Effect of Cyclophosphamide on Neural Tube Development in Chick Embryos

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ABSTRACT:
Cyclophosphamide is a nitrogen mustard alkylating agent. CP has potent immunosuppressive properties and issued clinically in a number of autoimmune disorders like Wegener’s granulomatosis, rheumatoid arthritis, nephritic syndrome, systemic lupus erythematosus and has also been used to prevent organ rejection after transplantation. In the present study fertilized eggs were administered with cyclophosphamide and the development of neural tube was studied after 21 days. The histological and gross features of neural tube were identified. Cyclophosphamide cytotoxicity results in depression of proliferation of cell activity associated with malformations and embryonic death. Injection of the drug causes depression of mitotic activity by day 2 which produces malformations.

INTRODUCTION:
The chick brain and nervous system starts developing from the neurectoderm 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.
Stage 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).

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 encephaloceles are possibly post closure defects (Padmanathan-May 2006) Case reports and epidemiologic studies have implicated widely differing therapeutic drugs as one of the causative factors for neural tube defects. 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. It is one of the most extensively studied anti-neoplastic agents. It is believed to be causing cross linking of DNA to play a critical role in anti-neoplastic properties (Mirkes, 1985).
The aim of this study is to demonstrate the effect of cyclophosphamide 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 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 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

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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 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 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.

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. 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 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 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 brain cells of foetuses obtained from CP treated mice showed an increased population of cells with typical apoptotic morphology.

The main effect of cyclophosphamide is due to its metabolite phosphoramidate mustard which is formed in cells that have low levels of ALDH (Aldehyde dehydrogenase). The metabolite forms DNA crosslinks 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.

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FIGURE LEGENDS:

Figure 1: Normal chick embryo
Figure 2: Undeveloped chick embryo after treatment with cyclophosphamide
Figure 3: Histology of normal chick embryo brain
Figure 4: Histology of drug treated chick embryo brain
FIGURES: