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Vitamin C as a Modulator of Bone Healing

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Vitamin C is a significant antioxidant and factor in collagen synthesis. Block in collagen synthesis is the reason for widely spread in past disease scurvy. Last decades vitamin C is an object of many scientific studies over bone and connective tissue repair.

In our study we present the results of vitamin C in situ application in fracture of mice femur. We prove the role of vitamin C as a modulator of bone repair.

Key words: vitamin C, bone, repair, healing, collagen, fractures.

Introduction

The role of vitamin C for human and animals is well known. It is a water-soluble micronutrient essential for human health and participates in nutrition for primates, some mammals (bats, guinea pig), birds and fish [6].

Vitamin C, also known as L-ascorbat or ascorbinic acid. Its formula is C6H8O6 and molecular weight is 176.13 g/mol (Fig. 1).

The significance of vit. C as an antioxidant is as substrate for ascorbinic peroxidase. In collagen synthesis it takes part as a cofactor and electron donor for 8 enzymes.

Human body cannot synthesize vit. C and its quantities in cell storage are limited. When there is a deficiency of that vitamin the collagen synthesis stops and scurvy occurs. The collagen without vit. C is unstable and cannot perform its function [1].

First description of scurvy was given by Hippocrates, about 400 years B.C. and first scientific attempt for explanation of the disease was made by J. Lind, surgeon in British navy, in 1753.

In 1928 the anthropologist Stefansson proves why the Eskimo do not suffer of scurvy.

From 1928 to 1933, the Hungarian research team of Joseph L Svirbely and Albert Szent-Györgyi and, independently, the American Charles Glen King, first isolated vitamin C and showed it to be ascorbic acid. The anti-scurvy compound was called Vitamin C.

From 1933 to 1937 Sir Walter Norman and Tadeus Reichstein, autonomously, succeeded in synthesizing Vitamin C. After synthesizing Vitamin C, Sir Walter Norman received the Nobel Prize in Chemistry [6, 8]. This accomplishment not only constituted

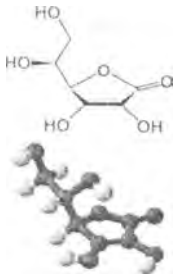

Fig. 1. Vitamin C structural formula

a valuable addition to knowledge of organic chemistry but also made possible the cheap mass production of Vitamin C for medical purposes.

1955 J.J. Burns showed that the reason why some mammals were susceptible to scurvy was due to the inability of their livers to produce the active enzyme L-gulonolactone oxidase (GLO).

Daily usage of vit. C is an object of a serious research but it's a fact that people consuming foods rich of vit. C are healthier and die rarely of chronic diseases.

It is required the daily usage of vit. C to be between 90 mg and 2000 mg.

Health organizations in different countries give similar data: Great Britain – 40 mg daily, Canada – 60 mg, United States – 60-90 mg, WHO – 45 mg daily [8].

Hipo- and avitaminosis C is characterized with disruption of mineral component of the bone. The skeleton over which calcium and phosphorus are piled is damaged. It is impossible these minerals to be adequately situated even if their intake is significant.

Bone healing is a scientific problem from decades [5]. There are several known factors which have influence over the process of bone repair. Some of them are shown on a diagram, others are age, fracture type, bone density, etc. (Fig. 2).

Continuity of the process depends on the extent of the trauma and the shortest period for initial bone healing in human is 3-4 weeks after the fracture [3, 4].

As we know bone healing process has several phases in which main part takes the periosteum. It is the source of precursor cells which differentiate later in chondroblasts and osteoblasts – the basic cells of healing bone.

First phase is the reactive phase. It begins with haematoma and ends with formation of granulation tissue of fibroblasts and aggregated cells (Fig. 3).

Next phase characterizes with formation of new bone also known as callus. Main part here has hyaline cartilage which adhere the fracture gap. The new bone is then changed by lamellar bone and the process is known as enchondral ossificatioin. Meantime collagen matrix is formed and after its mineralization the osteoblasts built new lamellar bone over its surface [2].

