The Effect of Desferoxamine on Bone Healing of Tibial Fracture with 2 Mm Defect in Sprague Dawley Rat

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ABSTRACT

Introduction. Desferoxamine is one of iron chelating drugs. With the chelation of iron, phosphatidylinositol -3-kinase inhibitor is inhibited so that the release of endothelial nitric oxide synthesis phosphorylation is uninhibited. We examined whether addition of desferoxamine would lead to fracture healing enhancement.

Material and methods. Twenty four *Spargue Dawley* mice were used in the study. Their tibias were fractured and 2 mm defect were created. The fractures were then fixated using intramedullary K-wire. They were then allocated into four groups: group I received nothing, group II, III, and IV received desferoxamine since the first, fifteenth, and twenty second day respectively. The radiological and histological scores were measured at the sixth week and analyzed using ANOVA test.

Results. There were significant differences in radiological and histological scores among groups. ($p = 0.024$ and $p = 0.007$ respectively)

Conclusions. Addition of desferoxamine since the fifteenth but not after the twenty second days enhanced fracture healing.

Keywords: desferoxamine, fracture healing, phostidylinositol 3 - kinase inhibitor, endothelial nictric oxide syntesis

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Efek Desferoksamin terhadap Penyembuhan Fraktur Tibia dengan Defek 2 mm pada Tikus *Sprague Dawley*

ABSTRAK

Pendahuluan. Desferoksamin adalah suatu *chelating agent*. Penarikan besi oleh desferoksamin menyebabkan kerja enzim *phosphatidylinositol -3- kinase inhibitor* terhambat. Hambatan enzim tersebut membuat phosphorilasi *endothelial nitric oxide synthesis* berlangsung tanpa hambatan. Penelitian ini menilai efek pemberian desferoksamin terhadap penyembuhan fraktur.

Bahan dan cara kerja. Dua puluh empat ekor tikus putih *Sprague Dawley* yang dipatahkan dan dibuat defek sebesar 2 mm pada tibianya diikutkan dalam penelitian. Setelah difiksasi intrameduler dengan menggunakan K-wire, tikus tersebut dialokasikan ke dalam empat kelompok. Kelompok I tidak mendapatkan terapi desferoksamin, kelompok II mendapat desferoksamin sejak hari pertama, kelompok III dan IV mendapatkan desferoksamin sejak hari ke lima belas dan dua puluh dua. Pada minggu keenam, dilakukan pengukuran skor radiologis dan skor histologis. Kedua skor tersebut dibandingkan antar kelompok dengan menggunakan uji ANOVA.

Hasil. Terdapat perbedaan yang bermakna antar kelompok dalam skor radiologis (p = 0,024) dan skor histologis (p = 0,007).

Simpulan. Pemberian desferoksamin sejak hari ke-lima belas tetapi sebelum hari ke-dua puluh dua mempercepat penyembuhan fraktur.

Kata kunci: desferokasmin, penyembuhan fraktur, *phostidylinositol 3 - kinase inhibitor*, *endothelial nictric oxide syntesis*

Introduction

Fracture healing involves migration, proliferation, and differentiation of numerous types of cells such as endothelial cells, fibroblasts, chondroblasts, osteoblasts, and osteoclasts. Bone formation occurs through two different processes, depending on the stability.[1] In absolute stability, bone formation occurs through intramembranous ossification. In relative stability, bone formation occurs through endochondral ossification.

In endochondral ossification, healing process begin with formation of hematoma. Hematoma contains angiogenesis factors.[2] Hematoma will also isolate tissue perfusion, leading to low oxygen pressure and regional hypoxia. Formation of callus, deposition of cartilage and bone will worsen the hypoxia.[3]

Vascular endothelial growth factor (VEGF) is a potent mediator in hypoxic conditions that can induce angiogenesis process. It accumulates in the hematoma after fracture.[4,5] Recent studies showed that VEGF might serve as an important mediator during angiogenesis and bone development process including differentiation of osteoblasts and osteoclasts recruitment.[6,7]

Endogenous VEGF is secreted from endothelial cells, fibroblasts and osteoblasts. VEGF stimulates endothelial cells to synthesize osteogenic factors, thus acting indirectly in bone formation. During the bone healing process, VEGF is required not only for the formation of blood vessels, but also for the callus formation and mineralization.[2] VEGF induces angiogenesis in endochondral ossification and normal fracture healing.[8,9] VEGF also plays an important role in fracture healing.[10,11]

The purpose of this study was to determine the effect of desferoxamine in fracture healing with bone defect of two millimeter.

