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Histological Assessment of the Effect of α-Tocopherol on Fracture Healing in Rabbits

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To investigate the effect of α-tocopherol (vitamin E) on fracture healing in rabbits, two groups of 10 rabbits were either injected with α-tocopherol (treated) or untreated (controls). The right femurs of both groups were fractured, and the treated group were injected intramuscularly with 20 mg/kg α-tocopherol daily for 5 days starting on the day of fracture. After 21 days, histological sections of the fractured region were examined and scored. Fracture healing had progressed further in the α-tocopherol group than in the control group. A statistically significant difference between the histological grading of fracture healing in the two groups was found. This difference may result from an antioxidant (α-tocopherol) effect on free oxygen radicals in the fracture area. We conclude that α-tocopherol may affect fracture healing favourably and might be useful as a therapeutic agent in clinical fracture management.

KEY WORDS: α-Tocopherol; Vitamin E; Antioxidant; Fracture healing; Rabbit; Histological assessment

Introduction

The reactive oxygen metabolites derived from normal cellular metabolism are highly toxic. Oxygen-derived free radicals cause tissue injury.[1,2] At the cellular level, injury caused by lipid peroxidation may range from increased permeability to cell lysis.[1,3]

In addition to tissue injury caused by extrinsic destructive forces, free oxygen radicals also have intrinsic destructive effects on tissue healing.[4,5] Necrotic tissues produced after trauma lead to inflammatory responses, causing the production of superoxide, a toxic oxygen metabolite, and consequently result in lipid peroxidation.[1] These effects of free oxygen radicals can be opposed by antioxidant defence mechanisms, which may be enzymatic or non-enzymatic.[1] Among the non-enzymatic defence mechanisms, α-tocopherol (vitamin E) is a lipid-soluble vitamin that is effective in the lipid phase of the metabolism.[1] α-Tocopherol protects cellular membranes from lipid peroxidation by converting free oxygen radicals into less reactive forms.[6]

There have been some reports of the effects of antioxidants after fracture.[7,8] In this study, the effect of α-tocopherol on fracture healing was investigated in rabbits.

Materials and methods

ANIMALS

A total of 20 white male New Zealand rabbits weighing 1.2 – 2.3 kg were used in this study, which was carried out in the Uludag University Experimental Animals Research Laboratory according to the ethical rules of the institution, permission being obtained from the Institutional Animal Ethics Committee of Uludag University, Bursa, Turkey. The necessary permissions were obtained. The rabbits were divided into two groups of 10, housed in individual cages and fed *ad libitum* during the 21-day experimental period.

EXPERIMENTAL PROCEDURE

One group of rabbits received 20 mg/kg α-tocopherol (Ephynal®, Roche, Switzerland) intramuscularly 1 h before starting the experiment, at the time at which the femurs were broken. A daily injection of α-tocopherol was given for 5 days thereafter. The control group did not receive any corresponding treatment.

After pre-anaesthesia with 0.25 mg/kg subcutaneous atropine sulphate (Haver®, Istanbul, Turkey), both groups of rabbits were anaesthetized with 0.2 mg/kg xylocaine (Rompun®, Bayer, Germany) and 20 mg/kg ketamine hydrochloride (Ketalar®, Eczacibasi, Istanbul, Turkey). When the appropriate depth of anaesthesia had been achieved, the right femur of each rabbit (experimental and control groups) was fractured by manual angulation stress, and closed fractures were produced. Within the same period of anaesthesia, femoral fractures in both groups were reduced by closed methods and fixed externally by cast immobilization, including proximal and distal joints adjacent to the fracture. There was no sign of severe pain in any animal. Cast immobilization probably prevented this.

HISTOLOGICAL ANALYSIS

All the rabbits were killed with intraperitoneal sodium pentobarbital (Pental Sodium®, IE Ulagay, Istanbul, Turkey) injection on day 21 of the experiment. Fractured femurs were separated from proximal and distal articulations, and soft tissues were completely cleaned away. These block femurs were kept in 10% neutral buffered formalin for 10 days and fixed. They were then decalcified in formic acid buffered with sodium citrate. Sections (6 μm) were taken from prepared paraffin blocks, stained with haematoxylin and eosin, Masson's trichrome and toluidine blue,[9] and examined by light microscopy. The fracture healing was evaluated using a five-point scale defined by Allen *et al.* (Table 1).[10]

STATISTICAL ANALYSIS

Histological scores were assessed using the Mann–Whitney *U*-test. Values were recorded as mean ± SD. $P < 0.01$ was considered significant.

Results

According to the criteria of Allen *et al.*[10] used to assess the histological slides prepared from the fractured femurs, there were four animals with complete bony union, five with incomplete bony union and one with complete cartilaginous union in the

TABLE 1:
Grading scale for histological assessment of fracture healing[10]

Grade	Histological assessment
0	Non-union
1	Incomplete cartilaginous union
2	Complete cartilaginous union
3	Incomplete bony union
4	Complete bony union

experimental group (Fig. 1). By contrast, in the control group, four animals showed incomplete bony union and six showed complete cartilaginous union (Fig. 2; Table 2).

Fracture healing was statistically significantly more advanced in the α-tocopherol group compared with the control group ($P < 0.01$).

