty and cleft palate occurred very rarely and all appeared in the embryos with an underdeveloped malformation.

A significant increase occurred in the number of hepatic megakaryocytes in all experimental treatments compared with control and untreated embryos (Fig. 6). Histological examination of blood showed a significant increase in the number of undifferentiated nucleated red blood cells in all experimental groups compared with control groups (not shown).

Electrophoretic Observations

Table 3 shows a comparison between relative percentages of five maternal serum proteins (albumin, $\alpha 1$ globulin, $\alpha 2$ globulin, β globulin, and γ globulin) in different treatments. The main results obtained from electrophoretic studies of serum proteins of pregnant mice on day 15.5 of their gestation were as follows: (1) There was a significant increase in albumin levels in experimental mice from the 3.5 and 4.5 day treatments. (2) There was a significant decrease in the value of $\alpha 1$ globulin in the

TABLE 1. Gross malformations observed in experimental, control, and untreated embryos after day 3.5, 4, 5.5, and 6.5 injection with lithium carbonate

Injection days	3.5			4.5			5.5			6.5		
Treatment types												
Observations	Experiment	Control	Untreated	Experiment	Control	Untreated	Experiment	Control	Untreated	Experiment	Control	Untreated
Total number of pregnant females in each treatment	9	6	6	9	6	6	7	6	6	12	6	6
Total number of embryos	76	66	72	89	68	74	67	70	76	112	71	77
Healthy embryos (%)	**43**$^{**,++}$ (**56.5**)	65 (98.5)	72 (100)	68$^{**,++}$ (76.3)	66 (97.1)	73 (98.7)	53$^{**,++}$ (79.1)	70 (100)	76 (100)	83$^{**,++}$ (74)	70 (98.6)	77 (100)
Resorbed embryos (%)	10$^{**,++}$ (13.2)	1 (1.5)	0	16$^{**,++}$ (18)	2 (2.9)	1 (1.3)	**2**$^{*,+}$ (**3**)	0	0	9$^{**,++}$ (8.03)	1 (1.4)	0
Embryos with lordosis (%)	5$^{**,++}$ (6.6)	0	0	3$^{*,+}$ (3.4)	0	0	**0**	0	0	3$^{*,+}$ (2.7)	0	0
Underdeveloped embryos (%)	11$^{**,++}$ (14.5)	0	0	2$^{*,+}$ (2.3)	0	0	1$^{*,+}$ (1.5)	0	0	14$^{**,++}$ (12.5)	0	0
Eye malformation (%)	7$^{**,++}$ (**9.2**)	0	0	**0**	0	0	**11**$^{**,++}$ **(16.4)**	0	0	**3**$^{*,+}$ **(2.7)**	0	0

The highlighted number shows significant different from other experiment values in each row $P < 0.008$.
*Different to control in each treatment $P < 0.01$.
**Different to control in each treatment $P < 0.001$.
$^{+}$Different to untreated in each treatment $P < 0.01$.
$^{++}$Different to untreated in each treatment $P < 0.001$.

TABLE 2. Measurements of embryo weights, placenta weights and embryo lengths in untreated, control, and experimental embryos injected at day 3.5, 4.5, 5.5, and 6.5 of pregnancy

Stage at injection	Observation	Embryos weight (g)	Placenta weight (g)	Embryos CR (mm)
3.5 day	Untreated	0.323 ± 0.039	0.139 ± 0.011	14.79 ± 1.14
	Control	0.413 ± 0.032	0.126 ± 0.014	14.78 ± 0.52
	Experiment	0.319 ± 0.046*,+,□	**0.135 ± 0.017***,+,□	**12.83 ± 0.75***,+
4.5 day	Untreated	0.323 ± 0.039	0.146 ± 0.011	14.84 ± 1.14
	Control	0.350 ± 0.03	0.124 ± 0.010	13.09 ± 0.51
	Experiment	**0.316 ± 0.044***,+,□	0.124 ± 0.018+,□	**12.93 ± 0.63**+,□
5.5 day	Untreated	0.323 ± 0.039	0.139 ± 0.011	14.79 ± 1.14
	Control	0.430 ± 0.026	0.156 ± 0.023	15.26 ± 0.82
	Experiment	0.316 ± 0.037*,+,□	0.129 ± 0.019*,+,□	**12.35 ± 0.78***,+,□
6.5 day	Untreated	0.323 ± 0.039	0.145 ± 0.011	14.79 ± 1.14
	Control	0.384 ± 0.047	0.146 ± 0.017	14.158 ± 0.32
	Experiment	0.297 ± 0.045*,+,□	0.127 ± 0.025*,+,□	**13.14 ± 0.71***,+,□

