



ARTÍCULOS ORIGINALES / *Originals*

RABBIT GROWTH PLATE MORPHOLOGY IN TEMPORARY BILATERAL BLOCKING

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Abstract

Blocking of the growth plate (GP) using plates with screws (tension band plating) is a modern method used to correct deformities and moderate leg length discrepancy in growing children. Determining the duration of temporary bilateral blocking without the occurrence of irreversible changes of GP is of paramount importance important.

Methods: Two-month-old Californian breed male rabbits (n=30) were exposed to bilateral blocking of the distal GP of the right femur locking plates with screws for 3, 5, and 7 weeks. The fixators were removed after 5 and 7 weeks in 18 rabbits and 3 weeks after that, animals were sacrificed. The contralateral limb was used as a control. Histological, histomorphometric, and X-ray analyses were performed.

Results: During GP blocking, its height gradually decreased. This decreased was more pronounced after 7 weeks. Destructive changes

progressed with an increase in the blocking duration. Three weeks after discontinuation of the bilateral blocking that lasted 5 weeks, the height of the GP significantly increased 1.2 times on the lateral side and 1.9 times on the medial side ($p < 0.001$) compared to the control. When blocking was discontinued after 7 weeks, the structure of the GP was partially restored after 3 weeks, the height of GP significantly increased 1.2 times on the lateral side, and 1.07 times on the medial side ($p < 0.01$) compared to the control.

Conclusion: Restoration of the structural-functional features of the GP after the removal of the plates depends on the duration of temporary bilateral blocking, which must be taken into account in the clinical setting.

Key words: temporary bilateral blocking, rabbit, guided growth, tension band plating, histology.

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Resumen

El bloqueo de la placa de crecimiento (PC) utilizando placas con tornillos (banda de tensión) es un método moderno utilizado para corregir deformidades y alteraciones moderadas en la longitud de las piernas en niños en crecimiento. Es de suma importancia determinar cuál debe ser la duración del bloqueo bilateral temporal sin que ocurran cambios irreversibles en la PC.

Métodos: Conejos machos de raza californiana de dos meses de edad ($n = 30$) fueron expuestos al bloqueo bilateral de la PC distal colocando placas del fémur derecho con tornillos durante 3, 5 y 7 semanas. Los fijadores fueron retirados después de 5 y 7 semanas en 18 de los conejos, y 3 semanas después los animales fueron sacrificados. La extremidad contralateral se utilizó como control. Se realizaron análisis histológicos, histomorfométricos y de rayos X.

Resultados: Durante el bloqueo de la PC, su altura disminuyó gradualmente. Esta disminución fue más pronunciada después de 7 semanas. Los cambios destructivos se incrementaron a medida aumentaba la duración del bloqueo. Tres semanas después de la interrupción del bloqueo bilateral que duró 5 semanas, la altura de la PC aumentó significativamente 1.2 veces en el lado lateral y 1.9 veces en el lado medial ($p < 0.001$) en comparación con el control.

Conclusión: La restauración de las características funcionales estructurales de la PC después de la extracción de las placas depende de la duración del bloqueo bilateral temporal, lo que debería tenerse en cuenta en el tratamiento clínico de estas alteraciones.

Palabras clave: bloqueo bilateral temporal, crecimiento guiado, placas de bandas de tensión, histología.

Introduction

The concept of “guided growth” is widely used in pediatric orthopedics and represents the influence on the functioning of bone the growth plates. There are various methods of growth plate blocking (permanent epiphysiodesis, stapling, PETS (percutaneous epiphysiodesis using transphyseal screws) technique, and temporary tension band plating). Tension band plating for temporary bilateral blocking is a modern method used to correct coronal and sagittal deformations of lower limb bones (unilateral blocking)¹⁻³ and moderate (2-5 cm) leg length discrepancies (bilateral blocking) in growing children.³⁻⁵ The advantage of this method, compared to the use of staples, is a less rigid growth plate blocking, which reduces the risk of damage to the growth plate.⁶ The

rate of complications for this method is less than 10%.⁴ In a multicentre study, the results of treatment of frontal deformities (varus and valgus) and leg length discrepancy in 126 children using plates with screws were analyzed. The authors noted complications in 20 (18%) patients, most often associated with implant failure (i.e.: migration and fracture) in 10% of the patients. The second most frequent complication was associated with premature closure of growth plate in 5 % of the patients.⁷ The authors noted that the use of growth plate blocking in patients just before the onset of skeletal maturity is a complex issue, and careful monitoring throughout the whole treatment period prevents most of the complications.

