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Effect of sodium valproate on neural tube development in chick embryos

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ABSTRACT

Valproic acid is an antiepileptic drug prescribed as monotherapy in newly diagnosed cases of Epilepsy. It is also useful in combating generalized Tonic-Clonic Seizures, Partial Seizures, and Myoclonic Seizures. It acts by increasing the levels of the Neurotransmitter GABA in the cerebrum. Valproate inhibits sustained repetitive firing induced by depolarization of cortical or spinal cord neurons. It produces small reductions of the low-threshold (T) Calcium current at clinically relevant but slightly higher concentrations than limit sustained repetitive firing. Reducing T currents may contribute to the effectiveness of Valproic acid against partial and tonic-clonic seizures and absence seizures respectively. *In vitro*, Valproate can stimulate the activity of the GABA synthetic enzymes, Glutamic Acid Decarboxylase and inhibit GABA degradative enzymes, GABA transaminases. However, studies show that they cause defects in the formation of neural tube if used during pregnancy. In the present study fertilized eggs were administered with Sodium Valproate and the development of neural tube was studied after 21 days. The histological and gross features of neural tube were identified.

Keywords: Sodium Valproate, Chick embryo, Neurulation.

INTRODUCTION

The chick brain and nervous system starts developing from the neuroectoderm nearly 20- 21 hours of incubation. At 23-26 hrs neural folds are visible in the region of head. Neural folds meet at the midbrain after 26hrs. By 33-38 hrs three primary brain vesicles are seen. After 40-45 hrs when the cranial flexure occurs five neuromeres of the hindbrain are distinct (1) and anterior neuropore closes. By 48 hrs posterior neuropore closes. 52-64 hrs the forebrain is lengthened and constrictions between brain parts deepened (Hamburger – Saunders).

Neural tube defects (NTDs) are one of the most common birth defects, occurring in approximately one in 1,000 live births. Failure of closure of neural tube during development results in anencephaly or spina bifida aperta but encephalocele is possibly post closure defects. Case reports and epidemiologic studies have implicated widely differing therapeutic drugs as one of the causative factors for neural tube defects.

An anti- epileptic agent has capacity to cause fetal abnormalities when administered to the pregnant women. Sodium Valproate produced dose-related teratogenic effects in rodents. Antiepileptic drug therapy must not be continued throughout pregnancy, as there is likelihood of foetal exposure to the antiepileptic drug.(S. Kaneko et al ;1983). Valproic acid causes central nervous system symptoms which include sedation, ataxia, tremor; rash .The aim of this study is to demonstrate the effect of Sodium Valproate in early stage chick embryos on neural tube development both before and after closure of neural tube.

MATERIAL AND METHODS

a) SELECTION OF EGGS

Well developed, mature and healthy fertile eggs were selected from the breeders from white leg horn (*Gallus gallus*). Excessively large or small eggs, cracked or thin shelled eggs were avoided because their will have difficulty in retaining moisture which is needed for proper chick development. Penetration of microorganisms increases in cracked eggs. Eggs should not be washed or wiped with clean cloth as it removes the protective coating and promotes the entry of microorganism. Rubbing and washing also

serves to force microorganism through the pores of the shell.

b) INCUBATION OF EGGS: It was done for a period of 24 hrs.The maintained temperature, 101 ° F for first week
102° F for second week
103° F for third week

Optimum growth for most of the species requires a relative humidity of 60% until eggs begin to pip, after which the relative humidity should be raised to 70%.The humidity was maintained inside the incubator by placing an open pan of water with suspending a piece of cloth from the water, proving wick action.

c) ADMINISTRATION OF TERATOGENIC AGENTS IN TO INTACT CHICK EMBRYO

At day 1, a small hole over the broad end of the egg was made using 22-gauge needle. 0.5 micrograms of Sodium Valproate was injected into the egg. Same dosage was given to another group of eggs after completion of 48 hours by using an insulin syringe. After administration of drug the hole was sealed with molten wax and the eggs were placed back into the incubator.

d) PROCESSING AND STAINING

After 21 days of incubation the eggs were broken and the embryo was collected and fixed in 10% formalin solution for 48 hrs. The brain tissue was separated, processed and stained with Haematoxylin and eosin stains. The slides were studied under the simple microscope and various features were identified.

e) DATA ANALYSIS

The data is analyzed statistically using SPSS software (version 17.0)

OBSERVATION AND DISCUSSION

Sodium Valproate has most influence on organogenesis stage of development where organs follow a distinct sequence of cell division, migration differentiation and cell death. The drug causes oxidative stress leading to apoptosis. Most frequently results in the failure of the neural tube closure (spina bifida) and may lead to reduced post natal cognitive function in addition to major congenital malformations(2).

