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The effect of bone marrow ablation on regional biomechanical properties of rat tibia

Kemik iliği ablasyonunun sıçan tibiasının bölgesel biyomekanik özellikleri üzerine etkileri

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Amaç: Normal sıçan tibiasının bölgesel (metafiz-diyafiz) biyomekanik özellikleri ve bir kırık iyileşme modeli olan kemik iliği ablasyonu sonrası bu özelliklerde ortaya çıkan değişiklikler incelendi.

Çalışma planı: Tibialarına kemik iliği ablasyonu uygulanan 24 adet Sprague-Dawley cinsi sıçan deney grubunu, hiçbir cerrahi işlem uygulanmayan sekiz adet sıçan kontrol grubunu oluşturdu. Tüm sıçanlardan proksimal metafiz, proksimal diyafiz, distal diyafiz ve distal metafiz örnekleri hazırlandı. Kontrol grubunda kompresif kuvvetler uygulanarak tibianın bölgesel segmentlerinde sertlik (E), güç (S_{maks}) ve dayanıklılık (toplam enerji absorbsiyonu, U) parametreleri ölçüldü. Deney grubunda ise ablasyonu takiben 1, 3, 7, 9 ve 15. günlerde kompresyon uygulanarak, ablasyonun tibianın bölgesel mekanik özelliklerinde meydana getirdiği değişiklikler incelendi.

Sonuçlar: Normal sıçan tibiasında anatomik bölgeler arasında en düşük E, S_{maks} ve U ölçümleri proksimal metafiz bölgesinden elde edilirken, en yüksek değerler E ve S_{maks} ölçümlerinde distal diyafiz, U ölçümlerinde ise proksimal diyafiz bölgelerinden elde edildi. Kemik iliği ablasyonu sonrasında tüm test değerlerinde 1-7. günlerde düşüş, 7-9. günlerde hafif bir artış ve 9-15. günlerde yeniden bir düşüş saptandı. Mekanik ölçüm parametreleri açısından iki grup arasında belirgin istatistiksel fark saptanırken ($p<0.05$), tibianın bölgeleri arasında anlamlı fark görülmedi ($p>0.05$).

Çıkarımlar: Normal sıçan tibiasında biyomekanik açıdan diyafiz en dirençli, proksimal metafiz ise en zayıf anatomik bölgedir. Kemik iliği ablasyonu sonrası meydana gelen metabolik olaylar, tibianın bölgesel mekanik özelliklerinde değişikliklere neden olmaktadır. Ablasyona bağlı intramedüller hasardan en fazla etki lenen bölgeler tibianın metafizer segmentleridir.

Anahtar sözcükler: Biyomekanik; kemik iliği/fizyoloji; kırık iyileşmesi/fizyoloji; sıçan; tibia.

Objectives: Regional (metaphyseal-diaphyseal) biomechanichal properties of normal rat tibia, and changes on these biomechanichal properties after bone marrow ablation, a model of fracture healing, were examined.

Methods: The study included 24 Sprague-Dawley rats that underwent tibial marrow ablation, and eight control rats with no surgical procedure. Proximal metaphyseal, proximal diaphyseal, distal diaphyseal, and distal metaphyseal samples were prepared from the tibias of all rats. In the control group, stiffness (elastic modulus, E), strength (maximum strength, S_{max}), and toughness (total energy absorption, U) parameters of the regional tibial segments were evaluated under compression loads. In the experimental group, compression was applied following bone marrow ablation on days 1, 3, 7, 9, and 15, and ablation-induced changes in the regional biomechanical properties were studied.

Results: The lowest E, S_{max}, and U values were obtained from the proximal metaphysis. The highest E and S_{max} values were from the distal diaphyseal, and the highest U values were from the proximal diaphyseal regions. In ablation-induced rats, decreases were observed in all the mechanical test values during days 1 to 7, followed by slight increases on days 7 to 9, and eventual decreases on days 9 to 15. There were significant differences between the two groups with respect to biomechanical parameters ($p<0.05$), but no significant differences were found between the tibial regions ($p>0.05$).

Conclusion: Biomechanically, the most resistant and the weakest anatomic regions of normal rat tibia are the diaphyseal region and proximal metaphysis, respectively. The metabolic changes occurring after bone marrow ablation lead to changes in the mechanical properties of the tibia. The most affected tibial segments from ablation-induced intramedullary injury are the metaphyseal segments.

Key words: Biomechanics; bone marrow/physiology; fracture healing/physiology; rats; tibia.