In another similar multicentre study involving 537 patients, the importance of the



patient's age for the successful correction of deformity was also emphasized, and the following complications were found: breakage of screws – 0.53% and limitation of movements – 1.12%.⁸ The time of the removal of the fixators is also an important factor of achieving optimal correction. In particular, in a retrospective analysis of 94 patients, it has been shown that an increase in a fixator lifespan by ≥ 6 months increases the risk of hypercorrection (odds ratio, 19.2; 95% confidence interval, 5.2-71.4; $p < 0.005$).⁹

Growth acceleration after unilateral growth plate blocking using plates with screws is one of the features that occurs after this procedure, especially in younger patients with high growth potential (younger than 14 years).⁶ However, this phenomenon is not well understood.¹⁰ In an experimental study in rabbits, which had a plate in the proximal tibia for 3 weeks, growth acceleration of the medial side was identified histologically 2 weeks after removal of the plate. Growth arrest was discovered 3 weeks after the plate was removed, when the columnar structure in the growth plate was restored.¹⁰

Consequently, determining the age for the initiation of application and the duration of the growth plate blocking without the occurrence of irreversible changes is definitely an important issue, the solution of which will increase the efficacy of the application of this method.

The aim of study was to evaluate the morphological changes of the distal growth plate of the femur of rabbits during and after temporary bilateral blocking using locking plates with screws.

Materials and methods

Animals

The experiments were carried out on two-month-old Californian breed male rabbits ($n=30$), with an average body weight of 1.5 ± 0.2 kg, in the experimental biological clinic of the Sytenko Institute of Spine and Joint Pathology, Ukraine. The animals were

kept in separate cages, with a 12-hour light period, and provided with a complete diet and water *ad libitum*. The experimental model was validated by N. Mast et al.¹¹ and consists of a temporary bilateral blocking of the distal growth plate of the femur using locking plates with screws (temporary bilateral blocking). During work with the animals, the requirements of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes,¹² and the Law of Ukraine “On the protection of animals against cruel treatment”¹³ were observed. The protocol for experiments on animals was approved by the Bioethics Committee at the Sytenko Institute of Spine and Joint Pathology (No. 116 dated 25.03.2013, No. 154 dated 27.06.2016).

Experimental design

All rabbits were exposed to temporary bilateral blocking.¹⁴ The contralateral limb was used as a control.

The animals were randomly divided into two groups. The first group ($n = 18$) was used to study the morphological structure of the epiphyseal cartilage after its bilateral blocking for 3, 5, and 7 weeks. In the second group ($n = 12$), removal of the fixators was performed under intravenous anesthesia, following temporary bilateral blocking, which lasted for 5 and 7 weeks. Three weeks after that, the animals were sacrificed to further study the morphological structure of the epiphyseal cartilage. After preliminary sedation with a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg), the animals were euthanized by intravenous injection of a lethal dose of phenobarbital (150 mg/kg). Later, both femoral bones were removed to be used for histological analyzes.

Surgical intervention technique

The animals underwent surgical intervention in aseptic conditions under intravenous anesthesia with a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg). The skin of the lower extremities was shaved and treated

with a betadine solution three times. Linear longitudinal incisions of the skin up to 1.5 cm in length were performed along the medial and lateral surfaces in the region of distal epimethaphysis of the femur. Extraperiosteal fixation, first of the medial side and then the lateral side of the distal growth plate of the femur was performed using blocking plates with two screws. One screw was introduced

into the epiphysis (Fig. 1A), and the other into the femoral metaphysis (Figure 1B). The location of the screws was controlled by X-rays (Fig. 1). The length of the screws fixing the plate did not exceed half the transverse size of the proximal femur in the frontal plane. Additionally, a Kirschner wire was introduced into the distal part of the operated and contralateral femoral bone¹⁴ (Fig. 1C).

