Teratogenecity caused due to Oral Administration of Neomercazole to Albino Mice

ASMA RASHID KHAN¹ & ASMATULLAH²

¹Department of Zoology, Govt College of Science, Wahdat Road, Lahore, Pakistan.
²Department of Zoology, University of the Punjab, Lahore, Pakistan

ABSTRACT

Neomercazole (carbimazole) is an antithyroid drug. Present study was designed to evaluate the teratogenic and embryotoxic effects of above mentioned drug given to mice during organogenesis. The dose groups used were 0.2, 0.4, 0.6 and 0.8 µg/gBW. The pregnant mice were exposed to these dose groups on days 8 and 12 of gestation. Fetuses were recovered on day 18 of gestation and were subjected to morphological, morphometric and skeletal studies. Morphological studies revealed anomalies like distorted axis, hydrocephaly, microphthalmia, open eye lid, agnathia, micromelia, syndactyly, subcutaneous hemorrhages and kinky tail. Moreover, there was overall decline in litter size and upsurge in percentage of fetal resorptions. Detailed study of skeletal parameter presented reduction in ossification in skull, ribs and limb region.

Keywords: Neomercazole, Carbimazole, embryotoxicity, teratogenicity.

INTRODUCTION

Ratio of occurrence of hyperthyroidism is 5:1 in women as compared to men and the chance of hyperthyroidism during pregnancy is 0.2% (Mestman, 1997). Giliron (1998) focused on two main causes of hyperthyroidism observed in pregnancy i.e., Graves' disease and gestational transient thyrotoxicosis. The prevalence of hyperthyroidism may represent 3-4% of all pregnancy. But these patients need immediate therapy with antithyroids and regular monitoring for signs of fetal and maternal hyperthyroidism and hypothyroidism (Patil- Sisodia & Mestman, 2010). Autoimmune fetal hyperthyroidism can generally be treated by optimizing therapy of mother, such as by increasing the dose of antithyroid drugs (Polak, 2011).

Antithyroid drugs (thionamides) are most commonly used for the therapy of an overactive thyroid (hyperthyroid). Propylthiouracil, methimazole, carbimazole and radioactive iodine are being used as effective antithyroids (Gupta et al., 1992) with potential teratogenic risk also. Most commonly occurring defect due to these antithyroid drugs is aplasia cutis congenita in new borns (Perger et al., 2011). Aplasia cutis congenita (a circumscribed absence of skin that usually involves scalp) is one of the major anomaly associated with methimazole exposure during pregnancy (Rodriquez- Garcia et al., 2011). Among other anomalies related to prenatal methimazole exposure are choanal and esophageal atresia, minor facial anomalies, and psychomotor delay (Clementi et al., 1999). A case report reveals embroyopathy caused by maternal thiamaole usage during first trimester of pregnancy. Embryopathy is mainly characterized by choanal atresia, esophageal atresia, minor dysmorphic facial features, growth retardation and delayed psychomotor development. The baby boy born to antithyroid treated mother at the age of 4 years showed delayed speech and language development (Ozgen et al., 2006).

Neomercazole (carbimazole) has been found to be very valuable against Graves' disease. Carbimazole is found to be effectual if given within the range of 20mg/day (minimum) and 40mg/day with minimum risk of iatrogenic hypothyroidism to treat hyperthyroidism (Page et al., 1996). Some patients are also given 30mg/day once methimazole (Mafauy et al., 1993). It is also found that drug transfer across the placenta and into the breast milk is higher for lipid soluble methimazole (an ultimate metabolite of carbimazole) than any other antithyroid drug (Kampmann & hensen, 1981). Kriplani et al. (1994) described a cohort study of 32 pregnancies with thyrotoxicosis and majority of mothers were using carbimazole. Problems observed were preterm labour (25%), pregnancy induced hypertension and one maternal death also. While 13% IUGR and 6% unspecified congenital anomalies were found in infants.

In view of the above mentioned literature, the present study was designed and carried out in
mice embryos because of their possible extrapolation in human application.

**MATERIALS AND METHODS**

8-10 weeks old mice weighing 30gms each were used during this research. Four dose groups i.e., NI, NII, NIII and NIV with Control groups were designed having 10 mice each.

Neomercazole (Carbimazole) was used as an antithyroid. The drug is soluble in water. The dose was prepared by diluting a tablet of 5mg in distilled water in such a way that each 0.1 ml of the solution contained desired dose. Four doses used during this experiment were 0.2, 0.4, 0.6 and 0.8µg/gBW, keeping in view minimum and maximum dose per gm body weight during human treatment. These doses were administered orally on days 8 and 12 of gestation. A control group was also managed alongside, which was administered as 0.1 ml of distilled water.