Materials and methods

This was a post-test only control group animal study. Twenty eight Sprague-Dawley rats weighted 200-250

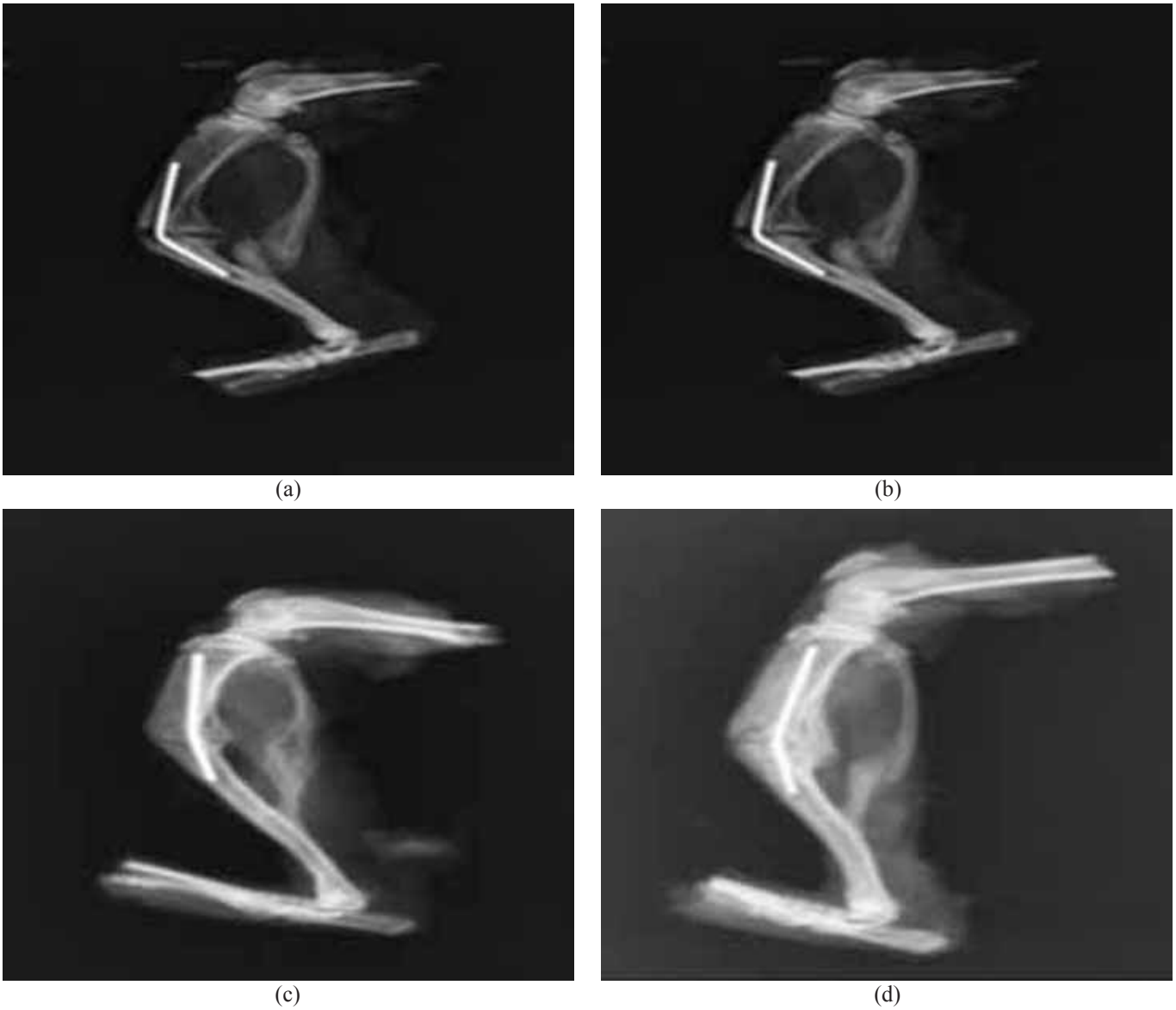
g were included in the study. The study was conducted at Animal Nutrition Laboratory, Fakultas Kedokteran Universitas Indonesia from June 2010 to September 2010. Rats with disability, infection, and protrusion of implant were excluded from the study.

