Teratogenic Effect Of Maternal Hypoglycaemia: A Study On Newborn Albino Rats

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Abstract. Hypoglycaemia was induced in primigravida Wistar albino rats during early pregnancy and its effect was studied on the newborn. On d 9.5 of gestation, 1.6 mU of Actrapid human insulin per gram body weight was infused intraperitoneally to two groups of animals. One group was allowed to become hypoglycaemic for one hour and subsequently restored to euglycaemic level while the other group was maintained at euglycaemic levels throughout the experiment and afterwards by intraperitoneal infusion of exogenous dextrose. The same experiment was repeated on d 10.5 with another set of animals. All the animals were allowed to deliver normally. Significant decrease in the mean body weight and crown rump length was observed among the neonates from hypoglycaemic mothers. Gross and histological examination showed that 4.61% neonate of hypoglycaemic animals induced on d 9.5 and 7.93% on d 10.5 had a patent foramen ovale. In one neonate of d 10.5, there was microphthalmia, aphakia and failure to develop neural layer of retina, and the optic nerve anlage was occupied by glial cells covered by connective tissue. The present study revealed that brief maternal hypoglycaemia during early pregnancy induced teratogenic effects on various unreported systems too, in the newborn albino rats and its implications in the human beings need to be taken into account.

Key words: Euglycaemia, Hypoglycaemia, Dysmorphogenesis, Microphthalmia and Aphakia.

Introduction:

In the management of Type 1 diabetes mellitus (Insulin dependant diabetes mellitus-IDDM), hypoglycaemia is frequently encountered as an unavoidable condition. The teratogenic effect of hypoglycaemia during the early period of human pregnancy is uncertain (Mills et al, 1988). Kawaguchi, et al (1994) reported significant growth retardation of the embryos but no teratogenic effect of maternal hypoglycaemia on the rat embryos. Administration of an optimum dose of insulin was reported to be successful in reducing malformation rates in infants born to diabetic mothers (Baker et al, 1981, and Sadler and Horton, 1983). On the other hand, the practice of intensive insulin therapy in Type 1 diabetic pregnant mothers poses a potential risk of life-threatening hypoglycaemia, the ‘Syndrome of Hypoglycaemia Unawareness’ due to loss of normal counter-regulatory hormonal responses to hypoglycaemia. Rosenn et al (1995) also reported similar conditions.


The present study is an attempt to find out any unreported teratogenic effect on the organs or systems of the neonates due to insulin induced brief maternal hypoglycaemia in albino rats.

Materials and Methods:

Fifty virgin female rats, Wistar albino strain, with an average age of 120 days, weighing 120 to 180 grams were procured from National Institute of Nutrition, Hyderabad, India. The animals were acclimatized in an artificially lighted room with a dark period of 11 hours, from 6 p.m. to 5 a.m. of the next day. The animals had free access to standard laboratory feed (ALINCO, New Delhi, India) and potable water.

The animals were housed overnight with normal males of the same strain. Mating was confirmed by the presence of sperms in the vaginal smear on the following morning. Midnight of the day of mating was designated as ‘day zero’ (d 0) of the
embryo development, the subsequent 24 hour period was considered as d 1, the first day of gestation (Kalter, 1968). Sperm positive females were isolated.

The sperm positive animals were divided into the following groups:
(a) Hypoglycaemic Group 1 (HG1): Hypoglycaemia induced on d 9.5-10 rats
(b) Hypoglycaemic Group 2 (HG2): Hypoglycaemia induced on d 10.5-10 rats
(c) Euglycaemic Group 1(EG1): Post-insulin euglycaemic on d 9.5-10 rats
(d) Euglycaemic Group 2(EG2): Post-insulin euglycaemic on d 10.5-10 rats
(e) Control group (CG): 10 rats

Actrapid human insulin (Torrent Pharmaceuticals Ltd, India), at the rate of 1.6mU/gm body weight in 5 ml of normal saline, was infused intraperitoneally via a scalp vein needle to each animal of HG1 on d 9.5 and to HG2 on d 10.5 of gestation respectively. An initial dose of 0.5 ml was infused, followed by the remaining amount spread over a period of one hour. After discontinuation of insulin administration, 10% Dextrose solution was infused to restore the blood sugar to pre-infusion levels within 30 minutes.

Similar amounts of insulin were infused to the animals of EG1 and EG2 on d 9.5 and d 10.5 of gestation respectively. The animals concomitantly received 10% Dextrose in water through another intraperitoneal catheter starting 4 minutes after the insulin infusion. Dextrose infusion was initiated with 30mg/Kg body weight/min and adjusted at intervals of every 15 min to maintain the blood sugar at pre-infusion levels.

Blood samples were collected from the animals’ tail veins prior to infusion; and at 15, 30, 45, 60, 75, 90 and 105 min from the start of infusion. Blood sugar of the samples was estimated with a glucometer (Reflolux Typ. 11721156, Boerhinger).

Neither insulin nor dextrose solution was administered to CG, the control group. The animals of all the groups were allowed to deliver normally at full term.

**Examination of neonates:**

The neonates were sacrificed by cervical dislocation under ether anaesthesia. Crown rump length (CRL) and body weights of the neonates were measured. External morphological features were examined and recorded. The neonates were fixed in 10% Formol Saline for 7 days. The internal organs of all the neonates were examined under stereoscopic dissecting microscope.

