

THE EFFECT OF PIRACETAM ON VALPROIC ACID INDUCED CONGENITAL MALFORMATIONS IN SWISS ALBINO MICE.

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ABSTRACT

Valproic acid (VPA) is an antiepileptic drug which is widely used in humans and is a well known teratogenic agent when used during pregnancy. Piracetam is a nootropic or cognitive enhancer drug used to treat cognitive impairment in aging, brain injuries as well as dementia. In the present study, these two drugs VPA and Piracetam were administered orally to Swiss albino mice in the doses of 400 mg/kg and 800 mg/kg body weight respectively from gestational day (GD) 6-11 in order to see the protective effect of Piracetam against VPA induced teratogenesis. The fetuses were collected on GD 18 after uterotomy and observed for gross malformations if any. In VPA treated group the malformations observed were exencephaly, cranioschisis, limb and tail defects, haemorrhage, resorptions and retardation. No such anomalies were observed in control and Piracetam treated groups. However, in VPA+Piracetam treated group some resorptions and growth retardation were noted. This group showed highly significant ($p < 0.001$) protection against the teratogenic effects of VPA treated group though the developmental parameters were significantly reduced ($p < 0.001$) in comparison to those of group I (control) and group III (receiving piracetam). These findings suggest that Piracetam, if given in higher doses might protect the development in utero against the teratogenic effects of VPA.

KEYWORDS

Valproic acid, Piracetam, mice, exencephaly, cranioschisis, tail defects

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INTRODUCTION

Valproic acid (VPA) is a broad spectrum anticonvulsant and is widely used in the treatment of various types of seizure disorders and some psychiatric problems. This is believed to affect the function of the neurotransmitter GABA in the human brain. Its mechanism of action includes enhanced neurotransmission of GABA (by inhibiting GABA transaminase). It also blocks the voltage-gated sodium channels and T-type calcium channels. These mechanisms make it a broad spectrum anticonvulsant drug. However, several other mechanisms of action in neuropsychiatric disorders have been proposed for VPA.¹

VPA is a well established human teratogen.² Its exposure during pregnancy may result in an increased incidence of neural tube defects and a number of other major and minor malformations.³⁻⁶ Review of literature reveals that effective teratogenicity of VPA is dose dependent and it is a potent inducer of malformations of multiple organs in rat, mice, rabbit, zebrafish, monkey etc.⁷⁻¹¹ In humans, intrauterine exposure to VPA has been associated with mental retardation, cognitive impairment, behavioral deficits and neurodevelopmental delay.¹²⁻¹⁵

Piracetam (2-oxo-1-pyrrolidine acetamide) is a cyclic derivative of gamma-aminobutyric acid (GABA) but it does not exhibit any GABA-like activity in animals. It is completely absorbed after oral administration. It is one of nootropic drugs, used in many countries to treat cognitive impairment in aging, brain injuries as well as dementia.^{16,17} These are claimed to improve the higher telencephalic integrative activities in the brain and restore deficient higher nervous system activity without affecting subcortical functions.¹⁸ Unlike other psychotropic drugs, nootropics do not cause sedation or stimulation and are completely devoid of toxic effects, even when administered in very high doses. Piracetam has been reported to possess cytoprotective, antioxidant, antihypoxic and microcirculation protective effects.¹⁹⁻²²

It is believed that Piracetam being labeled as a nootropic or smart drug could possibly play a significant role against teratogenicity. Therefore, the aim of the present study is to investigate whether Piracetam (with its proven cyto-protective and antioxidant properties) can reduce the intensity of teratogenicity induced in pregnant Swiss albino mice by VPA which is a known potent teratogen.

