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PRENATAL EFAVIRENZ INDUCED BEHAVIOURAL CHANGES IN SWISS ALBINO MICE

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ABSTRACT

B Objective - Efavirenz is a non nucleoside reverse transcriptase inhibitor (NNRTI) which forms an important component of highly active retro viral therapy (HAART).However its safety profile in pregnancy is unknown as there are reports of its teratogenic potential. Thus we plan to see the effect of efavirenz on the behaviour of mice pups when exposed in utero. **Methods** – Efavirenz was given to pregnant mice in dose of 50mg/kg and 100mg/kg and control mice distilled water from 6th to 15th day of gestation. The mice were allowed to deliver and the 8 weeks pups were subjected to behavioural tests like open field exploratory tests, elevated plus maze tests and behavioural despair tests. **Results** – The treated mice showed enhanced anxiety and depression and reduced exploratory and locomotor activity when subjected to these tests. **Conclusion** – Efavirenz causes neurobehavioral changes in mice when given to their mothers in pregnancy.

Key Words- Highly active retroviral therapy, depression, anxiety, exploratory, locomotor

INTRODUCTION

Efavirenz is a preferred non nucleoside reverse transcriptase inhibitor (NNRTI) for use against HIV-1 infection. It is a common component of Highly Active Antiretroviral therapy (HAART) used along with tenofovir and lamivudine to treat HIV patients. The advantage of efavirenz over other NNRTI is that it has a superior efficacy, better tolerability, once fixed day formulation and better activity in HIV /TB coinfected patients. [1, 2]

Although efavirenz has various advantages it is not considered a safe drug in pregnancy. It has been classified as class" D" drug which means animal studies and some human studies have demonstrated increased risk of congenital malformations and the drug should be used only when potential benefits outweigh the risks.[3]

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 monkeys. Efavirenz has also been shown to cause drowsiness, insomnia, dizziness and other CNS side effects. Psychiatric symptoms like depression and mania has also been observed in persons taking efavirenz for a long duration. [4-6]

There is a theoretical risk that efavirenz may affect neurodevelopment of the fetus if given in 2nd and 3rd trimester of pregnancy. However due to lack of neurobehavioral studies the risk of exposure to efavirenz in pregnancy remains speculative. Thus in our present study we intend to see the behavioural changes in mice exposed to efavirenz in pregnancy.

MATERIALS AND METHODS

Necessary clearance was taken from Institutional ethical committee before the start of this study. Swiss albino mice were taken for the present study. Female mice were mated with male mice in the ratio of 3:1 and the presence of sperm in the vaginal smear was taken as the 1st day of gestation (GD1).The treated pregnant mice was given efavirenz in the dose of 50mg/kg and 100mg/kg from 6th to 15th day of gestation by gavage. Similarly the control mice were given distilled water by the same route. The pregnant mice were allowed to deliver and the pups of these mice were reared up to 8 weeks after which these pups were subjected to a battery of behavioural tests.

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Table 1-Prenatal Efavirenz induced Open field exploratory changes in mice

Groups	No of fetuses	Ambulation (N)	Immobility time (sec)	Rearing (N)	Self Grooming	Fecal pellets (N)
Control (Vehicle)	16	53.8 ± 3.2	113.2 ± 2.9	10.1 ± 2.6	5.22 ± 1.47	2.5 ± 1.1
Efavirenz 50mg/kg	20	40.6 ±2.1**	15.2 ± 2.6**	7.2 ±1.8**	4.9 ± 2.1*	1.8 ± 1.2
Efavirenz 100mg/kg	20	34.6 ± 2.8**	172 ± 3.3**	5.7 ± 2.3**	3.8 ± 1.8**	1.6 ± 1.2

***p<.05, ** p<.01**

Table2-Prenatal Efavirenz induced Elevated plus maze changes in mice

Groups	Fetuses (N)	Time spent in Open Arm(sec)	Time spent in Closed Arm(sec)
Control	16	88.3 ± 10.5	211.6 ± 25.1
Efavirenz 50mg/kg	20	52.2 ± 8.4**	247.8 ± 22.4**
Efavirenz 100 mg/kg	20	46.4 ± 6.2**	253.6 ± 25.2**

***p<.05, **p<.01**

Table 3-Prenatal Efavirenz induced Behavioural despair changes in mice

Groups	Fetuses (N)	Immobility time(sec)	Frequency of immobility (N)
Control	16	125.64±10.13	10.73±2.06
Efavirenz 50mg/kg	20	165.37±14.42*	14.28±3.14*
Efavirenz 100mg/kg	20	175.4±15.16*	16.33±3.75**

