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Phosphodiesterase-5 inhibitors may facilitate bone defect recovery

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Abstract. – *Background and Objectives:* **Bone healing is still one of the most important problems of the oral and maxillofacial surgery procedures. This study was designed to evaluate the effect of sildenafil citrate (which is used for erectile dysfunction) on bone defect healing in an experimental animal model.**

Materials and Methods: **A total of 42 male Wistar-albino rats were randomly assigned to the control group (n=21) or the study group (n=21). The control group was fed on a standard laboratory diet until 12 h before surgery, whereas the study group received Sildenafil citrate via orogastric tube 10 mg/kg once a day for 30 days. Under anaesthesia, a 3x3x2 mm depth defect was made on tibia of each rat. 7 animals from each group were euthanised on postoperative days 7,15 and 30. Bone samples were taken for examination, histologically on day 7, by 3D dental tomography on day 15, and for bone strength resistance on day 30.**

Results: **Statistically significant differences were determined between the groups from the inflammatory and repair phase, with the healing process being more advanced in the Sildenafil group.**

Conclusions: **Sildenafil citrate can be used as a supporting factor to accelerate the healing process of bone. In future comprehensive studies will need to demonstrate the Sildenafil citrate affect on bone defect healing.**

Key Words:

Sildenafil citrate, Bone healing, Oral and maxillofacial surgery.

Introduction

Bone is a dynamic biological tissue composed of metabolically active cells that are integrated into a rigid scaffold. There are three primary stages of bone healing, the early inflammatory stage, the repair stage, the late remodeling stage.

The healing potential of bone is influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms. These factors are classified in two groups (local and systemic factors). Both of them variables influence the rate and degree of fracture healing. Local factors are type of bone, degree of trauma, vascular injury, degree of immobilization, etc. Systemic factors are age, nutrition, systemic diseases, hormones. A continuously occurring state of bone deposition, resorption, and remodeling facilitates the healing process[10].

A vascular response is necessary for bone defect healing. After third day of the defect or fracture there becomes a considerable increase in local blood flow which is associated with vascular proliferation in the formation of defect or fracture callus. Such changes in flow are likely to be secondary to locally controlled vasoreactivity of pre-existing vessels, as opposed to that of newly-formed angiogenic vessels.

Nitric oxide (NO) is a highly reactive free radical involved in inflammation, arthritis and fracture repair[6]. It has been shown to have significant effects on osteoblasts, osteoclasts and osteocytes[13].

The regulator role of NO in blood flow and perfusion pressure to isolate vascular beds in response to appropriate physiological stimuli is known. The source of NO in the healing process of bone may be the surrounding bone, endothelial cells, smooth muscle cells, neutrophils, recruited macrophage/histiocyte-type cells and chondrocytes[6].

Many of the biologic actions of NO are mediated by cyclic 3050 guanosine monophosphate (GMP) which is rapidly degraded by phosphodi-

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esterases (PDE). Thus, inhibiting PDEs will elevate NO level in tissues and this mechanism will proceed with the increasing of tissue nitric oxide synthase (NOS) levels. Animal studies showed that PDE type 5 is located in vascular pulmonary and visceral smooth muscle as well as skeletal muscle[5]. PDE-5 inhibitors act by blocking of PDE-5 mediated hydrolysis of cyclic GMP leading to a rise in intracellular GMP levels. Cyclic GMP is a downstream mediator of NO-induced smooth muscle relaxation[11].

Sildenafil, being a phosphodiesterase-5 (PDE-5) inhibitor, is a strong peripheral vasodilator[11,12]. Although the main indication of these drugs is erectile dysfunction. Sildenafil is shown to be effective in various pathological conditions via NO pathway[12]. In recent years, the use has been observed in plastic surgery clinics after flap surgery with the aim of increasing graft circulation[7]. Furthermore, PDE-5 inhibitors also have less selectivity ratio for PDE-11, which are widespread in skeletal muscle[8].

We hypothesize that PDE-5 inhibitors could increase blood flow via NO pathway in bone defect. This mechanism probably consists to the increase and arrangement of NOSs with PDE-5 usage.

Materials and Methods

All animal procedures were approved by the Committee for Animal Experiments of the Dicle University Medical Research Centre, Diyarbakır, Turkey. A total of 42 male Wistar-albino rats were randomly assigned to the control group (n=21) or the study group (n=21). Operation was performed under local anesthesia and a 3×3×2 mm depth bone defect was made on tibia of each rat. The control group was fed on a standard laboratory diet until 12 h before surgery, whereas the study group received Sildenafil citrate via orogastric tube 10 mg/kg once a day for 30 days.

