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Anatomy

KEY WORDS:

ZIDOVUDINE AND EFAVIRENZ INDUCED FETAL TOXICITY IN SWISS ALBINO MICE

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ABSTRACT

Introduction: Zidovudine and Efavirenz are anti HIV drugs which are used in pregnancy to prevent maternal to child transmission.
Material and Methods: Zidovudine and Efavirenz are given separately as well as together in dose of 50mg/kg to pregnant mice and its effect on fetal toxicity was recorded by measuring number of implants , live fetuses , resorption in a single mice. Weight of the fetus as well as its crown rump length was also recorded.
Observation: There is decrease in number of implants, live fetus and increase in resorption from group I to IV. Also there is decrease in fetal weight and crown rump length from group I to IV.
Conclusion: There is fetal toxicity induced by Zidovudine and Efavirenz and thus should be used with caution in 1st trimester of pregnancy

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is characterised by destruction of the host immune system, resulting in the development of opportunistic infections, rare neoplasm and death. The aetiological agent responsible for AIDS is the human immunodeficiency virus which can be transmitted congenitally, parenterally and by sexual contact.

Use of antiretroviral drugs during pregnancy has increased since the demonstration of reduction of mother to child transmission (MTCT) of HIV-1 first with zidovudine monotherapy and more recently with highly active retro viral therapy regimens (HAART). Based on these recommendations many women are receiving HAART and may enter pregnancy already receiving multiple antiretroviral agents.

Zidovudine (3-azido-3-deoxythymidine) is a synthetic thymidine analog with potent in vitro activity against a broad spectrum of retroviruses including HIV-1, HIV-2, and human T-cell lymphotrophic viruses (HTLV) I and II.[1] Zidovudine acts as against HIV virus by inhibiting its viral reverse transcriptase and by inhibiting its viral DNA chain elongation.[2] It is a constituent of HAART(Highly active antiretroviral therapy) used for preventing Maternal to child transmission(MTCT).It is classified as class C drug which means that it was found safe in animal studies but studies in human are inconclusive. Zidovudine has been demonstrated to be genotoxic in humans and exerts a dose related decrease of cell proliferation and differentiation, cytotoxic effects, metabolic disruption, increased cell mortality and mtDNA depletion on human cells in vitro[3,4,5]

Efavirenz is non nucleoside reverse transcriptase inhibitor which binds to reverse transcriptase resulting in allosteric inhibition of RNA dependent DNA polymerase. Efavirenz has been classified as a class D drug (evidence of risk, use only when potential benefits outweigh the risk). Use of Efavirenz in 1st trimester has shown to cause neural tube defects (anencephaly, Dandy Walker syndrome) and facial defects like microopthalmia, anopthalmia and cleft palate in Cynomolgous monkey.[6,7]

The study is done to evaluate the risk benefit ratio of Zidovudine and Efavirenz as they are used as fixed dose combination to prevent maternal to child transmission of HIV virus during pregnancy

MATERIAL AND METHODS

Prior approval of Central Animal Ethical committee, IMS, BHU was taken before the start of this study. The study was carried out on Swiss Albino female mice of 3 months of age weighing 20-25 g, which were caged in air conditioned animal house of the Department of Anatomy. Animals were kept in polypropylene cage floored with paddy husk to provide them bed. Animals were provided with animal feed and water ad libitum maintained in 12 h light/dark cycles at 26 ± 2^{0}C. Female mice were kept in mating with male mice in the ratio of 2:1 overnight and were inspected next morning for the presence of vaginal plug. The day of vaginal plug seen was considered as day 0 of gestation (GD 0). Weight of each female mice was taken on GD 0 and placed in an individual cage.

Pregnant Swiss albino mice had been divided into following three groups–

Control group (I): Mice treated with distilled water
Group II: Mice treated with Zidovudine (ZDV)
Group III: Mice treated with Efavirenz(EFV)
Group IV : Mice treated with ZDV+EFV

Group II was given ZDV in the dose of 50 mg/kg from day 6-15 of gestation by oral gavage. Similarly Group III was given EFV in the dose of 50 mg/kg again from 6-15 day of gestation by oral route. Group IV was given combined therapy of ZDV and EFV in the dose

of 50 mg/kg each from day 6-15 of gestation by oral route. Control group was similarly given distilled water by the same route on the same day of gestation.. On the 18th day of gestation, weight of mice of each group has been taken and then the mice were sacrificed by cervical dislocation. The foetuses were collected by laparotomy and examined for gross abnormalities, if any. The uterus of the sacrificed mice was looked for number of implants, number of live foetuses and number of resorptions. The fetal weight of each mice along with its crown rump length was also recorded.