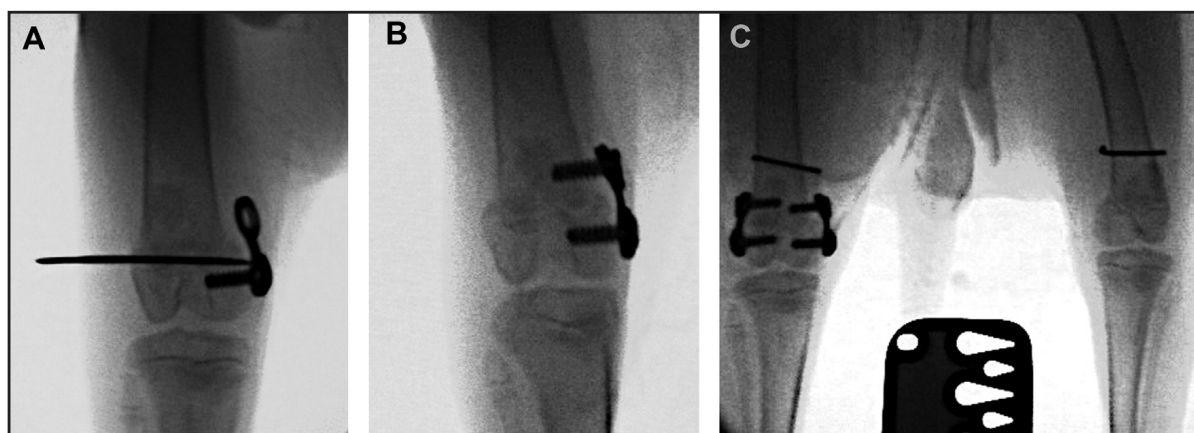


Figure 1. X-ray control during surgery of the bilateral blocking of the rabbit femur: one screw was introduced into the medial epiphysis (A), the other into the medial femoral metaphysis (B). Bilateral blocked limb and contralateral limb with Kirschner wire in the distal part of the femurs (C).

In the preoperative period and for 2 days after the operation, antibiotic prophylaxis with Cephazolin (5 mg×kg/day) was performed. In addition, the animals received treatment of the postoperative wounds with antiseptic solutions for the first 3 days following the operation.

Histological analysis

The rabbit femoral bones were fixed in 10% neutral formalin; after decalcification in 10% formic acid, the screws were removed and the distal parts were cut out. The material was dehydrated and poured into paraffin. Longitudinal histological 5-6 μ m thick sections (7 of each sample) were stained with hematoxylin and eosin. The analysis of the obtained histological preparations was carried out under a light microscope Olympus

BX-63 equipped with a digital camera DP73 (Olympus).

During histological analysis of epiphyseal cartilage, structural features of the resting, proliferating and hypertrophic zones were evaluated (Figure 2). The resting zone was determined to be between the subchondral bone and the region where chondrocytes are located in columns. The proliferation zone consisted of columns of chondrocytes, and the hypertrophic zone consisted of enlarged cells located between the proliferating zone and the primary spongiosa. Such delineation into zones is used according to previously conducted histomorphometry studies of epiphyseal cartilage in animals.¹⁵ In addition, the condition of the primary spongiosa zone was analyzed.

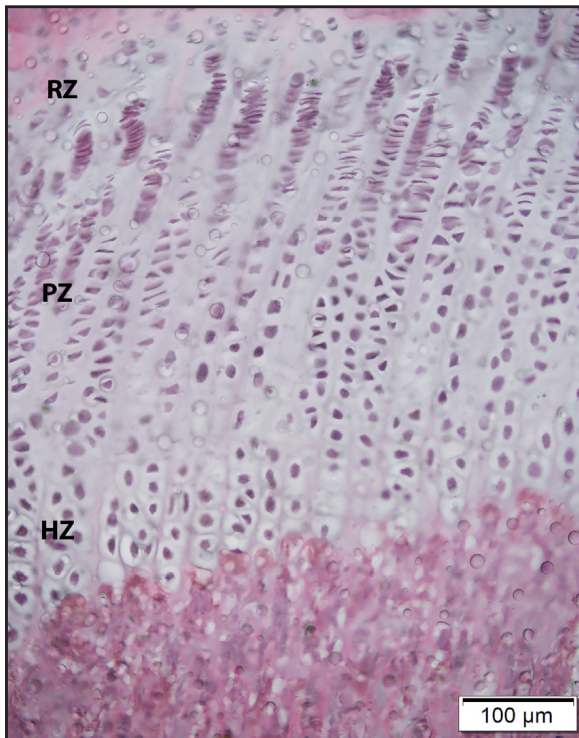


Figure 2. Histology of growth plate of a control rabbit. RZ-resting zone; PZ-proliferating zone; HZ-hypertrophic zone. Hematoxylin and eosin staining.

Histomorphometric study

The height of the epiphyseal cartilage in the medial, lateral and middle sides of the experimental (operated) and control limbs (measured in 7 longitudinal sections, 3 measurements for each medial, lateral and middle side) was measured using the software CellSens Dimension 1.8.1 (2013) for the microscope Olympus BX-63 (200x). Medial and lateral sides were determined to be at a distance of 2.4 mm from the central axis of the bone.

X-ray

The radiography (anteroposterior projection) of the hind limbs of rabbits was performed 1, 2 and 3 weeks after plate removal. The mechanical Lateral Distal Femoral Angle (mLDFA) was measured on each radiograph using the “Angle” software.

Statistical analysis

The obtained indicators are presented as

mean \pm standard deviation (SD). Paired t-test was used for compare mean values blocked and contralateral (control) limbs at the same period. Unpaired t-test was used for compare mean values of independent samples. One-way ANOVA was performed with Bonferroni correction for multiple comparisons with duration of bilateral blocking as factor. The difference between the mean values was considered statistically significant for $p < 0.05$. The IBM SPSS Statistics 20 software was used during the analysis.

