NEUROBEHAVIORAL PROFILE OF F_1 AND F_2 GENERATION MICE FOLLOWING ONE STAGE ZIDOVUDINE EXPOSURE THROUGH PREGNANCY AND LACTATION

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Abstract

Zidovudine is administered to pregnant women with HIV to prevent the spread of infection to their fetuses. Several animal studies reported behavioural alteration in zidovudine prenatally exposed offspring, possibly resulting from an action of this drug on CNS targets. The aim of the present study was to assess the neurobehavioral effects of one-stage zidovudine exposure during pregnancy and lactation in F_1 and F_2 generation of mice. Zidovudine (50 mg / kg/ day) was administered to female Swiss mice given orally from day 8 of gestation through pregnancy and up to day 10 of postnatal life. Control mice were given equal volume of distilled water. F_1 generation mice so produced, on attainment of 60-90 days age, underwent neurobehavioural testing for propensity towards anxiety and disturbances of learning and memory in the open field test, elevated plus-maze and Morris water-maze test. F_1 generation mice were crossed to produce F_2 mice. On attainment of 60-90 days, F_2 mice were also subjected to same neurobehavioral testings. Perinatal Zidovudine exposure caused significant decreased mobility suggesting certain degree of anxiety in F_1 mice whereas the contrary is true in F2 mice. No derangement of motor activity was detected at maturity in the zidovudine exposed generation of mice in F_2 mice. F1 mice, in the elevated plus maze test showed reduced entry in open arm whereas the entries are increased in F_2 mice. Similar increase in the number of entries in the closed arm lay F2 mice as compared to F1 mice was observed. Result of Morris water maze test showed decrease in learning capabilities in F_1 mice and regained learning ability in F_2. Overall results suggest that neurobehavioral functions are affected in F_1 generation and recovery in F_2 generation. Recovery in F_2 generation beyond normal profiles could be due to activation of oncogenes as a result of chromosomal alterations.

Key words: Perinatal, CNS, Elevated plus maze, Morris water maze test, Oncogenes, Chromosome.

Introduction

Zidovudine has been extensively used in pregnant HIV positive women to prevent transmission of infection to the baby (1,2). Zidovudine exposure during prenatal period is reportedly associated with alterations in brain morphology and function in the baby. Earlier experimental studies in mice and monkeys and clinical studies have indicated mitochondrial dysfunction and shortening of telomere length with prenatal exposure to various doses of zidovudine (3-8). A variety of abnormalities in neurobehavioral observations have been documented in rodents prenatally exposed to zidovudine (9-13). Other studies point to the transient nature of the functional damage in prenatal zidovudine exposed rodents (13). Rodents are altricial mammals with offspring born neurologically premature. The maturation takes place after birth over sometime. In human instances, the mother receives anti-HIV therapy during pregnancy through to the lactation period with relevant impact on the offspring. This has been imperative in designing the zidovudine schedule in mice through pregnancy to lactation (14). In the present study neurobehavioural screening of the offspring was carried out in matured offspring aged 60-90 days. The toxic profile of zidovudine on genetic mixed with development effect on fetus was studied by observing F1 offspring of zidovudine treated animals. Any pure genetic damage of transmissible nature was assessed by study of F2 generation of the originally exposed mice.

The present study was conducted on Swiss mice to assess the neurobehavioural effects of zidovudine exposure during pregnancy and till Post Natal Day (PND) 10. The next generation of mice produced by crossing of the perinatally exposed (F1) mice also underwent the same neurobehavioural tests to assess the effect of drug in the next generation.

Materials and Methods

Animal breeding and drug administration

After approval of Institutional Ethics Committee, the present study was conducted on inbred Swiss mice. Male and female mice weighing 25-30g and 80-90 days old were mated in the mating cages in the ratio of 1 male to 3 female mice. A 12 hours light-dark cycle, a room temperature 25 $\pm$ 2^{0}C, relative humidity of 45-55% was maintained. Five pregnant mice (Fo) were assigned as control and 5 pregnant mice (Fo) as treated group. The F1 generation mice produced were reared till 60-90 days age. F_1 generation mice are crossed to produce F_2 generation mice. Similarly, F_2 mice so produced were reared upto 60-90 days age..

