Placental changes induced by Trichloroacetic acid in rat

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Abstract- Histological changes in the placenta induced by Trichloroacetic acid(TCA) were studied in Charles Foster rat. TCA is one of the most common contaminants in drinking water formed following purification of water by chlorination process. TCA in the form of sodium trichloroacetate was administered by oral lavage to pregnant mother rats in the dose of 1000, 1200, 1400, 1600 and 1800 mg/kg body weight during the period of organogenesis i.e., from day 6 to day 15 of gestation. The placental and fetal changes were studied on day 19 of gestation. TCA resulted in fetal resorption as high as 92.15 % with 1800 mg/kg (P<0.001). A trend of decrease in the weight of placenta with the increased dose of TCA was observed along with hemangiomatous lesions, extensive hemorrhage and necrosis in the decidua basalis; infarction of the placenta; the breech and the hemorrhage along the Reichert’s membrane and reduced villigenesis. It is postulated that TCA induced placental changes resulted in embryonic anemia and subsequently embryonic anoxia, which forms the ground for reduced nutritional supply and O₂/CO₂ exchange which may alter the rates of the cell division, programmed cell death and cell to cell communication. These factors may be the root of alteration in the embryonic milieu which might have a direct bearing on the size, weight and architecture of the placenta and the fetus.

Key words – Placenta, Trichloroacetic acid (TCA) , humic and fulvic acid , anoxia , necrosis.

Introduction
Chlorine was introduced as a disinfectant to urban water supply to improve the hygienic quality of water by eliminating the waterborne bacterial pathogens and the consequent transmission of water borne diseases. Chlorine reacts exist in water as hypochlorous acid and hypochlorite ion (0.2-1.0 mg/L). They react with the natural organic compounds such as humic and fulvic acids to form several halogenated compound, Tibbet’s (1995). Both humic and fulvic acids are found in the soil resulting from the chemical and biological degradation of the dead organism. They are ubiquitous in the ground. They have been known to interact with the nutrients, toxic metals, radionucleotides and the halogens of the drinking water treatment to produce halogenated substances, among which chloroform and trichloroacetic acid are the most commonly found, WHO (1993). These are then directly introduced into public drinking water with obvious health consequences, Davies and Ghabbour (1998).

A tremendous research has been performed on the teratogenic mechanism on the embryo and the other associated tissue, since it is generally believed that the site of action of a chemical teratogen is an embryo itself and in the tissue that are directly or indirectly involved in the fetal malformations.

Chemical induced alteration on the placenta could by itself cause adverse effect in the embryo. Inadequate embryonic nutrients may influence the morphogenesis either on its own or by modulating the homeobox genes and protooncogenes involved in the prenatal development, Khera (1992).

In order to evaluate whether the detrimental effects caused by TCA are due to its action directly on the fetus by crossing the materno-fetal barrier or indirectly by altering the placental architecture, the present experiment was undertaken.

Material and Method
The study group inbreded Charles Foster strain of male and female rats of 85 to 120 days weighing to an average of 225 ± 60 g were included. They were obtained from the animal house, Department of Anatomy, Institute Of Medical Sciences, Banaras Hindu University. They were maintained in environmentally controlled rooms at temperature of 28-32 ° C, 40-60 % humidity, in the noise free environment and 12 hours light – dark cycle. The females and the males were placed individually in the solid bottom plastic cages. All the rats had access to water and animal diet ad libitum. The morning of finding sperm positive vaginal smear was defined as day 0 of gestation.

Trichloroacetic acid was obtained from LOBA CHEMIE PVT .LTD, (Mumbai). The solution made in distilled water, was adjusted to the ph 7.0- 7.5 by titration with sodium hydroxide. It was administered as a dose of 1000, 1200, 1400, 1600, and 1800 mg/kg body weight to pregnant mother rats during the period of organogenesis i.e., from day 6 to day 15 of gestation by oral lavage.

Each rat dams were euthanatized using overdose of ether anaesthesia, on the day 19 of gestation in order to avoid premature birth. Distilled water treated
controls were included in the test group. The gravid uterine horns were exposed by lower midline abdominal incision. The fetuses and the placenta were examined in situ. Each placenta was carefully examined for abnormality. They were then removed and preserved in 10% buffered neutral formalin for 3 weeks. They were dehydrated and embedded in paraffin. Serial section 6 m thick were cut and stained with hematoxylin and eosin. Observations were made by examining the serial section under optic microscope.

Statistics of the individual data were analyzed by using Ficher’s exact test. Difference between the dose group and the control groups were found using pair wise 't' test.