After intraperitoneal anesthesia using 40 mg/kgBW ketamine, the left leg of the rats was fracturized. The fracture was retrogradely fixated by intramedullary 1.0 mm Kirschner-wire. The rats were then randomly allocated into 4 groups: group one which received no desferoxamine, group two which received desferoxamine injection since the first day, group three which received desferoxamine injection since the second week, and group four which received desferoxamine at the beginning of the third week. Dose of desferoxamine used in this study was 30 mg/kgBW. All rats received similar postoperative treatment consisting of ampicillin and mefenamic acid 100 mg/kgBW/day divided into three dosing.

The rats underwent radiological evaluation at the sixth week. Radiological score was determined from the radiographs using the scoring system introduced by Tiedeman.[12] Following radiological evaluations, the rats were scarified for histological evaluation. Histological score according to Salkeld and Marino[13] was determined from the histological slide. The differences in radiological and histological score among groups were analyzed using one way ANOVA test.

Results

At the sixth weeks, two rats in the control group died,



(a) (b) (c) (d)

Figure 1. Radiographs at the sixth week of treatment. No callus was seen in group I (a). In group II, minimal callus was visible (b). The fracture line was hardly visible in group III (c) while it was still visible in group IV (d).

leaving 26 six rats for further analysis. Figure 1 showed the radiological imaging from each group while the radiological scores were outlined in table 1. ANOVA test revealed significant differences among groups. ($p = 0.024$) Post-hoc analysis test revealed that the difference occurred between group I and group III. ($p = 0.02$)

Histological finding in each group was shown in figure 2 and the scores were outlined in table 1. The ANOVA test revealed significant difference among groups with a p value of 0.007. Post-hoc analysis revealed that the difference occurred between group III and IV. ($p = 0.02$)