Only the healthy F_2 mice were chosen for the study as 60-90 days old as, many sick F_2 mice were also produced. Both the F_1 (n=10) and F_2 (n=10) along with control (n=10) mice were subjected to neurobehavioral study. Fo mice was treated with 50mg / kg /d Zidovudine (*Zidovir oral* solution, Cipla Drug Company, Goa, India), orally from day 8th of gestation through delivery till postnatal day 10. Perinatal drug dosing was as per Venerosi et al, 2000. Drug dosage was standardized in our laboratory after an initial dose of 70 mg/g/day where 100% fetal resorption was observed. Equal volume of distilled water were given orally to control mice. Principles of laboratory animal care (NIH publication No 86-23, revised 1985) guidelines were followed throughout.

Tests for neurobehavioral assessment

The two generations of mice underwent neurobehavioral studies to observe locomotor activity, anxiety and effects on learning and memory. The locomotor activities observed in an open field paradigm, anxiety was assessed in an elevated plus-maze and learning and memory was assessed in Morris water-maze test. The mice were divided into 3 groups- Group A comprises of the Control mice (n=10), Group B comprises of F1 mice (n=10) and Group C comprises of F2 mice (n=10).

Open field test

Locomotor activity was evaluated in an open field paradigm (15). Individual mice (n=10) were transferred from the home cage to an open field arena made of plywood and consisted of floor (96cm x 96cm) with high walls. The entire apparatus was painted black. The bottom of which is subdivided by 6mm thick white lines into 16 equal size squares. The test started by placing the animal in a corner of the apparatus. The behavior of the animal was then observed under red light. After each test, the apparatus was thoroughly cleaned with cotton pad wetted with 70% ethanol. Following behavioral parameters observed, ambulation (number of crossing the square boundaries with both forepaws), frequency of rearing (including wall rearing), grooming (rubbing the body or mouth with paws and rubbing the head with paws), total period of immobility (in seconds) and fecal pellets. Observations were made during 5 minutes on each mouse in each parameter.

Elevated plus maze

Adult mice were tested in a elevated plus maze (16) to assess anxiety. Four arms (15 x 5cm) of the maze were set at 90^0 angles in a plus-shaped design. Two opposing arms had high walls (12 cm) while the other two arms were devoid of rails of wall. The maze was elevated 25cm from the floor. The mice were individually examined in 5-min session in this apparatus. Each mouse was placed in the central platform facing one open arm. Dependent measures were recorded including, the number of times the mouse enter the closed and open arm and the time spent in open and closed arm.

Morris water maze test

Spatial learning and memory was tested in water maze (17). The maze consisted of a black circular pool (diameter 2.14m, height 80cm) filled to a depth of 44cm with water (25^0C). A circular platform (9 cm in diameter) was kept hidden 2cm below water level in the center of one of the quadrants. The platform remained in the same position during training days (reference memory procedure). At the beginning of each session, a random sequence of four starting poles along the perimeter of the pool was generated. All animals followed this sequence for that session. Each mouse was placed in the water facing the wall at the start location and was allowed 90 seconds to find the platform. The latency to reach the platform was recorded. If the mouse was unable to locate the hidden platform, it was lifted out and placed on the platform for 20 seconds.

Two sessions of four trials each were conducted on the first day of testing separated by 4 hrs and one session of four trials was conducted on the next day. After that the platform was removed and a Probe trial (without platform) was conducted 4 hr later. Each mouse was placed in the pool at the same randomly selected starting pole and swimming path was observed and time spent in the quadrant of the pool which initially contained platform was measured.

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

Statistical Analysis

Analysis of the data was done by using the SPSS version 12.00 software. Data are expressed as means $\pm$ SE values. The data were first analysed by non-parametric ANOVA- Kruskal Wallis (KW) test and followed by determination of Pair-wise significance difference using Multiple-Ranges Student Newman Kuel (SNK) Test. P-value <0.05 was taken as the level of significance.

