The Possible Prophylactic Role of Vitamin E on Methylprednisolone Acetate -Induced Femoral Head Necrosis

Thesis

Submitted in Partial Fulfillment
of Master Degree in Anatomy & Embryology

Presented By

Asmaa Wageh Tahef

M.B., B.Ch.

Under Supervision of

Prof. Dr. Nabila Yousef Abdel Halim

Professor of Anatomy & Embryology
Faculty of Medicine
Cairo University

Prof. Dr. Safwat Wadie Gerges

Professor of Anatomy & Embryology
Faculty of Medicine
Cairo University

Dr. Maha Khaled Abdel Wahed

Lecturer of Anatomy & Embryology
Faculty of Medicine
El-Fayoum University

Faculty of Medicine
EL-Fayoum University

2014

Acknowledgment

First and foremost of all thanks to Allah the most beneficial and merciful.

I am greatly honoured to express my sincere appreciation and deepest gratitude to my professor Dr. Nabila Yousef Abdel Halim, professor of Anatomy and Embryology, Cairo University for her instructive supervision, sincere encouragement and valuable advises. I am very much privileged and honoured to have her as my supervisor.

I wish to express my supreme thanks to Professor Dr. Safwat Wadie Gerges, professor of Anatomy and Embryology, Cairo University for his help, valuable guidance and keen supervision. To him I owe much than I can express.

My deepest gratitude and thanks for Professor Dr. Suhair Asaad Phillips, professor of Histology, Cairo University for her generous help and encouragement in image analysis and reading most of the histological data.

I would like to express my deepest appreciation to Dr. Maha Khalid Abd-Elwahed, lecturer of Anatomy, Fayoum University for her keen supervision, continuous support and precious guidance. I am deeply affected for her noble.

بسم الله الرحمن الرحيم

(وقل اعملوا فسيرى الله
عملكم ورسوله والمؤمنون)

Contents

Key words: femoral head, osteonecrosis, methylprednisolone acetate, vitamin E.

ABTRACT

Corticosteroid is a risk factor inducing femoral head osteonecrosis. The antioxidant Vitamin E could reduce such osteonecrosis. Sixty adult male albino rats were grouped into: G**I** (normal control), G**II** (Sham control), G**III**-MPSL subdivided into: **(III-A** -3 days) and (**III-B-**3 weeks), G**IV** (MPSL and vitamin E) subdivided into; (**IV-A-** 3 days) and **(IV-B-**3 weeks). **GIII** showed femoral head cortical and medullary pleopathological changes, which were reduced in **GIV** after vitamin E adminstration, being more in subgroup IV-B than IV-A demonstrating a duration dependency.

الكلمات الداله: (راس عظم الفخذ، تنخر العظم، اسيتات ميثيل البريدنيزولون، فيتامين هـ)

تعتبر الستبرويدات من احد العوامل المسببه لتنخر راس عظم الفخذ، و من المحتمل ان يستطيع فيتامين هـ المضاد للاكسده تقلبل هذا التاثير. و قد تم تقسيم 60 من ذكور الفئران البالغه اللى اربع مجموعات: المجموعه الاولى (الضابطه الطبيعيه)، المجموعه الثانيه (الضابطه الزائفه)، المجموعه الثالثه (اسيتات ميثيل البريدنيزولون) و تم تقسيمها الى مجموعتين فرعيتين (المجموعه الثالثه أ- 3 ايام) و (المجموعه الثالثه ب- 3 أسابيع)، المجموعه الرابعه (، اسيتات ميثيل البريدنيزولون- فيتامين هـ)) و تم تقسيمها الى مجموعتين فرعيتين (المجموعه الرابعه أ- 3 ايام) و (المجموعه الرابعه ب- 3 أسابيع). أظهرت المجموعه الثالثه تغيرات مرضيه فى كل من القشرة و تجويف نخاع العظم و قد تم تقلقل هذا التاثير بعد اعطاء فيتامين هـ فى المجموعه الرابعه و قد اشار ذلك الى التاثر بفترة التعرض.

