

## The Role of Vitamin C in Enhancement of Fracture Healing in Fracture with Periosteal Stripping at *Sprague-Dawley* White Rats Femur

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### ABSTRACT

**Introduction.** Fracture Healing still become a problem. There are many things to do to enhance and avoid the complication of fracture healing. Vitamin C is one of the ways to enhance the fracture healing. The purpose of this study is to know the role of vitamin C in enhancement the fracture healing in fracture with periosteal stripping at *Sprague-Dawley* white rats femur. The objective of the study is to know the role of vitamin C in enhancement of fracture healing in fracture with periosteal stripping at *Sprague-Dawley* white rats femur by using RUST score and histomorphometry.

**Materials and methods.** This is an experimental study. This study used 32 rats which were performed fracturization at the femur, periosteal stripping, internal fixation with K-wire and were given vitamin C supplementation orally in different dose, 5 mg, 10 mg, 20 mg and control. The evaluation at the second and fourth week radiologically using RUST score and histologically using histomorphometry.

**Results.** The RUST score evaluation at the second week showed that the result between groups were not statistically significant but at the fourth week the RUST score showed statistically significant result especially for the group receiving 20 mg vitamin C. The histomorphometry evaluation at the second and fourth week showed the enhancement of fracture healing by decreasing the amount of cartilage and increasing amount of woven bone along the study but were statistically not significant.

**Conclusions.** There is enhancement of fracture healing with supplementation of vitamin C as shown by with RUST score evaluation at the fourth week. Histomorphometry evaluation showed enhancement of fracture healing with supplementation of vitamin C. The larger dose of vitamin C give better histological grade but still not statistically significant.

**Keywords:** Enhancement of fracture healing, vitamin C, RUST score, histomorphometry

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## Peran Vitamin C Oral Terhadap Percepatan Penyembuhan Fraktur pada Fraktur dengan *Periosteal Stripping* di Femur Tikus Putih *Sprague Dawley*

### ABSTRAK

**Pendahuluan.** Penyembuhan fraktur merupakan masalah dalam ilmu orthopedi. Banyak hal dilakukan untuk mempercepat penyembuhan fraktur dan menghindari komplikasi penyembuhan fraktur. Salah satu cara yang diperkerikan dapat mempercepat penyembuhan fraktur adalah melalui pemberian vitamin C. Penelitian ini bertujuan untuk mengetahui peran vitamin C dalam mempercepat penyembuhan fraktur pada fraktur dengan *periosteal stripping* di femur tikus putih *Sprague-Dawley*.

**Bahan dan cara kerja.** Penelitian ini adalah penelitian eksperimental. Penilaian dilakukan terhadap 32 ekor tikus yang dilakukan frakturisasi pada tulang femur, periosteal stripping, fiksasi interna dengan K- wire dan diberikan dosis vitamin C yang berbeda-beda, 5 mg, 10 mg, 20 mg dan kontrol. Evaluasi dilakukan pada minggu kedua dan minggu keempat secara radiologis dengan skor RUST dan histologis dengan histomorphometry.

**Hasil.** Evaluasi skor RUST pada minggu kedua didapatkan hasil yang tidak bermakna tetapi pada minggu keempat didapatkan hasil yang bermakna secara statistik terutama pada dosis vitamin C 20 mg. Evaluasi histomorfometri pada minggu kedua dan keempat menunjukkan adanya percepatan penyembuhan fraktur dengan menurunnya jumlah kartilago dan meningkatnya jumlah *woven bone* selama penelitian tetapi secara statistik tidak bermakna.

**Simpulan.** Terdapat percepatan penyembuhan fraktur pada pemberian vitamin C dengan evaluasi skor RUST pada minggu keempat. Terdapat percepatan penyembuhan fraktur pada pemberian vitamin C dengan evaluasi histomorfometri tetapi secara statistik tidak bermakna. Pemberian vitamin C dengan dosis yang lebih besar menunjukkan gambaran derajat histologi yang lebih baik tetapi secara statistik tidak bermakna.