MATERIALS AND METHODS

Adult female Swiss albino mice (25-30 gm) were used in the present study after approval by animal ethics committee of Banaras Hindu University Varanasi, India. All experimental animals were housed under Standard laboratory conditions ($25^{\circ} \pm 2^{\circ}\text{C}$, 12 hr L/D cycle, 60 % RH) with free access to food and water in the animal house of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The mice were kept for mating in the evening and the presence of vaginal plug on the following morning was designated as day 0 of gestation. The pregnant mice were divided into four groups, each group contained eight mice. Group I served as control, group II received VPA 400 mg/kg/day from GD6-GD11, group III received Piracetam 800mg/kg/day from GD6-GD11 and group IV received VPA + Piracetam at the same dose and day mentioned earlier. All these drugs were given by oral route. All the groups of pregnant mice were sacrificed on day 18 of gestation by deep ether anaesthesia and fetuses and placenta were collected. The fetuses were blotted dry, weighed individually and grossly examined for any external malformations. The crown rump length (CR length) was measured with the help of a Vernier caliper.

RESULTS

The average weight of fetuses was 1.180 ± 0.153 gm in Group I (Control) and 0.796 ± 0.138 gm in Group II (VPA treated fetuses). In Group III

Table 1: Mean weight and CR length in control and treated fetuses collected on day 18 of pregnancy

Groups	No. of fetuses	*Mean $\pm$ SD of wt. (in grams)	**Mean $\pm$ SD of CR length (in mm)
Control	36	1.180 ± 0.153	24.13 ± 1.407
Valproic acid	42	0.796 ± 0.138	19.88 ± 2.154
Piracetam	30	1.107 ± 0.151	24.13 ± 1.814
Valproic acid + Piracetam	41	0.976 ± 0.136	22.12 ± 1.691

*F value= 55.39, df= (3, 152), $p < 0.001$; **F value= 51.29, df= (3, 152), $p < 0.001$



Fig. 1: Photograph showing fetuses from different groups: I- Control, II - VPA treated, III- Piracetam treated, IV- VPA + Piracetam treated

Table 2: Inter Group Comparison (Post hoc test)

Group	Weight	CR length
Control vs VPA	p<0.001	p<0.001
Control vs Piracetam (PCT)	p<0.05	p>0.05
Control vs VPA + PCT	p<0.001	p<0.001
VPA vs PCT	p<0.001	p<0.001
PCT vs VPA + PCT	p<0.001	p<0.001
VPA vs VPA + PCT	p<0.001	p<0.001

(Piracetam treated fetuses) the average weight was 1.107 ± 0.151 gm and in Group IV (VPA+ Piracetam treated fetuses) it was 0.976 ± 0.136 gm.

On comparison of the weight of fetuses of different groups, the reduction in weight of Group II (VPA treated fetuses) was highly significant in comparison to Group I as well as Group III ($p < 0.001$). The reduction in the weight of the fetuses of Group IV was highly significant ($p < 0.001$) in comparison to Group I as well as Group III, but in comparison to Group II, the weight was significantly increased ($p < 0.001$), (Table-1 & Table-2)

Table 3: Resorption in control and treated fetuses collected on day 18 of Pregnancy

Groups	No. of animals	No. of implantation	No. of dead/ Under developed fetuses	No. of fetuses	No. & % of Resorption
Control	8	63	2	59	2 (3.17)
Valproic acid	8	65	3	40	22 (33.84)
Piracetam	8	60	1	59	0 (0.00)
Valproic acid + Piracetam	8	62	0	53	9 (14.51)