Discussion

Tissue healing in living organisms after injury involves inflammation, repair and remodelling phases.[11] In the inflammatory phase, the first phase of fracture healing, polymorphonuclear leucocytes (PMNs), macrophages and mast cells migrate into the

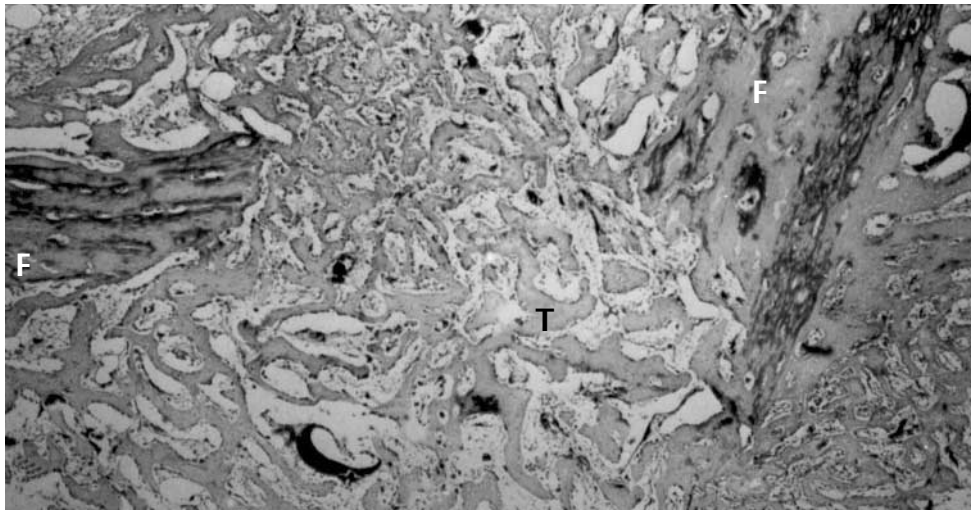

FIGURE 1: The union of fracture fragments (F) with newly formed bone trabeculae (T). Histological grade 4, α-tocopherol group, haemotoxylin and eosin, × 40

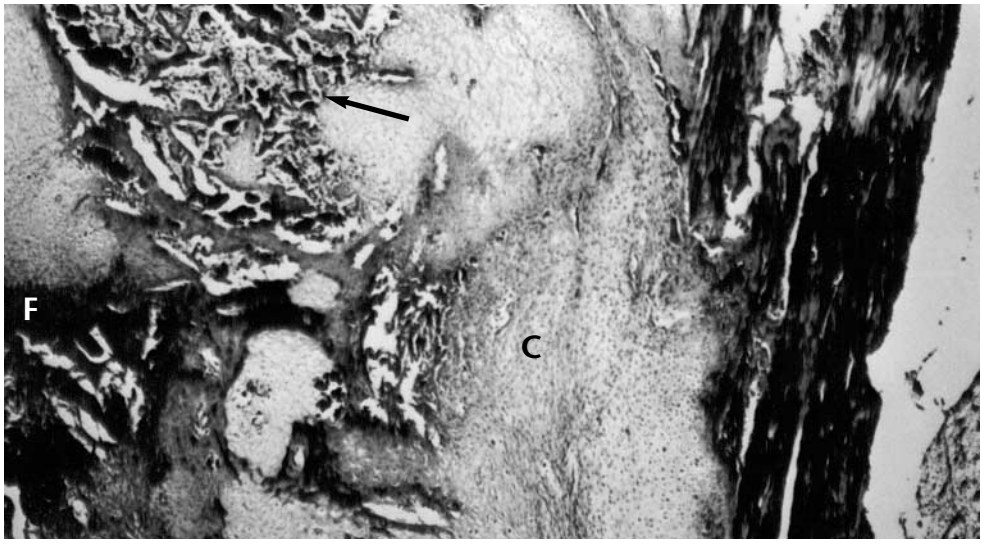

FIGURE 2: Cartilage islands (C) and endochondral ossification (arrow) between fracture fragments (F). Histological grade 3, control group, haemotoxylin and eosin, × 40

TABLE 2:
Histological assessments of the femurs of rabbits treated with α-tocopherol and controls, on day 21 after fracture, as a measure of fracture healing

	Animals assessed as grade (*n*)	
Mean histological grade	**α-Tocopherol group**	**Control group**
2	1	6
3	5	4
4	4	0
Mean ± SD	3.3 ± 0.67[a]	2.4 ± 0.51

[a]$P < 0.01$ compared with the control group.

fracture region.[12,13] The infiltration and activation of PMNs produces superoxide, which causes lipid peroxidation.[1] Free oxygen radicals, derived from PMN activation in the inflammatory phase, are reported to have negative effects on wound healing.[4,5]

Frost[14] considered that failures in the early phase of the healing process disturbed fracture healing. Further, Göktürk *et al.*[7] reported that free oxygen radicals resulting from PMN activation affected fracture healing negatively in an experimental animal model.

Free oxygen radical levels in the fracture haematoma of rabbits who received α-tocopherol have been found to be statistically significantly lower than the corresponding levels in rabbits who did not receive α-tocopherol therapy.[15] In view of this, we investigated the impact of α-tocopherol on fracture healing, especially in the inflammatory phase during which biological activity is highest.

Vitamin C, one of the non-enzymatic aqueous antioxidants, has been shown to have a positive effect on fracture healing[16,17] and to increase α-tocopherol levels by reducing its metabolism.[18,19] A significant increase in fracture healing in rabbits receiving α-tocopherol was detected in our study. A positive effect of α-tocopherol on fracture healing was also described previously using a rabbit model.[8] In a study in rats, however, no significant effect of α-tocopherol on tibial fracture healing was shown.[16] We believe that this apparent lack of an effect results from difficulties with the fixation of fractured rat tibiae and from the limited vascularization of the tibia, both of which have negative effects on fracture healing. For reasons of size we chose to use the rabbit femur, which enabled us to provide sufficient closed reduction and rigid fixation, important factors in fracture management.

We conclude that α-tocopherol has a positive effect on fracture healing and may be useful as a supportive agent in fracture cases. Further clinical and experimental studies of this effect are needed.

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