I. A NOVEL METHOD FOR THE IN VIVO VISUALIZATION AND INVESTIGATION OF THE MANDIBULAR PERIOSTEUM

The periosteum plays an important role in bone physiology, but observation of its microcirculation is greatly limited by methodological constraints at certain anatomical locations. This study was conducted to develop a microsurgical procedure which provides access to the mandibular periosteum in rats. Comparisons of the microcirculatory characteristics with those of the tibial periosteum were performed to confirm the functional integrity of the microvasculature. The mandibular periosteum was reached between the facial muscles and the anterior surface of the superficial masseter muscle at the external surface of the mandibular corpus; the tibial periosteum was prepared by dissecting the covering muscles at the anteromedial surface. Intravital fluorescence microscopy was used to assess the leukocyte-endothelial interactions and the RBCV in the tibial and mandibular periosteum. Both structures were also visualized through OPS and fluorescence CLSM. The microcirculatory variables in the mandibular periosteum proved similar to those in the tibia, indicating that no microcirculatory failure resulted from the exposure technique. This novel surgical approach provides simple access to the mandibular periosteum of the rat, offering an excellent opportunity for investigations of microcirculatory manifestations of dentoalveolar and maxillofacial diseases.

Publication:
Varga R, Janovszky Á, Szabó A, Garab D, Bodnár D, Boros M, Neunzehn J, Wiesmann HP, Piffkó J. A novel method for in vivo visualization of the microcirculation of the mandibular periosteum in rats. Microcirculation. 2014;21(6):524-31.

II. MICROCIRCULATORY ASPECTS OF BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW

Nitrogen-containing bisphosphonates induce osteonecrosis mostly in the jaw and less frequently in other bones. Because of the crucial role of periosteal perfusion in bone repair, we investigated zoledronate-induced microcirculatory reactions in the mandibular periosteum in comparison with those in the tibia in a clinically relevant model of bisphosphonate-induced medication-related osteonecrosis of the jaw (MRONJ). Sprague-Dawley rats were treated with zoledronate (ZOL; 80 i.v. μg/kg/week over 8 weeks) or saline vehicle. The first two right mandibular molar teeth were extracted after 3 weeks. Various systemic and local (periosteal) microcirculatory inflammatory parameters were examined by intravital videomicroscopy after 9 weeks. Gingival healing disorders (~100%) and MRONJ developed in 70% of ZOL-treated cases but not after saline (shown by micro-CT). ZOL induced significantly higher degrees of periosteal leukocyte rolling and adhesion in the mandibular postcapillary venules (at both extraction and intact sites) than at the tibia. Leukocyte NADPH-oxidase activity was reduced; leukocyte CD11b and plasma TNF-alpha levels were unchanged. Chronic ZOL treatment causes a distinct microcirculatory inflammatory reaction in the mandibular periosteum but not in the tibia. The local reaction in the absence of augmented systemic leukocyte inflammatory activity suggests that topically different, endothelium-specific changes may play a critical role in the pathogenesis of MRONJ. This model permits for the first time to explore the microvascular processes in the mandibular periosteum after chronic ZOL treatment. This approach may contribute to a better understanding of the pathomechanism and the development of strategies to counteract bisphosphonate-induced side effects.

Publication:
Janovszky A, Szabó A, Varga R, Garab D, Boros M, Mester C, Beretka N, Zombori T, Wiesmann HP, Bernhardt R, Ocsovszki I, Balázs P, Piffkó J. Periosteal microcirculatory reactions in a zoledronate-induced osteonecrosis model of the jaw in rats. Clin Oral Investig. 2014. (in press) IF (2013): 2,285

Janovszky A, Vereb T, Szabó A, Piffkó J. [Current approaches for early detection and treatment of medication-related osteonecrosis of jaw]. Orv Hetil. 2014;155(49):1960-6. (magyar nyelven)

Janovszky A. Microcirculatory aspects of bisphosphonate-related osteonecrosis of the jaw. 2014. (Ph.D Thesis)

III. EFFECT OF ISCHEMIC REPERFUSION ON THE PERIOSTEAL MICROCIRCULATION IN OSTEOPOROTIC RATS TREATED WITH BISPHOSPHONATE

Bisphosphonates are highly potent therapeutic approaches in osteoporosis, but their use may lead to local healing complications (e.g. in the jawbones). The role of periosteal events may be important in these reactions. The present study was conducted to examine whether the nitrogen-containing bisphosphonate zoledronate (ZOL) influences the tibial inflammatory consequences of hind limb ischemia-reperfusion (IR) injury in osteopenic rats. 12 week-old female Sprague-Dawley rats were ovariectomized (OVX) or sham-operated. Starting 4 weeks after the operation, weekly intravenous ZOL (80 µg/kg) or vehicle injections were conducted in the following 8 weeks. At the end of the protocol, a 60-min hind limb ischemia was induced by using a tourniquet which was followed by a 180-min reperfusion. Tibial periosteal polymorphonuclear neutrophil leukocyte (PMN) endothelial interactions were examined in the postcapillary venules by intravital fluorescence videomicroscopy. Systemic inflammatory parameters i.e. the adhesion molecule CD11b expression of PMNs (using flow cytometry) and plasma TNF-alpha levels were also determined. Limb IR induced significant increases in both PMN rolling and adhesion during the entire reperfusion period and these changes reached a similar extent in the sham-operated and ovariectomized rats. BIS treatment did not influence these events. TNF-alpha values showed similar degree of postischemic elevations in all experimental groups. CD11b expression increased in saline-treated sham-operated and OVX rats, but not in animals also treated with ZOL. Chronic ZOL treatment did not affect the limb IR-induced tibial periosteal microcirculatory consequences and TNF-alpha release, but reduced the inducibility of PMNs.