Result and Discussion

Zidovudine is administered during pregnancy in HIV positive women. Studies in animals have variously reported permanent damage to mitochondria in a variety of tissues, alterations in neurobehavioural profiles and overall growth of the organism.

The present study focused on effect of zidovudine exposure through prenatal to 10^{th} postnatal day on the neurobehavioral profile of the offspring. It is understood that murine nervous system development and maturation is optimum by 60^{th} PND (Postnatal day). In the present study, the period of drug exposure was extended till PND 10 to include more of neurogenesis period (14). Certain past studies have also suggested the transient nature of some of the toxic influences of the zidovudine exposure. The present study has been conducted in Swiss strain of mice while earlier studies had been in CD-1 mice, rats and monkeys.

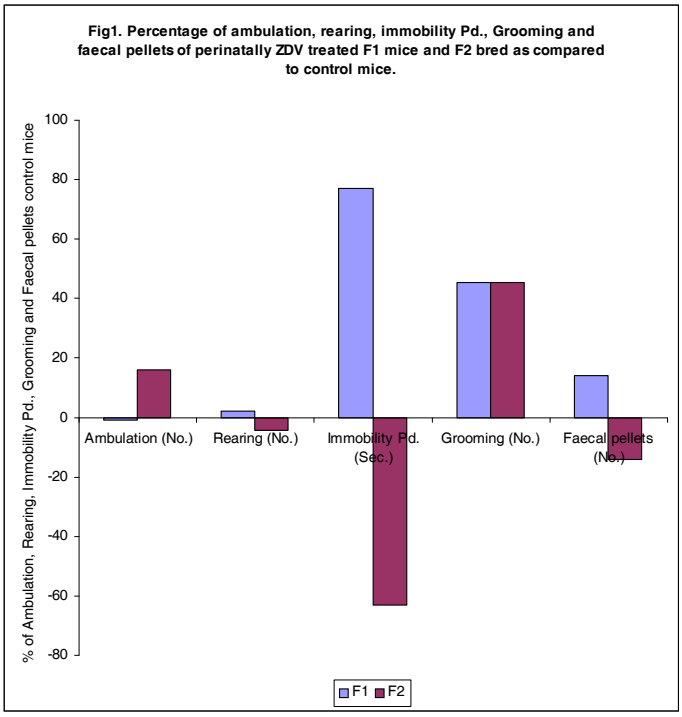
Open field test employs various observations that indicate components of anxious behaviour in tested animals. As apparent from Table-1 (a-b), the F1 generation of mice that were exposed to zidovudine during prenatal and 10^{th} PND singularly exhibited prolonged immobility profiles in this test. This was however not seen in F2 generation offsprings. On the contrary, the F2 generation had significantly reduced immobility profiles when compared to the unexposed control group of mice. Many of the tested behaviors under open field test are assigned to dopaminergic neurotransmission in associated brain areas (18-20). It appears that zidovudine exposure leaves significant and perhaps prolonged changes in this regard on the exposed F1 generation animals. The role of countering mechanism may be inferred at genetic level to explain marked reduction of immobility profile in F2 offspring from the results of the study. The parameters involving motor activity were not significantly different between either of F1 or F2 offspring compared to the unexposed control. It can be

Table 1a. : Effect of Zidovudine (50mg /kg x day 8 of gestation through delivery and PND 10) on open field behavior parameters (values in Mean ± SE).

Groups	Ambulation (Number)	Rearing (Number)	Immobility Period (Sec)	Grooming (Number)	Fecal Pellets (Number)
Control	118.50±15.37	27.60±4.04	67.60±21.58	2.2±0.55	1.40±0.60
F1 Generation	117.3±11.82	28.20±4.71	119.8±15.34	3.2±0.35	1.6±0.54
F2 Generation	137.3±3.0	26.4±50.7	26.4±5.09	3.2±0.62	1.2±0.59
K- value	1.141	0.412	15.429	3.57	0.372
p- value	0.565	0.814	0.000	0.217	0.830

Table 1b. : Group comparison study by Multiple Range SNK test for Immobility period

Groups	q	p-value
Control Vs F1	3.93	<0.01
Control Vs F2	4.33	<0.01
F1 Vs F2	5.55	<0.001



stated that no motor derangement was detectable at maturity in the zidovudine exposed generation of mice.

