Effect Of Low Doses Of Retinoic Acid On Spinal Cord Of Rat Fetuses

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Abstract: Retinoic Acid is an oxidative metabolite of Vitamin A, which plays a key role in many biological processes including growth and differentiation of epithelial tissues, vision, spermatogenesis and embryonic development. Retinoic Acid in a single low dose (7.5mg/kg body weight) was injected intraperitoneally to experimental group of albino rats on gestation day 11 (sperm +ve = 0 day). An equivalent amount of the vehicle (normal saline) was similarly injected to corresponding control group of rats. On 20th day of gestation, the rats were sacrificed and fetuses were taken out from both group of rats. On histological evaluation of spinal cord of treated group of fetuses, dilated central canal, distorted anteromedian fissure and infiltration of inflammatory cells were found. No such findings were observed in control group of rats.

Key Words: Rat, Fetus, Spinal Cord, Retinoic Acid

Introduction:

Neurulation and the subsequent growth and patterning of the neural tube is an immensely complex process. Patterning of the growing neural tube occurs in both the rostrocaudal axis and the dorsoventral axis under the influence of extracellular morphogens such as fibroblast growth factors, retinoic acid, Wnts, sonic hedgehog and bone morphogenetic proteins (Lee & Jessell, 1999; Briscoe & Ericson, 2001; Helms & Johnson, 2003).

Failure to complete closure of the neural tube at the rostral & caudal end leads to anencephaly and spina bifida (Copp et al. 1990). RA is a well known teratogen when administered to embryos and one of its many effects is to induce neural tube defects. The present investigation has been undertaken to study the effect of low doses of Retinoic Acid (RA) administered in pregnant rat on 11th day to see in what ways RA is involved in neurulation by examining histological aspect of Spinal Cord.

Material & Methods:

The total 40 Charles-Foster female rats used in the present investigation. Out of which 20 were treated with desired drug and 20 were kept as control.

Male & female Charles-Foster rats (150-200 gm) were placed together (1:3) overnight for mating & presence of sperm in the vaginal smear was taken as gestation day 0 (GD0).

RA (7.5mg/kg body weight) was administered intraperitoneally once in a single dose at 9.00 hr on gestation day 11 (GD 11). Control group of pregnant rats were administered similarly with equal volume of vehicle (normal saline). Both control & treated groups were sacrificed on GD 20 by ether anesthesia. In an average 4-6 fetuses were collected and fixed in 10% formalin. Fixed fetuses were washed thoroughly. Care was taken to remove spinal cord from both groups by laminectomy of vertebral column and immediately it was kept in 10% formalin for few weeks for fixation.

Then spinal cord was embedded in paraffin wax. After processing 5µm thick sections were cut with a rotary microtome. Staining was done with haematoxylin and eosin and slides were studied under light microscope.

Results:

On histological evaluation of spinal cord of treated group, it was observed that there was dilated central canal with disrupted ependyma because of which oedematous infiltration were present in grey column along with degeneration of cells (fig.1b). Then spinal cord was embedded in paraffin wax. After processing 5µm thick sections were cut with a rotary microtome. Staining was done with haematoxylin and eosin and slides were studied under light microscope.

Discussion:

The results described above suggest that retinoic acid even in the low doses is teratogenic. RA affects morphological organization & cellular behavior of developing spinal cord. Morphologically the general shape, shape of the lumen and spatial arrangement of the cell populations of floor plate and roof plate of spinal cord appears to be abnormal in treated group of rats.

A variety of cellular effects have been suggested as being responsible for the appearance of spina bifida following RA administration. These include vascular damage, malformation of the notocord, distortion of the neural folds, cell death in the neural tube, delayed posterior neuropore
Fig. 1.a. Photomicrograph of spinal cord of rat fetus of control group. a- Central canal, b- Ventromedian fissure, c- Grey column, d- White Column. H.E. X 40.

Fig. 1.b. Photomicrograph of spinal cord of rat fetus of treated group showing disorganized architectural pattern, cavity or vacuole formation (Arrow), dilated central canal (Arrow head), widened and distorted ventromedian fissure (Double Arrow head). H.E.X 40.

Fig. 2.a. Photomicrograph of ventromedian white column of spinal cord of rat fetus of control group. H.E.X 40.

Fig. 2.b. Photomicrograph of ventromedian white column of spinal cord of rat fetus of treated group showing cell proliferation along with inflammatory cells. H.E.X 40.

closure (Tibbles & Wiley, 1988; Kapron- Bras & Trasler, 1988a,b; Alles & Sulik, 1990 Padmanabhan, 1998). This may be the basis of findings (oedematous infiltration and disorganized architectural pattern) of present study. Evidence in favor of such a role comes from experiment involving the retinoic acid receptors (RARs), the nuclear transcription factors through which RA acts in the nucleus and the RA synthesizing enzymes. Thus RAR is expressed in the open neural tube and in the closed neural tube (Ruberte et al. 1991; Smith & Eichele, 1991; Smith, 1994; Chen et al. 1995).

The neural tube is initially a single layer of pseudostratified epithelium which then proliferates rapidly and differentiation of neurons takes place t on laterally in the mantle layer. There is a clear distinction between the densely packed proliferating cells of the ventricular zone and the more loosely arranged mantle zone in control group. Notochord induces proliferation of the neural tube. RA has a positive effect on cell proliferation (Crowe et al. 2003). Treated group shows increase in the numbers of differentiated neurons along with some inflammatory cells in the ventral mantle zone (ventromedian grey column).

Noticeable observations in the present study was widened and distorted anteromedian fissure accompanied by dilated & disrupted central canal. Excess RA causes mediolateral narrowing of ventral half of the neural tube in comparison with the dorsal domain which causes widened and distorted anteromedian fissure and simultaneously dilated
cental canal.

In few of previous studies the adverse effects of RA were found to be dose dependent at the different stages of embryo development. Mouse embryos exposed to excess RA (12 mg/kg) on day 7½ of gestation showed retardation of general development, abnormal differentiation of the cranial neural plate and abnormal development of the hindbrain (Morriss-kay et al., 1991).

References: