

Histopathological Changes in Multiple Fetal Tissues Exposed In-Utero to Zidovudine

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Abstract

Objective: Zidovudine, the first antiretroviral drug is used to prevent vertical transmission of HIV infection. Without any adequate proof of its safety to fetus, the drug was administered to pregnant women. The present experiment aims to study at the light microscopic level, the effect of zidovudine in fetus exposed in-utero to the drug.

Material and method: Sixty Swiss mice were divided into two groups of control (n=20) and experimental (n=40). A dose of 50 mg/kg/day was administered orally to experimental group and an equivalent amount of normal saline to control group. Drug was administered from day 8 to day 16 of gestation and on day 19 the animal was sacrificed. Fetus collected after laparotomy were fixed in 10% neutral formalin and then subjected to light microscopic study to assess the histopathological changes. H&E stained sections of liver, lung, kidney, brain and maternal ovary was analysed.

Result: Fatty degeneration of liver, degenerative changes in kidney section, dilatation of alveoli with thinning of alveolar wall, microcystic degeneration in cerebral cortex was observed. The maternal ovary of experimental group had small corpus luteum.

Conclusion: Multiple tissues are affected by in-utero administration of ZDV. Further study at ultrastructural level is needed.

Keywords: Zidovudine; Histopathology; Fetus; In-utero; Kidney; Brain

Introduction

Zidovudine (3'-azido 3'-deoxythymidine, azidothymidine, ZDV) is the first antiretroviral agent used to treat patients suffering from HIV-AIDS. Since 1994, this drug is also used to HIV positive pregnant women to prevent vertical transmission of the virus during pregnancy [1]. ZDV was an indispensable prophylactic drug especially to HIV positive pregnant women after a US Public Health Services Task Force recommended its use for reduction of perinatal HIV-1 transmission [2]. Despite the dramatic increase of ZDV in the perinatal use of ZDV, information about the microscopic consequences of use of ZDV to fetus, infants and children who were exposed to the antiretroviral drug in- utero is still meager [3-5]. The present study was therefore, carried out to investigate the prenatal effect of ZDV in fetus at the light microscopic level. In fact, the article may be considered as the continuation of teratogenic effect of ZDV published earlier by us [6-8].

Material and Methods

The present study was conducted in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi on 60 successfully pregnant Swiss mice. A room temperature of 25°C ± 2°C with relative humidity of 45-55% and 12 hr light-dark cycle was maintained. Animals were fed with readymade rodent pellet diet and water ad libitum. Zidovudine was purchased from pharmacy through antiretroviral therapy (ART) Centre, Institute of Medical Sciences,

BHU, Varanasi. Zidovudine Oral Solution USP (Zidovir), manufactured by CIPLA Ltd., Goa, India was used. One milliliter of oral solution contained 10 mg of active zidovudine. The pregnant mice were divided into two groups:

Group A: Control group (n=25)

Group B: Experimental group (n=40)

A dose of 50 mg/kg/day of zidovudine were administered orally to the experimental pregnant mice and an equivalent amount of normal saline was administered to the control group. First dose commenced on gestational day 8 and continued daily and the last dose was administered on gestational day 16. Mice were sacrificed on gestational day 19 by cervical dislocation. Fetus were collected by laparotomy. The entire fetus was fixed in 10% neutral formalin for not less than 10 days. One fetus per litter was randomly selected for microscopic study. Fetal tissues used in the study were kidney, liver, lung, brain and maternal ovary was studied. A 7 µm thick section was cut, stained with H&E and examined by light microscopy.

A prior permission was taken from the Institutional Animal Ethics Committee.

Result

Outcome of pregnancy in experimental group

Out of 40 experimental mice, 19 pregnant mice failed to gain weight after 5-6 days of treatment. These mice were observed for few more

days and sacrificed after constant weight was observed on 16th or 17th day of gestation. All the 19 (47.5%) mice had 100% fetal resorption characterized by dilated and highly vascular uterine horns (Figure 1). Three pregnant mice had bleeding per vagina on day 13th day of gestation. They were sacrificed and showed teratogenic changes in the fetus [6]. Remaining 18 (35%) pregnant mice carried the pregnancy to term which eighty six implantations were observed compared to 168 in the control group. The litter size were 4.53 ± 0.99 in the experimental group as compared to 6.5 ± 1.2 in the control (Figure 2 and Table 1).