Value represent mean ± SD; CR, crown-rump length. The highlighted numbers show significant different from other experiment values in each column $P < 0.05$.
*Different to control in each treatment $P < 0.05$.
+Different to untreated in each treatment $P < 0.05$.
□Different between control and untreated in each treatment $P < 0.05$.

TABLE 3. Relative percentages of five serum proteins in experimental embryos, control and untreated embryos from the day 3.5, 4.5, 5.5, and 6.5 treatments

Stage	Observation	Albumin (%)	α1 globulin (%)	α2 globulin (%)	β globulin (%)	γ globulin (%)
3.5 day	Untreated	43.3 ± 10.94	5 ± 2.79	15.77 ± 2.9	8.13 ± 4.03	32.2 ± 15.1
	Control	28.45 ± 0.255	8.3 ± 0.2	17.8 ± 2	8.6 ± 0.2	48.65 ± 11.7
	Experiment	**62.8 ± 0.16**	7.1 ± 1.41	11.8 ± 2*,+,□	4.3 ± 2	**15.6 ± 0.2**
4.5 day	Untreated	43.3 ± 10.94	5 ± 2.79	15.77 ± 2.9	8.12 ± 4	32.2 ± 5.4
	Control	30.05 ± 16.97	5.65 ± 3.29	19.9 ± 2.05	7.8 ± 4.05	44.6 ± 6.14
	Experiment	34.7 ± 9*,+,□	**5.3 ± 3.24***,+,□	12.2 ± 5.08*,+,□	22.4 ± 11.55	29.8 ± 2.65*,+,□
5.5 day	Untreated	43.3 ± 10.94	5 ± 2.78	15.77 ± 4.23	8.12 ± 4	32.2 ± 15.14
	Control	42.5 ± 0.2	2.6 ± 0.2	9.7 ± 0.2	4.9 ± 0.2	48.3 ± 0.2
	Experiment	31.13 ± 4.38	6.33 ± 3.92	11.76 ± 5.7	22.13 ± 3.13	36.63 ± 2.87
6.5 day	Untreated	43.3 ± 10.94	5 ± 2.78	15.78 ± 4.23	8.13 ± 4	32.2 ± 15.14
	Control	62.2 ± 0.2	8.4 ± 0.2	14.1 ± 0.2	2.8 ± 0.2	21.3 ± 0.2
	Experiment	50.5 ± 0.3	8.6 ± 0.2	11.65 ± 2.83	3.8 ± 1.17	38.05 ± 18.5

Values represent mean ± SD. The highlighted numbers show significant different from other experiment values in each column $P < 0.05$.
*Different to control in each treatment $P < 0.05$.
+Different to untreated in each treatment $P < 0.05$.
□Different between control and untreated in each treatment $P < 0.05$.

experimental mice of day 4.5. (3) There was a significant decrease ($P < 0.05$) in α2 globulin levels in experimental mice of days 3.5, 4.5. (4) The amount of β globulin increased significantly in the 4.5 and 5.5 day treatments. (5) The amount of γ globulin decreased in pregnant mothers of the day 4.5 treatments, and in the day 6.5 treatment increased significantly.

DISCUSSION

One critical factor is the stage of development at which embryos are exposed to teratogenic agents. Polifka and Friedman (1999) suggested that exposure later in gestation (after organogenesis, during growth stage of development) is less likely to produce fetal malformations although there are some exceptions. On the other hand, during very early stages of development after conception (i.e., preimplantation through implantation stages), exposures are unlikely to cause malformations (Schluter, 1971). It is clear that the embryo proper has not yet formed immediately after conception; therefore, before cells are differentiated to their specific developmental fates, a damaged cell can be replaced by any other in the embryo and normal development will proceed ("regulative development"). If significant cells are damaged or killed, the embryo will not survive. The results obtained in this study show that lithium carbonate may be teratogenic not only during the period of organogenesis but also in the early stages of development (preimplantation period). Results for treatment on day 3.5 of gestation confirmed that, despite the fact that the placenta and blood supply had not yet developed, lithium carbonate could diffuse into the developing embryo and cause malformations. Unfortunately, we did not measure lithium concentration in the maternal blood. However, Smithberg et al. (1984) reported in mice that the concentration of lithium carbonate after passing through the placenta reaches an amount almost equal to