Introduction

Avascular necrosis (AVN) is the result of interruption of the blood supply inducing death of the cellular components of bone resulting in bone destruction and collapse, articular dysfunction and severe pain. AVN usually involves the epiphysis of long bones, such as femur, humerus yet small bones can also be affected ***(Woo et al., 2006).***

The underlying pathology of AVN is unclear. In published literatures, AVN has been associated with a number of risk factors including corticosteroid use, alcohol consumption, immunosuppressive therapy, autoimmune diseases and rheumatoid arthritis .Yet patients who experience non-traumatic AVN usually have more than one risk factor, which indicates that the pathogenesis of non-traumatic AVN is probably multifactorial ***(Mont et al., 2000).***

Corticosteroids are the mainstay of therapy in most inflammatory and autoimmune disorders such as: rheumatoid arthritis and systemic lupus erythematosus (SLE). They are also included in most chemotherapy protocols. Corticosteroids play the leading role in non-traumatic cases of avascular necrosis of femoral head (ANFH). Therefore, ANFH is a potential major complication for large patient populations ***(Assouline-Dayan et al., 2002).***

Different percentages of ANFH encountered in the above mentioned diseases demonstrate that steroids act through different mechanisms of action. The most frequently emphasized theory is accumulation of fat embolies in intraosseous arterioles which lead to obstruction,

coagulopathies, and increased intraosseous pressure which could ensue in avascular necrosis similar to a compartment syndrome ***(Lieberman et al., 2003).***

Assouline-Dayan et al. (2002) observed that osteonecrosis occurs more commonly in patients who have received long-term courses of steroids; especially those patients who received long acting steroids, however, osteonecrosis also has developed in patients treated with short term courses of high dose steroids. Although the risk appears to increase with both the dose and duration of steroid treatment, it's difficult to predict which patients will develop osteonecrosis.

Oxidative stress, which has been implicated in numerous pathological conditions, including vascular injury has been reported to play a role in the pathogenesis of steroid-induced osteonecrosis. Apoptosis has also been suggested to be involved and is known to be induced by tissue oxidative injury (***Ichiseki et al., 2004).***

Ichiseki et al. (2005) reported that, oxidative stress affected the bone shortly after corticosteroid administration and before the development of osteonecrosis. Consequently, antioxidants may alleviate oxidative injury following corticosteroid administration and thus minimize osteonecrosis.

Numerous antioxidants have been reported, with antioxidant vitamins recently receiving particular attention. Antioxidant vitamins are already being used in conditions such as circulatory disorders in which oxidative stress is thought to play a role and they have been reported to

exert a considerable prophylactic effect in this context ***(Mikami et al., 2010).***

Iuchi et al. (2003) have demonstrated that vitamin E administration decreases the generation of active oxygen species and ameliorates the vascular endothelial dysfunction induced by steroid administration. In addition, ***Traber and Atkinson (2007)*** reported that vitamin E which is a fat-soluble substance in the body exerts potent antioxidant properties.

Furthermore ***Kuribayashi et al. (2010)*** reported that vascular endothelial damage by oxidative stress inhibits the anticoagulation system through reducing thrombomodulin and nitric monoxide (NO) production. Vitamin E administration can suppress vascular damage by reducing oxidative stress in the blood and blood vessels, through its antioxidant, membrane stabilizing and microcirculation activation.

In addition, ***Kuribayashi et al. (2010)*** studied the potency of vitamin E homologs against osteonecrosis and reported that alfa-tocopherol has the highest potency among the known vitamin E homologs and has the greatest potential for the prevention of osteonecrosis.

Aim of The Work

The aim of the present work was to induce femoral head necrosis using short and long treatment of corticosteroids (namely methylprednisolone acetate) and to study the role of anti-oxidant vitamin E on preventing or reducing this effect through:

- Light microscopic study.
- Image analysis technique.
- Statistical analysis of the obtained data.