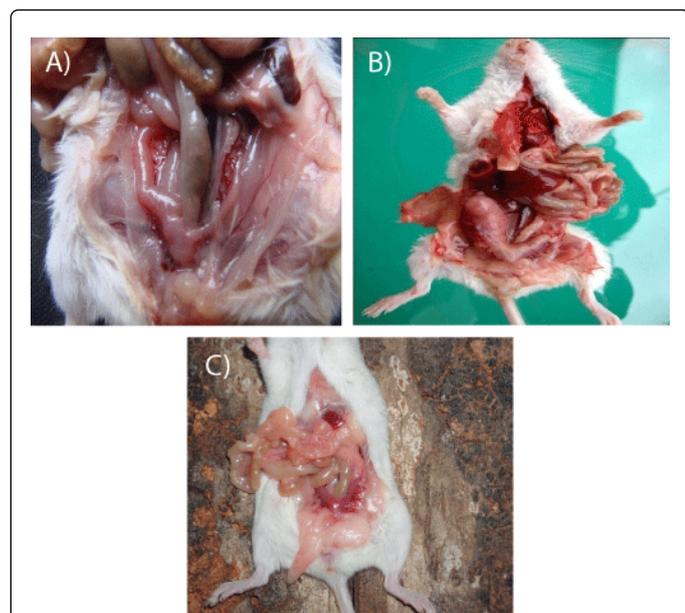


Figure 1: Dilated and vascular uterine tube in total resorption of fetus in experimental group.

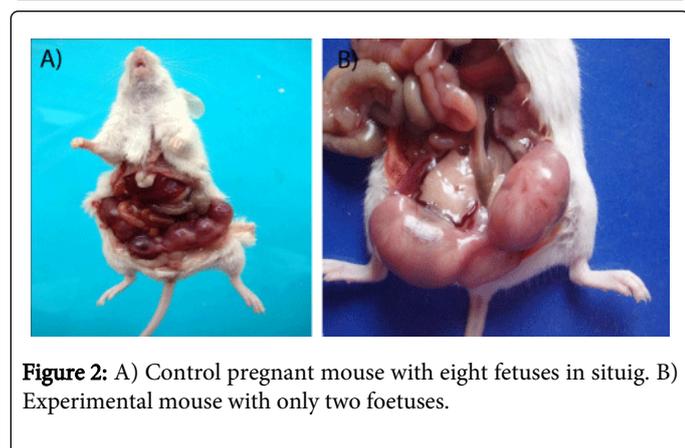


Figure 2: A) Control pregnant mouse with eight fetuses in situ. B) Experimental mouse with only two foetuses.

| | Control | Treated |
|---|-----------|------------|
| Total pregnant mice | 20 | 40 |
| No. of pregnant mice successfully carrying to term, GD 19 (%) | 20 (100%) | 18 (35%) |
| No. of pregnant mice with complete fetal resorption (%) | 0 | 19 (47.5%) |

| | | |
|--|---------------|-----------------|
| No. of pregnant mice with vaginal bleeding during pregnancy(%) | 0 | 3 (2.56%)* |
| No. of implantation sites | 168 | 86 |
| Total live fetuses | 166 | 77 |
| No. of dead fetus (%) | 0 | 2 (2.56%) |
| No. of resorbed implantation site (%) | 2 (2.7%) | 7 (8.9%) |
| No. of growth retarded fetuses (%) | 19 (11.3%) | 54 (70.1%) |
| No. of fetus with birth defects (%) | 0 | 3 (3.91%)* |
| Litter size | 6.5 ± 1.2 | 4.53 ± 0.99 |

Table 1: Zidovudine administration (50 mg/kg/day) from GD8 to GD 16 and outcome o pregnancy *ref 6.

Maternal weight gain during pregnancy

Mean weight of pregnant mice in the experimental group on day 0 of gestation was 25.91 ± 2.04 as compared to 25.58 ± 3.41 in the control group. Mean weight of pregnant mice on day 19 of gestation were 34.62 ± 3.63 and 37.28 ± 5.03 in experimental and control group respectively. However, the differences in the gain in weight were statistically not significant (Table 2).

| | Group | Total pregnant mice | Body weight in gm (mean \pm SD) | p-value |
|---------------------|---------|---------------------|-----------------------------------|---------|
| Body weight on GD0 | Control | 20 | 25.58 ± 3.41 | >0.05 |
| | Treated | 18 | 25.91 ± 2.03 | |
| Body weight on GD19 | Control | 20 | 37.28 ± 5.03 | >0.05 |
| | Treated | 18 | 34.62 ± 3.63 | |

Table 2: Weight gained by the control and experimental pregnant mice from GD8 to GD16.

Histopathological changes in fetal tissues exposed in-utero to Zidovudine

Light microscopic study was based on random selection of two fetus per litter of the experimental (n=36) and one or two fetus per litter of the control group (n=30).

