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Comparative study of drug damage caused on Cerebrum in Chick Embryos administered with Drugs - Cyclophosphamide and Sodium Valproate

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Abstract :

Cyclophosphamide

Cyclophosphamide is an alkylating agent and it comes under the category of Nitrogen mustards, which is used in the treatment different types of Cancers (Goodman and Gilman 9th edition 1996). As Malignant Cells (Cancer Cells) present with a Mutant or Absent p53 gene which fails to Stop Cell Cycle multiplication and do not allow the tissue to undergo Apoptosis. i.e, Programmed Cell Death (PCD), therefore the Cells multiply unlimitedly leading to the formation of Tumour.

To treat cancer this drug was Synthesized in 1854 and its properties was elaborately described in 1887. A general review about Cyclophosphomide was presented by Gilman and Philips(1946) and a similar review was provided by Ludlum and Tong(1985). This drug acts by cell cycle arrest, DNA repair and apoptosis, a specific form of nuclear fragmentation. Hemminki & Ludlam (1984) explained that it is generally agreed that Nitrogen Mustards interstrand cross-linking and is associated with Cytotoxicity. Gracia et al (1988) explained that Cyclophosphamide interstrands cross linkage of DNA leading to Retardation of Cell Division. The ultimate cause of cell death and related DNA damage was not known. Fisher (1994) expressed that Specific cellular responses include Cell Death .

Sodium Valproate

Valproate/Valproic acid (Depakene,et al 1978) is an antiepileptic drug which was approved by United States of America in 1978, after more than a decade of use in Europe(Goodman and Gilman 9th edition 1996).According to K.R Kristin Robinson April 2002 Valproic Acid/Valproate (Depakote) is a third drug that is prescribed as monotherapy in newly diagnosed cases of Epilepsy. It acts by increasing the brain's levels of the Neurotransmitter GABA. It is useful in combating generalized Tonic-Clonic Seizures, Partial Seizures, Myoclonic Seizures. Valproate causes birth defects; exposure during pregnancy is associated with about three times major abnormalities as mainly spina bifida with the risks being related to the strength of medication used and use of more than one drug. It causes "valproate syndrome". Characteristics of this valproate syndrome include triangle-shaped forehead, epicanthic folds with altered physical characteristics (dysmorphic features). It can cause side effects on the Gastrointestinal tract such as Diarrhoea, abdominal pain, nausea, and vomiting.

In the present study fertilized eggs were administered with Cyclophosphamide and Sodium Valproate during the process of incubation in two different sets of eggs. The development of Cerebrum was studied during 21 days of incubation. The gross features of Cerebrum were identified during different stages of development. Cyclophosphamide and Sodium valproate cause cytotoxicity results in depression of proliferation of cell activity in the Cerebrum associated with malformations and often leading to embryonic death.

Keywords: Cyclophosphamide**,** Sodium Valproate, Chick embryo, Cerebrum

INTRODUCTION:

The chick brain and nervous system starts developing from the ectoderm nearly 20- 21 hours of incubation.

Stage 7 : At(23 -26hrs) neural folds are visible in the region of head.

Stage 8 : (26 -29 hrs) Neural folds meet at the midbrain.

Stage 10 : (33-38 hrs) : Three primary brain vesicles are seen.

Sage 11 : After(40-45) hrs when the cranial flexure occurs five neuromeres of the hindbrain are distinct (V. Hamburger &H.L. Hamilton, 1951) and anterior neuropore closes.

By 48 hrs posterior neuropore closes. 52-64 hrs after the forebrain is lengthened and constrictions between brain parts deepened (Hamburger – Saunders).

The study of the Cerebrum in Chick embryo (Gallus gallus) is very important because it controls physical activities,maintenance of the balance of the organism, regulation of muscle tone (1,2). The chick brain consists of three mainparts cerebrum; cerebellum and medulla oblongata. The chick has large cerebral hemispheres and small cerebellar hemispheres (3). There are no significance differences that occur in the central nervous system of the birds compared to that mammal (4). The cerebral hemispheres were separated from cerebellum by a transverse fissure (5). The cerebrum in chick embryo of 20 days age consists of two components, gray matter and white matter. The gray matter is situated externally while the white matter is situated internally (6). Also cerebrum consists of cortex and medulla. The cerebral cortex shows six layers- They are:-Molecular layer, External granular lamina, External pyramidal lamina, Internal granular lamina, Internal pyramidal lamina, Multiform layer(7).Considering the

