ORIGINAL ARTICLE

Response to Injury and Estrogen in Vertebral Epiphyseal Plates and End Plates

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ABSTRACT

Although Estradiol (E2) has been reported to affect the proteoglycan synthesis and water content of the cartilage, its effects on epiphyseal and end plates of the intervertebral disc have not been widely explored. An experimental study using rabbit as a model was designed to observe the effects of estradiol on the epiphyseal plate and end plate of stab wounded intervertebral disc of young male domestic rabbits, with emphasis on changes in cell population and matrix. It was conducted at the Department of Anatomy, College of Physicians and Surgeons Pakistan, Regional Center, Islamabad. Animals in the control and experimental groups were operated upon and their intervertebral discs were injured to a depth of 4mm. Experimental group was given 5mg/kg intramuscular injection of E2 after the surgery. Animals (N=120) in control and experimentalgroupswere sacrificed after five (120 hrs.) and seven days (168 hrs.) of injury and intervertebral discs were harvestedfromboth groups (n=60).10µm thick sections were stained with Hematoxylin,Eosin and Alcian Blue stain. Quantitativeand qualitative data was recorded for the groups. E2 increased mitosis of chondrocytes significantly in epiphyseal plate. The end plate did not show a significant response. The results suggest that therapeutic administration of E2 can be mitogenic and may recruit cells to the intervertebral disc in response to injury which may have an effect on its response to injury and subsequent healing.
Keywords: Chondrocytes. Double- eyed chondrocytes. Epiphyseal plate. Estrogen..

INTRODUCTION

The intervertebral disc forms the cartilaginous articulation of the spine, enabling it to bend and twist in various directions. It comprises of two main regions; an inner nucleus pulposus, and, an outer collagenous structure of concentric lamellae encircling the nucleus, the annulus fibrosus[1]. During growth period of life typical epiphyseal cartilages with all layers described on growth cartilages of long bones are formed on the surface of these cartilaginous plates facing the vertebral bodies[2]. Vertebral end plates are the top and bottom portions of the vertebral bodies that interface with the intervertebral discs[3].

Estrogen, which is a generic term for the female sex hormones, can either be a naturally occurring steroid hormone,[4]or a synthetic non-steroidal variant, like diethylstilbestrol[5]. 17-β-Estradiol is the primary and most potent naturally occurring placental and ovarian estrogen in mammals[6]. Estradiol dipropionate (E2) is the most potent parenteral estrogen and has a prolonged action[7]. It has been reported that estrogen stimulates proteoglycan synthesis in rabbit chondrocytes in vitro and has a significant impact on the glycosaminoglycans and the water content of the intervertebral discs[8]. Estradiol has been found to significantly increase the synthesis of glycosaminoglycans in uterine connective tissue of the pregnant women[9]. Steroid hormones are used commonly to treat the injury to the intervertebral disc, both intramuscularly or epidurally[10].

Much published literature was not found for the regions of vertebral epiphyseal and end plate. So the current study was conducted to observe the response of estrogen, on the vertebral epiphyseal and end plates of experimentally stab wounded intervertebral discs, with special reference to changes in cell population and proteoglycan content of matrix, using rabbit as an experimental model.

MATERIAL AND METHODS

This experimental study was conducted at the Department of Anatomy, College of Physicians & Surgeons, Regional Center-Islamabad, Pakistan. The study was conducted in two main groups; Control group 'A' and experimental group 'B' with 60 animals in each group (N=120). Young male, 4-6 month old domestic rabbits (*OryctolagusCuniculus)* species were selected for the study. Each group was further subdivided into two subgroups (A1, A2 & B1, B2) of 30 animals each. Animals were fed water and food *ad libitum* and kept in the animal room having a normal fixed 12 hour day and night lighting cycle.

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Animals were anaesthetized with a combination of ketamine and xylazine. On the day '0', a median incision was given on the ventral abdominal wall from the sternum to the umbilicus of the anaesthetized animal. Dissecting in front of the spine between the paravertebral muscles, intervertebral disc was identified as a glistening white band between the two adjacent vertebrae. It was stabbed in a direction perpendicular to the direction of the spine to the depth of 4mm (Figure 1). Tip of the blade had been secured and restrained to make sure a uniform depth of injury upon all the animals and avoid injuring the spinal cord. Abdomen was then stitched in layers with catgut and silk. Animals in the experimental group (B1 & B2) were additionally given an intramuscular injection of Estradiol dipropionate (E2) 5mg/kg body weight, soon after the skin closure.