RESULT

The number of implants per dam was measured in each group and there was a consistent reduction in implants in all treated groups as compared to the control (P<.001)

Table 1: No. of implants per dam in different group of mice

Group	N	Mean	Std. Deviation	Std. Error	significance
1	6	8.5	0.54772	0.22361	
2	6	7.1667	0.75277	0.30732	0.12
3	6	6	0.63246	0.2582	0.04*
4	6	5.6667	0.5164	0.21082	0.00**

The number of live fetuses was observed in each group and there was a significant reduction in live fetuses per dam in all treated groups as compared to the control. (P<.001).

Table 2: No. of live fetuses per dam in different group of mice

Group	N	Mean	Std. Deviation	Std. Error	Significance
1	6	7.8333	0.75277	0.30732	
2	6	6.3333	0.8165	0.33333	0**
3	6	5.6667	0.5164	0.21082	0**
4	6	4.6667	0.5164	0.21082	0**

Number of resorption per dam were all measured and it was observed that there is increased resorptions per dams in all treated groups as compared to the control.(P<0.001)

Table 3: Number of resorptions per dam in different group of mice

Group	N	Mean	Std. Deviation	Significance
1	6	0.1667	0.40825	
2	6	0.5	0.54772	0.011*
3	6	1.5	0.54772	.001**
4	6	1.8333	0.40825	0**

There is a significant reduction in fetal weight in treated groups as compared to the control and the maximum reduction in weight was observed in TIII group. (P<0.001)

Table 4: Fetal weight of mice in different groups

Group	N	Mean (gm)	Std. Deviation	Significance
1	6	1.3	0.10954	
2	6	1.1	0.08944	.007*
3	6	1.05	0.08367	.001**
4	6	0.8667	0.08165	0**

Similarly the is a drop in crown rump length (CRL) in treated group which was highly significant as compared to the control and the maximum drop was observed in group TIII (P<0.001).

Table 5: Crown rump length of fetal mice in different groups

Group	N	Mean (mm)	Std. Deviation	Significance
1	6	22.5	1.3784	
2	6	20.5	0.54772	.008*
3	6	18.8333	0.75277	0**
4	6	17.5	0.83666	0**

DISCUSSION

Zidovudine and Efavirenz are both reverse transciptase inhibitor which are used as combination chemotherapy to prevent maternal to child transmission (Staszeweski S et al. 1999). The reason for their inclusion as component of HAART is as follows –

a. They have superior efficacy and tolerability as compared to other ARV.
b. They are available as part of once daily fixed dose combination.
c. They might have a low risk of both defects as compared to other ARV.

In the present study there was a decline in the number of implants per dams in the treated group from group I to IV. Similarly, there is a drop in number of live features from I to IV group as compared to control. The resorption rate increased significantly in the treated group.

Similarly in the present study, fetal weight and crown rump length of fetal mice showed a consistent decline from Group I to IV. These finding included reduction in fetal weight, decrease in crown rump length and drop in tail length in the treated group. These finding coincides with the study of Philip Toltzis and Tay Mourton, 1993 who also founded increase rate of resorption and embryo lethality and decrease crown rump length in ZDV treated mice. Thus increased fetal death and decreased fetal growth may result from decreased cell growth in both ZDV and EFV treated group. Both ZDV & EFV have inhibiting effect on cellular DNA polymerase to some extent.[8] This may result in inhibition of cleavage which might result in reduced number of cell in inner cell mass, thus causing increased fetal death and decreased fetal growth.

Mitochondrial damage by ZDV & EFV may also result in embryocytotoxicity as the mitochondrial maturity closely follows embryotoxicity. Less mature mitochondria leads to high level of cytotoxicity .[9]

CONCLUSION

Thus both Zidovudine and Efavirenz causes and embryo toxicity and lethality and when used in combination they demonstrate a supra additive effect and thus should not be given in Ist trimester to pregnant ladies.

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