Multifunctional Property of Cyclophosphamide, the present study was undertaken to observe and elucidate the changes in the Cerebrum after
administration of Drug. The changes were considered in relation to the anti-histogenesis or anti-mitotic activity of Cyclophosphamide by administering the drug into Fertilized Eggs. An attempt has been made to observe if the Drug passes through Placental Barrier leading to Malformations of Foetus. Such a study will help in treating Cancer Patients who are Pregnant. Cyclophosphamide has been experimented by few Scientist's on Laboratory animals like Chick Embryos etc. And their observations were noted. Case reports and epidemiologic studies have implicated widely differing therapeutic drugs as one of the causative factors for Cental Nervous system Malformations. A teratogenic agent has capacity to cause fetal abnormalities when administered to the pregnant women. (Teratog carcinog mutagen.1985;5(2):75-88). Cyclophosphamide (CPA) is one of the best studied teratogens; it produces primarily CNS and skeletal anomalies in humans and experimental animals. Sodium Valproate as an anti-epileptic agent has capacity to cause fetal abnormalities when administered to the pregnant women. Sodium Valproate produced dose-related teratogenic effects in Chick embryos. 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 Cyclophosphamide and 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 are selected from the breeders that are white leg horn (gallus gallus). Excessively large or small eggs, cracked or thin shelled eggs are avoided because they will have difficulty in retainingmoisture 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 disease organisms through the pores of the shell.

b) **INCUBATION OF EGGS**: Done for a period of 24hrs.The temperature should be
101 degree Fahrenheit for first week
102 degree Fahrenheit for second week
103 degree Fahrenheit 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 is maintained inside the incubator is maintained by placing an open pan of water with suspending a piece of cloth from the water, proving wick action.

c) **ADMINISTRATION OF CYCLOPHOSPHAMIDE AND SODIUM VALPROATE 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 cyclophosphamide is injected into the egg .Same dosage was given to another group of eggs after completion of 48 hours. It was done with an insulin syringe .Following drug administration; the holes are sealed with molten wax after which the eggs were placed back into the incubator.
Similarly 0.5 micrograms of sodium valproate is injected into the egg. Same dosage was given to another group of eggs after completion of 48 hours following the above steps.

d) **PROCESSING AND STAINING**: After 21 days of incubation the eggs are broken and the embryo is collected and fixed in 10% formalin solution for 48 hrs seperately for both the drugs. The brain tissue is separated, processed and stained with Haematoxylin and eosin stains. The slides are studied under the simple microscope and various features are identified.

e) **DATA ANALYSIS**: The data is analyzed statistically using SPSS software (version 17.0)

RESULTS AND DISCUSSION:

The normal chick embryo (Figure 1) has shown devastating changes after the administration of Cyclophosphamide (Figure 2). CP administration resulted in a dose dependent massive reduction in brain cells number as compared to the number of brain cells from control. CP-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. The histological study of normal chick embryo Cerebrum (Figure 4) was compared with the drug administered chick Cerebrum (Figure 5) at same age, which showed a gross loss in cellularity. The Microscopic Picture with H&E Stain showed reduced number of Neurons in all Layers of Cerebrum**.** 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 CP treated foetuses. The results of the present study corroborate both the possibilities. Brain cells obtained from CP treated foetuses upon incubation in vitro showed a decreased proliferative ability (cell number) as compared to brain cells of untreated foetuses. The cells of Cerebrum in foetuses obtained from CP treated chick embryos showed an increased population of cells with typical apoptotic morphology. The main effect of cyclophosphamide is due to its metabolite phospharamide mustard which is formed in cells that have low levels of ALDH (Aldehyde dehydrogenase). The metabolite forms DNA cross links between and within the DNA strands at guanine N7 positions which result in cell death. The

toxicity is greatest during the S or DNA synthetic phase of cell cycle.

