Retrospective study of the operative treatment of the pediatric distal forearm fractures – and examination of callus formation in mice fracture model

Ph.D. thesis

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INTRODUCTION

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide with widespread occurrence in various organs and diverse effects both in the nervous system and in the periphery. PACAP is strongly expressed in the central nervous system, where it exerts several effects such as it is a central regulator of circadian rhythmic activities, plays a role in memory formation and psychiatric processes, and is involved in central feeding control. It elicits these actions by binding the G-protein-coupled receptors, PAC1- and VPAC1/2, which also bind vasoactive intestinal polypeptide (VIP). PACAP is also known to be expressed in the retina, along with its receptors (PAC1, VPAC1 and VPAC2 receptors). Numerous studies have provided evidence that the neuroprotective effects are mainly mediated by the PAC1 receptor and diverging downstream pathways upon its activation.

Pituitary adenylate cyclase activating polypeptide is an evolutionary well conserved neuropeptide identified in hypothalamo-hypophyseal system. Two biological active forms, PACAP 1-38 and PACAP 1-27 exist with a short half-life *in vivo*. Since its isolation several peripheral organs have been proven to secrete and release the neuropeptide. PACAP plays a regulatory role in the development of gonads, chondrogenesis and teeth. PACAP has three main G protein coupled receptors; PAC1, VPAC1 and VPAC2 which induce the activation of adenylate cyclase, increase the intracellular cAMP concentration and can trigger the increase of PKA phosphorylation activity. Downstream target of this kinase classically can be the CREB transcription factor or in osteoblasts a possible target is the Runx2. However, it has been published that PACAP has various signalling crosstalks, for example, it has direct connection with the BMP, WNT, hedgehog or β-catenin signalling.

Although PACAP has been proven to be preventive against different harmful effects, little is known about its function in bone regeneration processes.

Bone development and regeneration have been widely discussed in the last two decades, although there are some molecular pathways which functions are not completely understood. Morphology and stability of long bones determine the size, strength and motility of animals. Subsequently, the proper inorganic and organic matrix production is responsible for the appropriate biomechanical function of long bones. Moreover, the matrix production and its composition are crucial for adequate limb development or for normal callus formation. Development of long bones or the osteoprogenitor-osteoblastic transformation is regulated by various signalling pathways such as BMP, HH and WNT signalization. The initiate steps of

osteogenesis are followed by the induction of proper matrix production which major organic component is Collagen type I, forming a specific architecture in cortical bone tissue. Orientation and thickness of collagen lamellas together with inorganic components grant the biomechanical stability of cortical bone and regulate the tensile strength of compact bone. Calcification processes of osteogenic tissues are also regulated by the osteoblasts which form calcium hydroxyapatite deposits between the collagen lamellas, and effect on the refractive resistance of this hard tissue.

For precise regulation of ECM production, osteoblast activation needs a specific timing and milieu which can be triggered by cytokines, growth hormones or other endocrine/paracrine pathways. BMP is one of the basic activator of bone development which can bind to BMPR and trigger the phosphorylation of Smad1/5 followed by its nuclear translocation. Termination of this pathway induces the production of bone specific molecules such as ALP (alkaline phosphatase), Osterix or even Collagen type I. Furthermore, it has been demonstrated that BMPs activates CREB transcription factor via the activation of PKA which also triggers the expression of molecules mentioned above. On the other hand, bone formation is regulated by Runx2 transcription factor which also can be activated by PKA and it regulates the expression of bone specific molecules and ECM components.

In the last decade more and more experiments proved the importance of PACAP in limb development. Although PACAP is a small neuropeptid, produced in the hypothalamo-hypophyseal pathway of central nervous system, peripheral release and function have also been proven. It has a pleiotrop function as PACAP regulates the development of different organs such as cartilage central nervous system or regulates the proper spermatoganaesis but it also has a protective function. Moreover, the presence of the neuropeptide has been shown in several peripheral tissues, such as in gonads, intestine, kidneys, or in human colostrum. It has been demonstrated that PACAP prevents the harmful effect of oxidative stress it triggers the activation of anti-inflammatory processes or it has a positive role during ischemic conditions. PACAP is also known to activate the immune system.