Results

Histological analysis

Three weeks after the bilateral blocking, it was found that the growth plate characteristic zones were preserved, but the histoarchitectonics was slightly disturbed from the medial and lateral sides. These changes manifested through the appearance of cell-free areas with slightly basophilic coloration and some disorganization of the columns in the proliferating zone. The resting zone was represented mainly by one (in minor areas – two) layer of elongated chondrocytes. In the hypertrophic zone, throughout the whole growth plate area of the operated limb, chondrocytes of round shape, shadow cells, and empty lacunae were observed. In the primary spongiosa, bone trabeculae located parallel to each other were identified at the medial and lateral sides, but their areas were slightly smaller than the middle part and the control limb. The overall height of the growth plate was irregular: it was slightly lower on the lateral and medial sides, but not significantly different when compared to the control limb, and it was decreased in the middle area by 1.1 times ($p < 0.05$) (Table 1).

Five weeks after the bilateral blocking, structural disorders were identified on the entire territory of the epiphyseal cartilage, with a greater manifestation of disturbances in the medial and lateral sides than the middle side. Additional findings include

Table 1. Growth plate height (μm) measurements at different period after bilateral blocking.

Side of growth plate	Time of blocking (weeks)						paired t-test			one-way ANOVA	
	3		5		7		weeks			blocked limb	control limb
	blocked limb	control limb	blocked limb	control limb	blocked limb	control limb	3	5	7		
Lateral	153.78 \pm 8.61	163.14 \pm 3.68	197.75 \pm 11.45	316.50 \pm 8.87	175.03 \pm 3.29	365.67 \pm 7.38	ns	p<0.001	p<0.001	p<0.01	p<0.001
Middle	119.86 \pm 3.63	134.54 \pm 1.71	172.07 \pm 6.37	239.67 \pm 7.19	146.00 \pm 2.99	307.27 \pm 5.54	p<0.05	p<0.001	p<0.001	p<0.001	p<0.001
Medial	183.07 \pm 9.57	192.06 \pm 4.79	186.08 \pm 7.97	306.48 \pm 9.58	204.36 \pm 5.85	409.04 \pm 6.92	ns	p<0.001	p<0.001	ns	p<0.001

Paired t-test; difference between the mean values of blocked and control limbs in the similar sides at the same period was considered statistically significant for $p<0.05$. one-way ANOVA test with the Bonferroni correction; difference between the mean values of limbs in the similar sites at the 3, 5 and 7 weeks was considered statistically significant for $p<0.05$.

absence of a resting zone in the isolated sites, chaotic location of the chondrocyte columns in the proliferating zone, the presence of single cells, shadow cells, and sites of cell-free matrix (Figure 3A). The hypertrophic zone was intermittent; the density of chondrocytes on the preserved sites was low. The invasion of blood vessels into the zone was noticed, with bone tissue forming around these blood vessels. The primary spongiosa zone was represented by fine-meshed bone trabeculae, which differed from the characteristic structure defined in the control limb. The histomorphometric analysis showed a decrease in the height of the growth plate compared to the control limb in all investigated sides: lateral – by 1.6 times ($p<0.001$); medial – by 1.6 times ($p<0.001$); middle – by 1.4 times ($p<0.001$) (Table 1).

Seven weeks after the bilateral blocking, marked destructive changes were detected throughout distal epiphyseal cartilage (Fig. 3C). There was an intermittency of the resting zone with the formation of isogenic groups of cells and areas with a slightly basophilic matrix free from cells that spread to the subchondral bone. In the proliferating zone, the columnar structure of the chondrocytes was disturbed, and the number of cells in columns was reduced. Areas of the matrix without cells

were found occasionally. The border with the hypertrophic zone was not clear. The hypertrophic zone was absent at some sites, and the proliferating zone transformed into the primary spongiosa zone where bone trabeculae were located chaotically. A distinctive feature was the expansion of intertrabecular spaces, in which cyst-like masses were observed. The height of the growth plate was uniformly significantly lower ($p<0.001$) compared to the control limb: by 2.1 times on the lateral side; by 2.0 on the medial side; and by 2.0 on the middle side (Table 1).

According to one-way ANOVA, it was found that after the bilateral blocking, the height on all sides of the growth plate varies unequally. The height of the middle of the growth plate increased by 1.4 times between weeks 3 and 5 of bilateral blocking, and decreased by 1.2 times between weeks 5 and 7. The height of the lateral side of the growth plate increased by 1.3 times between weeks 3 and 5 of bilateral blocking, but lacked significant changes between weeks 5 and 7. In the blocked limb, the height of the medial side of the growth plate did not differ at any time. In the control limbs, the height of the growth plate increased uniformly in all measured zones during the observation period.

