ORIGINAL ARTICLE

Response of Vertebral Bone Marrow to female sex hormone five days after Injury to Annulus Fibrosus

WAJID ALI CHATHA

ABSTRACT

Vertebral body has marrow cavities where hematopoiesis takes place. There are pluripotent stem cells in vertebral bone marrow and they may be recruited into the bone cells of the vertebra, form chondrocytes orcells of annulus fibrosus. Injury to vertebral disc is a common occurrence in both sexes; however, Estrogen has been reported to have beneficial effects in the postmenopausal women receiving hormone replacement therapy. An experimental study was designed to see the effects of estrogen on cell population of the bone marrow five days after the injury to intervertebral disc, using rabbit as an experimental model. Animals were divided into a control and experimental group of 15 each. Both groups received injury to the annulus fibrosus. Experimental group was given a calculated dose of estrogen after the injury. Animals were sacrificed and intervertebral discs along with marrow spaces were obtained 5 days after injury to the annulus fibrosus. The sections were stained with hematoxylin and eosin. Cells of the myelocyte series were found to be increasing in population in response to hormone therapy. It was concluded that estrogen may lead to recruitment of cells from bone marrow to the cell population of the annulus fibrosus and this maybe beneficial for its healing.

Keywords: Annulus Fibrosus; Bone marrow; Estrogen; Healing;Hormone Replacement Therapy; Injury;Intervertebral disc; Myelocytes; Vertebral body.

INTRODUCTION

The vertebrate body is organized segmentally and this becomes most obviously visible in the vertebral column, which consists of a series of vertebral bones and interconnecting joints and ligaments. During development, the vertebral column develops from the somites, the primary segments of the embryonic paraxial mesoderm. Anatomical, cellular and molecular aspects of vertebral column development have been of interest to developmental biologists for more than 150 years.[1]Hematopoietic failure has been observed in experimental animals following shock and injury. In humans, bone marrow dysfunction has been observed in the red cell component and characterized by a persistent anemia, low reticulocyte counts, and the need for repeated transfusions despite adequate iron stores.[2]Severely injured trauma patients often experience altered hematopoietic functions, manifested by an increased susceptibility to infection and the development of a persistent anemia[3].Bone marrrow stem cells have been used more recently for rejuvenating the myocardium after iscchaemic attacks and renal tubules following acute tubular necrosis[4,5].

The bone marrow acts as a reservoir for multiple stem cell populations, including hematopoietic stemcells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and very small embryonic-like cells (VSELs), which are mobilized at varying degrees into the peripheral circulation following injury.[6]Female sex hormone, Estrogen has been reported to have beneficial effects on healing of injured intervertebral disc and promote formation of granulation tissue.[7,8]Bone marrow-derived cells are multipotent cells that are capable of being chemoattracted to injured tissues, and release many cytokines and trophic factors[9].

Estrogen-replete women appear to maintain higher intervertebral discs compared to untreated post-menopausal women.[10] It has been shown that menopause has a negative effect on bone and on intervertebral discs. Estrogen has a beneficial effect of preserving the health of collagen-containing tissues, including the intervertebral disc. Estrogen should be seen as the first-choice therapy for bones and other collagen-rich tissues, such as intervertebral discs, because it maintains homeostasis of the bone-remodelling unit[11].

Bone marrow responds to injury in a variety of ways and to study effects of injury to cell population of vertebral bone marrow and its modification by a female sex hormone, a basic study was designed using rabbit as an experimental model.

METHODOLOGY

An experimental study was planned between the two groups; Control group 'A' and an experimental group

Correspondence to Dr. Wajid Ali Chatha, Assistant Professor Anatomy, College of Medicine, Arar Kingdom of Saudi Arabia E-mail: drchatha@gmail.com

'B' with 15 animals in each group (N=30). Young male rabbits were selected for the study. Food and water was provided to the animals *ad libitum* during the course of experiment.