On day 18 of gestation, the treated dams were weighed and anaesthetized with anaesthetic Ether. The dams were given midline incision in the abdomen and uteri were exposed. The number of implantations and resorptions were recorded. Fetuses were removed from the uteri. The fetuses were placed in Bouins fixative for 48 hours after being weighed. After 48 hours, fetuses were transferred to 70% ethanol. Various morphological anomalies were studied in the regions of craniofacial, trunk, limbs, tail and axis. Finally these were microphotographed with the help of digital camera. The morphometric analysis included fetal weight, crown rump length, head circumference, eye circumference, lengths of fore limb, hind limb and tail. The head and eye circumference values (p=mm) were calculated for each fetus with the help of computer based programme Ellipse circumference calculator (CSGN 2006). The morphometric data was subjected to AONVA by using SPSS software.

Fetuses were preserved in 95% ethanol by Richmond & Bannett (1938) method (skeletal preparation). These fetuses were eviscerated through small abdominal incision and all thoraco-abdominal organs were removed, then these were shifted to 2% KOH solution for the complete removal of flesh. On appearance of bones, foetuses were placed in Alizarin Red for 30 min. The deeply stained foetuses were then shifted to 1% KOH until the skeleton become clearly visible through surrounding tissue and finally cleared in 20% glycerinated 1% KOH. The stained specimens were preserved in 50% ethanolic glycerol for microscopic observations and macrophotography (Kawamura et al., 1990).

**RESULTS**

During present study, maternal toxicity showed a rise in maternal weight of vehicle control. While in treated groups slight rise in weight was observed as compared to the control then gradual decline in weight with increase in dose i.e., 4.1% increase in 0.2µg/g BW, 3.9% in 0.4 µg/g BW, 4.1% in 0.6 µg/g BW and 2.09 % in 0.8µg/g BW. Detailed study of fetal toxicity described increase in malformations in dose treated groups as compared to the control. Similarly resorption rate was also found high in higher dose groups as compared to control (Table 1).

![Table 1: Effects of Neomercazole on maternal weight and fetal toxicity after maternal exposure on days 8 and 12 of gestation.](image)

In this exposure, pregnant mice were treated with Neomercazole on day 8 and 12 of gestation. Recovered fetuses of the dose group 0.2µg/gBW, showed morphological deformities such as kyphosis microcephaly, open eye lid and hemorrhagic spot (Fig., 2). Where as in 0.4µg/gBW anomalies observed were hydrocephaly, dysplasia of hind limb (Fig., 3) and kinky tail . Malformations expressed in dose group 0.6µg/gBW included kyphosis, open eyelid, agnathia, hind limb dysplasia (Fig., 5), malrotated limb (Fig., 4), hemorrhagic spots and degenerate tail. Dose group N-IV (d) (0.8µg/gBW) showed abnormalities like hydrocephaly, syndactyly, microphthalmia (Fig., 6), hind limb micromelia and hemorrhagic spots (Table 2).
Fig., 1-6: Macrophotographs of 18 days old fetuses recovered from mothers followed by treatment with different doses of neomercazole on days 8 and 12 of gestation. Fig.,1: Vehicle control ; Fig.,2: 0.2µg/g BW; Fig.,3: 0.4µg/g BW; Fig.,4&5: 0.6µg/g BW; Fig.,6: 0.8µg/g BW. MC, microcephaly; MO, microphthalmia; HG, hygroma; KY, kyphosis; K, kinky tail; MR, malrotated limb; Hfl, hyperflexion of forelimb; MM, micromelia; Yellow Star, limb dysplasia, Arrow, haemorrhagic spots.

Skeletal parameter of control foetuses showed normal calcification in both axial and appendicular skeleton including vertebral column, ribs, limbs and digits. On the contrary skeletal preparations of foetuses recovered from 0.4µg/gBW dose group indicated less ossification in hind limb region. While there was complete absence of ossification in skull, fore limb, hind limb and ribs of fetuses recovered from dose group 0.8µg/gBW (Fig., 7)

Fig., 7: Fetal skeleton showing varying degree of ossification. A, Vehicle control skeleton showing well developed ossified skeleton; B, skeleton of 0.4µg/gBWNemercazole treated fetus presenting reduced ossification in hind limb region ; C, skeleton of 0.8µg/gBWNemercazole treated fetus presenting complete absence of ossification in skull, forelimb, hindlimb and ribs.
Table 2: Morphological anomalies induced in 18 days old foetuses recovered from pregnant mice treated with different doses of Neomercazole on days 8 and 12 of gestation.