Liver: Fatty degeneration was observed in the experimental group as compared to normal histology in the control (Figure 3).

Kidney: Marked degenerative changes were observed in the glomerulus, both the layers of Bowman's capsule as well as the tubules. Control group showed no change in the histological architecture of kidney (Figure 4).

Lungs: Alveoli were dilated and some areas of lung field showed rupture of alveoli in the experimental group. Normal lung parenchyma observed in the control group (Figure 5).

Brain: Microcystic degeneration was observed in the cerebral cortex in the experimental group. Sections were normal n the control group (Figure 6).

Maternal ovary: The size of the corpus luteum were small as well as less in number in the experimental group whereas the corpus luteum are large in the control group (Figure 7).

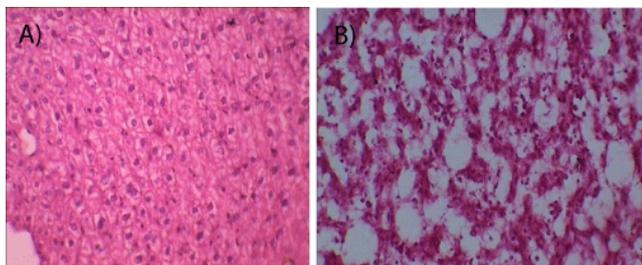


Figure 3: A) Photomicrograph of section of fetal liver (control), H&E; 400X. B) Photomicrograph of section of fetal liver (experimental) showing disruption of normal lobular architecture with features of macro and micro steatosis (white arrow), H&E; 400X.

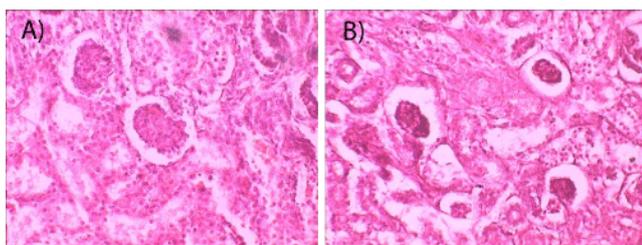


Figure 4: A) Photomicrograph of section of fetal kidney (control), H&E; 400X. B) Photomicrograph of section of fetal kidney (experimental) showing features of glomerulosclerosis (white arrow), mild tubular necrosis along with a few mixed inflammatory cellinfiltrates in the interstitium, H&E; 400X.

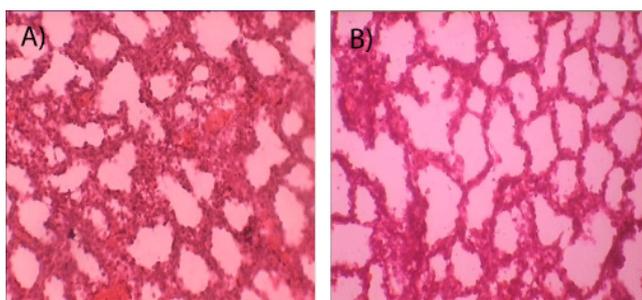


Figure 5: A) Photomicrograph of section of fetal lung (control), H&E; 400X. B) Photomicrograph of section of fetal lung (experimental) showing evidence of atelectasis, interstitial oedema with focal areas of type-2 pneumocyte hyperplasia (black arrow heads), H&E; 400X.

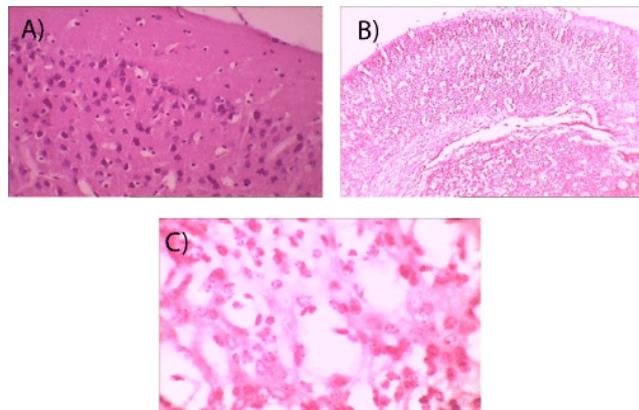


Figure 6: Photomicrograph of section of fetal brain (control), H&E; 400X. Photomicrograph of section of fetal brain (experimental) showing microcystic degeneration (black arrow heads), H&E; 400X & 1000X.