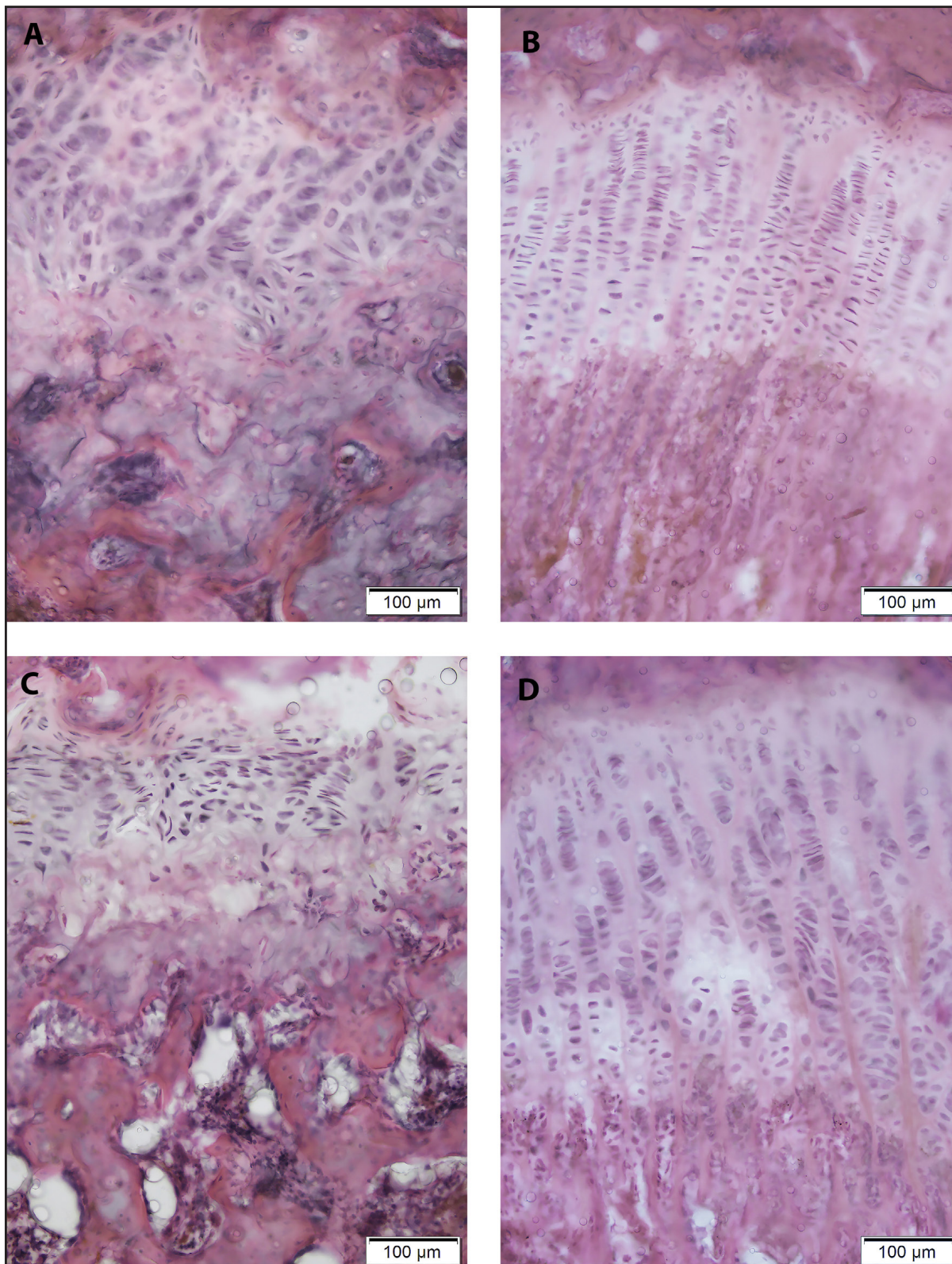


Figure 3. Histological analysis of distal femoral growth plate of the rabbits. Destructive changes in the growth plate after 5 (A) and 7 (C) weeks of bilateral blocking using blocked plates and restoration structure of growth plate in 3 weeks after the discontinuation of 5- (B) or 7-week-long temporary bilateral blocking (D). The appearance of new short chondrocyte columns in proliferative zone (B and D). Hematoxylin and eosin staining.