At the start of the experiment, animals were anaesthetized with a combination of ketamine and xylazine and laporotomy incision was given on the ventral abdominal wall. The posterior abdominal wall was explored for intervertebral disc. Disc was stabbed in a horizontal direction to the depth of 4mm. Abdomen was then closed. Animals in the experimental group were additionally given an intramuscular injection of Estradioldipropionate (E2) 5mg/kg body weight, soon after the skin closure.

Animals were sacrificed after 120 hours (5 days), for collection of the vertebra. Collectedvertebrae was fixed in 4% buffered formalin and processed for paraffin embedding after decalcification. 10µm thick coronal sections were made and stained with hematoxylin and eosin stain for microscopic observations.

Quantitative data was recorded for number of erythrocytes, monocytes, myelocyte series of cells, fat cells, megakaryocytesand total number of cells in a predefined unit area of the vertebral body.Data thus obtained was analyzed using SPSS.A p-value of equal to or less than 0.05 was taken as significant level of difference between the means of the cell population (TableI).

RESULTS

In the cell population that formed the spectrum of the study, it was found that number of erythrocytes increased significantly in the hormone treated group. The cells of the Myelocyte series increased significantly. Population of the dividing myelocytes also increased significantly in the experimental group.Total number of cells increased significantly in the experimental group. Monocytes also increased significantly. Results for the fat cells and megakaryocytes population in the vertebral bone marrow were not found to be statistically significant.

Fig.1: A Megakaryocyte seen in the control group.

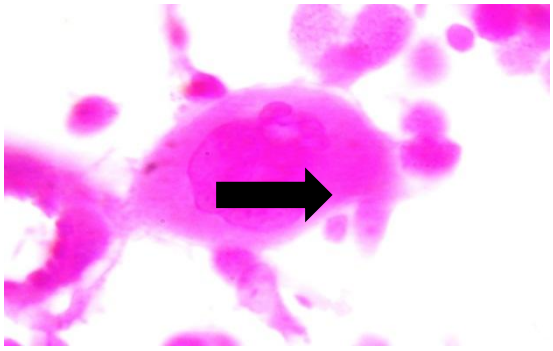


Table 1:Mean cell count in the control and experimental groups of the various cells found in the vertebral bone marrow.

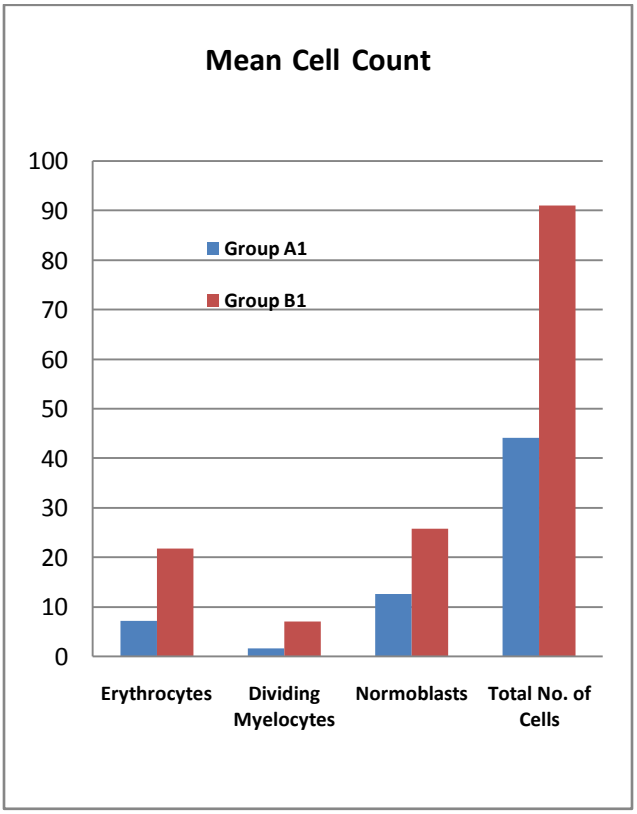


Fig. 2: A low powered micrograph showing the region of the cancellous bone with marrow cavities (A) in a section of intervertebral disc.