Review of literature

Long bones have a shaft, called the diaphysis, and two expanded ends; each called an epiphysis**.** In growing person, the diaphysis is separated from each epiphysis by the epiphyseal plate of cartilage. The articular surface of the epiphysis is covered with hyaline cartilage. The portion of bone between the diaphysis and the epiphysis is called the metaphysis. It extends from the diaphysis to the epiphyseal line. In the shaft, almost the entire thickness of the bone tissue is compact; at most, only a small amount of spongy bone faces the marrow cavity. At the ends of the bone, the reverse is true. Here the spongy bone is extensive, and the compact bone consists of a thin outer shell **(Fig. 1)** ***(Ross et al., 2003).***

Closer observation of the spongy bone reveals branching bony trabeculae and spicules projecting from the internal surface of the compact bone into the marrow cavity. There are no Haversian systems in spongy bone, but there are irregular arrangements of lamellae. These contain lacunae housing osteocytes that are nourished by diffusion from the marrow cavity, which is filled with bone marrow ***(Gartner and Hiatt, 2006).***

The diaphysis is covered by a periosteum except where tendons and muscles insert into the bone. There is no periosteum on the surfaces of bone covered by articular cartilage. The periosteum is a non-calcified, dense, irregular, collagenous connective tissue covering the bone on its external surface and muscles are inserted into it. Periosteum is composed of two layers, outer fibrous and inner cellular. Bone contains a central

cavity (marrow cavity), which houses the bone marrow. Bone marrow exists as two types: red bone marrow, in which blood cells are formed and yellow bone marrow, composed mostly of fat. The central cavity of a bone is lined with endosteum, a specialized thin, connective tissue composed of a monolayer of osteoblasts ***(Gartner and Hiatt, 2006).***

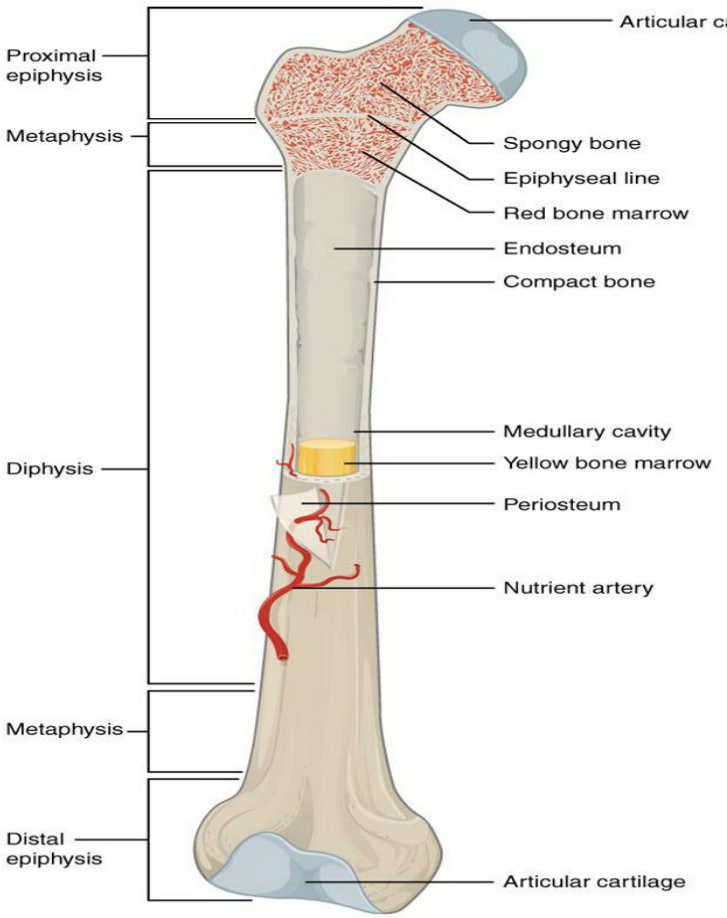

Fig. 1: Structure of a typical long bone. The diaphysis (shaft) of a long bone contains a large marrow cavity and has proximal and distal ends (epiphyses). The expanded part of the diaphysis near the epiphysis is the metaphysis. Except for the articular surfaces that are covered by hyaline (articular) cartilage, the outer surface of the bone is covered by a fibrous layer of connective tissue called the periosteum. The outer portion of the bone has a solid structure and represents compact bone. The interior of the bone exhibits a spongy configuration and represents spongy (cancellous) bone ***(Qouted after, http://cnx.org/content/m46281/latest/).***

Microscopic structure of Bone:

Bone is a specialized connective tissue composed of intercellular calcified material, the bone matrix, and three cell types: osteocytes, which are found in cavities (lacunae) within the matrix; osteoblasts, which synthesize the organic components of the matrix; and osteoclasts, which are multinucleated giant cells involved in the resorption and remodeling of bone tissue ***(Junqueira and Carneiro, 2005).***