As seen in Table–2(a-c) the observation in elevated plus maze test gives a further examination of anxiety behaviour. It was seen that compared to the control, F1 generation did not show greater preference for entry in open arm as also the F2 generation animals. Despite this the F1 mice spent much lesser time in closed arm and more time in open arm. These durations were less differentiated in case of F2 mice which showed increased number of entries in both closed and open arm, compared to either the control or the F1 generation. The observation indicates overall increased activity rather than anxiety (21). The entries in arm are particularly enhanced in F2 generation mice even above the control levels. This points to some genetic mechanism to counter decreased entry in F1 generation following exposure to pre and perinatal zidovudine.

The results of Water maze test displayed in Table 3(a-b) show significant differences in the probe trial. This indicates memory and learning. Decreased spending of time in this security quadrant was seen both in the F1 and F2 generation of mice. There was no indication whatsoever of recovery between F1 to F2 generation. Summarily, the learning process was seen as a change in escape latency at successive sessions tested in water maze test. The controls exhibited decreased successive latency profiles. Such a change was lost in zidovudine exposed F1 generation indicating deficit of learning. On the contrary, the F2 appeared to regain learning ability suggesting some reparative phenomena through change of generation. The ability to adopt new platform (working memory) was increased in F1 generation but the working memory was diminished in F2 generation mice. The probe trial test which tells reference memory profile shows that both the generation of the zidovudine exposed mother have decline of reference memory.

The overall result from evaluation at PND 60 may represent adult situation. The differences in observation parameters as compared to other studies (11,13,21) may be assumed to be based in differences of strain and other independent variables like dose of the drug and selection of only physically healthy looking offspring. The indication is that neurobehavioural functions do get affected in F1 generation of mice exposed to zidovudine through out pregnancy and till 10th PND akin to human situation. We have not examined recovery profiles of F1 generation over different periods of lifetime. The observation in F2 generation of mice indicates operation of genetic mechanism to counter the damage suffered in F1 generation. Indication of genetic mechanisms getting activated and apparently rendering recovery beyond normal profiles as that of controls, seen in F2 generation

Table 2a. : Effect of Zidovudine (50 mg/kg x Day 8 of gestation through delivery upto PND 10) on elevated plus maze behavior (values in Mean ± SE)

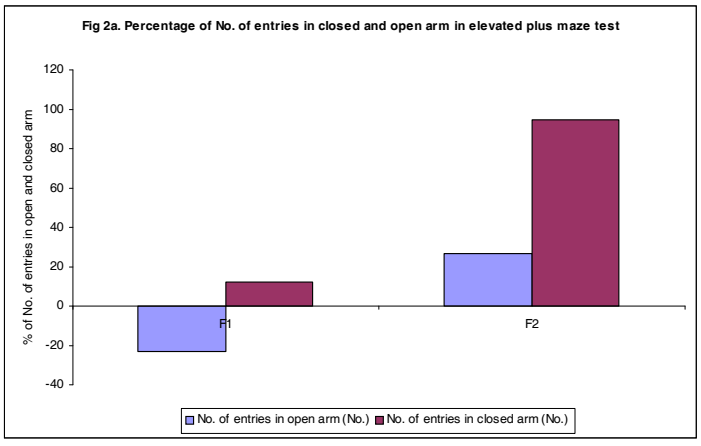
Group	No. of entries in		Time spent in	
	Open arm	Closed arm	Open arm (sec)	Closed arm (sec)
Control	11.20±5.75	7.5±1.80	82.00±15.65	218.0±15.65
F1 mice	8.60±1.49	8.40±1.24	127.8±22.47	171.20±22.41
F2 mice	14.20±0.96	14.60±0.90	160.90±40.43	181.10±11.86
K-value	8.418	11.287	3.743	2.875
p-value	0.015	0.004	0.154	0.238