Dislocated fractures of the distal forearm are the most common injuries in childhood Traditionally, these fractures have been treated by closed reduction and casting, but, due to the intrinsic instability of these fractures in the cast, many authors prefer surgical treatment to prevent redislocation. Unstable distal forearm fractures are usually treated by closed reduction and minimal invasive fixation. Operative osteosynthesis technique of pediatric wrist fractures are optimally minimally invasive, spare the physis and maintain an acceptable and painless

reduction. Unstable, angulated fractures of the distal radius can be treated by several methods: with insertion of Kirschner wires (K-wires), with dorsally inserted titanium elastic stable intramedullary nails or with short titanium elastic stable intramedullary nailing. Different types of operative fixation have been advocated, including plates and external fixator. The surgical gold standard for the treatment of distal metaphyseal or dia-metaphyseal radial fracture in childhood is closed reduction and K-wire fixation. Although there are many variations of the percutaneous pinning Muller and Kapandji techniques are most commonly used worldwide. K-wire fixation also carries many potential complications and disadvantages. Kirschner-wire related complications are well known in the literature: migration of the pins, superficial infections, damage of the physeal plates, skin irritation and insufficient biomechanical stability to maintain the reduction without casting.

Internal fixation with elastic stable intramedullary nailing (ESIN) has increased in popularity. Not only can it improve the quality of a closed reduction but it also increases stability of the fixation, thereby avoiding the necessity of cast immobilization and a lower risk of malunion. Disadvantages of ESIN relate to it is invasive nature and include the potential for injury to structures at the nail entry points, infection (superficial and deep), and the need for subsequent surgery for implant removal. The nail is inserted from the distal dorsal metaphysis.

Short elastic nailing is a modified elastic nailing method for the operative treatment of severely displaced paediatric distal metaphyseal or dia-metaphyseal radial fractures. With two short pre-bent, elastic titanium nails inserted in a retrograde fashion proximal to the distal radial physis. It provides very stable stabilization without the need for casting. The nails do not touch the physeal plates so the possibility of postoperative physeal arrest is reduced.

AIMS OF THE STUDY

1. Investigation of the femur in WT and PACAP KO mice.
2. To study the effects of PACAP in bone regeneration in a newly developed surgical fracture method. To compare the bone healing of the wild type and PACAP deficient mice. Tibias were scanned by micro-CT to confirm fracture and to study the bone healing (callus formation) process. Bone structure-related parameters were the bone volume, bone surface and bone mineral density were measured. Specimen from the callus were investigated by Western blot, RT-PCR.
3. To find the indication place of the three different operative technique. The fracture distance can determinate the operative method.
4. To evaluate the degree of axial deviation after the metal removal.
5. Different surgical techniques were analysed different aspects.
6. Distance of the fracture line from the radiocarpal surface, the diameter of the distal epiphysis of the radius, and the cumulative diameter of the distal epiphysis of the ulna and radius were measured on the primary x-ray.

MATERIALS AND METHODS

Tibia fracture model in mice

Animal

Twenty-four mice were 3-month-old PACAP HZ breeding, and backcrossed with CD1 mice. Wild type (WT) (n=12) and PACAP knockout (KO) (n=12) mice were three generation littermates and half of them were females and males. Mice were fed and watered *ad libitum* with standard feed pellets and tap water, kept under 12/12 h of light/dark cycles at 20-22°C and 40-60% humidity. All experiments were approved by the animal ethics committee of the University of Pecs (BA02/2000-24/2011). Genotyping was performed from tail samples with Phire Animal Tissue Direct PCR Kit (Thermo Fischer Scientific, Waltham, MA, USA)

Whole limb Alizarin staining

Hind limbs of mice were removed and only the skin was eliminated. The entire limb was washed in PBS three times and fixed in a 4:1 mixture of absolute ethanol and 40% formaldehyde. After washing in alcohol limbs were incubated in 30 mM KOH solution for 3 days, 60 mM KOH for 2 days and 80 mM KOH solution for 1 day till the tissues become completely glossy. Remnant of KOH was removed by washing in PBS three times and samples were stained with Alizarin Red.

Three point bending test

Three point bending tests were performed on mouse femur bones using a Chatillon TCD225 tensile test machine. The center part of the bones was loaded by a 5 mm diameter cilindrical head. Velocity of the head was 2 mm/min during the experiments. Value of the loading force at crack (F_m) as well as the value of the bending deformation at crack (Δl_m) were evaluated from the deformation-force diagrams.