1-Cellular structure: The cells of bone are osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts

Osteoprogenitor are spindle-shaped and have a pale-staining oval nucleus and scant pale-staining cytoplasm. They are located in the inner cellular layer of the periosteum, lining Haversian canals, and in the endosteum **(Fig. 2).** These cells, derived from embryonic mesenchyme, remain in place throughout postnatal life and can undergo mitotic division and have the potential to differentiate into osteoblasts. Moreover, under certain conditions of low oxygen tension, these cells may differentiate into chondrogenic cells. These cells are most active during the period of intense bone growth ***(Gartner and Hiatt, 2006).***

Osteoblasts are responsible for the synthesis of the organic components of bone matrix (type I collagen, proteoglycans, and glycoproteins). Osteoblasts are exclusively located at the surfaces of bone tissue, side by side, in a way that resembles simple epithelium **(Fig. 3).** When they are actively engaged in matrix synthesis, osteoblasts have a cuboidal to columnar shape and basophilic cytoplasm. When their synthesizing activity declines, they flatten, and cytoplasmic basophilia

declines. Some osteoblasts are gradually surrounded by newly formed matrix and become osteocytes ***(Junqueira and Carneiro, 2007).***

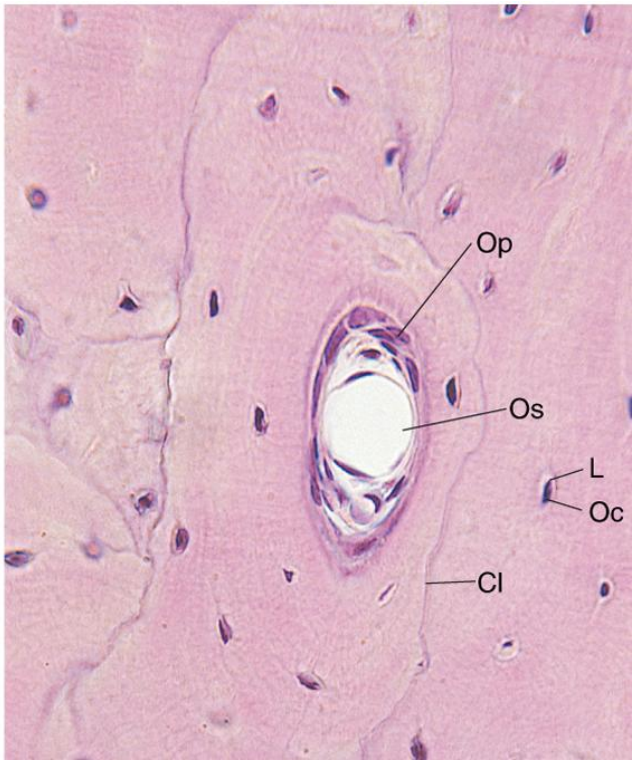

Fig. 2: A Light micrograph of decalcified compact bone (×540). Osteocytes (Oc) may be observed in lacunae (L). Also note the osteon (Os), osteoprogenitor cells (Op), and the cementing lines (Cl) ***(Quated after, Gartner & Hiatt, 2006).***

Osteocytes, which derived from osteoblasts, lie in the lacunae **(Fig. 3)** situated between lamellae of matrix. An osteoblast becomes an osteocyte when the cell is encased by osteoid matrix (uncalcified bone matrix) that it synthesizes itself. Lacunae and canaliculi form around the osteocyte and its cytoplasmic processes, respectively. Thus, each osteocyte lies in its own lacuna and contacts its neighboring osteocytes cytoplasmically, through canaliculi ***(Hirose et al., 2007).***