<table>
<thead>
<tr>
<th>Dose groups</th>
<th>Axis %</th>
<th>Brain%</th>
<th>Eye%</th>
<th>Snout%</th>
<th>Limbs %</th>
<th>Claws%</th>
<th>Skin %</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>----</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>Hind limb dysplasia( 2.22)</td>
<td>----</td>
<td>-----</td>
<td>Degenerat e(1.12)</td>
</tr>
<tr>
<td>0.2µg/g BW</td>
<td>Kyphosis (1.16)</td>
<td>Microceph aly(3.8)</td>
<td>Open eyelid(2.5)</td>
<td>-----</td>
<td>----</td>
<td>Hemorrha gic spots(12.8 )</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>0.4µg/g BW</td>
<td>Hydroce ph aly (6.9)</td>
<td>Open eyelid(2.5)</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>-----</td>
<td>Kinky tail(2.7)</td>
</tr>
<tr>
<td>0.6µg/g BW</td>
<td>Kyphosis (4.2)</td>
<td>Open eyelid(2.5)</td>
<td>Agnathia(1 .4)</td>
<td>Hind limb dysplasia( 2.7)</td>
<td>----</td>
<td>----</td>
<td>Hemorrha gic spots (2.5%)</td>
<td>Kinky tail(1.41)</td>
</tr>
<tr>
<td>0.8µg/g BW</td>
<td>microce ph aly (8.3)</td>
<td>Microphth almia (11.6)</td>
<td>----</td>
<td>Micromelia (10)</td>
<td>Syndactyly (3.3)</td>
<td>Hemorrha gic spots (6.6)</td>
<td>-----</td>
<td></td>
</tr>
</tbody>
</table>

Morphometric observations during this research showed a (p<0.001) significant reduction in weight, crown rump length brain and eye circumference and fore and hind limb lengths and tail lengths as compared to the control group foetuses (Table 3).

Table 3: Effects of Neomercazole on different parameters of foetuses after maternal exposure on days 8 and 12 of gestation.

<table>
<thead>
<tr>
<th>Dose Groups Δ</th>
<th>Fetal weight</th>
<th>Fetal CRL</th>
<th>Fetal Brain circumference</th>
<th>Fetal eye circumference</th>
<th>Fetal fore limb</th>
<th>Fetal hind limb</th>
<th>Fetal tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>1663± 9.39</td>
<td>22.51± 0.68</td>
<td>22.90± 0.033</td>
<td>7.07± 0.056</td>
<td>6.92 ±0.017</td>
<td>7.77± 0.00512</td>
<td>10.892 ±0.0104</td>
</tr>
<tr>
<td>N-I</td>
<td>892.1± 9.55</td>
<td>16.15± 0.035</td>
<td>22.16±0.357</td>
<td>6.77±0.018</td>
<td>5.6110±0.01531</td>
<td>6.0990 ±0.0198</td>
<td>9.918±0.0227</td>
</tr>
<tr>
<td>N-II</td>
<td>801.8± 0.55</td>
<td>13.31± 0.055</td>
<td>16.21±0.044</td>
<td>4.43±0.032</td>
<td>5.00±0.00277</td>
<td>5.7910 ±0.021</td>
<td>7.0080±0.01104</td>
</tr>
<tr>
<td>N-III</td>
<td>635.62± 29.70</td>
<td>10.99±0.342</td>
<td>14.41±0.048</td>
<td>3.66±0.011</td>
<td>4.206±0.3256</td>
<td>4.206±0.03256</td>
<td>6.4520±0.01143</td>
</tr>
<tr>
<td>N-IV</td>
<td>369.87±38.66</td>
<td>8.73± 0.661</td>
<td>10.16±0.025</td>
<td>3.08±0.039</td>
<td>3.026±0.1861</td>
<td>3.426±0.235</td>
<td>4.5278±0.388</td>
</tr>
<tr>
<td>ANOVA (Inter goup Comparison)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*** Significant difference (p<0.00)
DISCUSSION

Present study showed that Carbimazole induced overall reduction in growth rate. It included significant reduction (p<0.001) in different parameters like weight, crown rump length, brain size, eye size, limb size and tail size. Growth rate displayed retardation with increase in dose concentration exposure time. As neomercazole lowers the thyroid secretion and rapidly crosses placenta and ultimately cause fetal hypothyroidism. Clinical hypothyroidism is associated with high incidence of fetal loss, reduction in fetal weight and congenital system malformation. This condition is complemented with preterm delivery, poor vision development and neurodevelopmental delay (Su et al., 2011). El- Bakry et al., (2010) reported that in an animal study where methimazole was orally administered to albino rats in drinking water from gestation day 1 to lactation day 21. New borns showed severe growth retardation along with different deformities.