Fracture model

Mice were anesthetized with Euthasol 1% solution 7 µl/10 g by intra-peritoneal injection. Open fracture technique was performed as follows: a 1-cm-incision was made on the anterior aspect of the both proximal parts of the crural region under aseptic surgical technique. Muscles and periosteum were sharply incised. Fracture in the proximal third of tibia was made. The surface of the scalpel was labelled 0.5 mm distance from the cutting edge to ensure the standard depth

of the incision. Fracture location was 5 mm distal from the proximal articular surface of the tibia and the depth was 0.5 mm.

Micro-Computed Tomography (Micro-CT)

Femur CT studies obtained by using this micro-CT (Skyscan 1176 mikro CT). Radiograps were used to analyze the cortical thickness of the femur in ventral and dorsal aspect and femur densitometry of PACAP knockout (KO) and wild type (WT) mice was also examined. Density was measured in the region of greater trochanter. Tibias were scanned by micro-CT to confirm fracture and to study the bone healing process.

Light microscopical analysis

Both tibias were harvested after sacrificing the mice with an overdose of pentobarbital sodium 3, 7 and 21 days after surgery (100 mg/kg bw). Tibias were dissected and additional tissues were removed and washed in PBS three times and fixed in a 4:1 mixture of absolute ethanol and 40% formaldehyde. Bones were decalcified in 4% EDTA for four weeks till bones became soft. Then samples were dehydrated in ascending alcohol row and embedded in paraffin. Five μm of serial sections were made. After rehydration hematoxilin eosin staining was performed.

RT-PCR, Western blot

After removing additional tissues from the tibia surface, callus was precisely dissected approximately 2 mm^3 sample was removed. Tissues were cryo-ground in liquid nitrogen and dissolved in Trizol. PCR products were analysed by electrophoresis in 1.2% agarose gel containing ethidium bromide. Actin was used as internal control. Optical density of Western blot signals was measured by using ImageJ 1.40g freeware and results were normalised to that of control samples.

Statistical analysis

All data are representative of at least four independent experiments; data are mean values. Statistical significances between controls (unoperated sides) and operated sides were determined by one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test.

Human study

Between January, 2009 and December, 2018 a retrospective clinical study was performed at the Surgical Division of the Department of Paediatrics, Medical School, University of Pécs, Hungary. Gender of the patients, different types of surgical techniques, number of postoperative X-rays, date of metal removal and degree of axial deviation after the metal removal were evaluated. The radiological results were assessed for all patients at final follow up. Distance of the fracture line from the radiocarpal surface, the diameter of the distal epiphysis of the radius, and the cumulative diameter of the distal epiphysis of the ulna and radius were measured on the primary x-ray. Based on these parameters, results of the different surgical techniques were analysed by Pearson correlation test.

The dia-metaphyseal transition zone was defined as the large square based on the cumulative diameter of the distal epiphysis of the radius and ulna, minus the small square based on the diameter of the distal epiphysis of the radius alone. We focused on the distance of the fracture from the articular surface, which may determine the surgical procedure applied.

RESULTS

Tibia fracture model in mice

Anatomical morphology of bones of hind limb of PACAP KO mice is not altered significantly. No differences were detected in the length of femur or tibia, as well as no other anatomical disorders were visible. Nonetheless, the staining intensity was similar in both experimental group, thinner parts were visible generally in the diaphysis of the femur of gene deficient mice. Although the outer size of bones of gene deficient mice looked thinner in cross sections of middle portion of femur was thicker than Wild Type (WT) with HE staining. Based on the CT scan the diameter of bone marrow cavities was wider in WT animals. Length of the femur had no alterations in PACAP KO mouse, as well as the posterior cortical area of the bone or the sagittal diameter of femur had no significant differences. The anterior cortical bone was significantly thicker in the gene deficient animals than in the WT. The transverse diameter of the diaphysis of the femur was significantly shorter in PACAP KO mice. The further analyzes of the CT results did not show any other significant alterations but the density of the femur at the level of greater trochanter was tendentiously lower in PACAP gene KO animals, which suggest other matrix differences of this animal.

The force which was needed to result a complete fracturing in PACAP KO femur was significant lower. Reflection of the femurs had no significant differences in either genotype, although the PACAP deficient mice had tendentiously higher inclination.