In the literature, the reported dose of sildenafil to be administered varies from 3 to 20 mg/kg per day in animal models. A dose of 10 mg/kg Sildenafil achieves possible systemic protective effects in rats. Higher doses (over 20 mg/kg) may have greater vasodilatatory effects but they may also have side effects such as hypotension, diminished tissue perfusion, and excessive anti-inflammatory response, whereas doses lower than 10 mg/kg is less or not effective at all. Therefore, we chose to administer Sildenafil as a single daily dose[9] of 10 mg/kg.

The bone defect healing was examined radiologically and histologically at 3 phases: 1 – inflammatory phase; 2 – repair phase; 3 – remodelling phase[10].

Histologic Examination

At the end of the inflammatory phase (day 7), four longitudinal sections were taken 6-7 microns in thickness at 20 micron intervals from the paraffin blocks in which the bone samples obtained from 7 rats from the study group and 7 rats from the control group (total 14) had been embedded. These sections were then evaluated separately (Tables I, II; Figure 1A,B).

Radiological Examination

For the repair phase (day 15) following up callus formation, the bone samples were evaluated by cone beam densitometer three dimensional dental tomography (I–CAT Imaging Science International, Hatfield, PA, USA) (mg/mm^2).

Bone Strength Resistance Test

At the remodelling phase (day 30), the bone samples were attached to a Universal Test Machine (Testometric Co Ltd., U.K.) for bone strength resistance.

Before fracture testing, bones were immersed into distilled water at 37^{o}C (Nüve Incubator EN 500, Ankara, Turkey) for 24 hours. Then, all of the bones were mounted in plastic rectangular molds with a self-curing acrylic resin (Takilon, WP Dental, Germany). The bones were loaded in a Universal Testing Machine (TSTM 02500; Elista Ltd. Şti., Turkey). Testing machine adjusted using a 10 mm diameter stainless steel rod at a speed of 0,5 mm/minute. All the bones were loaded until fracture was occurred. The fracture force (Newton) was recorded for each specimen.

Thus it was aimed to evaluate the results throughout all the phases, of the effect of Sildenafil on bone defect healing, which was seen to start in the inflammatory phase.

Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows (SPSS inc., Chicago, IL, USA). The Mann-Whitney U test was used for statistical analysis of the inflammatory phase results of the control and Sildenafil groups (Tables I, II). A statistically significant difference was found between the groups ($p < 0.01$).

Table I. Histologic Examination Results of the Tibia of Control Group Rats (day 7).

Increase in hyperemia and number of capillaries:	Rat no	Section 1	Section 2	Section 3	Section 4
Absent 0	1	0	0	0	0
Present.....+	2	+	0	+	+
	3	0	0	0	0
	4	0	0	0	0
	5	+	0	0	0
	6	0	0	+	0
	7	0	+	0	0
Changes in fibrous tissue	1	+	+	+	++
No fibrous tissue 0	2	+	+	+	+
Predominance of fibrous tissue	3	+	+	+	++
and a low rate of cartilage tisssue .....+	4	+	+	+	+
Equal amounts of fibrous tissue	5	+	++	+	+
and cartilage tissue .....++	6	+	+	++	+
	7	+	+	+	+
Cartilage and bone tissue changes	1	0	+	+	+
No cartilage tissue.....0	3	+	+	0	+
Cartilage and a small amount	4	0	+	0	+
of immature bone.....+	5	+	0	0	0
Equal rates of cartilage and	6	+	+	0	0
immature bone.....++	7	0	0	+	0
Predominantly immature bone and					
a low rate of cartilage .....+++					

Table II. Histologic Examination Results of the Tibia of the Sildenfil Study Group Rats (day 7).

Increase in hyperemia and number of capillaries:	Rat no	Section 1	Section 2	Section 3	Section 4
Absent 0	1	+	+	+	+
Present.....+	2	+	+	+	+
	3	+	+	+	+
	4	+	+	+	+
	5	+	+	+	+
	6	+	+	+	+
	7	+	+	+	+
Changes in fibrous tissue	1	++	++	++	++
No fibrous tissue 0	2	++	++	++	+
Predominance of fibrous tissue	3	+	+	++	++
and a low rate of cartilage tisssue .....+	4	++	++	+	++
Equal amounts of fibrous tissue	5	+	++	++	++
and cartilage tissue .....++	6	++	++	++	+
	7	+	++	++	++
Cartilage and bone tissue changes	1	+++	++	+++	++
No cartilage tissue.....0	2	++	++	++	++
Cartilage and a small amount	3	+++	+++	++	+++
of immature bone.....+	4	++	++	++	+++
Equal rates of cartilage and	5	+++	+++	+++	+++
immature bone.....++	6	+++	++	++	+++
Predominantly immature bone and	7	+++	++	+++	+++
a low rate of cartilage .....+++					