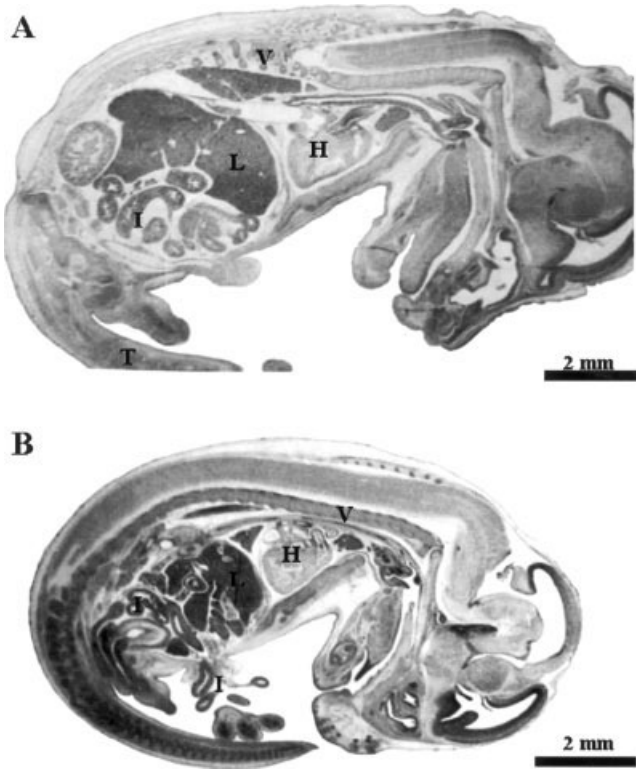

Fig. 2. Stereomicrograph to show comparison between normal and lordosis embryos in sagittal section. **A:** Untreated embryo with normal curvature at day 15.5 of gestation. **B:** Lordosis embryo with high intense curvature in vertebral column at day 15.5 of gestation; mother lithium carbonate injected at day 3.5 of pregnancy. H, heart; I, intestine; L, liver; T, tail; V, vertebral column.

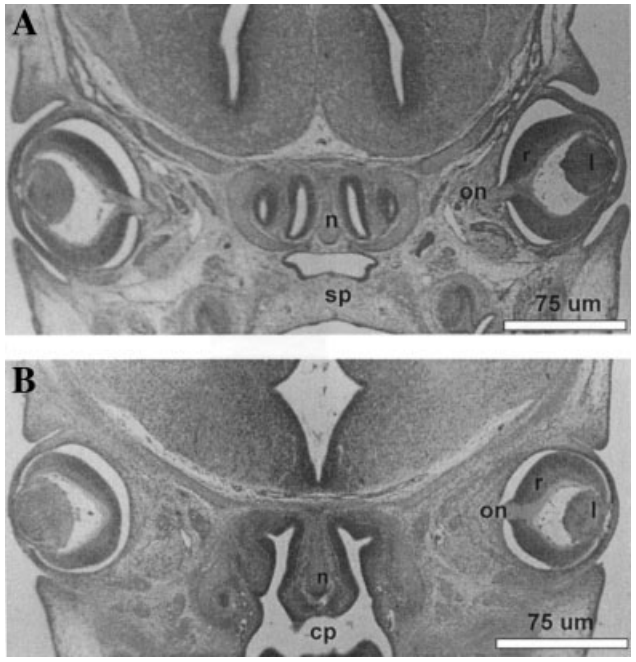

Fig. 3. Strereomicrograph to show comparison between normal and microphtalmia embryos in cross section. **A:** Control embryo with normal eye at day 15.5 of gestation. **B:** Malformed embryo with microphtalmia, and cleft palate at day 15.5 of gestation; mother lithium carbonate injected at day 5.5 of pregnancy. L, lens; R, retina; ON, optic nerve; N, nasal; CP, cleft palate; SP, secondary palate.