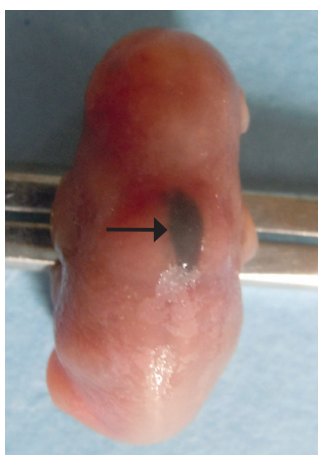


Fig. 2 A

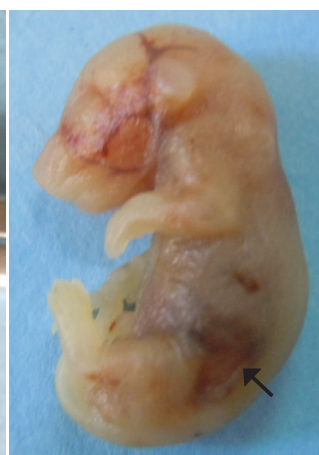


Fig. 2 B

Fig. 2 A & 2 B: showing widespread subcutaneous haemorrhage (arrow) in group II



Fig. 3: Showing increased vertebral flexion (arrow) in group II

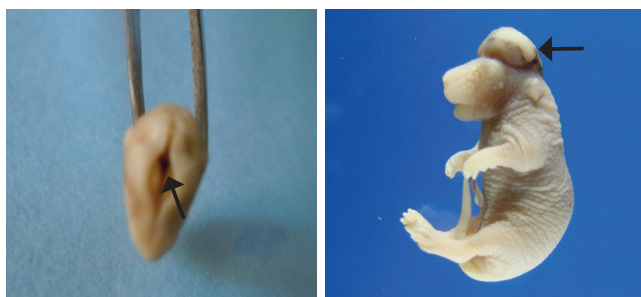


Fig. 4 A

Fig. 4 B

Fig 4 A: Showing Cranioschisis (arrow) and **4 B:** Showing Exencephaly (arrow) in group II

The average CR length of fetuses was 24.13 ± 1.407 mm in Group I (Control) and 19.88 ± 2.154 mm in Group II (VPA treated fetuses). In Piracetam treated fetuses the length was 24.13 ± 1.814 mm and in VPA+ Piracetam fetuses it was 22.12 ± 1.691 mm.



Fig 5: Showing rudimentary tail (arrow)

In accordance with the reduction in weight, the CR length of the fetuses was also reduced. In comparison to the Group I (Control fetuses) the reduction in length in Group II and IV was highly significant ($p < 0.001$) but in Group III it was not significant ($p > 0.05$). The reduction in length of Group II and IV was highly significant ($p < 0.001$) in comparison to that of Group III ($p < 0.001$). The significance of reduction was maximum in Group II followed by Group IV in comparison to Group I and III.

The resorption in Group II and Group IV treated mice was observed to be 33.84% and 14.51% respectively while 3.17% resorption was observed in Group I. No resorption was observed in Group III mice (Table 3).

In control group as well as Piracetam treated group no malformations of fetuses was observed. In VPA treated group highly significant decrease

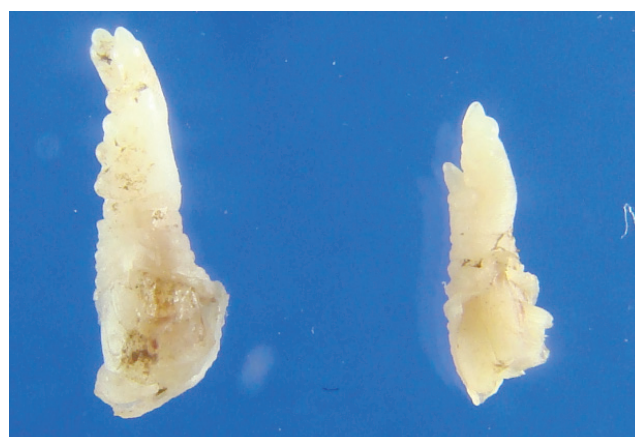


Fig 6: Showing syndactyly of 3rd and 4th digits

in the weight and length of fetuses was observed (Table 1 & 2 and Fig. 1). In addition, marked subcutaneous haemorrhage was seen in most of the fetuses (Fig. 2A, 2B). Hyperflexion of the vertebral column was a common feature (Fig. 3). The fetuses exhibited cranioschisis as well as exencephaly (Fig. 4A, 4B). The tail defects included short tail, rudimentary and complete absence of tail (Fig. 5). The limb defects included adactyly, ectrodactyly, brachydactyly as well as globular paw with syndactyly of 3rd & 4th digits (Fig. 6).