ALP expression in callus formation

ALP expression was measured on 3 different days of bone regeneration. On day 3 of callus formation mRNA expression of ALP was barely detected in control WT samples but showed an elevated ALP mRNA expression in WT callus. On the other hand, we demonstrated a higher ALP mRNA in PACAP KO tibia compared with PACAP KO callus samples. Similar expression pattern was detected on day 7 of callus formation. Reduced ALP expression was detected in callus of WT animals at the end of the healing process. In PACAP KO samples decreased mRNA expressions were shown on different days of callus formation. Interestingly, protein expression of ALP was not in correlation with its mRNA expression. In WT tibias almost undetectable ALP protein level could be seen, while in PACAP KO tibia higher expression of ALP was detected. Callus formation increased the ALP protein expression on

days 3 and 7 and started to reduce at 21 day of healing in WT mice. On the contrary, no elevation in protein expression was detected in PACAP KO callus on days 3, 7 and 21 of healing process.

Organic matrix production in bone fracture healing processes

Proper collagen type I production is also necessary for callus formation, therefore, we analyzed its expression. mRNA expression of collagen type I was low in non-fractured tibia and in callus of WT mice, with decreased expression on days 7 and 21. PACAP KO mice have higher collagen type I mRNA expression compared to WT. Protein expression of collagen type I was not in correlation with its mRNA expression. Low expression was detected in WT animals. On day 3 elevated expression was detected, peaking on day 7 and decreased to day 21 of callus formation. Expression of collagen type I was elevated in PACAP KO mice compared with WT controls, showing a further elevation on day 3. A lower increase was shown in PACAP KO mice on day 7, compared with the callus of WT animals. To day 21 of callus formation it was significantly reduced but remained higher than in callus samples of WT rodents.

BMP2,4,6 expression in callus formation

As BMPs are among the most important factors in callus formation and BMP signalling has direct connection with PACAP signalization we investigated different elements of BMP signalling. mRNA expression of BMP2 was elevated on day 3 of callus formation in WT animals but started to decrease from day 7 and reduced to day 21 of healing. In PACAP KO mice no alteration was detected in samples on days 3 and 7 but significant decrease was shown on day 21. BMP4 mRNA expression increased on all investigated days in WT animals. Weak BMP6 signals appeared in WT samples with and increased intensity in callus samples on days 3 and 7. On day 21, BMP6 expression decreased to the level of WT bone. On the other hand significant decrease was detected on days 7 and 21 in PACAP KO mice compared with the unoperated side. Smad1 mRNA expression increased in WT callus for days 3 and 7 and then decreased for the end of callus formation. In PACAP KO callus some elevation was detected only on day 3 but reduced signal was detected on days 7 and 21.

BMP2 protein expression was increased on days 3 and 21 in WT callus samples with no changes on day 7 of healing. On the contrary, a significant decrease was detected on day 3 in PACAP KO callus compared with the untreated bone, while no alterations were detected on days 7 and 21. BMP4 protein expression was elevated in WT and PACAP KO callus on day 3. On days 7 and 21 increased BMP4 protein expression was detected in callus samples of WT animals, but a significant elevation was detected in PACAP KO mice only on day 3, with no alterations

on days 7 and 21. BMP6 protein expression showed a significant elevation on the fractured side on the 3 investigated days in WT mice. On the contrary, an elevation was detected on day 3 followed by a significant reduction to end of regeneration in PACAP KO mice. Downstream target of BMP signalling is Smad1, which did not alter in WT callus samples, but showed a strong increase in PACAP KO rodents at the end of bone regeneration.

Human study

Out of the 196 children, stabilization of the distal radial fracture were treated by K-wire in 139/196, dorsal inserted ESIN in 44/196 and short ESIN in 13/196 patients. Cast immobilizations were used in all patients treated by K-wire. The first week, a short removable splint was applied in dorsal inserted ESIN treated group. Cast immobilization was not applied for patient treated by short ESIN. Control X-ray image was required on average in 4.3, 2.6 and in 3.4 times per cases after K-wire, dorsal inserted ESIN and short ESIN treatment. The average time of metal removal was significantly shortest (3.8 months), following stabilization with K-wires. The time of the implant removal was equal in patients of treated with different type of elastic nailing, (average 6 months). In children treated with K-wire, axial deviation of less than 5° was seen in 118 patients, 5-10° deviation in 15 patients, while deviation was above 10° in 6 children. One patient required further reconstruction, due to residual deformity. In the dorsal inserted ESIN group, less than 5° axial deviation was found in 37 patients and 5-10° in 7 patients. In all 13 children treated with short ESIN, axial deviation was measured to be less than 5. The fracture distance from the radiocarpal surface was on average 23.7 mm in the children treated with K-wire, 45.6 mm treated with dorsal inserted ESIN and 32.7 mm treated with short ESIN respectively.