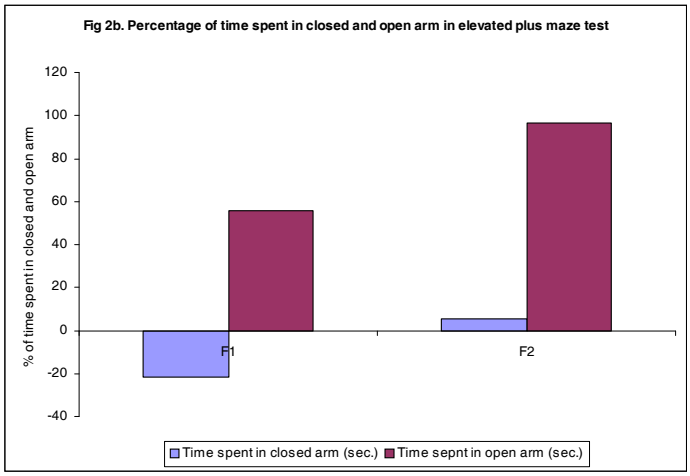
Table 2b. : A posthoc Group comparison study (SNK test) of the number of entries in open arm.

Groups	q	p-value
Control Vs F1	0.91	NS
Control Vs F2	3.81	<0.05
F1 Vs F2	4.25	<0.001

Table 2c. : A posthoc group comparison study (SNK test) of the number of entries in closed arm

Group	q	p-value
Control Vs F1	0.48	NS
Control Vs F2	4.26	<0.01
F1 Vs F2	5.85	<0.001





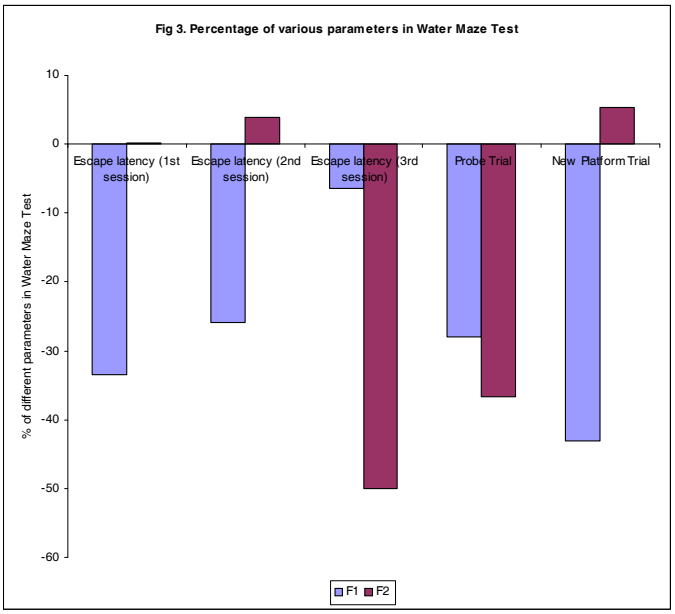
is the strong indication available in this study. Such a phenomenon is seen when free- radical stress leads to increased level of antioxidant enzyme within the cell as a restorative mechanism (22,23).We have examined only neurobehavioural parameters however; zidovudine exposure is understood to cause diverse effect throughout the organism. The consequence of genetic activity to

Table 3a. : Mean duration of time taken in three sessions of Escape latency, time spent in the quadrant during Probe trial and time taken in the new platform trial (Mean + SEM)

Group	Escape latency (sessions)			Probe trial	New platform trial
	I	II	III		
Control	16.48± 4.83	14.72±5.54	11.82±3.02	33.95±1.87	15.52±3.02
F1 mice	10.97±3.57	10.90±2.51	11.07±3.54	24.42±2.96	8.82±1.95
F2 mice	16.50± 3.24	15.30±6.42	5.9±3.14	21.5±3.19	16.35±5.52
K-value	2.131	0.066	2.259	7.210	1.960
p-value	0.375	0.967	0.323	0.027	0.374

Table 3b. : Posthoc group comparison study for Probe trial

Groups	q	p-value
Control Vs F1	4.14	<0.01
Control Vs F2	3.63	<0.05
F2 Vs F2	1.27	NS



counter or repair deranged fine process provides unlimited vistas for research and understanding. It may be naive to speculate but it seems akin to gene amplification phenomena in activation of oncogenes as well (24,25).

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