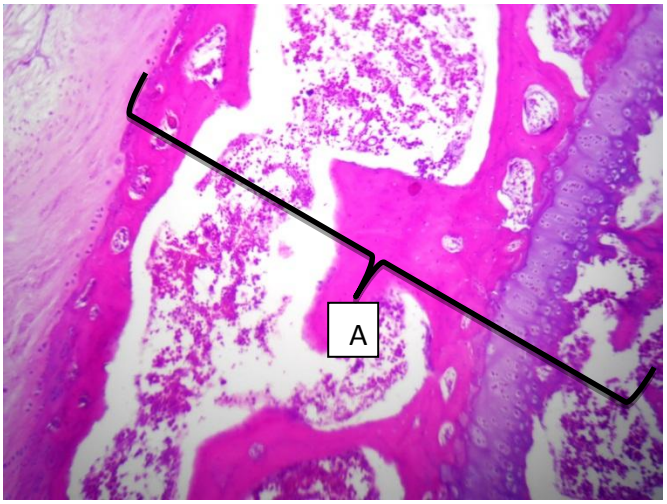


Fig. 3: A high powered micrograph showing dividing myelocytes (broad arrows) and a monocyte (thin arrow).

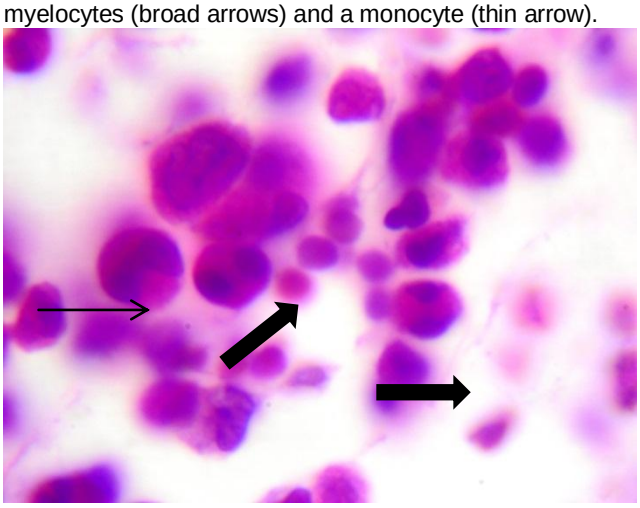
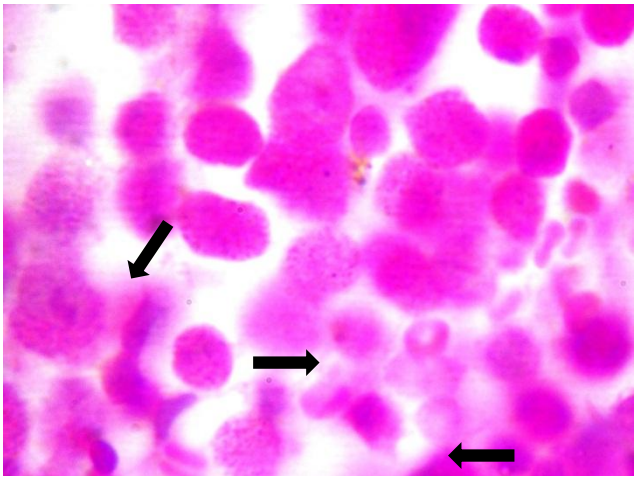


Fig. 4:A high powered micrograph showing the granulocytes (broad arrows) in a section of the vertebral bone marrow.



DISCUSSION

The blood cells begin their lives in the bone marrow from a single type of cell called the pluripotential hematopoietic stem cell, from which all the cells of the circulating blood are eventually derived. The different cells of the myelocyte series are myeloblast, promyelocyte, megakaryocyte, neutrophil myelocyte, young neutrophil metamyelocyte, “band” neutrophil metamyelocyte, polymorphonuclear neutrophil, eosinophil myelocyte, eosinophil metamyelocyte, polymorphonuclear eosinophil, basophil myelocyte, polymorphonuclear basophil[12].