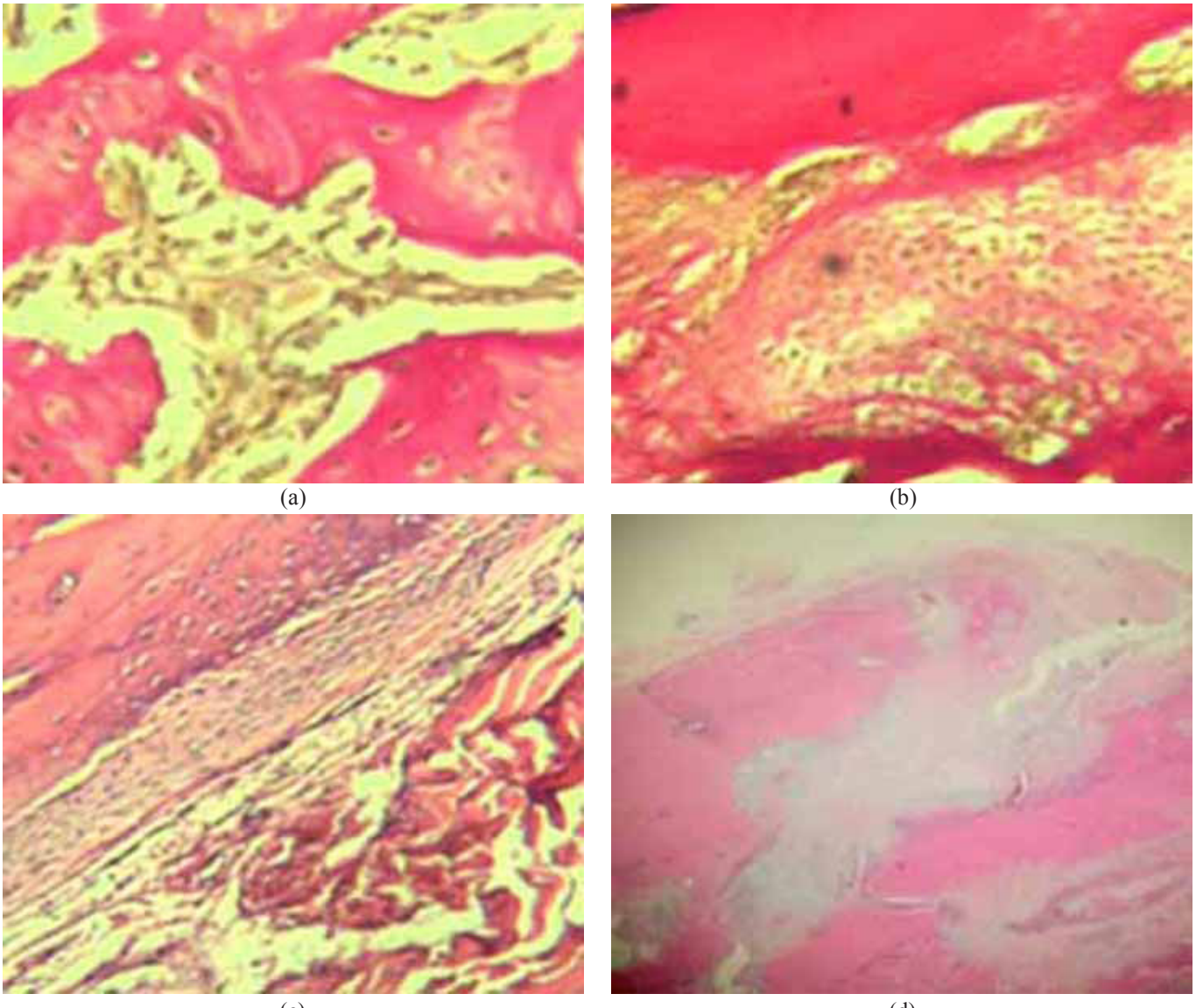
Table 1. Radiological and histological score of each group

Group	N	Radiological score (Mean ± SD)	Histological score (Mean ± SD)
I	6	4.2 ± 1.32	12.66 ± 1.50
II	6	6.5 ± 1.87	13.50 ± 1.87
III	6	7.5 ± 1.97	15.66 ± 1.21
IV	6	6.0 ± 1.67	12.83 ± 1.16

Discussions

Both mechanical and biological factors play important roles in fracture healing. Mechanical factor provide immobilization for the fracture while biological factor stimulate and enhance the vascularization.

In our study, the mechanical factor was provided by fixation with intramedullary K-wire. Fixation with intramedullary K-wire provides relative stability for the fracture, although it could not control rotational



(a) (b) (c) (d)

Figure 2. Histologic finding in each group. Compared to group I (a), II (b), and IV(d), group III (c) showed more mature bone.

instability.

We found that the healing process was most advanced in group treated with desferoxamine on the fifteenth day. On the fifteenth day, the process of neovascularization begins. Desferoxamine increases endothelial nitric oxide synthesis (eNOS) phosphorylation in endothelial cells which was inhibited by phosphatidylinositol 3 -kinase inhibitor. Phosphatidylinositol 3 -kinase inhibitor requires Fe in order to work, therefore chelating of Fe by desferoxamine inhibits the enzyme. After the 22 day, the endothelial cells have been matured, so addition of desferoxamine play little role.

Conclusions

Addition of desferoxamine since the fifteenth but not after the twenty second days enhanced fracture healing.

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