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Bone marrow ablation in rats has been described as a fracture healing model.[1-3] The healing potential of the bone is influenced by many biochemical, biomechanical, cellular, hormonal and pathological mechanisms.[1,2] Fracture healing is a repair process where the fractured bone tissue is restored to an original bone tissue with a complete biomechanical integrity.

The bone is a dynamic and biological tissue, consisting of metabolically active cells. The mechanical properties of the bone depend on the intensity, nature and material characteristics of the trabecular structure of the bone.[4] The trabecular bone has a complex architectural structure. Furthermore, the typical micro-nature and mechanical properties of the trabecular bone highly differ even in various regions of the bone itself.[5] Due to its complex characteristics, several models have been established in order to demonstrate the relationship between the nature of the bone and its mechanical properties (elastic modulus, maximum strength, etc.).[4-8] Although regional mechanics of the cortical or trabecular bones have been examined in detail in several experimental [9,10] or cadaver [11-14] studies, changes in the biomechanical characteristics of the bone associated with the bone marrow ablation have not been studied yet. The objective of the present study was to examine the regional (metaphyseal-diaphyseal) biomechanical properties of the normal rat tibia, and daily changes on these biomechanical properties after bone marrow ablation, which is a fracture-healing model. With this hypothesis, we considered that the mechanical properties of different anatomical regions would be different, and the use of bone marrow ablation would reduce the regional mechanical properties of the tibia.

Material and method

The study included 32 Sprague-Dawley male rats (250 to 390 grams) based upon the approval by the Ethical Committee. Eight of the rats were randomized to a control group in order to examine the biomechanical properties of normal rats, and none of them underwent any surgical procedure. The rats in the experimental group (n=24) were tranquilized with ketamine hydrochloride (80 mg/kg) (Ketalar, Pfizer Ilac Sirketi Ltd. Istanbul) and xylazine 12 mg/kg (Rompun, Bayer Türk Kimya Sanayi Ltd., Istanbul). After the right lower extremity was shaved and washed, it was stained with povidone-iodine, and draped for standard surgical methods. For bone marrow ablation, as described by Suva et al.[15] a 1 cm long medial incision was made through the tibial periost from the tibial proximal, and a hole was made in the cortex by means of a 2 mm surgical drill almost 2 mm below the epiphyseal. After being damaged by the medullary intravenous vein catheters (20-23 G), they were washed by serum physiologic saline and sucked by vacuum, and the content of the marrow was aspirated. Repeating this procedure 2 to 3 times, the marrow was completely depleted. The skin was sutured by a non-resorbable material (3/0 ethilon), and then closed by an adhesive surgical drape.

The rats in the experimental group had no restriction on food and motion from the bone marrow ablation until their sacrifice. Following the surgical procedure, five rats at each day at days 1, 3, 7 and 9, and four rats at day 15 were killed by a high dose of tiopental sodium (Pentothal, Abbott

SpA, Aprilia LT, Italy). The right tibias of rats were removed, and their soft tissues were debrided. They were kept in a freezer (-20 °C) until the time of the biomechanical test, and before the test they were resolved at a room temperature within a serum physiologic saline.

In order to demonstrate the effect of ablation damage on the biomechanical properties of the tibia, samples of proximal metaphyseal (p), proximal diaphyseal (d1), distal diaphyseal (d2), and distal metaphyseal (b), each being at most 5 mm long were prepared using a mini electrical saw. The samples' surfaces were finished using a high-speed grinding disk to obtain an appropriate surface for axial loading. The surface of the p profile of the tibia was considered a triangle while the surfaces of both diaphyseals (d1, d2) and b profile were considered a circle; the height, length, and diameter of each sample were measured; and then their areas were calculated, and recorded for biomechanical assessments (Figure 1).

During biomechanical assessments, all samples were deformed by a 2.5 mm/min axial compression using an electromagnetic testing equipment (Instron, Series IX Automated Materials Testing System, Instron Corporation, C anton, Massachusetts, USA).[6,8,16] The data were plotted on a graph where the horizontal axis represents the "changes" (ε) and the vertical axis represents the "stress" (S). Then, the biomechanical properties of the material were calculated using the time-deformation graphics. Three mechanical parameters were used:

Module of Elasticity (E): It is the linear part of the curve ($E=tg\cdot=S/\varepsilon$). Elasticity module is one of the parameters defining the strain characteristics of a material. An increased module of elasticity indicates that the material can be easily strained while decreased elasticity shows that it can be hardly strained.