The bone marrow is the primary site of the hematopoiesis and hence a place to look for a variety of pluripotent cells. This study was designed to observe the effect of injury to the annulus fibrosus and the female sex hormone, estrogen, on the vertebral body marrow cell population five days after the injury.

Osteoclasts are also derived from the mature monocytes and macrophages.[13]Osteoblast, chondrocytes and fibroblasts all arise from the monocytes. These chondrocytes and fibroblasts form the major population of the vertebral endplates and annulus fibrosus of the intervertebral disc. Herniation of the disc is a common problem that may require medical or surgical intervention. Not much literature was found on the healing of annulus fibrosus or the vertebral bone marrow. The mitogenic effect of estradiol can have a clinical implication in the period of healing of the intervertebral disc. The promotion of mitogenesis on monocyte population may result into formation of more annulus forming cells and the same may be directly recruited from the bone marrow.

CONCLUSION

Injury to the intervertebral disc is a common phenomenon especially after the fourth decade of life. Incidence can be more in the women due to child bearing and in males doing heavy manual labor. Whatever the etiology of the injury, it may resolve spontaneously or require surgical or medical intervention. The role of estrogen shows promising results in this study and the same shall be explored in further studies.

REFERENCES

1. Scaal M. Early development of the vertebral column. Semin Cell Dev Biol. [In press].[Available online 10 November 2015]. Cited 20 Nov 2015. Available at; http://www.sciencedirect.com/science/article/pii/S1084952115002499.
2. Livingston DH, Anjaria D, Wu J, Hauser CJ, Chang V, Deitch EA. et al. Bone Marrow Failure Following Severe Injury in Humans. Annals of Surgery. 2003; 238(5):748-53.
3. Wu JC, Livingston DH, Hauser CJ, Deitch EA, Rameshwar P. Trauma Inhibits Erythroid Burst-Forming Unit and Granulocyte-Monocyte Colony-Forming Unit Growth Through the Production of TGF-β1 by Bone Marrow Stroma. Annals of Surgery. 2001;234(2):224-32.
4. Orlic D, Kajstura J, Chimenti S, Jakoniukl, AndersonSM, Li B. et al., Bone marrow cells regenerate infarcted myocardium. *Nature. 2001;* (410) 701-5.
5. Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. Journal of Clinical Investigation. 2003;112(1):42-49.
6. Rennert RC, Sorkin M, Garg RK, Gurtner GC. Stem cell recruitment after injury: lessons for regenerative medicine. Regenerative medicine. 2012;7(6):833-50.

7. Chatha WA, Khan MY, Minhas LA. Effects of exogenous estrogen on granulation tissue: a chronology of first week in injured intervertebral disc. Pak J Med Health Sci. 2014; 8(1): 2-7.
8. Chatha WA, Khan MY, Minhas LA. Role of Exogenous 17-β Estradiol in injured Intervertebral Disc. Pak J Med Health Sci. 2014; 8(3): 736-740.
9. Li Y, Hua X, Hua F, Mao W, Wan L, Li S. Are bone marrow regenerative cells ideal seed cells for the treatment of cerebral ischemia? Neural Regeneration Research. 2013;8(13):1201-9.
10. Baron YM, Brincat MP, Galea R, CallejaN.HumReprod. Intervertebral disc height in treated and untreated overweight post-menopausal women.2005 Dec;20(12):3566-70.
11. Calleja-Agius J, Muscat-Baron Y, Brincat MP. Menopause Int. Estrogens and the intervertebral disc. 2009 Sep;15(3):127-30.
12. Guyton, Arthur C. Textbook of medical physiology / Arthur C. Guyton, John E. Hall.—11th ed. 2006. Elsevier Inc. Philadelphia.
13. Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, et al. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;87(18):7260-64.