Animals in the Group 'A1' & 'B1' were sacrificed after 120 hours (5 days), while those in Group 'A2' & 'B2' were sacrificed after 168 hours (7days) of operation, to collect the IVD. Harvested IVD was fixed in 4% buffered formalin. Harvested IVDs were then processed for paraffin embedding and10µm thick coronal sections were cut (Figure 2). Tissue was stained with hematoxylin and eosin stain for recording quantitative data. Alcian blue staining was done to record intensity of staining for proteoglycan content of the two regions. Intensity was graded as "Absent, Mild, Moderate and Deep" on an ascending scale.

Quantitative data was recorded as the mean cell count per defined unit area of the intervertebral disc, with standard error, while the qualitative data was recorded as percentage of observations between the subgroups. SPSS software was used for applying student's t-test to detect significant difference ($p \leq 0.05$) in the means of cell population between the subgroups.

RESULTS

One hundred and twentyintervertebral disc specimens were obtained fromthe rabbits that were operated for the study. None developed wound infection during the course of study. A unique phase of dividing chondrocytes was observed and termed "Double Eyed Chondrocyte"(Figure 3). (New term coined by the Principal Author). It represented a chondrocyte having two nuclei and sharing the same intense basophilic territorial matrix, with or without an intervening cell membrane visible. Since the matrix around these cells was found very uniformly and intensely basophilic, these cells were believed to have divided recently, and were therefore, taken as a marker of mitotic activity in the region where they were observed.

In the individual comparison of control groups (A1 & A2) it was observed that in the epiphyseal plate, number of 'double eyed' chondrocytes was significantly more in the subgroup A2. However, total number of chondrocytes was significantly more in subgroup A1. In the end plate, number of 'double eyed' chondrocytes and total number of chondrocytes was significantly more in subgroup A1. In the animals sacrificed after 120 hrs. in control (A1) and experimental group (B1) number of double eyed' chondrocytes was significantly more in the experimental subgroup in the region of end plate and epiphyseal plate.

In the control group of 120 hrs. (A1) and experimental group of 168 hrs. (B2) it was found that number of 'double eyed' chondrocytes in the epiphyseal plate was significantly more in the subgroup B2. However it was inverse for endplate region. In the annulus fibrosus number of chondrocytes and other cells was significantly more in the subgroup B2. Number of fibroblasts was significantly more in the subgroup A1. Number of macrophages and other cells was significantly more in the subgroup A1 in the NP. In the granulation tissue number of fibroblasts with clear nucleus was significantly more in the subgroup B2. Number of macrophages, neutrophils and vessels were significantly more in the subgroup A1. Total number of cells was significantly more in the subgroup B2.

When comparing control group of 168 hrs. (A2) with experimental group of 120 hrs.(B1), it was found that the number of 'double eyed' chondrocytes was significantly more in the subgroup B1 in the epiphyseal plate and end plate regions.

When the control group of 168 hrs (A2) was compared with experimental counterpart (B2), it was observed that number of 'double eyed' chondrocytes in the epiphyseal plate was significantly more in subgroup B2. Total number of chondrocytes was also significantly more in the subgroup B2.

When the experimental groups (B1 & B2) were compared with each other total number of chondrocytes was significantly more in subgroup B2 in the epiphyseal plate. In the endplate number of 'double eyed' chondrocytes was significantly more in the subgroup B1.

In staining grades, which were recorded as percentage of total observations, it was observed that the epiphyseal plate stained more in the deep category after 7days (subgroup B2). However in case of endplate all the subgroups stained dominantly in the mild category (Figure 4 & 5).

