Histological and Immunohistochemical study on the effect of overdose of vitamin A intake on th tibia of young and adult albino rats

Thesis Submitted For Partial Fulfillment of M.SC. Degree in Histology

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Dedication

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To all of them and to **Amira & Yara**, I dedicate my work.

Hanaa Mohammed Jouda

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بحث مقدم توطئة للحصول على درجة الماجستير في الهستولوجي

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List of abbreviation

Cellular retinoic acid binding protein II ---------- (CRABP-II)

Cellular retinol binding protien I ---------- (CRBP-I)

Ethylene-di amine-tetra-acetic acid ---------- (EDTA)

Growth hormone ---------- (GH)

Hormone replacement therapy---------- (HRT)

Insulin like growth factor-I---------- (IGF-I)

Recommended dietary allowances---------- (RDA)

Retinoic acid---------- (RA)

Retinoic acid receptors α, β and γ ---------- (RARα, RAR β, RARγ)

Retinoid X receptor α ---------- (RXR α)

Retinol activity equivalent ---------- (RAE)

Retinol binding protein---------- (RBP)

Histological and Immunohistochemical study on the effect of overdose of vitamin A intake on the tibia of young and adult albino rats

Abstract

Introduction: Vitamin A is a fat soluble vitamin. Some individuals receive more than the recommended daily allowance of vitamin A due to dietary habits or use of dietary supplements that contain vitamin A. This study aimed to investigate the effects of vitamin A overdose on the epiphyseal plate structure in young rats and on the structure of the metaphysis of adult rats.

Materials and methods: Seventy male albino rats were divided into two main groups. Group I (young group) consisted of 40 rats -four weeks old- that were subdivided into four equal subgroups. Subgroup (Ia) was used as control. Rats of subgroups Ib, Ic and Id were given vitamin A at a dose of 100000 IU/100 gm/day for five days. These rats were sacrificed at the sixth, eighth and twelfths day from the beginning of the experiment respectively. Group II (adult group) consisted of 30 adult rats that were subdivided into three equal subgroups. Subgroup (IIa) was used as control. Rats of subgroup IIb and IIc were given vitamin A at a dose of 80 mg/kg/day for two weeks (group IIb) and three weeks (group IIc). Animal were sacrificed at the end of these two and three weeks respectively. The proximal ends of tibiae of the hind limbs were taken, processed and examined by light and scanning

electron microscopes. Morphometric and statistical studies were also done.

Results: Vitamin A overdose had significant and progressive serious effects on epiphyseal plate in young rats and on cancellous bone structure in metaphysis of adult rats.

Conclusion: Vitamin A overdose proved to be toxic as it induced premature closure of epiphysis in young rats and osteoporosis in adult rats.

Key wards: vitamin A overdose, epiphyseal plate, osteoporosis, rat.

INTRODUCTION

Vitamin A is a fat-soluble vitamin also known as retinol (active form of vitamin A). Vitamin A is important in the production of retinal pigments, which promotes good vision, especially in dim light. Also it maintains healthy teeth, skeletal and soft tissues, mucous membranes, and skin **(*Hamrik and Counts, 2008)*.**

Vitamin A plays a major role in induction and control of epithelial differentiation in mucous secreting and keratinizing tissues**.** Unfortunately, its use in disorders of keratinization such as psoriasis, and in precancerous skin conditions has been limited by a series of toxic effects known collectively as hypervitaminosis A **(*Cashin and Lewis, 1984)*.**

Dietary vitamin A is obtained from preformed vitamin A (or retinyl esters), which is found in animal food (liver , milk , kidney and fish oil), fortified food and drug supplements. It is also obtained from provitamin A carotenoids from plant sources, especially carrots. Dietary vitamin A is available mainly as preformed vitamin A in western countries and as provitamin A carotenoids in developing countries**.** After absorption, its active form; retinol is transported via chylomicrons to the liver. Then, it is either stored as retinol ester or re-exported into the plasma in combination with retinol-binding protein for delivery to tissue sites. The body absorbs retinoids and vitamin A very efficiently, as it lacks the mechanisms to destroy excessive loads. Thus, the possibility of toxicity exists unless intake is carefully regulated ***(Maqbool and Stallings, 2008)*.**

For most people, up to 25,000 IU (7500 μg) of vitamin A per day is considered safe. However, people over age 65 and those with liver disease should probably not be supplemented with more than 15000 IU per day, unless supervised by a doctor. In pregnant women, the maximum safe intake is being re-evaluated. However, less than 10,000 IU (3,000 μg) per day is generally accepted as a safe dose. Birth defects could result from large dose of vitamin A ***(Azais and Pascal, 2000).*** Some individuals receive more than the recommended daily allowance (RDA) of vitamin A due to dietary habits or use of dietary supplements that contain vitamin A ***(Heather et al., 2005).***

To control vitamin A deficiency, large therapeutic doses are administered in developing countries to women and children, who are often undernourished. Nevertheless, little attention has been given to the short-term kinetics (i.e., after absorption but before storage) of a large dose of vitamin A. Moreover, appropriate dosing regimens have not been systematically evaluated to ascertain the quantitative improvement in vitamin A status of the women and children who receive these supplements **(*Penniston and Tanumihardjo, 2006).***

Emerging evidence suggests that sub toxicity without clinical signs may be a growing concern, because intake from preformed sources of vitamin A often exceeds the recommended dietary allowances (RDA) for adults, especially in developed countries. Osteoporosis and hip fracture are associated with preformed vitamin A intakes that are only twice the current RDA ***(Penniston and Tanumihardjo, 2006).***

In man, the symptoms of hypervitaminosis A include, among others: headache, desquamation of skin, alopecia and alterations of mucous membranes leading to conjunctivitis and rhinitis. The effect of hypervitaminosis A on bone is presented as tenderness and restricted

movement. In children, radiological changes have been observed ***(Colin et al., 1984).*** Vitamin A toxicity in the infant from dietary over dosage, was recognized as periostitis with rare progression to premature closure of the lower limb epiphyses. Most cases of vitamin A - induced premature epiphyseal closure (physeal obliteration) occur in pediatric dermatologic patients given vitamin A analogues ***(Rothenberg et al., 2007).***

Aim of the work

The aim of this work was to investigate the effect of high doses of vitamin A, on the structure of the epiphyseal plate of tibia of young male albino rats and on the structure of metaphysis of tibia of adult male albino rats.