DISCUSSION

VPA is a widely used antiepileptic drug. It is known to reduce fetal weight drastically and is an established teratogen.² This is known to cross the placenta and cause a wide spectrum of congenital anomalies.³⁻⁹

In the present study, incidence of resorption observed in VPA treated (Group II) and VPA + Piracetam treated (Group IV) mice was found to be 33.84% and 14.51% respectively while 3.17% resorption was seen in controls (Group I). No resorption was seen in Piracetam treated mice (Group III). Incidence of resorption in VPA + Piracetam treated mice was significantly low in comparison to VPA treated mice. The absence of resorption in Group III may be a chance factor.

The average weight and CR length of Group I, Group III and Group IV fetuses was significantly higher in comparison to Group II treated fetuses ($P < 0.001$). The teratogenic mechanism of VPA seems to be multifaceted as it is reported to affect the cytoskeleton, cell motility, zinc, methionine, homocysteine and glutathione metabolism.²³⁻²⁷ It also affects the peroxisome proliferation-activated interaction and gene expression.^{28,29} It has been shown to reduce vitamin E level³⁰ as well as folic acid. The deficiency of which is proved to be responsible for teratogenicity, especially

neural tube defects as it acts as a coenzyme in reduction, DNA synthesis, protein metabolism and methyl donor reactions.³¹⁻³³ Its lack can cause DNA hypomethylation and DNA fracture and thus abnormal gene productions.³⁴ Under these effects, the intrauterine growth retardation and the related weight reduction appear to be associated with developmental toxicity effect of VPA.³⁵⁻³⁷ Haemorrhage, the blanket effect of VPA can be explained on the basis of its effect on methionine, homocysteine and glutathione metabolism. Increased vertebral (lumbar) flexion, limb defects and tail defects may be explained on the basis of its effect on cytoskeleton, cell motility and production of free oxygen radicals. The neural tube defects such as cranioschisis and exencephaly may be explained on the basis of loss of heterozygosity of gene critical development for which folic acid deficiency seems to be responsible. All these multifaceted effects of VPA pooled together may be responsible for VPA induced battery of congenital malformations. No particular effect can be assigned to any particular anomaly.

In the group treated with Piracetam the length of the fetuses was approximately equal to those of the controls though there was slight difference in weight. Piracetam improves mitochondrial function where conversion of food energy to the energy of ATP occurs.³⁸ This helps in the oxidation of toxic compounds by mitochondrial enzymes. Thus the increased amount of antioxidants neutralise the effects of free oxygen radicals produced by VPA decreasing the number of apoptotic and dead cells.³⁹ Hence, Piracetam protects against the induced deleterious effects of VPA on a given subject.

Piracetam stimulates prostacyclin synthesis which has vasodilator as well as platelet aggregation inhibitory properties.⁴⁰ Due to this effect there is decreased adhesion of red blood cells to endothelium. This facilitates movements of erythrocytes through the circulation providing sufficient oxygen to the cells whether developing or developed. Thus it also neutralizes the toxic effects of free oxygen radicals.⁴¹

In the present study, in the group where Piracetam has been administered along with VPA, the congenital malformations are significantly reduced ($p < 0.001$) in comparison to those of only VPA treated group. It could not completely eliminate the effect of VPA which is a potent teratogen. The reason may be that the dose of Piracetam was not sufficient enough to protect against VPA. So, for proper protective function there has to be a proper balance between the dose of teratogen "VPA" and the dose of protective agent "Piracetam". It may be possible that higher doses of Piracetam would have protected the treated subjects. This suggests that Piracetam if given in higher doses in pregnant epileptic patients might protect the conceptus against the deleterious effects of VPA or may be other antiepileptic drugs also.

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