Observation and result

Control Placenta, day 19 of gestation

A discoid shape placenta was observed. The placenta had two surfaces. The convex surface attached to the uterine musculature and the concave surface faced the embryo. It was thickest at its centre and, its thickness decreased at its periphery. The embryonic surface formed an acute angle with the uterine surface of the placenta at the periphery (Fig.1). Each placenta had an umbilical cord, which arose from its centre containing the uterine vessels clearly visible within the substance. The mean weight of placenta was 732.23mg (table 1).

Treated placenta, day 19 of gestation

The 1000 mg and 1200mg TCA/kg treated placenta revealed no abnormality on gross inspection, nevertheless, multiple foci of hemorrhagic area were observed with 1400 mg TCA/kg and above dose treated placenta. While the mean weight of 1000 and 1200mg TCA treated placenta were 679.80 and 545.93, respectively, showed no significant change in comparison to the controls, the mean of 1400 mg TCA/kg placenta was 402.15, which was significantly reduced when compared to the controls, (p<0.05). At 1600 and 1800 mg TCA/kg body weight, the treated placenta had a mean of 384.95 mg (P<0.01) and 302.43 mg (p<0.001), respectively showing a significant reduction when compared to the controls. A trend of decrease in the weight of the placenta with the increase in the dose of TCA was, thus observed.

Histological changes in the placenta

According to Cinquetti et al (1983), the chorioallantoic placenta of the rat has two regions; the junctional zone and the labyrinth. The junction zone was composed of (a) outer giant cells that separated the exteriorly located decidua basalis and (b) inner trophospongium consisting of a highly packed basophilic spangioblast cell. In the decidua basalis, the maternal blood spaces were lined by cytotrophoblast and syncitiotrophoblast. The trophospongium contained sparsely distributed large necrotic areas, each enclosing a vascular space surrounded by concentric layers of degenerated cells.

Fig. 1. Diagrammatic representation of the of chorion-allantoic placenta of a rat from sagittal section, Khera (1992).
Cellular strands from the trophospongiosum extended into the labyrinth. In the labyrinth, the communication network of the maternal lacunae containing maternal erythrocyte could be distinguished from embryonic capillaries lined by the endothelial cells having, deeply stained basophillic and semilunar – shaped nucleus in cross section (Fig. 3A and Fig. 3B). The materno embryonic nutritional and gaseous exchange, is believed to take place in the labyrinth through the trophoblastic layer which begins to develop on day 9 of pregnancy and consist of three layers of overlapping cyto and syncytiotrophoblasts and an embryonic capillary endothelium, Hernandez-Verdun et al (1974).

Cytotrophoblasts lines the basal venous receptacle which is a flattened cavity, located at the deepest part of the labyrinth. The maternal blood circulates from towards the embryo, through intricate vascular spaces in the decidual tissue between the mural trophoblast and ultimately in the vascular network adjacent to the surface of the Reichert’s membrane. Jollie et al, (1990) suggested that the maternal blood in the network and the Reichert’s membrane are separated by the fenestrated and the attenuated processes of the trophoblasts. The embryonic or the vitelline vascular system vessels containing nucleated erythroblast lined the interior of the visceral layer of the yolk sac (Fig. 5A). The parietal layer had two parts; the capsular parietal yolk sac -A portion related to overlying mural trophoblast and decidua capsularis and the placental parietal yolk sac. A portion that covers the fetal surface of the choorioallantoic placenta : the yolk sac endodermal epithelium was separated from the portion chorionic ectoderm (trophoblast) by a singularly thick basement membrane termed the Reichert’s membrane.

**TCA treated placenta, day 19 of gestation :**

The placenta of the 1000mg TCA /kg showed similar architecture to that of the control (Fig. 2A and fig.2B). With slightly high dose i.e., 1200 mg TCA/kg, the cytrophoblasts around the embryonic capillaries were undergoing degeneration (Fig.4B). The trophoblasts in the trabeculae showed necrotic foci as well as degeneration of the trophoblastic barrier, which resulted in the admixture of the embryonic erythroblast with the maternal erythrocytes.

The 1400, 1600 and 1800 mg TCA /kg dose produced changes mainly at the following sites (1) cytotrophoblastic lacunae separating the maternal circulation from the embryonic circulation in the labyrinth;(2) the Reichert’s membrane and (3) the mesometrial surface of the placenta.