Publication:
Under review

IV. CONTROLLED-RELEASE OF ANGIOGENIC FACTORS TO PREVENT THE DEVELOPMENT OF MEDICATION-RELATED OSTEONECROSIS OF THE JAW (MRONJ)

Various carrier (calcium sulphate- or polyasparaginic acid-based) were applied to test their applicability and effects on healing processes in the mandibular bone. After the tooth extraction, one of these carriers was implanted in twenty 12-week-old male Sprague-Dawley rats under ketamine (25 mg/kg ip) and xylazine (75 mg/kg ip) anesthesia. 5 carriers of 10 tested showed a relative longer applicability, but a fast polymerization

tendency in the extraction socket. Six weeks after tooth extraction, mandible samples were taken to investigate with μCT (in progress). The carriers applied did not influence bone regeneration and bone height reached the the mandibular ridge. Kinetics will be tested in further in vitro experiment, using the above mentioned carriers.

IV. PREVENTIVE EFFECT OF LIGHT AND SHOCK WAVE THERAPY ON THE DEVELOPMENT OF MEDICATION-RELATED OSTEONECROSIS OF THE JAW (MRONJ)

60 12-week-old male Sprague-Dawley rats were randomly allotted into intravenously vehicle- or BIS-treated (zoledronate) groups. At the third week of the protocol, molar teeth were extracted on the right side of the mandible under ketamine and xylazine anesthesia. A part of the animals were treated lovally with one of the adjuvant approaches (808 nm or 930 nm laser, LED or extracorporal shock wave therapy). 6 weeks after the invasive dental procedure, the incidence and the severity of the gingival lesions were estimated under an operating microscope. Osteonecrosis of the jaws were examined with μCT. Periosteal tissue and bone samples were taken for further histological and molecular biological investigation (immunohistochemistry, PCR, Western blot).
The adjuvant approches applied did not induce any mucosal reactions. At the site of tooth extraction, however, gingival healing was disturbed in the BIS-treated animals, while LLLT reduced significantly the severity of the lesion.μCT scanning revealed osteonecrosis in BIS-treated animals, while the adjuvant approches applied decreased significantly the incidence of the lesion. After the adjustment of reactions, the above mentioned special procedures will be performed, however, because of some factors (e.g. numerous samples, limitid availability of PCR instrument) these are time-consuming processes.

Conclusions:
The adjuvant approaches applied reduced significantly the incidence and the severity of gingival lesions and osteonecrosis.

V. LIMITED EFFICACY OF ISCHEMIC PRECONDITIONING IN OVARIECTOMIZED RATS.

Potential preconditioning-dependent and independent effects of estrogen supplementation. Our aim was to examine the effects of ischemic preconditioning (IPC) on the local periosteal and systemic inflammatory consequences of hindlimb ischemia-reperfusion (IR) in Sprague-Dawley rats with chronic estrogen deficiency (13 weeks after ovariectomy, OVX) in the presence and absence of chronic 17ß-estradiol supplementation (E2, 20 μg kg−1, 5 days/week for 5 weeks). Here, sham-operated (non-OVX) animals served as controls. As assessed by intravital fluorescence microscopy, rolling and the firm adhesion of polymorphonuclear neutrophil leukocytes (PMNs) gave similar results in the Sham+IR and OVX+IR groups in the tibial periosteal microcirculation during the 3-h reperfusion period after a 60-min tourniquet ischemia. Postischemic increases in periosteal PMN adhesion and PMN-derived adhesion molecule CD11b expressions, however, were significantly reduced by IPC (2 cycles of 10’/10’) in Sham animals, but not in OVX animals; neither plasma free radical levels (as measured by chemiluminescence), nor TNF-alpha release was affected by IPC. E2 supplementation in OVX animals restored the IPC-related microcirculatory integrity and PMN-derived CD11b levels, and TNF-alpha and free radical levels were reduced by IPC only with E2. An enhanced estrogen receptor beta expression could also be demonstrated after E2 in the periosteum. Overall, the beneficial periosteal microcirculatory effects of limb IPC are lost in chronic estrogen

deficiency, but they can be restored by E2 supplementation. This suggests that in females endogenous estrogen may play a role in the anti-inflammatory protection provided by limb IPC. The IPC-independent effects of E2 on inflammatory reactions should also be taken into account in this model. Publication is under review.