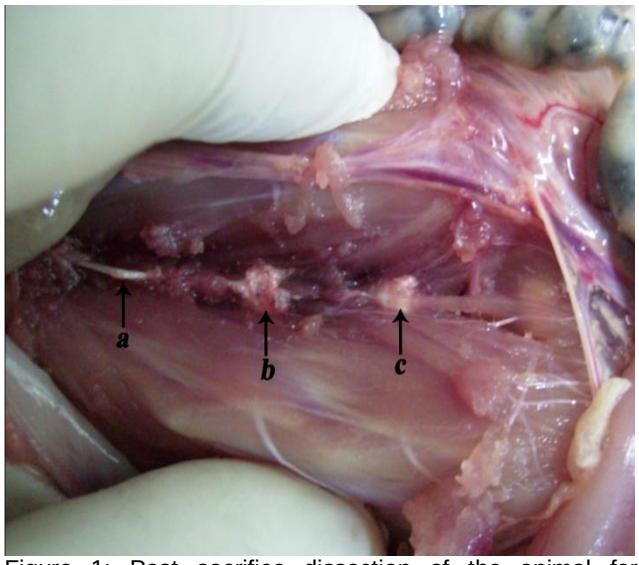

Figure 1: Post sacrifice dissection of the animal for harvesting the intervertebral disc. Arrow (a) points to the anterior longitudinal ligament, arrow (b) points to the injured intervertebral disc and arrow (c) points to the normal intervertebral disc of the animal.

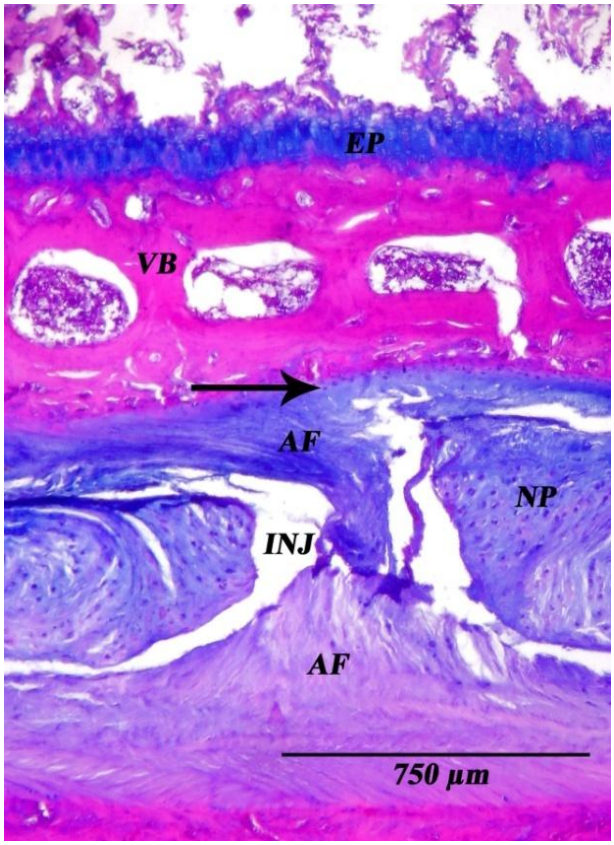

Fig. 2: A low powered photomicrograph of coronal section of intervertebral discshowing regions of epiphyseal plate (EP), vertebral bone (VB), nucleus pulposus (NP), annulus fibrosus (AF) and injury (INJ). Arrow points to the very thin region of end plate. (H & E and Alcian Blue Stain)

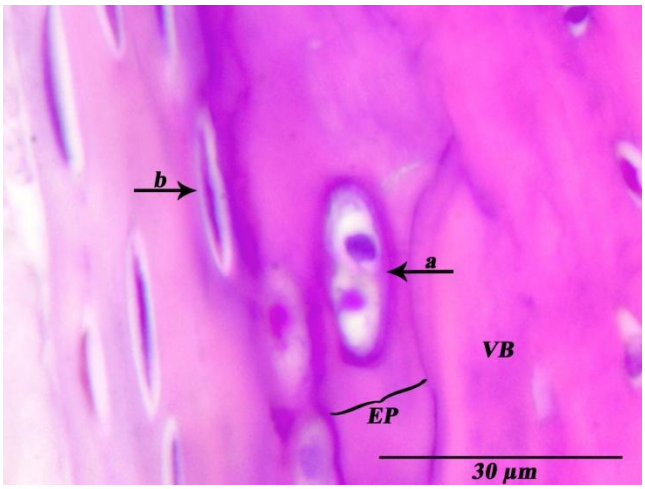

Fig. 3: A 'double eyed' chondrocyte, representing the mitotic activity, sharing the same intense basophilic territorial matrix (arrow 'a') in the end plate (EP).Fibroblasts of annulus fibrosus (arrow 'b') and vertebral body is also seen (VB). (H & E stain)