Three weeks after the 5-week-long temporary bilateral blocking, the epiphyseal cartilage of the operated limb was continuous (Figure 3B). The resting, proliferating and hypertrophic zones were clearly observed. However, the resting zone was intermittent, especially in the middle side, and contained isogenic groups of up to 4 round-shaped chondrocytes. The areas of the cell-free matrix, which were found in all zones of the epiphyseal cartilage, were characterized by mosaic coloration—from slightly basophilic to sharply eosinophilic. In the proliferating zone, the columns of chondrocytes were apparent, some of which were not parallel to each other, and at different angles to the limb axis. From the medial side, there were shorter columns that contained between 8 and 12 cells. They

began from the resting zone and ended, not reaching the hypertrophic zone. The structure of the chondrocytes, located in the columns of the proliferating zone of both extremities, was typical – they had a triangular shape, flattened nuclei and a basophilic coloration. The hypertrophic zone did not differ from the control limb in terms of its structure. In the primary spongiosa zone of both limbs, bone trabeculae were located parallel to the bone axis.

According to the results of the histomorphometric analysis, the height of the epiphyseal cartilage was uneven: significantly higher than the values of the control limb on the lateral (by 1.2 times) and medial (by 1.9 times) sides ($p < 0.001$), without changes in the middle side (Table 2).

Table 2. Growth plate height (μm) measurements in 3 weeks after temporary bilateral blocking.

Side of growth plate	Investigation time (weeks)				paired t-test		unpaired t-test	
	5-week-long temporary bilateral blocking		7-week-long temporary bilateral blocking		weeks		blocked limb	control limb
	blocked limb	control limb	blocked limb	control limb	5	7		
Lateral	362.34 \pm 5.80	295.15 \pm 4.62	359.93 \pm 12.49	321.51 \pm 6.41	$p < 0.001$	$p < 0.01$	ns	$p < 0.01$
Middle	265.29 \pm 5.80	261.79 \pm 6.27	251.34 \pm 6.62	262.75 \pm 6.18	ns	ns	ns	ns
Medial	530.68 \pm 24.14	284.92 \pm 5.41	389.27 \pm 7.53	362.87 \pm 4.56	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$

Paired t-test; difference between the mean values of blocked and control limbs in the similar sides at the same period was considered statistically significant for $p < 0.05$. Unpaired t-test; difference between the mean values of limbs in the similar sites at the 5-week-long and 7-week-long temporary bilateral blocking period was considered statistically significant for $p < 0.05$.

Three weeks after the 7-week-long temporary bilateral blocking, continuous epiphyseal cartilage was observed on the longitudinal histological sections of the operated limb (Figure 3D). In contrast with the control limb and the limb that underwent 5-week-long temporary bilateral blocking, the resting zone was almost impossible to identify. In the proliferating zone from the medial and lateral sides, the columnar

structure of chondrocytes was disturbed: isolated cells, shadow cells, and columns of differing heights were observed. Cell-free areas with uneven coloration of the matrix were found throughout the whole length of the epiphyseal cartilage of the experimental limb. However, they were much less common in the control limb. The hypertrophic zone was rather similar to the control limb by structure. Invasion of blood vessels from the primary



spongiosa was observed. In the primary spongiosa zone, most of the bone trabeculae that were observed were parallel to the axis of the bone. However, they were much shorter than the ones in the control limb, and some had a looped structure. According to the results of the histomorphometric analysis, it was determined that on the middle side, the height of the growth plate did not differ from the value in the control limb, but on the lateral and medial sides it had increased by 1.2 and 1.07 times respectively ($p < 0.01$) (Table 2).

According to one-way ANOVA, three weeks after the plate removal following the 7-week-long temporary bilateral blocking, the height of the growth plate on the medial side was 1.4

times less than the value after 5-week-long temporary bilateral blocking. In the middle and lateral sides of the growth plate, no differences were found between groups with different blocking periods. In the control limbs, the height of the growth plate increased during the observation period in the lateral and medial sides, without changes in the middle side.

X-ray

There were no differences in the mL DFA in the operated limb after 1, 2, and 3 weeks after the plates' removal when compared with the control for all periods of measurement (Fig. 4). Earlier, we obtained similar data when measuring the mL DFA while implementing bilateral blocking for 3, 5, and 7 weeks.¹⁶