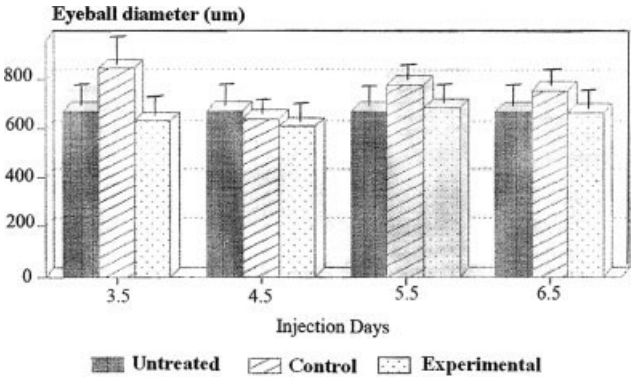

Fig. 4. Comparison between eyeball diameters after different treatments. Values expressed as mean ± SD.

that of blood serum. The presence of resorbed embryos reported here, common among the malformed embryos with an especially high rate after exposes at 3.5 and 4.5 days of gestation, confirm that lithium carbonate has significant effects at these stages. While the precise mechanism of this teratogenic effect is unknown, it is clear that receiving lithium carbonate at the beginning of organogenesis may block development, leading to resorption. The significant decrease in placental weight in some experiments reported here probably affects its nutritive role, leading to resorption or at least growth retardation. Among the treatment days, results from treatment on day 4.5 showed no effect on embryo placental weight or crown-rump length. It is clear that the action of lithium is stage-dependent. Our literature search showed there is no report investigating whether other teratogens given at similar time-points (day 4.5) behave in a similar way. We suggest that differences may be correlated with developmental events taking place in the embryo at the time of treatment. At stage 4.5 day (around the morula stage), embryos are beginning implantation; embryos are not yet well attached to the uterine wall and no placenta has developed.

The risk of embryonic or fetal damage depends not only on the agent involved and developmental stage but also on host susceptibility, dose, and other factors. In the literature, we reviewed different experimental animals with various responses to the exposure of lithium carbonate are reported. Experimental work in animals often uses agents in dosages that are many times greater than those likely to occur in humans (Polifka and Friedman, 1999). Among the vertebrates, studies show that pregnant mice are more resistant to the embryotoxic and teratogenetic potential of lithium (Schluter, 1971; Hansen et al., 1990). Therefore mice were sensitive only at higher concentrations (Hansen et al., 1990). This is one of the reasons why we used a high dosage of lithium carbonate in our experiments. Measuring LD50 for the Balb/c mice determined the level of embryotoxic and

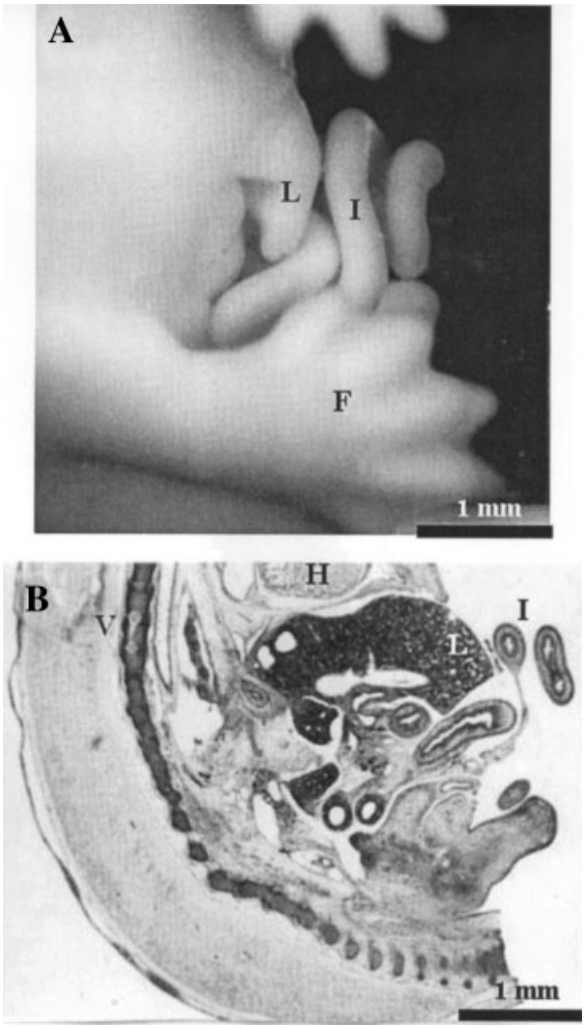

Fig. 5. Stereomicrographs of embryos in day 15.5 of gestation injected at day 5.5 of gestation, showing exohepatic malformation. **A:** Whole mount embryo. **B:** Mid-sagittal section embryo. H, heart; I, intestine; L, liver; V, vertebral column; F, foot.