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. The normal chick embryo (Figure 1) has shown devastating changes after the administration of Sodium Valproate (Figure 3). Sodium Valproate administration resulted in a dose dependent massive reduction number of cells in Cerebrum as compared to the number of brain cells from control. The Microscopic Picture with H&E Stain showed reduced number of Neurons in all Layers of Cerebrum**.**

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 .The histological study of normal chick embryo brain tissue (Figure 4) was compared with the drug administered chick embryo brain tissue (Figure 6) at same age, which showed a gross loss in cellularity. The loss in the cellularity could be attributed to two factors:

A decrease in proliferation of brain cells and 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 number of cells in Cerebrum of untreated fetuses. Sodium Valproate produced dose related Teratogenic effects. The cells of Cerebrum in foetuses obtained from SV treated chick embryos showed an increased population of cells with typical apoptotic morphology

The final conclusion is that the chick embryos treated with cyclophosphamide have shown more growth retardation compared to the embryos treated with sodium valproate

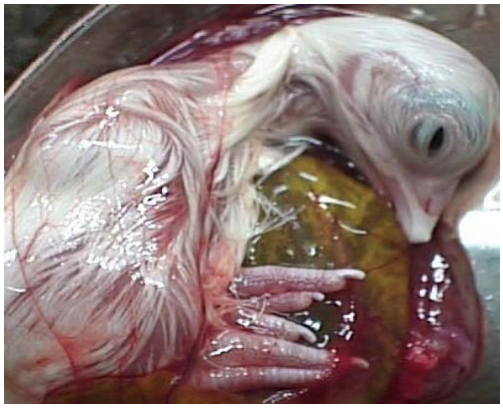

Figure 1: Normal chick embryo

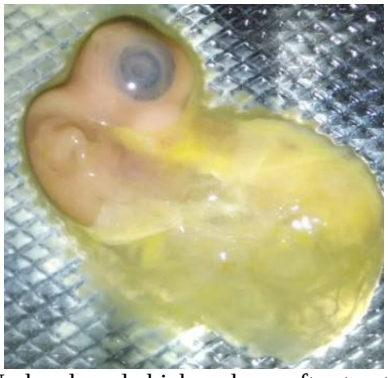

Figure 2:Undeveloped chick embryo after treatment with cyclophosphamide

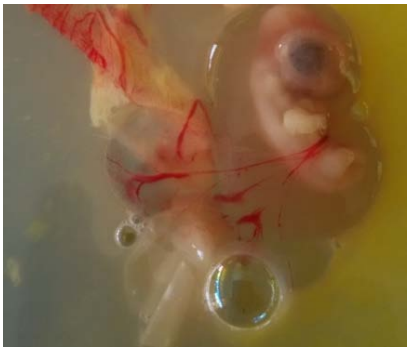

Figure 3: Undeveloped chick embryo after treatment with sodium valproate

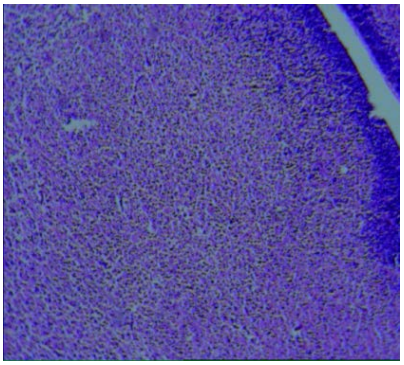

Figure 4: Histology of normal chick embryo brain

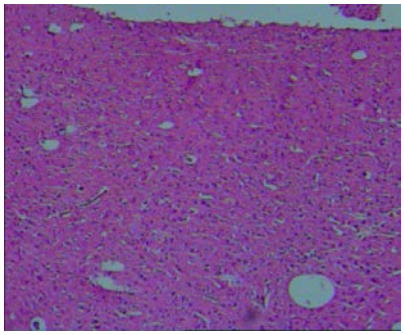

Figure 5: Histology of cyclophosphamide treated chick embryo brain

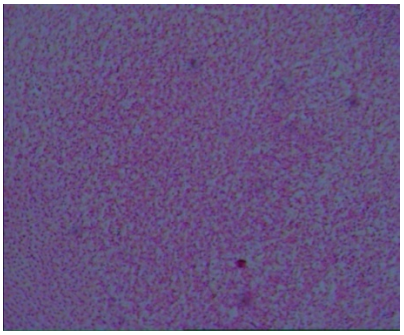

Figure 6: Histology of sodium valproate treated chick embryo brain

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