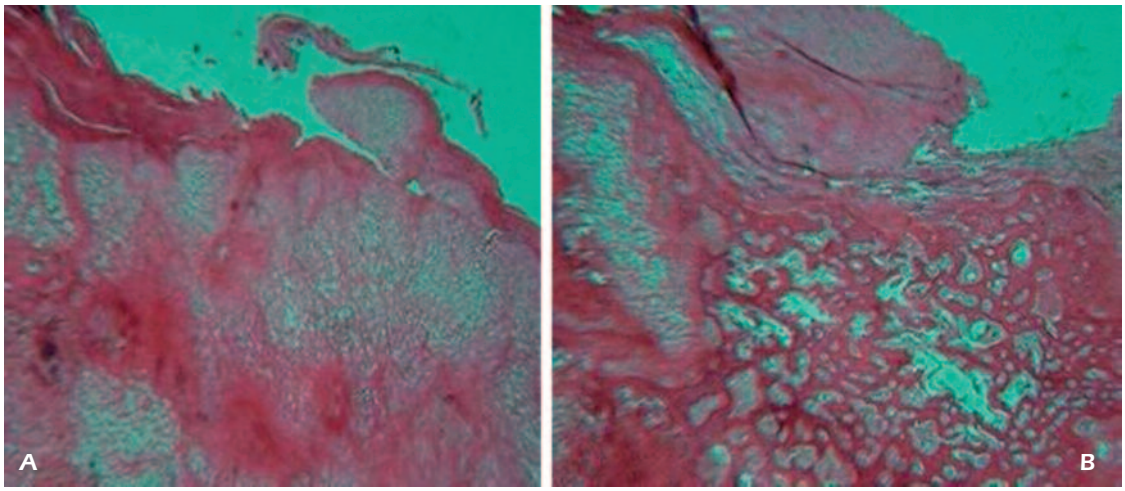

Figure 1. *A,* Defect healing in a control group rat (day 7). *B,* Defect healing in a Sildenafil group rat (day 7) showing intense fibrous tissue and a small amount of cartilage tissue. (Haematoxylin eosine × 160).

Student's *t* test was used for statistical analysis of independent groups of the results of bone examination by cone beam densitometer three dimensional dental tomography (Table III). A statistically significant difference was found between the groups ($p < 0.0001$).

Student's *t* test was applied to the measurements obtained for bone strength resistance by applying force to the samples up to breaking point (Table IV). No statistically significant difference was determined between the groups ($p > 0.05$).

Results

At the end of seventh day rats were sacrificed. In the histological examination, an increase was observed in the capillary vascular number and volume. Cartilage and bone volume in Sildenafil group was larger than control group (Tables I, II; Figure 1,A,B). 3D three dimensional dental tomography evaluation at the fifteenth day revealed that Sildenafil group had higher bone density than control group (Table III). Additionally Sildenafil group's bone strenght resistance was higher than control group (Table IV).

Discussion

The effect on bone healing is an interesting current topic[2,3]. There are several published studies on the effects of PDE-5 inhibitors on fracture healing. However, to the best of our knowledge there are no studies on bone defects arising from various pathologies in the oral and maxillofacial areas.

Bone healing is directly related to blood circulation. Sildenafil citrate increases blood flow by maintaining the vascular smooth muscle surface vasodilation of nitric oxide (NO). Achieving increased blood flow from NO angiogenesis and vasodilation is beneficial to defect[1]. On day 14 vascular improvement reaches the highest level of angiogenesis, which then accelerates the changing of cartilage callus to bone callus[4].

In the group to which Sildenafil citrate had been given, one of the most important factors of the positive effect seen in the fourth week in the repair phase, particularly the transition of cartilage callus to bone callus, is that it is connected to the effect of increased angiogenesis and vasodilation. This effect of Sildenafil citrate is thought to be mediated by NO. One of the impor-

Table III. Results of the repair (callus) phase (day 15).

Rat No	1	2	3	4	5	6	7
Control group	92	87	89	99	103	79	81
Sildenafil group	134	143	156	161	224	131	149

Table IV. Results of the remodelling phase (day 30).

Rat No	1	2	3	4	5	6	7
Control group	75.3	63.7	69.4	70.2	94.6	62.2	71.4
Sildenafil group	82.5	65.9	78.2	76.5	119.0	71.9	79.0

tant factors in fracture healing in the inflammation phase is the formation of granulation tissue. Fibroblasts migrating into haematoma and the collagen produced and other extracellular matrix proteins, take on one of the most important roles of the formation of fibrous callus. In a study on cell cultures from fibroblasts obtained from human Peyronie plaque, Sildenafil citrate was seen to decrease collagen 1 synthesis and miyofibroblast differentiation *in-vitro*[14].

Conclusions

We hypothesize that PDE-5 inhibitors could increase blood flow via the NO pathway in a bone defect. This mechanism probably consists to the increase and arrangement of nitric oxide synthase with PDE-5 usage. Further clinical studies are needed to clarify the beneficial use of Sildenafil citrate on bone defect healing.

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