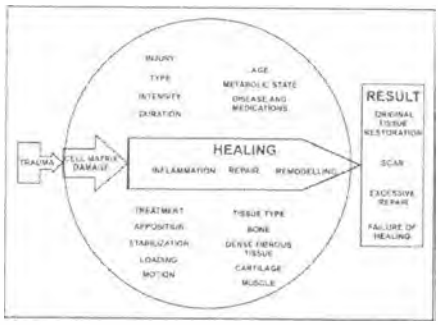

Fig. 2. Variables that influence bone healing

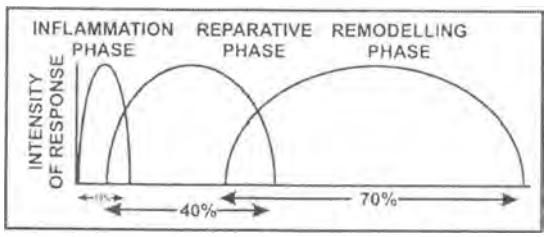

Fig. 3. Phases of bone healing process

Last phase, or remodelling, is the exchange of the trabecular bone with compact bone.

Experimental fractures are extremely difficult for standardization [1, 7]. Fracture models are made in mice, rats, rabbits, dogs, sheep, cats and calves. Although that mice and rats bones do not repeat the human model of regeneration they are used widely in orthopaedic practice [1]. The results cannot be translated directly over human and they are only orientative. Main challenge is fracture stabilization.

There are four methods of experimental fracture creation – manual, three point bending, guillotine and with osteotomies.

The evaluation of the bone healing in experimental fractures is based on several methods as fluoroscopic, histological, mechanical testing, osteodensitometric, biological markers of bone formation.

The aim of our study is to estimate the role of vit. C in bone healing process.

Material and Methods

Our model is based over 70 experimental fractures of mice femur. We divided it into 3 groups.

First group contains 30 animals which fracture sites were injected with 1ml 30% solution of ascorbinic acid immediately after fracture. Their diet after fracture was rich of vit. C – up to 2 grams daily (Table 1).

Second group contains also 30 animals with fractures but without initial injection of vit.C in fracture site. They were only given before and after fracture occurrence, food extremely rich of vit. C. The intake was 1,5 – 2 grams per day.

Control group contains 10 mice. They have only fractured femora without any special diet. The evaluation is made by manual test and X-ray study. The days are 7th, 14th, 21st and 28th after fracture occurrence.

After dissection and visual estimation material for histology study is taken.

Results

First evaluation was made at the 7th day. There were no X-ray signs for new bone formation. Manual testing showed pathologic movements.

New bone formation is seen at 14th day in mice treated with vit. C in difference with control group, in which this callus formation is shown after 3rd week. At the 14th day there is still pathologic mobility (Fig. 4).

Third week characterizes with expressive callus formation and lack of mobility of the fragments.

T a b l e 1. Material and Methods

TOTAL 70 FRACTURES		
I GROUP 30 Fractures	II GROUP 30 Fractures	III GROUP 10 Control
Vit. C Injection	–	–
Vit. C Rich Diet	Vit. C Rich Diet	Normal Food

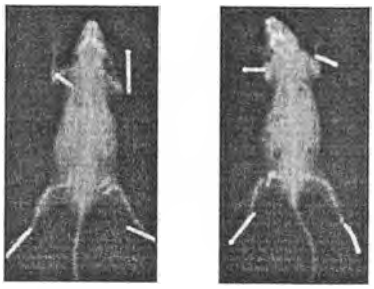

Fig. 4. Second week callus formation in Group I (left) and lack of callus formation in control group

Callus formation has bigger size in group injected with vit. C (group I) and especially in those cases with bigger dislocation of the fragments.

Group with only vit. C rich diet (group II) has more strength of bone callus but the callus is not as elastic as in control group.

After the 4th week there are not pathologic movements during manual testing. X-ray graphies show that the bone callus density is close to the trabecular bone density. There is no relation between callus size and the duration of the bone healing process.

Discussion

Best performance of callus formation and bone healing showed mice injected with vit. C solution and fed with rich of vit. C food. In parallel with control group bone callus in mice from Group II (only vit. C rich diet) was not so tensile, but more solid.

We believe that vit. C has positive influence over bone healing and fastens the process of bone repair. It takes part in the process at the stage between first and second phase, between the first and second week after fracture. Its usage immediately after fracture occurrence, in doses up to 50 times more than normal daily intake, would fasten the process of collagen skeleton formation and bone healing.

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