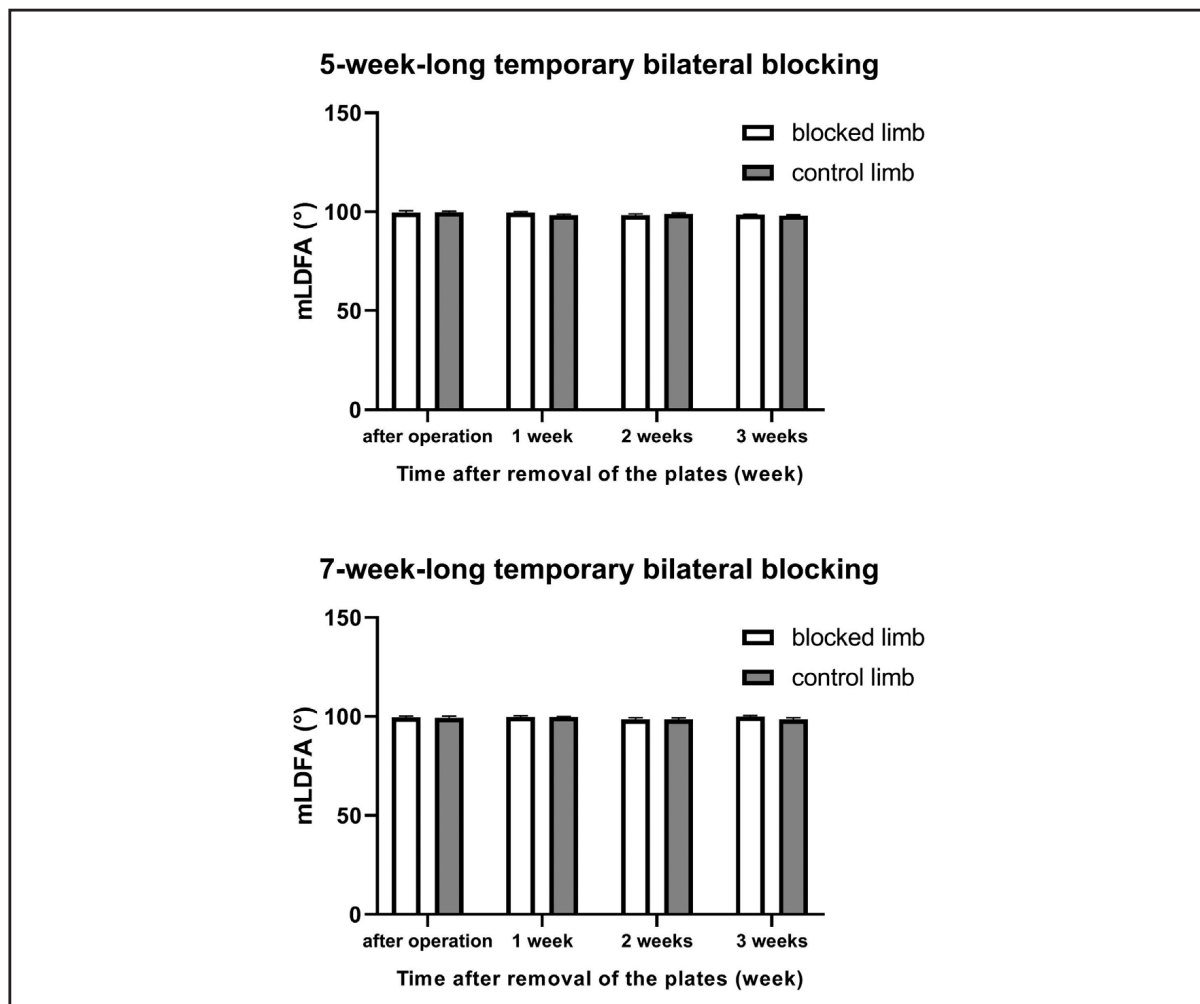


Figure 4. Analysis of the mechanical lateral distal femoral angle (mL DFA) 1-3 weeks after the discontinuation of the 5- or 7-week-long temporary bilateral blocking. There were no statistically significant difference between the blocked and the control limbs for all terms (paired t-test).

Discussion

The method of temporary unilateral blocking of the growth plate of long bones with the purpose of correction of the femoral or tibial deformities in children using plates with screws was adopted relatively recently – since 2007.¹⁷ Therefore, the number of clinical trials, especially for the analysis of follow-up results, is limited. Identified complications, in particular the premature closure of the growth plate, prompted us to conduct an experimental study to determine the possibility of restoring the functionality of the growth plate after removal of the plates.

We found that 3, 5 and 7 weeks after the temporary bilateral blocking, the height of the epiphyseal cartilage and the primary spongiosa zone gradually decreased compared to the control limb, indicating a delay in the longitudinal growth of the bone. We noted that growth was suspended in the medial side of the growth plate after 3 weeks, in the lateral – after 5 weeks, and after 7 weeks in the middle side. Destructive changes (histoarchitectonics disorders, changes in cell density, etc.) had progressed with the increase of the blocking duration.

