PRENATAL ZIDOVUDINE INDUCED SKELETAL CHANGES IN SWISS ALBINO MICE

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ABSTRACT: OBJECTIVES: Prevention of mother to child transmission (MTCT) of HIV virus remains a great challenge in front of us. Zidovudine is one of the drugs recommended in prevention of transmission. But its safety profile in pregnancy is still not established. We have planned this study to see the effect of Zidovudine on skeletal system of mouse embryo. **METHODS:** Pregnant mice were given Zidovudine by oral route in doses of 50mg/kg&100mg/kg and similarly control mice were given distilled water from day 6th to 15th day of gestation. The foetuses were collected after sacrificing the mother on 18th day and they were subjected to Alizarin red staining to see the skeletal changes. **RESULTS:** The treated mice offspring shows osteopenia and bone demineralization in a dose dependent manner. **CONCLUSION:** Restrain should be exercised while prescribing Zidovudine to pregnant mothers as it can harm the bones of their offspring's.

KEY WORDS: Mother to child transmission, HIV, Osteopenia, Demineralization

INTRODUCTION: Zidovudine is a synthetic thymidine analog with potent activity against a broad spectrum of retroviruses including HIV-1, HIV-2, and human T-cell lymphotropic viruses (HTLV) I and II. It was the first drug to demonstrate clinical efficacy in patients suffering from HIV-1 infection. It was approved by FDA in 1987 for treatment of Acquired Immuno Deficiency Syndrome (AIDS) ¹. The current recommendation from the Centre For Disease Control (CDC) are that Zidovudine therapy be offered to pregnant mothers with HIV and normal CD4 counts to prevent mother to child transmission (MTCT) transmission of HIV.

Zidovudine acts against HIV virus by inhibiting its viral reverse transcriptase and by blocking its viral DNA chain elongation. Host cellular DNA is also inhibited but only at a higher concentration of 100 times than those required to inhibit viral reverse transcriptase².

Animal studies have shown high and rapid placental transfer of Zidovudine and it readily crosses human placenta^{3,4}. It may hinder early embryonic development as it inhibits DNA replication and thus retards cell division^{5,6}. It is classified as class "C" drug which means that it was found safe in animal studies but studies in human are inconclusive⁷. Zidovudine has been shown to cause hematological abnormalities like anemia and neutropenia in mice by causing bone marrow depression. Zidovudine has been demonstrated to be genotoxic in humans and exerts a dose related decrease in cell proliferation and differentiation, cytotoxic effects, metabolic disruption, increased cell mortality and MT DNA depletion on human cells in vitro^{8,9}.

The objective of our study is to see the effect of zidovudine on skeleton and bone mineral density of mice offspring's when given prenatally to them.

MATERIAL AND METHODS: Permission of Institute ethical committee was taken before the start of the experiment. Swiss albino mice (25-30gm) were taken from animal house of Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University. Animals were kept in 12:12 hr

light and dark cycle and fed standard pellet diet and tap water ad libitum. The female mice were kept with the male mice in ratio of 2:1 and vaginal smear positivity for sperms were taken as 1st day of gestation (GD1). The pregnant mice were divided into 3 groups. The 1st group was designated as control and was given distilled water by gavage from day 6th to 15th of gestation. The 2nd and 3rd groups were called as treated and were given Zidovudine in the doses of 50 mg/kg and 100 mg/kg by gavage on same days of gestation. On 18th days the female dams were sacrificed by cervical dislocation and fetus was dissected out by uterotomy.

For studying the skeletal anomalies, the fetuses were skinned out, eviscerated and then fixed in 70% alcohol.

- a. The eviscerated specimen was kept in 1% KOH solution in the volume of approximately 10 times of the volume of fetus. Solution was changed daily till all the left tissues were dissolved and bones became clearly visible.
- b. The specimen was then transferred in 1% KOH solution containing a few drops of 0.1 % alizarin red solution in water. The fluid was changed daily till the bones were properly stained red and became visible.
- c. The solution was replaced by 40% glycerol solution in water for 24 hours.
- d. It was further replaced by a solution of 80% glycerol in water for 24 hours.
- e. Finally, the 80% glycerol was replaced by 100% glycerol in which the specimen was preserved after giving a second change.

RESULTS: Zidovudine given in low dose (50mg/kg) caused osteopenia and low bone mineral density (BMD) in the mice offspring as compared to the controls. Few rib fractures were seen. (Fig 2) When given in high doses (100mg/kg) Zidovudine caused heavy osteoporosis with extremely low bone mineral density as seen in deficient staining of bones by alizarin red. There were few sites of blotchy ossification. (Fig. 3).

DISCUSSION: Zidovudine exerts its teratogenic effect by inhibiting cell proliferation and cell differentiation thus causing hindrance in growth and development. Also Zidovudine has been shown to lower BMD and cause osteopenia (12.9% to 34%) in patients of HIV who were taking this drug for more than 1 year. Zidovudine can cause bone demineralization by inhibiting DNA polymerase gamma which causes mtDNA depletion and drug toxicity. This can lead to high levels of serum lactate which can further lead to low BMD. Zidovudine has also been found to increase osteoclastogenesis in a cultured mouse macrophage preosteoclast cell line by increasing promoter activity of tartrate resistant acid phosphatase (TRAP) and by augmenting binding and function of nuclear transcription protein NF-kappa B.

Zidovudine has been found to be highly effective in preventing mother to child transmission (MTCT) of HIV virus. It is regularly given to pregnant mothers infected with HIV either alone or in combination with other antiretroviral agents to prevent MTCT. But its safety profile in pregnancy is yet to be established. It is classified as a class "C" drug which should be used when potential benefits outweigh the risk. Zidovudine has a good placental transfer in humans and so is capable to harm the foetus due to its genotoxic effects on cells. This causes a dilemma to the treating physician whether to use this drug during pregnancy.

ORIGINAL ARTICLE

We found out that the effect of Zidovudine in causing osteomalacia and low BMD is not limited to the particular organism but can affect its progeny as well with deleterious effects. So caution needs to be exercised and proper counselling needs to be done before prescribing this drug to an infected pregnant lady.

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Fig. 1: Alizarin Red staining of control mice

Fig. 2: Alizarin Red staining of Zidovudine (50mg/kg) treated mice showing osteopenia and rib fracture

Fig. 3: Alizarin Red staining of Zidovudine (100mg/kg) treated mice showing osteomalacia and rib fusion

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