Based in our results the fracture distance determinate the operative technique. In our department K-wire fixation was indicated, when the fracture distance was shorter than the width of the distal radius. Dorsal inserted ESIN procedure was chosen when the fracture distance was higher than the cumulative diameter of the radius and ulna. Transition zone fractures were treated differently depending on the surgeon, but the SESIN technique was used in the fractures of transition zone. There were significant differences between the axial deviations at the time of implant removal. The highest incidence of axial deviation was measured in patients treated by K-wiring. The differences in the time of implant removal were also significant, the shortest time was measured in K-wire treated group. No further differences were found in our series.

DISCUSSION, CONCLUSIONS

Tibia fracture model in mice

Although PACAP KO mice do not show apparent macroscopical alterations, several disorders have been detected in the absence of PACAP in different tissues during development, such as in teeth, testes and ovaries. Lack of PACAP also influences neuronal behaviour of these animals, and results alterations in neuronal development. Not only has the absence of the neuropeptide but its addition also affected the differentiation of chondrocytes, neurons and mammary epithelial cells. We have demonstrated earlier that PACAP increases Ca^{2+} release in osteoblasts and induces their differentiation. Further investigation of PACAP KO mice femur showed several signalling alterations and morphological changes. Femur cortical thickness and diameter of its bone marrow cavity were altered in these animals. Moreover, collagen type I secretion and ALP expression and activation elevated in PACAP KO femurs. PACAP importance in various stresses has been detected in the last decade, such as it compensates the harmful effects of oxidative stress and mechanical load in chondrogenesis. It also has a protective function in retinopathy and ischemic conditions. All of these facts indicate that PACAP can have important functions in fractured bone remodelling and healing.

Investigation of callus formation in mouse models is widely used to follow bone healing with different fracture induction. Closed tibia fracture model is well described and involves the use of stainless steel fixation pins. Disadvantages of this model are the lack of both axial and rotational stability with using a pin, the high risk of knee dislocation, and the potential for intramedullary cavity damage. For standardization of callus position we developed a well reproducible surgical fracture method. We used 24 animals from WT and PACAP KO groups and analyzed callus formation with CT and histological staining which showed a precisely positioned callus formation. This way callus samples can be removed from the bone and standardized comparability with molecular biological methods is guaranteed.

Activation of different factors is important during bone regeneration to induce proper organic and inorganic matrix production. One of the best indicators of callus formation is the increased ALP expression and activation. PACAP affects ALP function and its lack results in an increased ALP expression and activation in femurs of PACAP KO mice. Interestingly, significant mRNA reduction was detected in PACAP KO callus samples. Similarly to our previous results, we hypothesized that PACAP influenced post-transcriptional processes. In normal callus formation of WT animals ALP protein level continuously decreased to the end of bone regeneration, similarly to normal callus formation of dogs. On the contrary, the higher ALP level in PACAP

KO animals did not reduce to the end of bone healing, indicating a disturbed callus formation in PACAP KO mice. Togari et al. have proven that different neuropeptides and their receptors have an effect on ALP function and bone metabolism. In this study we provided additional data that PACAP has a fine tuning function in proper bone regeneration, which can be a direct effect through PKA-Runx2-ALP axis or indirect via ß-catenin-WNT pathways.

Collagen type I formation, part of the organic matrix of PACAP KO bone was found to be disturbed, and showed significant elevation resulting thickened collagen lamellas in the osteons. In normal callus formation expression of collagen type I showed a peak and its expression normalised to the end of bone formation. Similar results were detected in our model which represents normal healing process in WT mice. Collagen expression in PACAP KO mice was significantly higher during callus formation. Alteration of WNT pathways is proven to alter proper collagen secretion and results in osteoporosis. As mentioned above WNT-ß-catenin pathway has a crosstalk with PACAP signalling, which further strengthens the hypothesis that PACAP regulation has a balancing function in proper collagen secretion. Furthermore, lack of PACAP shifts the ECM production towards the organic matrix expression.

