Amino acids and fracture healing

IMPACT PARAGRAPH

Main objectives and results

The main goal of the research described in this dissertation is to investigate the role of amino acid metabolism, specifically the arginine-citrulline-nitric oxide metabolism, on the fracture healing process and the development of complications during bone healing, such as delayed healing and nonunion development. This metabolism can influence the fracture healing process via three distinct ways. Firstly, through stimulation of the metabolism, amino acids are formed from proteins to act as precursors for collagen, one of the main constituents of bone tissue. Secondly, one of the most relevant enzymes in this metabolism is the inducible nitric oxide synthase. This enzyme influences the regulation of inflammation, which is especially important during the first phase of the bone healing cascade. Disturbances during this phase often lead to complications later on during the healing process. Finally, the angiogenic reactivity is stimulated via several enzymes, and is of importance for optimal vascularization of the newly formed bone.

The most important findings described in the thesis are firstly that the combination of a femur osteotomy with periosteal injury induced by electrocauterization result in a highly reproducible and reliable mouse model for investigating bone healing and bone healing difficulties. Subsequently, this model was used to investigate a disrupted amino acid metabolism which was shown to lead to an almost complete absence of bone healing, and which is concurrent with an adverse effect on the inflammatory reaction during bone healing. Contrariwise, when stimulating the amino acid metabolism by additional oral supplementation, the normal physiologic bone healing process shortened by approximately 30% and a stimulated collagen formation and a beneficial inflammatory response were observed.

Finally, in patients with long bone nonunions who underwent autologous bone grafting treatment, the levels of several amino acids and genes related to the arginine-citrulline-nitric oxide metabolism obtained during the surgical procedure, were found to be able to act as a predictor for defining the success of treatment outcome.

Scientific, ethical and societal impact

In the last decades, a wide range of different murine fracture healing and delayed union and nonunion models were developed. The model described earlier in this thesis resulted in better controlled biomedical condition as compared to other fixation techniques and the obtained tissues could be used for biomechanical, biochemical, genetic, radiographic and histological analysis. Recently, a new generation of comparable plates is developed in which polyether-ether-ketone is coated in titanium. Using these plates, every animal can then be monitored multiple times and without the need of euthanasia for data collection. This way,

research can be even more in compliance with the 3R principle of reduction, replacement and refinement in animal testing and can drastically lower the amount of animals needed for experimentation.
Mainly elderly patients with (hip) fractures suffer from a substantial loss of skeletal muscle mass (and subsequent function). Citrulline supplementation stimulates skeletal muscle and total body weight in both experimental and clinical studies. The increase in body weight in our amino acid supplementation study, after the reduced weight loss during the first postoperative days indicates an advantageous recovery and rehabilitation period. This taken together with the 30% shortened healing period until the bone parts are united may indicate that citrulline can improve postoperative recovery in the frail elderly population.
In literature, the treatment for fracture nonunions is structured in a pentagonal concept describing the five key stones for adequate treatment: local mechanical stability, active bone cells (osteoblast and osteoclasts), the presence of an adequate scaffold, sufficient vascularity at the fracture site and lastly the presence of growth factors necessary for callus formation. The findings in our study strongly suggest that if proven in the clinical setting, the nutritional status of the patients should also be taken into account, thus transforming the treatment into a hexagonal concept. To be able to finalize this concept, studies into the amino acid metabolism should thus be conducted in patients that are prone to developing an impaired fracture healing such as multitrauma patients or in the frail elderly with an already marginal nutritional status.

The results described in this thesis can have a huge clinical potential and are of great interest for clinicians, dieticians, industrial partners and mainly the patients, but also for fellow reseachers working in the field of bone healing metabolism. Therefore the research in this thesis is already (partially) published in peer-reviewed scientific journals such as *Bone*, the *Archives of Orthopaedic and Trauma Surgery* and *European Cells and Materials*, and have also been presented at several distinguished national and international conferences such as the *European Society of Tissue Regeneration in Orthopaedics and Traumatology* and the *International Society for Fracture Repair*.
Within the Maastricht University Medical Center, the importance of an adequate non-union treatment is recognized. Recently, a multidisciplinary outpatient clinic has started in which the findings from several clinical and translational studies is put to practice by trauma-, orthopaedic and plastic surgeons in the treatment of patients with nonunions.