In the labyrinth the cytrophoblasts surrounding the capillaries showed degeneration (Fig. 4C and Fig.4D). The trophoblast illustrates multiple necrotic foci and disintegration of the trophoblastic barrier that separates the blood in the sponge like maternal lacunae from the blood in the meshwork of the embryonic capillaries, resulting in the admixing of the maternal erythrocyte and fetal erythroblast (Fig.2 C and Fig.4C (arrows). Furthermore, “hemangiomatous” lesions of the maternal vascular lacunae in the labyrinth were observed. Hemangiomatous lesion were defined to

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### Table 1: Histological changes in the placenta following exposure to TCA at sacrifice on day 19 of gestation

<table>
<thead>
<tr>
<th>Treatment (mg/kg /day)</th>
<th>Control</th>
<th>1000</th>
<th>1200</th>
<th>1400</th>
<th>1600</th>
<th>1800</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of litters examined</td>
<td>5 with 2 embryos / L</td>
<td>5 with 2 embryos / L</td>
<td>5 with 2 embryos / L</td>
<td>6 with 2 embryos / L</td>
<td>6 with 2 embryos / L</td>
<td>5 with 1-2 embryos / L</td>
</tr>
<tr>
<td>No. of placenta Showing lesion/ Total examined</td>
<td>0/10</td>
<td>0/10</td>
<td>1/10</td>
<td>11/12</td>
<td>12/12</td>
<td>10/10</td>
</tr>
<tr>
<td>a. Lesions in the trophoblastic trabeculae in the labyrinth</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>b. Occurance of fetal hemorrhage in the maternal circulation</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>c. Reichert’s membrane breech and hemorrhage along its length</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>d. Peripheral zone of the placenta, hemorrhage and necrosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>e. mesometrial zone of placental necrosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Mean weight of the Placenta ± S.D.</td>
<td>732.23 ± 16.39</td>
<td>679.80 ± 16.48</td>
<td>545.93 ± 18.82</td>
<td>402.15 ± 15.32</td>
<td>384.95 ± 9.73</td>
<td>302.43 ± 2.74</td>
</tr>
</tbody>
</table>
Fig. 2 Photomicrograph of a Chorioallantoic placenta of a rat, on day 19 of gestation. A: Placenta of control rat, showing myometrium, major maternal venous spaces, decidua basalis, trophospongium, and labyrinth. B: Placenta of 1000 mg TCA/ kg treated group, having similar architecture as the control. C: 1400 mg TCA treated placenta, having a rupture of trophoblastic barrier (arrows) that extend through the width of the labyrinth, hemorrhage in the mesometrial region( * ) and necrosis in the peripheral region ( . ). D: 1800 mg TCA/ kg treated placenta showing extensive hemorrhage ( * ) and necrosis in trophospongium.

Fig. 3: A: Labyrinth of a control rat (day 19 of gestation) showing trophoblastic trabeculae consisting of cytotrophoblasts (thin arrow) and syncytiotrophoblast (thick arrow), fetal capillaries lined by endothelial cells (FC) containing fetal erythroblast (arrow head) and maternal lacunae (ML) containing maternal erythrocytes (arrow). x 600.
those areas which exhibited dysplasia of the maternal labyrinth into cluster of microhematomas or blood communicating caverns. The caverns were broken following degeneration of the trophoblasts and were lined with trophoblastic stroma that contained fewer embryonic capillaries (Fig.4C and Fig. 4D). The maternal lacunae that had escaped the serious assault were filled with plasma and contained fewer erythrocytes,(Fig. 4D). Extensive areas of hemorrhage could be seen within the labyrinth, (Fig.5A).

The Reichert’s membrane continuity was broken at places (Fig. 5A and Fig. 5B), and hemorrhage along its whole length could be seen , (Fig 5A Rm). There was reduction in the villous formation showing suppressed villigenesis, (Fig.5A).

The mesometrial surface of the placenta showed extensive area of necrosis to a varying depth, and hemorrhage , (Fig.4C, 4D and Fig. 5C,5D).

Discussion
Relation of the lesion with the teratogenecity
TCA was administered for 10 consecutive days, since the continued dose for ten consecutive days did not produce any explicit signs of maternal toxicity, therefore it was continued as such throughout the experiment.

In the present study a significant resorption rate was observed at 1000 mg TCA/kg treated rat which increased to a highly significant rate at 1800 mg TCA/kg treated rat. Moreover reduction in the mean fetal weight and fetal abnormalities were observed at 1000 mg/ kg and above dose . Since the placenta forms the main barrier to the exogenous toxins administered to the mother, it might be the target organ on which the drug acts, subsequently producing the detrimental effects on the embryo secondary to placental changes.