Maximum stress (Smax): It indicates the maximum stress that can be achieved in a material. When this value is exceeded, deformation is irreversible. The higher the maximum stress, the higher the stability of the material, i.e. a lower maximum stress indicates lower stability for the material.

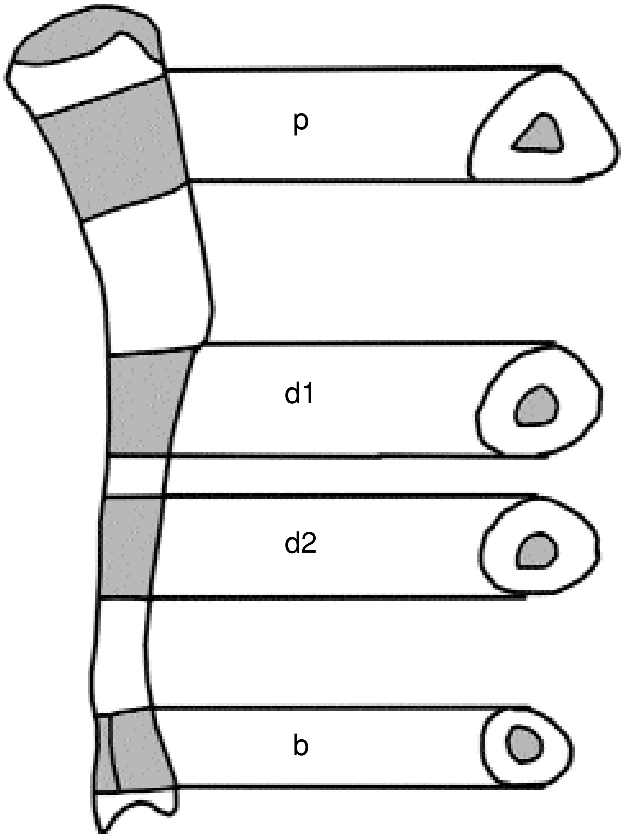

Figure 1. Sampling for biomechanical test from the ablation-induced tibia and surface forms of the samples. (p) Proximal metaphyseal, (d1) proximal diaphyseal, (d2) distal diaphyseal, (b) distal metaphyseal.

Strain energy per unit (U): It represents the entire area remaining under the stress-strain curve during the deformation of the material. The magnitude of the value gives the measure of a material's ability to absorb the deformation energy. Increased values indicate tougher material while decreased values indicate a less tough material.[16]

For comparison of the data from the biomechanical test by days, variance analysis (ANOVA), and for matched multiple comparison tests, Student's t test was used.

Results

The mechanical test results for each rat are provided in Table 1. In the control group, the highest values for E and Smax assessments were from the d2 region (901.4±248 MPa and 73.1±18.3 Mpa respectively). While the E value in the distal diaphyseal (d2) region was significantly different than the p ($p<0.001$), d1 ($p=0.004$) and b ($p=0.009$) assessments, a significant difference was found only in the comparison with the p value in the Smax assessment ($p<0.001$, Table 1).

During the strain energy per unit (U) assessments, the d1, d2 and b values were all similar (10.3±3.6 N/m2, 9.0±2.9 N/m2, and 10.2±4.6 N/m2 respectively, $p>0.05$). For all mechanical assessment parameters, the lowest mechanical test results were obtained in the assessment of the p value (Table 1).

Although a statistically significant difference was found during the paired comparisons of the values from the anatomical regions of the tibia, comparison of entire anatomical regions yielded no significant difference ($p>0.05$, Table 1).

The mechanical test results obtained at day 1 after the ablation in the experimental group showed an increase in all segments of the tibia compared to the results of the control group. Although such an increase was found in all mechanical test values, it was statistically significant in all Smax values ($p<0.05$), but none of the increased U values reached to such significance ($p>0.05$). During the assessment of the elasticity module (E), increases were significant in all, but d2 (Table 1).