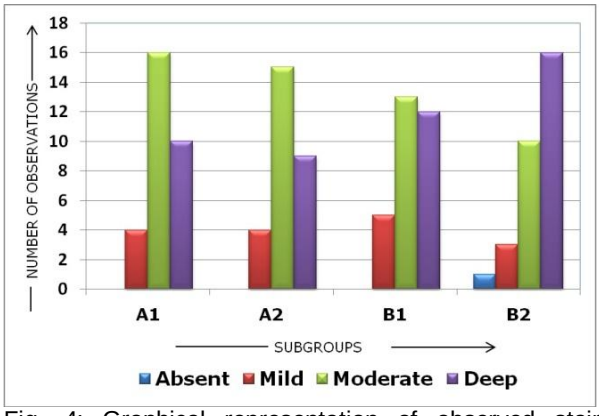

Fig. 4: Graphical representation of observed staining grades of the matrix that has been stained with alcian blue stain to detect the proteoglycan content of the matrix in the region of epiphyseal plate.

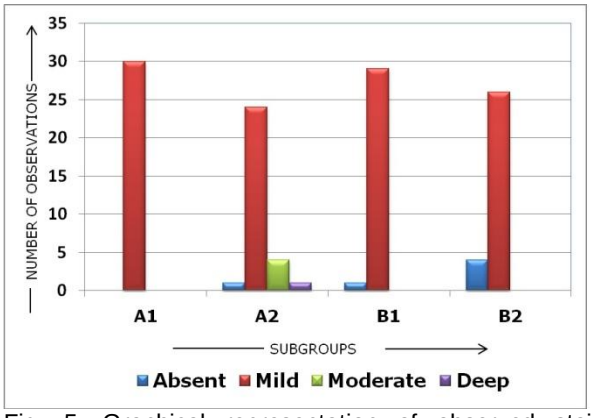

Fig. 5: Graphical representation of observed staining grades of the matrix that has been stained with alcian blue stain to detect the proteoglycan content of the matrix in the region of end plate.

DISCUSSION

The current study was designed to observe the effects of estrogen, beneficial or otherwise, using its parenteral form estradiol dipropionate (E2), on injured intervertebral disc using rabbit as an experimental animal. It was postulated thatestradiol dipropionate will have improve the hydration of the regions, cause deposition of glycosaminglycans and will be mitogenic in the observed regions.

Epiphyseal plate and end plate have chondrocytes as dominant cells in the regions. Therefor population of 'double eyed' chondrocytes was taken as a marker of mitotic activity in these regions. It was found to be increased, in experimental subgroups, in the region of epiphyseal plate. Total chondrocyte population also increased in the experimental subgroups. When injured, the mitotic activity increased resulting in increase of 'double eyed' chondrocytes with a similar increase in the total number of chondrocytes in the area.

In the endplate a significant drop in the mitotic activity after seven days was observed. A falling level of blood estrogen level might be a reason for this finding, but this was anomalous to the findings in the epiphyseal plate. This fact, therefore,needs further evaluation in a separate study.

Total cell population increased in experimental subgroups as compared to control subgroups and this was expected as the E2 had been having a growth promoting effect on the cells in general.

By looking at the staining grades of the epiphyseal plate, it is suggested that fresh matrix is laid down in epiphyseal plate in response to E2. In case of endplate it is observed that the cells are only transiting from the endplate to the annulus but its proteoglycan (PG) content is not increased in response to E2. Another explanation for this might be the thinness of the region of the end plate and the available observation technique. Electron or high resolution microscopy shall be able to shed more light on this observation.

Observed staining grades for the content of proteoglycans did not suggest a massive lay down of matrix, and it is expected, as proteoglycan was not excessively being laid down within a week of healing response. Since the qualitative data was recorded as percentage of total observations, it cannot be relied on as statistically significant.

CONCLUSION

This study provided insight into population of chondrocytes in response to injury and administration of estradiol. Estradiol dipropionate (E2), a potent form of estrogen, had effects on the epiphyseal plate recruiting more cells into the intervertebral disc.

Chondrocytes are the cells that synthesize collagen and other matrix molecules and Estradiol dipropionate exerted a beneficial effect on them

In the light of above observations it is deduced that improving mitosis and recruitment of more cells after injury and administration of E2 may provide the injured disc with cells to help in healing.

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