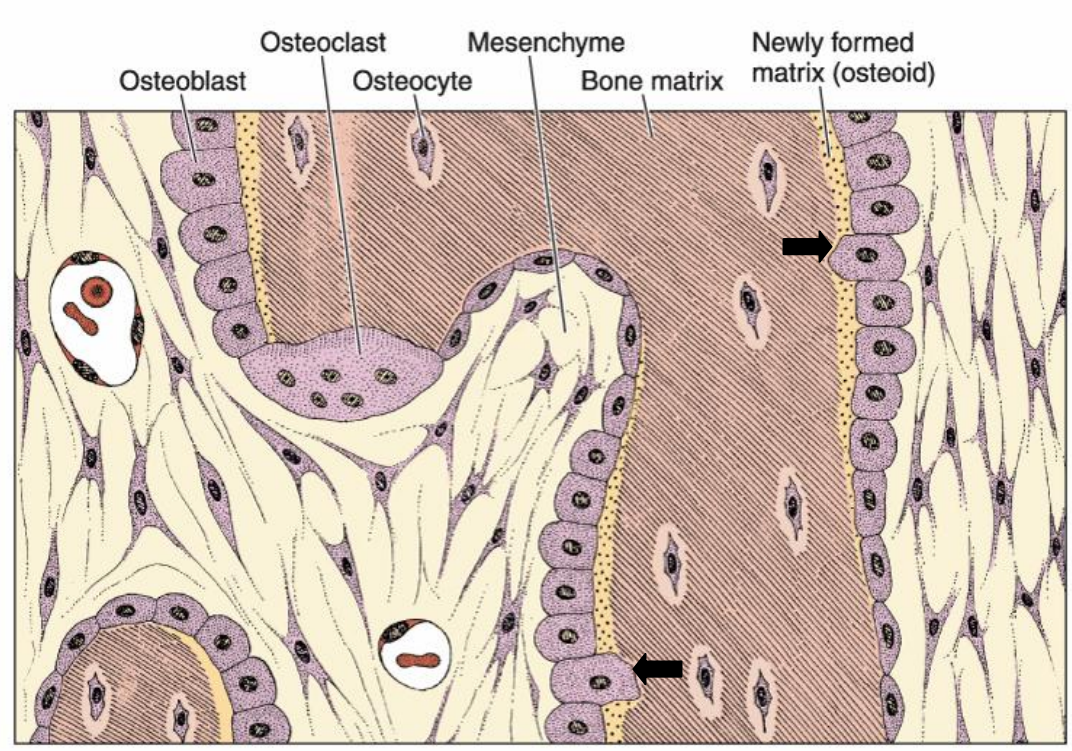

Fig. 3: Diagram showing Osteoblasts, which forms a strand of matrix that traps cells. As this occurs, the osteoblasts gradually differentiate to become osteocytes. The arrows indicate osteoblasts being trapped in newly formed bone matrix ***(Quated after, Junqueira and Carneiro, 2007).***

Although osteocytes have reduced proliferative activity and are not capable of mitotic division, they are actively involved with the maintenance of the bony matrix. Some of the osteocytes die during remodeling, but most probably return to the state of osteoprogenitor cells or persist as osteocytes for a long time ***(Jiang et al., 2007).***

Osteoclasts are very large, branched motile cells derived from the fusion of bone marrow-derived mononucleated cells and are responsible for bone resorption. They are multinucleated cells with fine finger like cytoplasmic processes and are rich in lysosomes. Osteoclasts lie in resorption craters known as Howship's lacunae on bone surfaces or in

deep resorption cavities called cutting cones. These bone cells can only resorb mineralized bone matrix ***(Kollet et al., 2007).***

2-Bone matrix: consists of organic and inorganic components. The association of organic and inorganic substances gives bone its hardness and resistance ***(Balcerzak et al., 2006).***

Organic Component of bone matrix, constituting approximately 35% of the dry weight of bone, includes fibers that are almost exclusively type I collagen, makes up about 80% to 90% of the organic component of bone. Type I collagen in bone is highly cross-linked, which prevents it from being easily extracted and causes the matrix to be acidophilic. ***Inorganic Component*** of bone, which constitutes about 65% of its dry weight, is composed mainly of calcium and phosphorus along with other components, including bicarbonate, citrate, magnesium, sodium, and potassium. Calcium and phosphorus are arranged in an ordered fashion along the type I collagen fibers; they are deposited into the gap regions of the collagen but also are present along the overlap region ***(Gartner and Hiatt, 2006).***

Specific structural glycoproteins are also present in the bone matrix. Bone glycoproteins may be responsible for promoting calcification of bone matrix. Other tissues containing type I collagen are not normally calcified and do not contain these glycoproteins ***(Junqueira and Carneiro, 2007).***