In the recent course of study various morphological defects were studied including head /brain anomalies like microcephaly, hydrocephaly, exencephaly, open eye and microphthalmia, hygroma; kyphosis, kinky tail, malrotated limb, hyperflexion of forelimb, micromelia, limb dysplasia(Fig 1-6). These results are in agreement with the case history in which maternal treatment with methimazole (an ultimate metabolite of carbimazole) caused neonatal hypothyroidism. As a consequence of which abnormalities of central nervous system like incomplete maturation of neuronal and glial cells, reduction in synaptic densities and myelin deficiency (Wong & Leung, 2001). In another animal study where methimazole induced fetal mental retardation as it produced deleterious effects on neural growth and development like reduced synaptic activity, delayed myelination and alteration in neurotransmitter level (Kormilas et al., 2010). El- bakry et al. (2010) reported some anomalies and developmental deformities in the cerebral cortex and cerebellar regions of brain due to methimazole (ultimate metabolite of carbimazole). These degenerations became more obvious and widely spread at the 3rd postnatal week. Consequently due to these deformations reduced growth in neurons of these regions was observed. In an experimental work, female rats were fed with the same drug from day 1 till 21st day of lactation in order to induce hypothyroidism. This condition resulted severe growth retardation in neurons of cerebral and cerebellar region. Basically these factors produce mal-development of neuron and dendrites in different brain regions of fetuses (Ahmed et al., 2012). Komoike et al. (2013 ) worked on fertilized egg of zebra fish. Eggs were grown on culture plates containing methimazole. The embryos showed iridic and retinal coloboma, loss of pigmentation, hypoplastic hind brain, turbid tissue in fore brain, swelling of notochord and curly trunk. Histological sections also showed delayed development and hypoplasia of whole brain and spinal cord along with severe disruption of retina.

Another major defect of the recent study was skin haemorrhagic spots, which is supported by a review of case history of 29 females given carbimazole during their pregnancy. Outcomes of the study revealed various anomalies like skin defects (62%), oronasal anomalies (48%), facial dysmorphism (38%), gastrointestinal anomalies (33%) and abdominal wall defect (19%). Out of 27 babies studied in this review, 3 had aplasia cutis (a major skin defect) (Ting et al., 2013). In another study where maternal therapy with methimazole induced a skin defect in the fetus called aplasia cutis congenital (absence of skin fold) (Lollegen et al., 2011). Six cases of embryopathy were reported due to use of carbimazole in the first trimester. The anomalies included facial dysmorphism, patent omphalomesentric duct, aplasia cutis congenital, choanal atresia with aorta coarctation (Koenig et al., 2010).

Research work also showed skeletal defects which clearly indicated that ossification and mineralization of skeleton was reduced with higher doses and more exposures (Fig 7). These results are being justified by the findings of Gripp et al. (2011) who analysed a number of anomalies after carbimazole administration to pregnant albino rats from 10th day of gestation till parturition. This treatment proved to be embryotoxic as different fetal anomalies were observed like clinodactyly of the fifth finger. Growth retardation was observed particularly in long bones (tibia and ulna). In another study experimental rats were orally given carbimazole throughout the pregnancy. Results showed reduction in crown rump length of fetus as well as reduction in thickness of epiphyseal growth plate in ulna and tibia of treated fetuses (Shaikh et al., 2013). Amara et al. (2012) described an experimental study on rats, who were administered methimazole. Fetuses ultimately showed reduced femur length. Reduction in body weight was also observed. Calcium and phosphorus level also declined in bones of the treated fetuses.
CONCLUSION

Purpose of this study is to raise awareness about potential teratogenicity of carbimazole. Above study indicates that carbimazole exerts potentially adverse effects on development as it is reducing the size of different body organs as well as inducing skeletal defects. Therefore, it is suggested that this drug should be prescribed with extreme care and there must be better reporting of congenital anomalies in children of women with Graves disease with or without in utero exposure to antithyroid drug.

REFERENCES


initial treatment of hyperthyroidism. *Clin. Endocrinol (Oxf).* **46(2)**: 240.


