Teratogenic effects of dilantin on thoraco-abdominal organs of developing chick embryos

M Singh, G L Shah & K P Singh
Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India
Received 25 August 1999; revised 3 May 2000

Congenital anomalies on some viscera like heart, liver and kidney have been investigated in chick embryos after a single injection of dilantin (3 mg/egg), a known antiepileptic drug, on 4th day of incubation. On 19th day of incubation, chick embryos were collected to observe the gross malformations and histological changes in heart, liver and kidney. On gross examination, visceroptosis (29%), thin anterior abdominal wall (28%), ectopia cordis (10%) and dextrocardia (1%) were observed. Histological examination of the kidney revealed glomerular degeneration in kidney while in liver, dilated central veins with degenerated hepatocytes were present. Longitudinal section of the heart showed thicker musculature specially of ventricles with a narrower lumen in comparison to that of the control. The results indicate teratogenicity of dilantin in developing chick embryos.

Phenytoin (diphenylhydantoin sodium, dilantin) is one of the most frequently prescribed antiepileptic agent. Due to its basic action to block Na\(^+\) and Ca\(^{2+}\) channels\(^1\) it also has other clinical uses in the treatment of various CNS disorders\(^2\) including cardiac arrhythmias. Chronic prenatal exposure to phenytoin during critical period of somatic and neural development in rodents\(^3\) induces various congenital anomalies including cleft palate\(^4,6\), limb deformities like ectrodactyly and digital hypoplasia\(^7,11\), hydronephrosis and tubular degeneration\(^12\) and malformations of CNS like alteration in cerebellar cytoarchitecture and loss of purkinje cells\(^13,15\). Similarly, clinical studies also prove the validity of phenytoin as a potent teratogenic agent if selected for chronic treatment of epileptic mothers during pregnancy\(^16\). In humans, reports indicate that a large number of infants whose epileptic mothers were under prolonged phenytoin treatment during pregnancy presented with Fetal hydantoin syndrome (FHS) with characteristic physical features like craniofacial, skeletal, ocular and cardiac abnormalities, nail and digital hypoplasia, microencephaly, cerebellar ataxia with deficit in pyramidal and purkinje cells which ultimately lead to mental retardation\(^9,17,18\).

Therefore the present study has been aimed to investigate if phenytoin can induce any thoraco-abdominal anomalies in chicks.

Materials and Methods
Fertile eggs of white leghorn chicks were incubated at 37\(^\circ\) ± 1\(^\circ\) C and 65-85% RH. The total number of eggs used were one thousand out of which seven hundred and fifty were treated with the drug and two hundred and fifty were treated with an equal volume of normal saline as controls.

Eggs were turned twice a day by a mechanical device throughout the period of incubation. Each experimental egg was injected with 3 mg of dilantin (Epsolin-Cadila) [3 mg/egg] on 4th day of incubation with a tuberculin syringe\(^19\). Similarly, control eggs were treated with normal saline on day 4 of incubation.

The chicks were collected on 19th day of incubation by breaking the eggs and were observed for gross malformations of heart, liver and kidney. The viscerae collected after observation were fixed in 10% neutral formaline for further processing for histological observations after staining with H and E.

Results
On gross examination of treated chick embryos only 55% embryos were found to be viable and 45% were dead, while in control only 10% embryos were dead. Of treated viable embryos, total visceral defects was 68%, out of which, visceroptosis 29%, thin anterior abdominal wall 28%, ectopia cordis 10% and dextrocardia 1% were found. In the specimen, where there was no visceroptosis, the anterior abdominal wall had sparse hairs. Visceroptosis was more frequent in abdominal region (Fig. 1a). In those treated specimen where the anterior abdominal wall
Fig. 1—Dilantin treatment on embryonic day 4 showing gross appearance of thoraco-visceral anomalies in chick embryos. [(a) visceroptosis (b) poorly developed hairs on ventral body wall, and (c) haemorrhagic spots over atrial region of heart].

Discussion

Administration of dilantin during embryonic period of developing chick induced ectopia viscera in thoraco-abdominal region and degeneration of hepatic cells and renal glomeruli. So far, there is paucity of literature on hydantoin induced malformations of thoraco-abdominal viscera in chicks. The results obtained from human studies substantiate the present observation. Haemorrhagic disease in newborn children has been observed in the offspring of those pregnant mothers who were exposed to hydantoin anticonvulsant therapy during first trimester of pregnancy. In the present study haemorrhagic spots over atrial region of heart after single injection of dilantin was also observed corroborating well with the findings of Stevenson and Gilbert.

Collins et al. have reported forelimb ectrodactyly induced by single ip administration of sodium diphenylhydantoin. In addition to ectrodactyly other malformations like cardiovascular defects, hydronephrosis, absence of kidney(s) (anephrosis) and skeletal deformities have also been reported in mice. In the present study also renal glomerular degeneration and hepatic cells degeneration have been reported. Limb anomalies and cardiac anomalies in hydantoin exposed chicks have been reported. The present findings are in agreement with those of Stevenson and Gilbert.

It is clearly documented that each organ goes through its most susceptible stage early in its differentiation and thus various organs may become susceptible in succession. In chick, embryonic differentiation begin almost simultaneously with incubation, the undifferentiated stage being passed within genital tract of mother and all major organogenesis begins by 48 hr of incubation. Hence any drug injected before or during first few hours of incubation, will present itself in sufficient amount during susceptible period causing disturbance in normal embryogenesis. The FHS, per se, probably represents derangement of embryogenesis and organogenesis.

Buehler et al. have reported that children with more clinical signs of the FHS have a lower level of activity of epoxide hydrolase, an enzyme involved in epoxide detoxification. It has been hypothesized that formation of epoxide intermediate during the metabolism of phenytoin, rather than the specific drug itself, may be the teratogenic agent which may impair the fetal malformations. In the present study it may also be hypothesized that the drug used is mediating through its metabolites. Further, studies
Fig. 2a-d
Fig. 2—Paraffin sections of kidney, liver and heart. (a) control kidney, H&E × 268; (b) treated kidney showing dilation of tubules (arrow) along with clumping of degenerated glomeruli (arrow head), H&E, × 268; (c) control liver H&E, × 168; (d) treated liver showing dilated central vein (CV), derangement of cytoarchitectural pattern and degeneration of hepatic cells, H&E, × 256; (e) L.S. of control heart; (f) treated heart showing thick ventricular musculature of the hypertrophied heart, and (g) treated heart showing hypertrophied ventricular elongation of the heart with haemorrhagic spots (arrow head) H&E, × 4.16.
are required on the dilantin plasma level, much remaining to be done in this aspect.

Thus, it may be concluded that dilantin or other hydantoin anticonvulsant agents may interfere directly or indirectly the embryogenesis of developing chick embryos. It is also summarized that even a single administration of dilantin during the critical period of embryogenesis produces congenital anomalies on thoraco-abdominal organs in developing chick. Thus, teratogenic effects of antiseizure agents necessitate careful consideration of proper clinical care of epileptic women during pregnancy. Therefore, therapeutic recommendation of dilantin during pregnancy may again be questioned.

References
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