**Kata kunci :** Percepatan penyembuhan fraktur, vitamin C, skor RUST, histomorfometri

### Introduction

Fractures contribute to increased medical cost and loss of productivity.<sup>1</sup> An estimated six million fractures occur every year in the United States, and up to 10 % of the fractures may result in non union.<sup>1</sup> In Indonesia, 9.1% of traffic accidents resulted in fracture, therefore as the incidence of accidents is increasing, the incidence of fracture is also expected to increase.<sup>2</sup>

The incidences of delayed and non union are still high. Delayed and non union cases are difficult to overcome and cause high medical cost.<sup>3</sup> Therefore, many authors attempt to enhance fracture healing based on diamond concept of fracture healing.<sup>4,5</sup>

Vitamin C is one of the ways to enhance fracture healing.<sup>6</sup> The role of vitamin C as cofactor in hydroxylation of amino acid to form triple helix collagen which is essential for formation of immature callus in bone healing.<sup>6-9</sup> Vitamin C is also as an important antioxidant in reducing free radicals which result from

the inflammation of fracture.<sup>7-9</sup> Deficiency of vitamin C will increase insulin like growth factor binding protein-1 (GFBP-1) as well as growth factor binding protein-2 (GFBP-2) and decrease activity of insulin like growth factor-I (IGF-I).<sup>10</sup> The functions of IGF-I are to stimulate collagen synthesis, bone forming, and maintain the homeostasis of the bone.<sup>11</sup>

The assessment of fracture healing in clinically activities and in the studies is still a subjective process without gold standard.<sup>1,12</sup> In this study, the enhancement of fracture healing assess by radiograph with RUST score and histologically with histomorphometry.

This study aim was to know the effect of vitamin C in enhancement of fracture healing in fracture with periosteal stripping in different dose of vitamin C. The hypothesis was there would be significant differences in enhancement fracture healing in different dose of vitamin C supplementation.

Tabel 1. Analysis of the RUST Score at second and fourth week

Variabel	Second week		Fourth week	
	Median	P	Median	P
Skor RUST kontrol	4.5(4-8)	0.467	4.5 (4-6 )	0.023
Skor RUST vit C 5 mg	4.0(0-4)		4.0 (4-6)	
Skor RUST vit C 10 mg	4.5(4-6)		4.0 (0-4)	
Skor RUST vit C 20 mg	6.0(4-8)		8.0 ( 8-8)	

**Materials and methods**

This study was an experimental study. This study was undertaken within the period of December 2012-April 2013, towards Spargue Dawley white rats in animal laboratory of Nutritional Department Universitas Indonesia. Minimal sample size using Federer formulation were 8 rats for each group. The groups divided into four groups with different dose of vitamin C supplementation, 5 mg, 10 mg, 20 mg and control group. The vitamin C supplementation was given orally. Rats were fed with usual food without any additional of vitamin C in the food. Inclusion criterias were healthy male rats, aged 12 weeks and weighted 250-350 grams, Rats with impairment and infected lower extremity were excluded.

The rats were randomized into four groups, each group contained of 8 rats. The first group was control, while

the second, third, and fourth group was given vitamin C supplementation of 5, 10, and 20 mg respectively. The rats were anesthetized and fractured transversely in the mid diaphyseal of femur by using small saw, performed periosteal stripping 4 mm to proximal and 4 mm to distal from the fracture site and then fix with intramedullary K-wire sized 1.2 mm. After 2 weeks, four rats in each group were sacrificed and then assessed by radiograph using RUST score and histologic assessment using histomorphometry. In the RUST score evaluation, the assesment of fracture healing used the presence or absence of callus and a visible fracture line at the total four cortices visible on the anteroposterior and lateral radiographs. The RUST score value for a set of radiograph of 4 (definitely unhealed) to 12 (definitely healed). In the histomorphometry, the assessment of fracture healing in this study used four variabels: percentage of cartilage,