The eyeball along with the forebrain and surrounding tissues were prepared for paraffin section. Serial sections, 15 micrometers in

<table>
<thead>
<tr>
<th>Maternal Group</th>
<th>Neonate Number</th>
<th>Litter Size Mean</th>
<th>Weight (Gm) Mean ± SD</th>
<th>CR Length Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>60</td>
<td>6.0</td>
<td>5.72 ± 0.44</td>
<td>5.20 ± 0.07</td>
</tr>
<tr>
<td>EG1</td>
<td>65</td>
<td>6.5</td>
<td>5.70±0.39</td>
<td>5.16±0.09</td>
</tr>
<tr>
<td>EG2</td>
<td>63</td>
<td>6.3</td>
<td>5.69±0.31</td>
<td>5.11±0.06</td>
</tr>
<tr>
<td>HG1</td>
<td>67</td>
<td>6.7</td>
<td>4.75±0.49</td>
<td>4.79±0.20</td>
</tr>
<tr>
<td>HG2</td>
<td>59</td>
<td>5.9</td>
<td>4.08±0.53</td>
<td>4.50±0.12</td>
</tr>
</tbody>
</table>

(++ p < 0.001 vs EG1 & EG2

(+ + p > 0.10 vs HG1

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thickness, were prepared and stained with H/E stain and with Osmium tetroxide stain. Histological findings of the specimens were studied and compared.

**Statistical analysis :**

All data were prepared as group mean values with standard deviation (± S.D.). Inter-group differences in the prevalence of morphologic lesions and all other comparisons were made using unpaired ‘t’ tests.

**Observations and Results :**

The maternal mean blood sugar levels in HG1 and HG2 fell rapidly from the pre-infusion level of 130±5 mg/dl to 55±5 mg/dl by 15 min of insulin infusion. The blood sugar level then declined gradually to 35±2 mg/dl in the next 45 min. Within thirty minutes after the withdrawal of insulin infusion with concomitant exogenous dextrose, the blood sugar level returned to euglycaemic level. However, in the EG1 & EG2, the blood sugar was maintained at the pre-infusion levels throughout the period of experiment lasting 60 min and thereafter.

The mean litter size was not significantly different amongst the various groups. There was significant decrease in the mean neonatal weight and CRL in the offspring of HG1 & HG2 as compared to that of the EG1 & EG2 (Table No. 1). However, there were no statistically significant differences in the mean weight and CRL of the neonates from CG and EG1 & EG2 or between the litters of HG1 and HG2.

Dissection revealed patent foramen ovale in the hearts of 3(4.61%) neonates from HG1 and in 5(7.93%) neonates from HG2 (Fig. 1). The hearts from all the other groups were found normal. One neonate (1.59%) from HG2 had microphthalmia of the right eye (fig. 3), while no such malformation was found in the eyes of other neonates (Fig. 2).

**Histological findings :**

Histologically, there was absence of the neural layer of the retina with aphakia in the microphthalmic neonate belonging to group HG2. The optic nerve fibres could not be identified by optical microscope. Glial cells covered by connective tissue occupied the mass representing the optic nerve (Fig. 4).

**Discussion :**

Normal development in rats during the early phase of organogenesis of embryogenesis depends on uninterrupted glycolysis and its interruption leads to dysmorphogensis (Freinkel et al, 1983 and Buchanan et al, 1985).

In the present study, the findings from the neonates of HG1 and HG2 were compared with those of CG, EG1 and EG2, to relegate the reported controversy regarding embryotoxic effects of insulin. Sadler and Horton Jr (1983) and Buchanan et al (1986) reported insulin to be non-teratogenic in different experimental animals including the rat.

Duration of the induced maternal hypoglycaemia, in earlier studies, was more prolonged as compared to the hypoglycaemia that occurs in the treatment of human patients (Hannah and Moore, 1971, and Okeda et al, 1992). In human clinical studies, however, the incidence of birth defects induced by hypoglycaemia could not be clearly ascertained (Mills et al, 1988).

Buchanan et al (1986) reported ‘gross development anomalies’ in albino rat embryos subjected to brief maternal hypoglycaemia. In another study, Kawaguchi et al (1994) declined to attribute any birth defect to maternal hypoglycaemia. In the present study however, 3 neonates (4.61%) from HG1 and 5(7.93%) from HG2 group had patent foramen ovale.

Sadler (1995) reported microphthalmia, chorioretinitis, retinal dysplasia and cataract due to maternal infection during embryonic period of gestation. Gene mutation is also reported to cause microphthalmia (Muller and Young, 1998). In this study, however, one (1.59%) neonate had microphthalmia with failure of development of the neural layer of the retina. Consequently, the nerve fibres in the optic nerve could not be demonstrated by light microscopy and the mass of the optic nerve was composed of glial cells covered by connective tissue. There was aphakia in the same case. Such malformations are attributed to disturbances in cell interaction between the lens placode and optic
vesicle (Larsen, 1997), one of the factors may be maternal hypoglycaemia.

Although the high percentage of malformations may be attributed to the small sample size, it is evident that maternal hypoglycaemia during early organogenesis causes teraogenic effects in the rats. The applicability of the present findings in human beings may be debatable, but it is an indication that precautionary measures are needed in the treatment of Type-1 diabetic mothers.

References:

Fig. 1

Fig. 2
Photomicrograph of the eye of control neonate in coronal plane. Haematoxylin & Eosin stain x13.2 L = Lens, R = Retina, On = Optic nerve.
Fig. 3

Fig. 4
Photomicrograph of the Optic nerve in Fig. 3. Note the absence of nerve fibres. Osmium tetroxide stain x330. Gc = Glial cell.