Three weeks after the 5-week-long temporary bilateral blocking, complete restoration of the morphological structure of the epiphyseal cartilage, including the primary spongiosa zone, took place. An increase in the height of the growth plate on both sides in the operated limb (by 1.2 times on the lateral side, by 1.9 times on the medial side) was established, whereas after 5 weeks of bilateral blocking, the height of the epiphyseal cartilage decreased over its entire length, compared with the control limb (Table 1-2). In a similar study in rabbits, which used unilateral growth plate blocking of the distal femoral bone with nonabsorbable filament and screws for 4 weeks, restoration of the length of the blocked bone after incision of a non-absorbable filament was established after 4 weeks.¹⁸ In another study, during an

experiment on rabbits, a growth rebound was detected in the proximal tibia region 2 weeks after the removal of the plate, which was used to block the growth plate for 3 weeks, on the side where the plate was located.¹⁰ In our study, the growth plate was blocked over longer periods (5 and 7 weeks) compared to Martínez GS. et al.¹⁸ and Corominas-Frances L. et al.¹⁰ However, we also found a significant increase in the growth plate on both sides after removing the plates in both groups; whereas the maximum increase in growth was identified on the medial side in the group with a shorter blocking period (5 weeks).

In our experiment, when we removed the plates and discontinued the bilateral blocking of the distal growth plate of the rabbit femoral bone after 7 weeks, the structure of the epiphyseal cartilage was partially restored in 3 weeks, but did not become completely identical to the control limb, which we associate with an increase in the blocking time. During this process, the growth of the bone in length due to the functioning of the growth plate occurred, as evidenced by the characteristic structure of the hypertrophic and primary spongiosa zones. The height of the epiphyseal cartilage in the operated limb was greater in comparison with the control one: after removing the blocked plates, it was 1.2 times larger on the lateral side, and 1.07 times larger on the medial side ($p < 0.01$) (Table 2).

Growth of the limb in length occurs due to the activity of different zones of the epiphyseal cartilage, mainly due to the degree of hypertrophy of the chondrocytes (40-50%), and only 10% depends on the proliferation of the chondrocytes.¹⁹ One of the reasons for this is that the hypertrophied chondrocytes become precursors of about 50% of the osteoblasts, which are subsequently involved in the endochondral bone formation.^{20,21} In our study, starting from week 5 of blocking, the hypertrophic zone almost disappeared; presumably this is explained by the discovered decrease in the height of the epiphyseal



cartilage in weeks 5 and 7 of blocking compared to the control limb.

In addition, the identified disorders in the primary spongiosa zone can also be related precisely to the disorders in the process of hypertrophy of chondrocytes. In experimental studies of the effect of compression on the epiphyseal cartilage of the proximal part of the tibia of rabbits (aged 13 weeks), reduction in expression of collagen types II and X was identified in week 6 of the experiment, which may indicate a decrease in the number of proliferative and hypertrophied chondrocytes, respectively, due to compression.²² In addition, the proliferation of the chondrocytes in the resting zone is affected by hypertrophied chondrocytes through the secretion of Indian hedgehog. That is, in the absence of hypertrophied chondrocytes, the proliferation of the chondrocytes in the resting zone is also affected.²³ At the same time, 3 weeks after the discontinuation of the 5- or 7-week-long temporary bilateral blocking, the restoration of the structure of the proliferating zone and the appearance of new short chondrocyte columns have been established.

In our study, the age of the animals at the beginning of the experiment was 8 weeks. It is known that the maximum rate of growth of the femur of rabbits occurs in the first 4 weeks of life and at weeks 8-10 it slows down and then reaches a plateau. Also, the growth rates of the right and left femoral bones are not significantly different.²⁴ In the distal part of the femur of the rabbit, the growth plate is completely closed at the age of 19-24 weeks.²⁵ That is, in our study, 3 weeks after the 7-week-long temporary bilateral blocking, the age of the animals was 18 weeks, and the

bone growth was almost complete. However, an increase in the growth plate height was established 3 weeks after the 7-week-long temporary bilateral blocking compared with the control limb. This may indicate that when the method of temporary bilateral blocking is used, restoration of the growth plate function may be possible even in the case of prolonged growth inhibition.

According to our previous X-ray study, the mLDFA after the bilateral blocking was implemented for 3, 5 and 7 weeks did not differ from the mLDFA in the control bone¹⁶. Histologically, we had established that the suppression of growth occurred earlier on the medial side than on the lateral and middle sides. After the removal of the plates, the greatest increase of the height of the growth plate occurred on the medial side. This phenomenon possibly compensates for the growth suppression caused by temporary bilateral blocking. However, it did not lead to bone deformation, which we confirmed by measuring the mLDFA, which did not differ from the mLDFA in the control bone.

Temporary bilateral blocking leads to the development of structural abnormalities in the growth plate, which cause inhibition of its function. Restoration of the structural-functional features of the growth plate after the removal of the plates depends on the blocking period, which must be taken into account in the clinical setting.

Conflicto de intereses: los autores declaran no tener conflicto de intereses.

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