A biphasic response with two reduction phases was observed in the curve of the experimental group after the ablation. A reduction was observed in all the mechanical test values on days 1 to 7, followed by remarkable increases on days 7 to 9, and eventually

Table 1. Biomechanical test results for all tibia segments before and after the ablation

		Control	Experimental Group (n=24)					
		Day 0 (n=8)	Day 1 (n=5)	Day 3 (n=5)	Day 7 (n=5)	Day 9 (n=5)	Day 15 (n=4)	*p* ANOVA
Elasticity module (E) (MPa)	p	410.6±200.6**	582.0±146.6	424.7±118.0	424.1±143.7	524.8±98.9	356.0±91.2	p=0.02
	d1	538.3±176.5*	786.7±162.9***	635.1±113.9	432.6±95.0	724.3±126.4	691.9±190.2	p=0.008
	d2	901.4±248.0	929.2±75.4	921.3±113.9	798.5±182.5	842.2±200.3	696.4±52.6***	p=0.297
	b	578.5±175.3*	915.7±151.2***	528.9±92.6	597.8±68.5	1112.6±267.3‡	701.0±165.3	p<0.0001
Maximum stress (S$_{maks}$) (MPa)	p	39.8±16.5$^{\#}$	70.2±10.8***	56.8±14.9	46.5±13.6	50.2±13.6	38.6±14.6	p=0.013
	d1	59.6±24.2	68.0±7.8***	57.4±8.8	61.0±15.6	60.3±15.1	64.5±22.3	p=0.837
	d2	73.1±18.3	96.1±5.2***	72.6±6.9	64.1±16.4	97.2±20.3***	77.5±16.0	p=0.027
	b	64.4±13.3	87.9±5.6***	70.4±7.4	78.9±7.1***	75.9±17.1	55.5±11.9	p=0.002
Strain energy per unit (U) (N/m^2)	p	6.9±2.2	10.1±4.4	8.5±2.6	12.2±4.9***	13.4±6.3***	4.8±1.4	p=0019
	d1	10.3±3.6	13.5±3.6	10.6±3.9	9.1±4.2	9.2±4.1	6.4±2.0***	p=0.146
	d2	9.0±2.9	10.6±5.2	8.4±2.4	9.4±4.4	11.4±5.0	9.7±3.6	p=0.843
	b	10.2±4.6	11.9±3.8	9.8±4.1	12.6±4.6	11.5±6.2	8.3±2.9	p=0.734

The following p values were obtained by Student's t-test. *p<0.05; **p<0.001, comparison of the results for d2 with other anatomical regions during the E assessments in the control group; #p<0.001, comparison of results for d2 with other anatomical regions during the Smax assessments in the control group; ***p<0.05; ‡: p<0.001comparison between the control group and post-ablation results.

decreases again on days 9 to 15 after the ablation. The final assessments yielded results similar to baseline values (Figure 2). In order to evaluate the net effect during the 15 days following the ablation, the mechanical test results of the rats in the experimental group at day 15 were also compared with the control group. No statistically significant difference was observed during the comparison (p>0.05, Table 1).

Discussion

Any material with applied forces undergoes deformation, straining in accordance with the direction of the force. Given the stress and strain values of a material being calculated, and their relationship expressed in a graphic, the mechanical properties (E, Smax and U) of a material can be determined independent of its shape.[6,8,16] The module of elasticity is an indication of the straining characteristics while

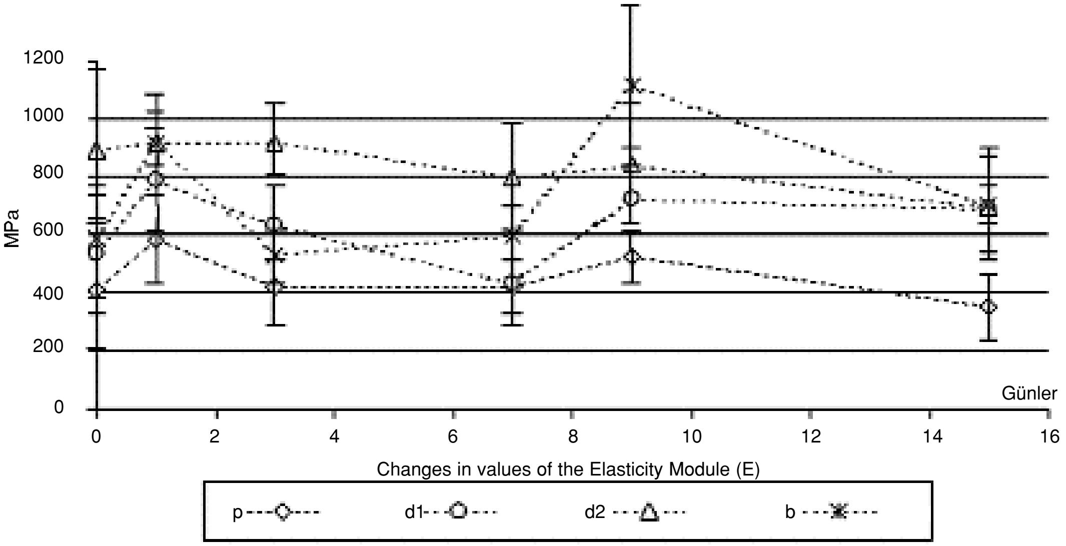

Figure 2. Changes in the values of the Elasticity Module (E) in rats with bone marrow ablation by day (p) Proximal metaphyseal, (d1) proximal diaphyseal, (d2) distal diaphyseal, (b) distal metaphyseal.