The normal chick embryo (Figure 1) has shown devastating changes after the administration of Sodium Valproate (Figure 2). Sodium Valproate administration resulted in a dose dependent massive reduction in brain cell number as compared to the number of brain cells from control. Sodium Valproate induced cytotoxicity manifested by dose dependent disturbance of cell-cycle resulted in an overall depression of proliferation activity clearly associated with the occurrence of malformations and embryonic death (3). The histological study of normal chick embryo brain tissue (Figure 3) was compared with the drug administered chick embryo brain tissue (Figure 4) at same age, which showed a gross loss in cellularity.

The loss in the cellularity could be attributed to two factors:

- (1) A decrease in proliferation of brain cells and
- (2) Induction of cell death in the brain cells of drug treated embryos.

The results of the present study corroborate both the possibilities. Brain cells obtained from Sodium Valproate treated fetuses upon incubation *in vitro* showed a decreased proliferative ability (cell number) as compared to brain cells of untreated fetuses. Sodium Valproate produced dose-related Teratogenic effects. The brain cells of foetuses obtained from SV treated mice showed an increased population of cells with typical apoptotic morphology(4).

FIGURE LEGENDS



Figure 1: Normal chick embryo



Figure 2: Undeveloped chick embryo after treatment with sodium Valproate

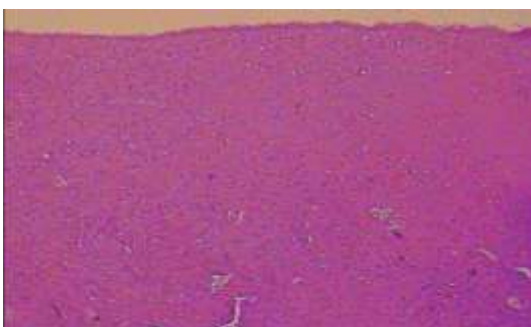


Figure 3: Histology of normal chick embryo brain

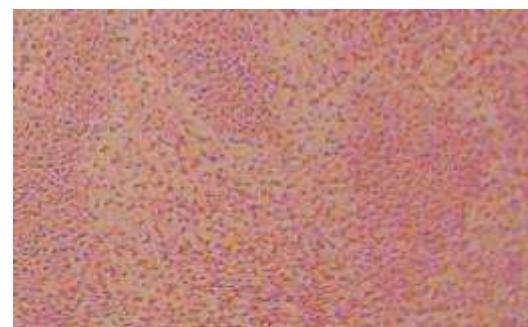


Figure 4: Histology of drug treated chick embryo brain

REFERENCES

- [1] V. Hamburger & H.L. Hamilton; 1951 ,page 55-57
- [2] Katie Alexandra Lloyd. "A scientific review: mechanisms of Valproate-mediated teratogenesis" Oxford university press, Bioscience Horizons (1-10): volume 6, 2013.
- [3] Kelly PG, Regan CM. "study on Valproate-induced perturbations of neurulation in the explanted chick embryo." Toxicology.71 (1-2):137-44. 1992.

- [4] Kaneko.S, K.Otani, Y.Fukushima, T.Sato, Y Nomura & Y.Ogawa “transplacental passage and half life of sodium Valproate in infants born to epileptic mothers” British Journal of Clinical Pharmacology, 15, 1983.
- [5] Ornoy A. Valproic acid in pregnancy: how much are we endangering the embryo and fetus? Reproductive Toxicology.28 (1):1-10. 2009
- [6] Alexander.F.W “Sodium Valproate and pregnancy.” Arch. Dis. Child., 54, 240. 1979.
- [7] Dickinson et al. Transmission of Valproic acid (Depakene) across the placenta: Half-life of the drug in mother and baby. J. Pediatr., 94, 832. 1979.
- [8] Dickinson's group (1979) and Nau's group, “Transmission of Valproic acid (Depakene) across the placenta: Half-life of the drug in mother and baby”. J. Pediatr., 94, 832. 1982.
- [9] Cecilie M Lander “Antiepileptic drugs in pregnancy and lactation.” 2006.
- [10]N Adab, U Kini et al. “The longer term outcome of children born to mothers with epilepsy”Journal of Neurology ,Neurosurgery Psychiatry;75:1575-1583,2004.
- [11]Somsak Tiamkao “The efficacy of intravenous sodium Valproate and phenytoin as the first-line treatment in status epilepticus: a comparison study”, Bio Medical Central, Neurology, 13:98, 2013.
- [12]G.Mawer et al. studied “Pregnancy with epilepsy: Obstetric and neonatal outcome of a controlled study” 2010.