IJTA International Journal of Therapeutic Applications
ISSN 2320-138X

COGNITIVE CHANGES INDUCED BY PRENATAL EXPOSURE TO EFAVIRENZ IN SWISS ALBINO MICE

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ABSTRACT

Background- Efavirenz is a nonnucleoside reverse transcriptase inhibitor with a proven efficacy against HIV-1 virus. Continuous use of this drug has shown to cause cognitive impairment and memory defect.

Aims- The safety of efavirenz on fetus when given to mother is yet to be established. So in this experiment we try to observe the cognitive changes in offspring of mothers exposed to efavirenz in utero.

Methods- Efavirenz was given to pregnant mice in dose of 50 mg/kg and 100mg/kg and distilled water to control mice from 6th to 15th day of gestation by oral route. The mice were allowed to deliver and the pups were reared upto 8th week after which they were subjected to Morris water maze test.

Results- The efavirenz treated mice took significantly increased time to reach the hidden platform and thus exhibited a significant defect in spatial learning and memory.

Conclusion- Efavirenz causes cognitive changes in developing embryo if exposed during pregnancy.

Key words- cognitive, spatial learning, memory

Prevention of mother to child transmission (PMTCT) remains one of the important challenges in an HIV infected pregnant female especially in resource constrained settings. Low socioeconomic structure, high illiteracy rate, high cost of anti retroviral (ARV) treatment and apathetic attitude of health care industry remains some of the important reasons for ill compliance of antiretroviral treatment. During pregnancy the antiretroviral drugs have been advocated for prevention of transmission to the child but the safety paradigm of many agents are still in dark.

Efavirenz has been approved by FDA as 1st line drug for HIV since 1999. It is a non nucleoside reverse transcriptase inhibitor (NNRTI) which is an important component of highly active retroviral therapy (HAART) [1, 2]. Due to few reports of its teratogenicity in animal and human studies it is now classified as class 'D' drug which should be used only if the potential benefits outweigh the risks[3]. The recommendations of both United States and European guidelines state that it should not be used in pregnancy especially in 1st trimester because of its propensity to cause neural tube defects (NTD) [4]. However the newer WHO guideline says that efavirenz is a better choice than nevirapine as a component of HAART in pregnancy due to its low cost, lesser toxicity, single dose regimen and effectiveness in HIV-TB co-morbidity [5].

Efavirenz is a neuroactive drug and it can easily cross blood brain barrier. The concentration of efavirenz in CNS is higher than any other ARV agent[6]. Due to its high concentration neuropsychiatric symptoms are commonly seen in almost 50% of the users. The common symptoms include depression, mood changes, irritability, dizziness and impaired concentration [7, 8]. Due to paucity of reports we took up the present study to see the effect of efavirenz on cognitive changes in mice offspring exposed in-utero.

MATERIALS AND METHODS

Prior approval of Institute ethical committee was taken before the start of the study. Swiss albino mice were obtained from the departmental animal house for the present study. Female mice were mated with male mice in the ratio of 3:1 and sperm positivity in vaginal smear was taken as day 0 of gestation. The control mice were given

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distilled water and the treated group was given efavirenz in doses of 50mg/kg and 100mg/kg from 5th to 16th day of gestation. The dams were allowed to deliver and the pups were reared up to 8 weeks after which they were subjected to Morris water maze test used for learning and memory assessment.

measured. On completion of the probe trial, a black platform that extended 1 cm above the surface of water will be placed in a quadrant other than that chosen for the submerged platform. Each mouse will then be given four trials of 90s to locate it. The latency to reach the platform will be recorded (working memory procedure)

Table 1-Effect of prenatal efavirenz on cognition of mice offspring (Morris water maze test)

Groups	Fetuses	Time to reach hidden platform from poles(sec) (values are mean ± S.D)				
		1st pole	2nd pole	3rd pole	4th pole	Reverse actualisation
Control	16	15.16±3.5	9.48±2.11	11.36±2.63	12.76±2.47	17.34±3.75
Efavirenz (50mg/kg)	20	22.25±4.2*	21.12±3.24**	23.34±3.60**	23.84±3.04**	28.56±3.91**
Efavirenz (100 mg/kg)	20	25.59±4.7**	25.23±3.75**	26.57±3.71**	25.46±3.96**	34.47±4.69**

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

Morris water Maze test-

The maze consists of a black circular pool (Diameter- 2.14m, Height-80cm) filled with water (25°c ± 1°c) to a depth of 44cm. Eighth week offspring of mice will be tested for spatial learning and memory. Initially all the pups will be exposure to water maze for 1 minute without platform. Next day a circular platform (Diameter-9cm) will be kept hidden 2 cm below water level in the centre of one of the quadrants. The platform remains in the same position during training days. At the beginning of each session, a random sequence of four starting poles along the perimeter of the pool will be generated. All animals will follow this sequence for that session. Each mouse will be placed in the water facing the wall at the start location and will be allowed 90 seconds to find the hidden platform. The animal will be allowed 20s rest on the platform. The latency to reach the platform will be recorded. If the mouse will not be able to locate the hidden platform it will be lifted out and placed on the platform for 20s. The procedure will be repeated for all the four start locations. Two sessions of four trials each will be conducted on first day of testing at the interval of four hours and one session of four trials will be conducted on the next day. After that, the platform will be removed and a probe trial (w/o platform) will be conducted four hrs later. Each mouse will be placed in the pool at the same randomly selected starting pole and swimming path and time spent in the quadrant of pool that initially contained platform will be observed and

RESULTS

The time taken by the mice to reach the hidden platform from all the poles was significantly higher in the treated group as compared to the control. Also the time taken to find the reverse platform (reverse actualisation) on day 3 was also significantly higher in the treated group as compared to control depicting loss of memory and spatial learning in them. (Table 1)

DISCUSSION

Efavirenz has been shown to reduce memory and cognitive impairments in humans. Studies have also shown that efavirenz if given to rats caused memory defects and increase susceptibility to stress. There is also evidence of impairment of aversive memory, cognitive defects and inducement of anxiogenic behaviour by repeated use of efavirenz.It has been suggested that increase of proinflammatory cytokines and inhibition of brain creatine kinase activity in cerebellum, hippocampus, striatum and cortex leads to defect in cognition, memory and depressive like behaviour in rats.[9]

The Morris water maze test is the most widely used, well documented, standard and validated test for observing cognitive changes in rodents.[10] In our experiment we found that there is a defect in spatial learning and memory in mice exposed prenatally to efavirenz. This deficit was dose dependent and seems to increase with increase in dose.

Efavirenz has a good placental transfer ratio and the drug is able to cross the CNS barrier easily. It might be possible that efavirenz on reaching the embryo may cause an increase in proinflammatory cytokines like interleukin-1β and tumor necrosis α and decrease in brain creatine kinase activity. Increase of these biochemicals in brain may lead to cognitive defects by stimulation of the HPA axis.[10]

So efavirenz should be cautiously prescribed to pregnant females as it can cause cognitive impairment and delayed milestone in their children.

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