As BMPs are important indicators of callus formation, we focused on the expression of BMP2, 4 and 6, which have important functions in bone remodelling processes. Interestingly, significant reduction was detected in their mRNA expression suggesting the same theory that PACAP has post-transcriptional effects. BMP2 protein expression was reduced in PACAP KO callus, but significant elevations were detected in its expression in WT rodents. BMP4 protein expression was higher in WT groups in bone healing but an early decrease was detected in PACAP KO mice callus. BMP6 is one of the BMPs which has an important function in mature bone homeostasis and in healing. In WT callus expression of BMP6 continuously increased but an early reduction was detected in PACAP KO callus, followed by strong decrease at the end of bone regeneration. On the contrary, Smad1 expression elevated to the termination of bone healing. These results also suggest an active but PACAP-BMP independent Smad1 activation during bone regeneration. Lack of PACAP disturbs the normal BMP release and enhances the early regeneration via BMP4 and 6 expression. Accumulation of these cytokines is not stimulated in the lack of PACAP and results in a delayed and PACAP independent triggering of Smad activation. These data support the indirect fine tuning function of PACAP on BMP signalling, controlling proper callus formation and regeneration.

In our study we developed a precisely reproducible fracture method that enables standardized molecular analysis. We provided evidence that PACAP has an important function in proper

bone regeneration. The lack of the neuropeptide disturbs normal organic and inorganic matrix production and alters normal BMP activation in callus formation.

Human study

The current study was designed to evaluate the differences in clinical and radiographic outcomes between three different (K-wire, dorsal inserted ESIN, short ESIN) methods. Percutaneous pin fixation is most commonly used for unstable distal radial, or distal forearm fractures in paediatric patients. This method has several advantages: it is minimally invasive, has short operative time, implant removal is not technically demanding and it does not require any special instrumentation. K-wire fixation has some complication as well: migration of the wires, damage to the growth plate, skin irritation and infection. This technique is usually necessary to wear short, or long cast after the reduction and fixation.

Dorsal insertion nailing is also an accepted possibility fixation for the distal dia-metaphyseal radial fractures. Generally it is difficult to bend the nail before the final position, which is the key point of this method. If the fracture distance too close to the physis the risk of the dorsal cortical damage is relatively high. It is very important to verify the optimal level of the fracture when the dorsal inserted ESIN technique is indicated. This type of minimally invasive technique usually does not require cast immobilization.

SESIN technique is a novel method for fixation of the unstable distal dia-metaphyseal radial fracture in children. SESIN procedure has a risk of complications such as: injury of the superficial branch of the radial nerve, iatrogenic injury of extensor pollicis longus tendon, and injury of the physis if the insertion points are incorrect. The authors suggested this technique for the dia-metaphyseal fracture because of the fixation in this zone is difficult, usually too distal to treat by dorsal inserted ESIN, and too proximal to fix with a K-wire. The authors concluded that short ESIN is an effective, safe and stable form of stabilization for unstable fractures of the distal third of the radius. A number of different treatment options have been published previously in the literature. Some of them suggest the transepiphyseal fixation methods. The main complication of these procedures is iatrogenic physeal injury and subsequent secondary physeal arrest and deformity. Almost all of the previous studies have concluded technical problems and high rates of different complications. Two articles published excellent results after the treating this special fracture type. One of this technique does not respect the physis. The other type of the fixation the short ESIN technique respects the physis.

In this study we focused on the distance of the fracture from the articular surface, which may determine the surgical procedure applied. We haven’t found any previous studies which focused

on the measurement of the fracture distance from the articular surface. Based on our results we found that the fracture distance determines the operative method. If the fracture distance is shorter than the width of the distal epiphysis of the radius the recommended treatment is K-wire fixation based on our retrospective study. If the fracture distance is higher than the cumulative width of the distal epiphysis of the ulna and radius the optimal treatment is dorsal inserted ESIN according to our results. In the study period short ESIN technique was used just in the distal dia-metaphyseal radial fractures. If the level of the fracture distance was measured between the distal radius epiphysis width and cumulative width of the radius and ulna, stabilization was performed with the presented three different methods. K-wire fixation is a gold standard method for the distal metaphyseal radial fracture, some authors suggest treating the transition zone fractures this way as well. Based in our results the short ESIN is a safe and physis-sparing procedure for stabilizing the unstable dia-metaphyseal radius fracture. We have not observed any complication for the method in our series. short ESIN wasn't used in any other level fractures, just in the transition zone.

PUBLICATIONS

1. Publications related to the thesis

Józsa G, Szegeczki V, Pálfi A, Kiss T, Helyes Z, Fülöp B, Cserháti C, Daróczi L, Tamás A, Zákány R, Reglődi D, Juhász T (2018) Signalling alterations in bones of pituitary adenylate cyclase activating polypeptide (PACAP) gene deficient mice. Int J Mol Sci. 27;19(9). pii: E2538. doi: 10.3390/ijms19092538 (IF: 3.687)

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Cumulative impact factor: **8.705**

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