***p < .05,**p< .01**

a. ***Open-field exploratory test:***

An open-field apparatus made of plywood measuring 60.96X 60.96X60.96 cm will be used to record the locomotor activity and to test the open-field exploratory behavior of mice. The floor of the apparatus will be divided into 16 evenly spaced squares surrounded by opaque high walls of 60.96 cm. The entire apparatus will be painted black except the 6 mm wide white lines that divide the floor into 16 squares. The open field apparatus will be illuminated by a 100W bulb focusing onto the field from a height of about 100cm from the floor. The entire room except the open field will be kept dark during the experiment. In the novel test situation, each animal will be centrally placed in the test apparatus for a maximum test period of 5 min to observe the following behavior: (1) Ambulation- the number of squares crossed by the mice; (2) Rearing- the number of times mice stood on its hind limbs (supported and unsupported); (3) Self- grooming- the number of responses of grooming, scratching, licking and washing made by each individual mice; (4) Fecal boli- the number of fecal boli exuded by each individual mice. Before each trial, the floor and the walls are cleaned with cotton soaked in 70% alcohol

b. Elevated plus-maze test:

The plus maze consists of two opposite arms 50X10cm, connected with central square (10X10cm), giving the apparatus a shape of plus (+) sign. One arm, painted white, will be kept open, whereas the other enclosed with a 40 cm high wall and painted black together with the wall. The maze will be kept in a dimly lit room and will be

elevated 50 cm above the floor. The experimental animals will be placed individually in the centre of the maze facing towards the enclosed arm. An arm entry will be recorded when all four paws of the mice enter the arm. Like the open field apparatus, the floor and walls of the open and enclosed arms will be cleaned with 70% alcohol before each trial.

c. Behavioral despair test-

To evaluate the depression status in mice this maze will be used. The individual mice will be placed in a circular glass chamber, 45 cm in diameter containing 25 cm depth of water, so that mice could not touch the bottom of the cylinder with its hind limbs or climb over the edge of the chamber. Two swim sessions will be conducted, an initial 15 mint pretest, followed by a 5 min test 24hrs later. The period of immobility (remain floating in water without struggling) and frequency during 5 min test period will be noted and evaluated.

Mann Whitney U test was applied for statistical analysis and to calculate the level of significance.

RESULTS

In the open field exploratory test the efavirenz treated mice showed significant decrease in ambulation, rearing, grooming and increased immobility as compared to control denoting decreased locomotor activity and enhanced anxiety in the treated pups.

In the elevated plus maze test , the treated mice spent significantly more time on enclosed arms as compared to controls signifying decreased exploratory and increased anxiety levels in the treated pups.

In the behavioural despair test, the treated mice showed decreased frequency and period of mobility in the water denoting increase in depression in the treated offspring.

DISCUSSION

Efavirenz has been shown to cause congenital malformation in animals as well as human beings. However, the incidence rates of these defects vary from 0% to 22.6% with mean of 2.9%. The incidence rates of neural tube defects (NTD) in previous studies are further diminished with the incidence rate as low as 0.08%. [5]The posterior neuropore closes at 28 days in human being and so NTD's usually occurred due to exposure to efavirenz in the 1st trimester.[6] However, exposure to efavirenz in the second and third trimester has not yet been linked to any CNS developmental or any behavioural anomaly in the offsprings.

We found an increase in anxiety and depression levels and decrease in exploratory and locomotor activities in the pups of mice treated with efavirenz during pregnancy. Previous studies have also demonstrated increase in depression and stress levels in mice exposed to efavirenz.[7, 8] The increase in proinflammatory chemokines and tumor necrosis factor alfa (TNF-α) in serum of those mice were reasoned to be the cause of depression. It may be possible that these proinflammatory chemokines and tumor necrosis factor α may pass from mother to fetus and may cause subtle damage to developing cells in cerebrum and imbalance in the neurotransmitter level resulting in these behavioural anomalies. Further, these anomalies are so subtle that these may not be able to be diagnosed by ultrasonography and may be manifested at adolescent life.

It has also been seen that people on anti retroviral agents are prone to risk behaviours like unplanned pregnancies, reduced use of folate supplementation, cigarette smoking and alcohol use further making them more susceptible to congenital malformation.[9, 10]

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