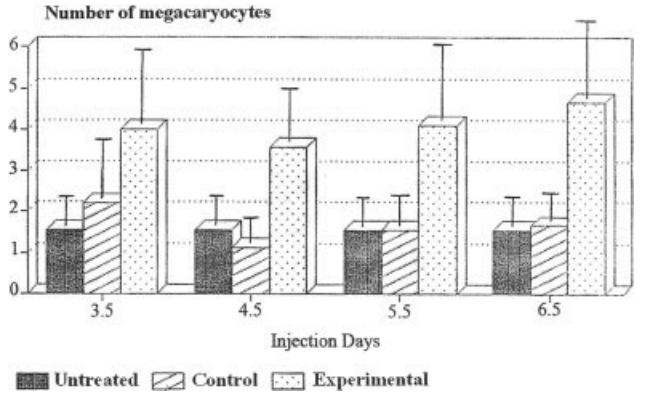

Fig. 6. Comparison between the numbers of megacaryocytes in different treatments. Values expressed as mean ± SD.

maternal toxicity (300 mg/kg b.w.) for lithium carbonate. This similar amount of teratogenicity had also been determined by other researchers on the same strain (Balb/c mouse), although the experimental work was for later stages of development. The aim of using this amount in the present work was to show that laboratory animal studies often provide a means of identifying compounds with teratogenic potential before humans have been harmed. Especially important is our finding that exposure at earlier stages can be harmful, as that stage of development has previously been considered unlikely to be affected. Unfortunately, it is usually impossible to extrapolate data from animals to a clinical situation involving an individual pregnant woman except in a very general way.

Malformation of the vertebral column was the other problem in experimental embryos, which appeared as lordosis in these embryos. This kind of malformation has been described by Marathe and Thomas (1986) and Sharma and Rawat (1986). Several studies (Busa, 1988; Cooke et al., 1989; Kao and Elinson, 1989; Maslamski et al., 1992) suggested that lithium has a role in determining the anterior–posterior direction of the body plan. The table of developmental stages in the mouse shows that embryos on day 9 normally show lordosis, while the curvature of the embryos reduces gradually at later stages (Theiler, 1972). Retaining lordosis on day 15.5 confirmed that these embryos have been retarded in growth at least in external appearance.

In the case of eye malformations, it is clear that the stages when lithium carbonate was administered did not include stages when eyes were undergoing later development. From our results, it can be concluded that lithium carbonate probably affects the germinal layers of the eye fields at early stage, which causes some disorders during eye development at later stages. Among our findings, it is not clear why lithium has not affected eye tissues when treated on day 4.5. The results for eye malformations are confusing. In the case of eyeball malformation, while the results (Fig. 4) show significant differences between control and experimental animals, no significant difference is apparent between untreated and experimental animals. The interesting finding is a significant increase in control animal measurements compared with untreated animals on exposure days 3.5, 5.5 and 6.5 in this case. It may be concluded that normal saline solution causes these significant changes. In the case of embryo weights, normal saline solution apparently produced a significant increase in body weight. Further consideration is needed to prove this hypothesis. Our data also showed significant differences in retinal thickness and lens diameter between control and experimental animals on exposure days 3.5 and 5.5, but no significant change is demonstrated on exposure days 4.5 and 6.5.

It is clear that the expansion of the growing liver within the septum transversum is related to the excavation of the neighbouring body wall; however, it is not clear whether destruction of the ectomesodermal wall occurs independently or is caused by the expansion of the liver mass.