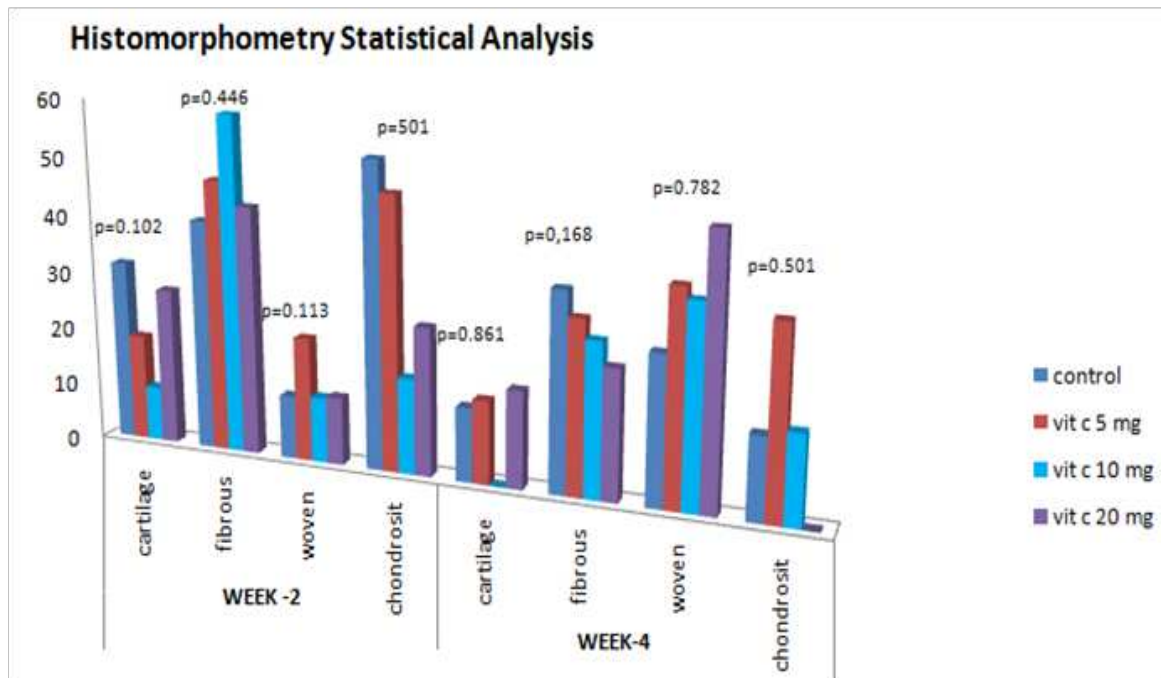


Figure 1. Histomorphometry analysis at week-2 and week-4

woven bone, fibrous tissue and chondrocyte number. We performed the same thing to the rest of the rats at fourth week. We calculated all the callus area at the proximal and distal of fracture site used J image program. We made a percentage of cartilage area, fibrous tissue area and woven bone area and also the of chondrocyte number from all the callus in the week two and in the week four. The calculation of percentage of cartilage area, fibrous tissue area, woven bone area and chondrocyte number are compared between week two and week four. The acceleration of fracture healing was happened if there were the higher level of percentage of woven bone and the lower level of percentage of cartilage and fibrous tissue in the week four.

Data were collected and then analyzed using Statistical Package for Social Sciences (SPSS) version 19.0. Statistical significance if p value less than 0.05.

## Results

There were 32 white rats, eight rats in each group. All the rats were alive, only two rats were excluded because of wire protrusion.

Table 1 summarizes of the RUST score at second week and fourth week. The score was statistically significant at fourth week with significant different between control, 5 mg, and 10 mg group to 20 mg group with p score of 0.046, 0.011, 0.014 respectively.

In the histomorphometry analysis, at second week statistically no significant,  $p < 0.05$  but histologically there were higher histological grade of the groups which were given vitamin C supplementation. In the groups with vitamin C supplementation, there was process of mineralization from histological assessment compare to the control group.

At fourth week, the histomorphometry evaluation statistically no significant,  $p < 0.05$  but higher histological grade in the group with vitamin C supplementation. In the group with vitamin C supplementation there were higher percentage of woven bone, and the lower percentage of the cartilage and fibrous tissue.

## Discussions

The subject characteristic in this study is the body weight. In the statistical analysis, there was no significant result so there was no effect of body weight of rats to the result of this study.

The assessment of fracture healing in daily clinical activities and studies is still subjective assessment without gold standard.<sup>1,3</sup> Radiological Union Scale for Tibia (RUST) score was more valid and reliable compare to the conventional assessment.<sup>13</sup> In this study assessment

of radiological with RUST score were done to all the groups in second week and fourth week. In second week, the analysis of RUST score was statistically no significant. As we know that RUST score was made base on cortical bridging and the fracture line that can be seen. After the second week of fracture healing in rats, we can see histologically the cartilage in a great amount and cellular component and also a small part of cartilage begin to calcify.<sup>4</sup> Based on that, on the radiograph examination there was no cortical bridging. On the other hand, at fourth week there was a combination between calcified cartilage and new woven bone. Assessment of fracture healing, beside use radiograph examination, also can use micro computed tomography ( $\mu$ CT) or Faxitron radiograph.  $\mu$ CT is a mini model of CT that usually used by the radiologist but the system could measure until micrometer. The other function of  $\mu$ CT are: assessing the mineralized callus assessment, trabecular volume, and cortical volume.<sup>13</sup> Therefore, the  $\mu$ CT can be used to assess the enhancement of fracture healing.