Smax shows the stability and U, the amount of energy absorbed during straining, i.e. toughness.[16]

To change the shape of a material, stretching, axial loading or rotating forces can be applied. In many studies, mechanical properties of the trabecular bone were obtained using the uniaxial compressive and tensile strengths.[7,9,10,17,18] In our study, we used only compression to determine the regional biomechanical properties of the tibia as we employed rats and the specimens derived by separating the tibia into segments were very small.

Several models have been developed combining various methods in order to reveal the mechanics of the bone.[6,9,10,13,14,19,20] Some of these studies are experimental models, and although they were performed with dogs [6,19] and rats [9,10,20,21] , no previous study provided the regional mechanical properties of the rat tibia and examined those mechanical properties following the bone marrow ablation. In our study, the tibia of the rat was divided into four anatomical regions in order to demonstrate the mechanical properties of separate anatomical regions. Among these anatomical regions, the highest mechanical results were obtained in d2 for E and Smax assessments, and d1 for U assessment (Table 1). The d1 and d2 of the tibia are cortical regions (Figure 1). Furthermore, the lowest results were obtained in the p assessments for all mechanical assessment parameters (E, S_{max} and U) (Table 1). Based on these results, the diaphyseal of the tibia is mechanically the most resistant region whereas the proximal metaphyseal is the weakest region.

The bone marrow ablation in rats has been employed as a model of intramembranous fracture healing in rodents due to its fast healing process and independency from the bone cortex.[1,2,15] In this model, there exists a repair process where the cartilage phase is lacked due to damaging in the tibial bone marrow and primary mineralization is induced by endosteal new bone formation.[22] The bone marrow ablation effectively induces the bone formation-resorption process.[1-3,15] Studies have comprehensively demonstrated that in rats with bone marrow ablation, an extratibial bone resorption takes place on the post-ablation days 1 to 7, and new bone formation on days 7 to 9, and osteoclastic resorption in the intramedullary bone on days 9 to 15.[2,15] Similarly, in our study we obtained a biphasic response where a decrease occurred in the mechanical test results between days 1 and 7, followed by a mild increase between days 7 and 9, and subsequently a decrease again until day 15 in ablation-induced rats (Figure 2, Table 1). Those results indicate that the metabolic changes occurred during the healing process of 15 days in ablation-induced rats are also directly reflected in the biomechanical properties.

The changes in the mechanical assessments reflecting the resorption and new bone formation associated with ablation in the bone marrow were detected in all tibial segments (p, d1, d2 and b). However, mechanical changes associated with ablation by days had a statistically significant difference only in the metaphyseal segments (p and b) except the U value of b (Table 1). It suggests that the metaphyseal segments in the ablation-induced tibia are the most affected regions. Lack of a statistically significant difference in the b value in spite of the fact that the U is a variable dependent on the E and Smax in the biomechanical assessments may be associated with the size (diameter, cortical thickness, etc.) of the bone on which the mechanical test was applied.

An analysis of the net biomechanical effect in all of the bone segments resulting from the change after 15 days following the ablation showed no significant difference (Table 1). It indicates that the changes in the mechanics associated with the damage incurred in the bone marrow returned to normal at the end of the healing process of 15 days.

In conclusion, biomechanically the diaphyseal region is the most resistant and the proximal metaphyseal region is the weakest anatomic region of normal rat tibia. The metabolic changes occurring after bone marrow ablation lead to changes in the regional mechanical properties of the ablation-induced tibia. In the experimental group, the biphasic reduction in the E, Smax and U values as a result of the damaging in the bone marrow indicates that stability is reduced in all tibial segments, it can be easily strained and it has a lower toughness. The most affected tibial segments from ablation-induced intramedullary injury are the metaphyseal segments (p and b). The mechanical weakening related with ablation is fixed at the end of a healing process for 15 days.

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