- **I-Bone:**

Bone is a specialized type of connective tissue that is together with cartilage, make up the skeleton. These tissues serve three functions: (a) mechanical support and site of muscle attachment for locomotion, (b) protection for vital organs and bone marrow and (c) a metabolic reserve of ions, especially Ca and phosphate ***(Meghji, 1992).***

The cavities of the long bones and vertebrae contain the bone marrow, where blood cells are formed. There are two types of mature bone; cortical and cancellous bone, which differ morphologically and functionally. Cortical bone which is compact in nature, is dominating in the diaphysis of long bones giving mechanical strength to the skeleton. Cancellous bone is found in the trabecular bone network in the vertebrae, pelvis and in the epiphyses of the long bones. Despite its smaller volume, the surface of the cancellous bone is larger than the cortical surface. It is in close contact with the bone marrow, and is considered to be metabolically more active than cortical bone **(*Marks and Odgren, 2002)***.

Epiphyseal plate of long bones:

The physis is the region that separates the epiphysis from the metaphysis. It is the zone of endochondral ossification in an actively growing bone or the epiphyseal scar in a fully grown bone ***(Adler, 2000).***

Longitudinal growth occurs at the epiphyseal plate. In the growth plate, immature cells lie toward the epiphysis, called the resting zone, with more mature chondrocytes in the proliferating zone and large chondrocytes in the hypertrophic zone adjacent to this. During

childhood, the growth plate matures. Its total width decreases and eventually disappears at the end of puberty with complete replacement by bone along with cessation of longitudinal growth. In specific disorders, timing of epiphyseal fusion is advanced or delayed ***(Emons et al., 2011).***

Cell proliferation in the epiphyseal plate is regulated by a number of different factors (e.g. insulin like growth factor-I). The extracellular matrix is rich in glycosaminoglycans, proteoglycans and type II collagen, whereas especially at the edges type I and type VI collagen can be also observed ***(Milz et al., 2002).***

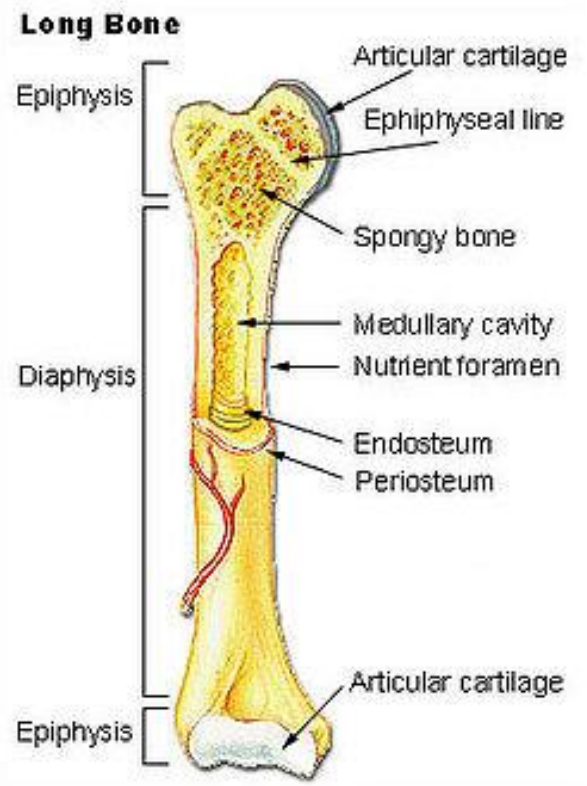

Diagram showing the structure of long bone *(Marieb and Hoehn , 2007).*

Effect of nutrition and hormones on bone:

Nutrition is important as it provides the basis for bone growth and repair. It is required by bone for two separate functions: nutritional requirement of any tissue (protein, vitamin C and zinc for growth and repair of collagen matrix), and chemical compounds (one of which is calcium) that give the bone its unique supporting and protective properties. Nutrition is important for final height of bone. This may be exemplified by the retarded bone growth and the late onset of puberty of a child suffering from intolerance of gluten. In addition, nutritional disorders have been proved to affect hormonal secretion. Growth hormone (GH) and insulin like growth factor-I (IGF-I) have different target cells in the epiphyseal growth plate. Growth hormone stimulates the slowly dividing prechondrocytes in the germinative cell layer while IGF-I promotes the clonal expansion of a GH-primed cell in the proliferative cell layer. Thyroid hormone was found to block the IGF-I induced clonal expansion, probably in the late proliferative layer, and stimulate chondrocyte maturation ***(Lampl et al., 1992).***

Most of the vitamins and minerals could affect bone metabolism. Vitamin K has been documented to improve bone metabolism and structure, decrease bone loss and to increase bone biomechanical properties ***(Huang et al., 2001).***

Calcium (Ca^{++}) has been known as an essential hardening ingredient of bone ***(Love, 2003)***. The Ca^{++} sensing receptors was reported to act as a growth factor in various cells. In osteoblasts, Ca^{++} sensing receptors stimulate osteoblasts proliferation, differentiation and mineralization. In osteoclasts, Ca^{++} sensing receptors stimulated