In this study we performed periosteal stripping that made vascularization of the bone damaged and caused impairment of fracture healing. As we know that the roles of vitamin C are as cofactor of collagen formation, antioxidant, the capillary endothelium protector and to maintain IGF-I activities to make the process of collagen formation, callus formation, bone matrix mineralization, and bone formation run well.<sup>7-10</sup>

The result of radiological assessment with RUST score in fourth week was statistically significant, and then to know which group that gave greatest effect among the groups in enhancement of fracture healing, post hoc test with Mann-Whitney test was performed. The result was 20 mg vitamin C supplementation gave the greatest effect. Based on the result, we can use RUST score in assessing enhancement of fracture healing in fourth week.

The accurate histological measurement in fracture healing is important. Quantitative histological analysis (histomorphometry) has been proven as a good measurement to assess the fracture healing.<sup>14,15</sup> Repeated histomorphometry measurement in different time should count the tissue and cell that has role in fracture healing. The measurement in total or percentage to the fibrous tissue, cartilage, and woven bone described the enhancement of fracture healing. Resorption time of cartilage was a sensitive index to assess the fracture healing.

In this study the measurement of cartilage percentage, fibrous tissue percentage, woven bone

percentage and chondrocyte count were performed. In the histomorphometry evaluation during the study at second week and fourth week to all the groups, the result were tend to decrease in percentage of cartilage, fibrous tissue, and chondrocyte count. On the other hand there was increase in percentage of woven bone. Martos<sup>8</sup> in his study stated that supplementation of vitamin C will increase the histological grade, the same as the study of Sarisozen<sup>9</sup> In this study there were increase of histological grade of the groups with supplementation of vitamin C by the increase of percentage of woven bone and the percentage of cartilage and fibrous tissue decreased. Supplementation of vitamin C would increase the activity of IGF-I so the production of collagen and osteoblast increase.

In the histomorphometry evaluation at second week, there was no statistically significant differences between control group and vitamin C supplementation group. According to Einhorn<sup>3</sup> at second week of rats fracture healing the cartilage were found in great amount and there was cellularity increase, also a small amount of mineralization of cartilage. In this study, there was also the increase of cartilage mineralization but statistically no significant. The same things were also found in the other studies with vitamin C supplementation in fracture healing that stated the supplementation of vitamin C would increase histological grade. There were two reasons why the result of histomorphometry evaluation was not significant. First, the food was given to rats probably contained enough vitamin C for fracture healing process although there was no supplementation at all. The second reason was the vitamin C worked with IGF-I, IGF-I itself was affected not only by vitamin C but also by growth hormone, IGF-I bindings protein and also substance that released by osteoblast.<sup>16</sup>

In the histomorphometry evaluation at fourth week statistically no significant but there was an increase of woven bone histologically, especially at the group with 20 mg vitamin C supplementation. Based on that, there was enhancement of facture healing and the same with the studies which performed by Martos<sup>8</sup> and Sarisozen<sup>9</sup> In this study there also found the decrease of cartilage percentage which give assessment of enhancement of fracture healing but statistically no significant.

Besides the decrease of cartilage, fibrous tissue and increase of woven bone, apoptosis of chondrocyte could be as a good indicator for enhancement of fracture healing. Chondrocyte apoptosis can be evaluated by tunnel assay.<sup>15,17</sup> In this study, we were not performed this evaluation because of budget limitation, time

and materials. In evaluation effect of vitamin C supplementation in fracture healing, it should be performed the biomechanical test and blood level of vitamin C analysis. We suggest to perform the tunnel assay, blood level of vitamin C and biomechanical test for the next study.

### Conclusions

There was a tendency of enhancement of fracture healing histologically with histomorphometry evaluation by vitamin C supplementation and also in RUST score evaluation at fourth week, although statistically no significant. The higher dose of vitamin C supplementation gave the better enhancement of fracture healing compared to the lower dose. Vitamin C is still needed for the fracture healing.

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