One of the most widespread side-effects of lithium therapy in humans is an increase in the number of cir-

culating granulocytes, mostly neutrophils, although effects on other lineages have also been reported (Boggs and Joyce, 1983). Lithium appears to increase the level of pluripotent hematopoietic stem cells, either indirectly stimulating the release of cytokines and/ or directly by acting on stem cells (Boggs and Joyce, 1983; Ballin et al., 1998; Chasis, 2004). Chasis (2004) reported quantitative data in normal mice showing that between embryonic day (E) 12.5 and 16.5 primitive erythroblasts become progressively enucleate in the circulation. According to the abundance of nucleated RBCs at E 15.5 in experimental specimens reported here, the presumption is that, at the beginning, lithium decreases the production of these cells (Duhm and Becker, 1979). Therefore, to compensate for the lack of RBCs, the embryo is continually making them, and because it takes a long time for RBC differentiation to be completed, the RBCs observed are mostly nucleated. A significant increase in megakaryocytes in the liver is reported here, but the cause remains presently unknown. The direct effect of lithium on the early stages of vascular development has been reported in the chick embryo (Giles and Bannigan, 1999). However, we could not see the abnormality in the mouse embryo, even though it might be expected at the early stages of mouse development, as Giles and Bannigan (1999) discovered that extra-embryonic avasculogenesis appears at early stages of chick embryo development rather than at later stages. But as we discussed before, host susceptibility and other dependent factors are also important.

Protein electrophoresis is normally used to evaluate, diagnose, and monitor a variety of diseases and conditions. Previous reports about probable effects on blood both in embryos and mothers were the reason to evaluate serum proteins at least in mothers to investigate any changes in the level of the proteins and their possible relation to malformations. Changes in the protein levels of maternal blood serum, especially when the mother is pregnant, are more complex. Changes may result from the effect of lithium on maternal liver (as a main source of plasma protein synthesis) and also on the immune system, which may affect the normal function of the liver. The levels of serum globulin were highly variable and did not correspond to different days of treatment. It was not possible to measure the fetal or embryo serum globulin and due to time constraints it was not possible to carry out histological work on maternal organs, which remains to be done in future studies.

Although there are various reports about the teratogenic effects of lithium carbonate in animals, the discussion on the teratogenic effects of lithium in world literature has not yet reached a final conclusion (Kucera, 1996). Kucera (1996) believes that the teratogenicity of lithium has not been proved unequivocally. It is not clear whether the problem results from choosing different dosages of lithium carbonate during experimental work or using different ways of lithium treatment, or it may result from dealing with nonacute experiments. While some studies suggest that lithium is not a strong teratogen in humans (Giles and Bannigan, 2006), others highlight the risk when lithium administration is applied to pregnant women, especially during the first 3 months of pregnancy.

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LITERATURE CITED

Allagui MS, Hfaiedh N, Vincent C, Guermazi F, Murat JC, Croute F, Elfeki A. 2006. Changes in growth rate and thyroid and sex-hormone blood levels in rats under sub-chronic lithium treatment. Hum Exp Toxicol 25:243–250.

Allakhverdiev VD, Smol'nikova NM. 1983. Toxic effect of lithium salts on the pregnant rat and on the prenatal development of the fetus. Farmakol Toxikol 46:108–110.

Ballin A, Lehman D, Sirota P, Litvinjuk U, Meytes D. 1998. Increased number of peripheral blood CD34 C cells in lithium-treated patients. Br J Haematol 100:219–221.

Boggs DR, Joyce RA. 1983. The hematopoietic effects of lithium. Semin Hematol 20:129–138.

Busa WB. 1988. Roles for the phosphatidyl inositol cycle in early development. Philos Trans R Soc (Lond) B 320:415–426.

Chasis JA. 2004. Mammalian primitive erythrocyte: neither fish nor fowl. Blood 104:1–2.

Cooke J, Sysmes K, Smith EJ. 1989. Potentiation by the lithium ion of morphogenetic responses to a Xenopus inducing factor. Development 105:549–558.

Duhm J, Becker BF. 1979. Studies of lithium transport across the red cell membrane. J Membr Biol 51:263.

Fujii S, Oku H, Takahashi R, Kanbara Y, Sugasawa J, Ikeda T. 2007. Optic nerve dysfunction secondary to long-term use of lithium carbonate. Jpn J Ophthalmol 51:79–81.

Garcia C, Mayaudon H, Dupuy O, Leberre JP, Bordier L, Bauduceau B. 2007. Silent thyroiditis in a patient under lithium therapy. Rev Med Interne 28:46–47.

Giles JJ, Bannigan JG. 1997. The effects of lithium on neurulation stage mouse embryos. Arch Toxicol 71:519–528.