Khera (1992 ) states that the development of the labyrinth in the placenta depends on the coherent simultaneous growth of three differential components;

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**Fig. 4** Labyrinth of the control placenta showing honey comb appearance. A. B: 1200 mg /kg treated placenta showing foci of trophoblastic necrosis (arrow). C: 1400mg /kg showing hemangiomatous lesion, disruption of trophoblastic barrier (white arrow), dilated maternal lacunae filled with microhematomas and containing few RBC, along with plasma filled areas around the fetal capillaries ( * ).D: 1800mg/kg placental labyrinth showing extensive foci of degeneration (thin arrow), dilated and elongated maternal lacunae, empty or filled with very few RBC (white arrow). x 200.
Intercommunicating maternal vascular lacunae.

Branching embryonic capillaries extended from the allantoic villi.

The trophoblast that separates the maternal and the embryonic blood, and through which materno-embryonic exchange takes place.

These three components are integrated into interconnected trabeculae that forms a sponge like network, which gives it a “honey-comb appearance” (Fig. 2A and Fig. 3A).

TCA at 1000 mg/kg body weight and above dose group seemed to disrupt the synchrony in the development of the cytotrophoblast, maternal vascular lacunae, and embryonal capillaries in the labyrinth. The materno-fetal surface area for exchange were reduced. These changes are likely to modulate nutritional and homeostatic conditions in which the embryo develops and may thus influence its viability and growth. Serious placental changes are known to cause the adverse effects on the embryonic developments. Failure of villous formation at the endometrium chorioallantoic border and the absence of endometrial invasion by the chorionic cells have been reported to result in embryonic death and abortion in donkey-in-horse pregnancy established by embryo transfer, Allen (1982). A reduction in the surface exchange area and decreased parenchymal tissue in human and sheep placentas has been reported as the major determinant of the embryonic growth, Aherne and Dunnill (1966); Kulhanek et al (1974); Molteni et al (1978); Teasdale, 1984. Uteroplacental embolization in pregnant sheep caused intrauterine growth retardation (IUGR) that resulted from decreased oxidative metabolic rate in the embryo, Clapp et al (1981) and Block et al (1990). Placental lesions such as thrombus formation, infarction have been reported to play an important role in spontaneous abortions of humans, Ornoy et al (1981).

The well apparent gross as well as histopathological changes in the embryo at 1400 mg/kg body weight were seen (Fig. 4A). The most prominent change was the decreased number of villi. The villi were smaller, more compact and were distributed in a more regular pattern. The fetal vessels were also smaller and fewer in number. The intervillous space was filled with a dense network of fetal vessels and connective tissue, with little free space. The maternal vessels were more abundant and larger in diameter. The trophoblastic layer was thinner and more lucent.

Fig. 5. A: 400 mg /kg treated placenta showing breech in the reichert’s membrane (arrow) and hemorrhage along its entire length (Rm). Reduced villi, and large hematoma in the labyrinth. B: Peripheral edge of the 1800 mg/kg treated placenta with broken reichert’s membrane (arrow), necrosed and sloughed placental tissue with large area filled with plasma (*). C: Necrosis in the trophospongium (white *). D: Necrosis of the mesometrial surface of the placental tissue (*). x100.
kg body weight and above dose group were always found associated with the respective presence and absence of the placental lesions. This association between the placenta and the embryo at these doses needs further analysis. However it would be thoughtless to discard the placental changes as being one the factors causing alteration in the fetal development. A severe blood loss of the embryonic could cause embryonic anemia and, subsequently, embryonic hypoxia. Anoxia at lower dose induces apoptosis, Kam et al. (2000). Khera (1992), states that the loss of the embryonic blood with the subsequent ischemia could reduce the nutritional supply and O$_2$/CO$_2$ exchange which may alter the rates of the cell division, programmed cell death and cell to cell communication. TCA enhancing these factors may be the root of alteration in the embryonic milieu which might have influenced the organs development that have taken place during the period of organogenesis during the dosing of TCA, i.e., heart, kidney etc. and may have thus have a direct bearing on the size, weight and architecture of the placenta and the fetus.

References
12. Khera KS. Extraembryonic Tissue changes Induced by 2,3,7,8 tetra chlorodibenzo-p-Dioxin and 2,3,4,7,8- pentachlorodibenzofuran With a note on dierrection of maternal blood flow in the labyrinth of C57BL /6N Mice. Teratology, 1992;45:611-627.