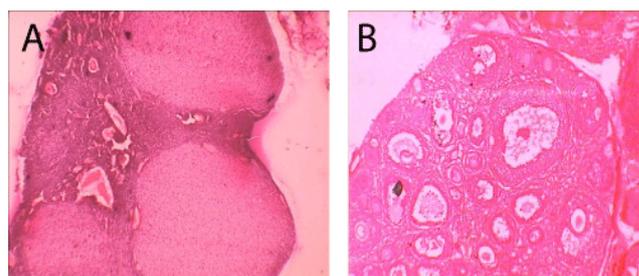


Figure 7: A) Photomicrograph of section of maternal ovary (control) with normal corpus luteum (white arrow), H&E; 100X. B) Photomicrograph of section of maternal ovary (experimental) showing atretic corpusluteum (white arrow), H&E; 100X.

Discussion

Histopathological changes observed in multiple tissues of fetus could be the result of the mechanism of action of Zidovudine. ZDV is structurally related to endogenous nucleoside thymidine, differing in the replacement of hydroxyl group at the 3'- position of the ribose ring with an unreactive azido (N₃) group. Therefore, ZDV has a strong affinity for DNA polymerase β and γ . Thus, it acts by inhibiting reverse transcriptase enzyme [9]. DNA polymerase α and β are capable of incorporating ZDV-monophosphate into DNA and cause chain termination [10].

Preferential incorporation of ZDV into telomeric DNA and cause shortening from 22 Kb to 2 Kb in DNA of lung, liver and brain tissues from ZDV-exposed newborn mice [11]. Poirier et al. observed incorporation of ZDV into DNA of fetal liver; lung, heart, skeletal muscle, brain, testis and placenta were observed when ZDV was infused to pregnant rhesus monkeys which varied between 29 and 1944 molecules of ZDV/106 nucleotides. ZDV was also found to be threefold higher in fetal blood and amniotic fluid than those in maternal blood [12]. Bialkowska et al. were first to identify the oxidative damage to nuclear DNA in fetal liver and kidney tissues of

pregnant swiss mice and patas monkeys. Increased level of 8-oxo-2'-deoxyguanosine, a reactive oxygen species, may contribute to the mechanism of transplacental carcinogenesis [13]. In human samples too, Olivero et al. and Meng et al. observed incorporation of ZDV in DNA of cord blood cell of newborn infants exposed in-utero to ZDV by using anti-ZDV radioimmunoassay [14,15]. Using trypan blue exclusion technique, Slamenova et al. reported the cytotoxic effects of ZDV in human embryonic lung fibroblasts and colonic Caco-2 cells. They observed that 3 hr treatment of cells with ZDV was moderately cytotoxic for the cells [16].

We observed small litter size which is in consistent with previous researchers who observed small litter size in mice and rat [17,18]. In a cohort study in pregnant women an increased risk of low birth weight (<1500 g) in 2% of exposed infants and premature delivery in exposed fetuses was observed [19,20].

Neurobehavioural changes reported by different authors [21,22] suggest a cerebral effect of ZDV. We had also observed neurobehavioural changes in the same experiment [7]. In- vitro study of brain cells from fetal mouse exposed to ZDV showed slightly more cytotoxic effect as compared to other tissues of the body – like limb-bud or different organ rudiment [23]. Even a single exposure of 150 mg/kg ZDV along with tracer amounts of ¹⁴C-ZDV in pregnant rats produced a higher concentration of ZDV in fetal brain than in spinal cord [24].

There are numerous reports on mitochondrial alteration in fetus and infants exposed in-utero to ZDV. One of the earliest to report observed visual impairment, respiratory and cardiac rhythm disorders, epilepsy, and deterioration of cognitive and psychomotor abilities in children exposed prenatally to ZDV [25]. In another study, mitochondrial DNA was observed to be damaged in liver tissues [26]. In yet another study of prenatal exposure of 40 mg/day of ZDV in patas monkeys showed damage of cerebral mitochondria [27]. It was opined that affected mtDNA polymerase γ can gradually lead to dysfunction and cellular toxicity. And, the clinical manifestations of the mitochondrial toxicity resemble those of inherited mitochondrial diseases like hepatic steatosis, lactic acidosis, myopathy, nephrotoxicity, peripheral neuropathy and pancreatitis [28].

We observed myriad histopathological changes in multiple fetal tissues of mice which although could not be compared in to with the previous works done by numerous researchers, however, our result can project a composite picture of the side effect of ZDV affecting almost all tissues of the body. We would suggest an electron microscopic study of the same to confirm the ill effect of the drug in a more detail way in different species. Lastly, the gravity of the toxic effect of ZDV in children exposed perinatally to ZDV can be proven in years to come as the children born to mothers exposed to ZDV could be all below 20 years since it was recommended for use in 1994 only 2.

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