Giles JJ, Bannigan JG. 1999. The effect of lithium on vascular development in the chick area vasculosa. J Anat 194:197–205.

Giles JJ, Bannigan JG. 2006. Teratogenic and developmental effects of lithium. Curr Pharmacol Design 12:1531–1541.

Hansen DK, Walker RC, Grafton TF. 1990. Effect of lithium carbonate on mouse and rat embryo in vitro. Teratology 41:155–160.

Jurand A. 1980. Malformations of the central nervous system induced by neurotropic drugs in mouse embryos. Dev Growth Differ 22:61–78.

Jurand A. 1988. Teratogenic activity of lithium carbonate: an experimental update. Teratology 38:101–111.

Kao KR, Elinson RP. 1989. Dorsalization of mesoderm induction by lithium. Dev Biol 132:81–90.

Kucera V. 1996. Is lithium a teratogen? Cas Lek Cesk 135:27.

Lewicki M, Paez H, Mandalunis PM. 2006. Effect of lithium carbonate on subchondral bone in sexually mature Wistar rats. Exp Toxicol Pathol 58:197–201.

Marathe MR, Thomas GP. 1986. Embryotoxicity and teratogenicity of lithium carbonate in Wistar rat. Toxicol Lett 34:115–120.

Manhas B, Kumar R. 1988. Toxic effects of lithium carbonate on chick embryos. J Anat Soc India 37:38–44.

Maslamski JA, Leshko L, Busa WB. 1992. Lithium-sensitive production of inositol phosphates during amphibian embryonic mesoderm induction. Science 256:243–245.

Parivar K, Zeynali B. 1995. Teratogenic effects of lithium carbonate on development of mouse embryos. J Sci I Iran 6:80–88.

Polifka JE, Friedman JM. 1999. Clinical teratology: identifying teratogenic risk in humans. Clin Genet 56:409–420.

Scheinfeld NS, Faad MD, JD. 2004. Teratology and drug use during pregnancy. www.emedicine.com/med/topic3242.htm.

Schluter G. 1971. Effects of lithium carmine and lithium carbonate on the prenatal development of mice. Naunyn Schmiedebergs Arch Pharmakol 270:56–64.

Sharma A, Rawat AK. 1986. Teratogenic effects of lithium and ethanol in the developing fetus. Alcohol 2:101–106.
Sharma SD, Iqbal M. 2005. Lithium induced toxicity in rats: a haematological, biochemical and histopathological study. Biol Pharm Bull 28:834–837.
Simard M, Gumbiner B, Lee A, Lewis H, Norman D. 1989. Lithium carbonate intoxication. A case report and review of the literature. Arch Intern Med 149:36–46.
Smithberg M, Dixit PK. 1982. Teratogenic effects of lithium in mice. Teratology 26:239–240.
Smithberg M, Dixit PK, Singer L. 1984. Uptake and transfer of lithium in pregnancy and lactation in the mouse. Proc Soc Exp Biol Med 175:164–168.
Tandon A, Bhalla P, Nagpaul JP, Dhawan DK. 2006. Effect of lithium on rat cerebrum under different dietary protein regimens. Drug Chemic Toxicol 29:333–344.
Theiler K. 1972. The house mouse, development and normal stages from fertilization to 4 weeks of age. New York: Springer.
Tsaltas E, Kontis D, Boulougouris V, Papakosta VM, Giannou H, Poulopoulou C, Soldatos C. 2007. Enhancing effects of chronic lithium on memory in the rat. Behav Brain Res 177:51–60.
Weinstein MR, Goldfield M. 1975. Cardiovascular malformations with lithium use during pregnancy. Am J Psychiatry 132:529–531.
Weinstein MR, Goldfield M. 1969. Lithium carbonate treatment during pregnancy. Dis Nerv Syst 30:828–832.
Wright TL, Hoffman LH, Davies J. 1971. Teratogenic effects of lithium in rats. Teratology 4:151–155.
Yonkers KA, Litle BB, March D. 1998. Lithium during pregnancy: drug effects and their therapeutic implications. CNS Drugs 9:261–269.
Zarnescu O, Zamfirescu G. 2006. Effects of lithium carbonate on rat seminiferous tubules: an ultrastructural study. Internat J Androl 29:576–582.