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Evaluation of matrix metalloproteases as early biomarkers for bone regeneration during the applied Masquelet therapy for non-unions

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ABSTRACT

Introduction: In the current study, we sought to determine if serum concentrations of MMPs correlate with bone regeneration occurring during the course of the Masquelet-therapy and to identify if MMPs may serve as early biomarkers reflecting successful bone regeneration and tissue remodeling.
Material and methods: This study was designed as a prospective clinical observer study. We compared serum samples over the time of treatment, as a matched-pair analysis, from 10 patients who were treated successfully with the Masquelet-therapy (Responder) with 10 patients who did not respond to the Masquelet-therapy (Non-Responder). The quantitative measurement was performed with Luminex Performance Human High Sensitivity Assays according to manufacturer's instructions. The lab technician performing the Luminex assays was blinded to both patient data and clinical outcome.
Results: Analysis of the expression pattern of MMP-2, -8 and -9 showed significant differences between groups. Two days after the first step of the Masquelet therapy Responder showed peak values of MMP-8 and MMP-9 that where significantly higher ($p = 0.003$ and $p = 0.042$, respectively) than in Non-Responder. In contrast serum levels of MMP-2 were lower after the first step of the Masquelet therapy in the Non-Responder group. The ratio of MMP-9 and MMP-2 was significantly higher in the Responder group two days after step I ($p = 0.031$) as well as 4 weeks after step II ($p = 0.030$).
Conclusion: The findings of the current study emphasize the potential role of MMPs as biomarkers in bone remodeling. In particular, a distinct expression of MMP-2 correlates with successful bone regeneration, whereas initial overexpression of MMP-2 serum levels might identify patients that have a higher risk for a poor outcome of the Masquelet-therapy. Furthermore, we were able to introduce the serum analysis of the ratio of MMP-9 and MMP-2 as promising novel modality for early prediction of the outcome of the Masquelet therapy. Further analysis of this ratio over time subsequent to the second step might serve as an early indicator of a favorable response to the induced membrane technique.
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Introduction

Complex cellular and molecular processes determine physiological fracture healing and deviations can lead to delayed or failed fracture healing [1]. Non-unions occur in up to 30% subsequent to long bone fractures thereby remaining a common and persistent complication [2,3]. Failed fracture healing prolongs the duration of the disease and leads to severe limitations in the quality of life [4]. Treatment of non-unions with a large size defect remains a considerable challenge for caregivers. Thus, the induced membrane technique, also known as Masquelet-therapy, was established [5–7]. The Masquelet-therapy is an osteoinductive, osteoconductive and osteogenic procedure [7] that induces bone regeneration [6,7].

Matrix metalloproteases (MMPs) play an important role during tissue regeneration and bone remodeling as part of the bone healing process [8]. They belong to a family of 23 zinc-dependent proteolytic enzymes that have initially been described by their ability to cleave the extracellular matrix (ECM) [8]. Since then it became evident that these enzymes have a wide role in tissue

Abbreviations: MMP, matrix-metalloproteases; ECM, extracellular matrix; BMP, bone morphogenic protein; PMMA, polymethyl methacrylate; CRP, C-reactive protein.

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remodeling, in particular MMPs contribute to angiogenesis, regulation of cell growth, release of growth factors and response to inflammatory chemokines [9]. During the remodeling of bone and cartilage MMPs contribute through osteoclast recruitment, osteoblast survival, bone resorption and formation [8,10]. Hence, they have been introduced as possible consistent biomarkers of bone remodeling [8,11]. Dominant roles during bone regeneration were postulated for MMP-2, MMP-8 and MMP-9 [8,12]; in particular studies utilizing knockout animals showed bone development defects and reduced bone size once MMP-9 and MMP-2 were missing [8]. Furthermore, it was implicated that the biological function of MMP-8 involves a variety of tissue remodeling processes associated with inflammatory conditions [12]. Despite recent studies emphasizing the clinical relevance of MMPs in context with bone homeostasis [8] clinical evidence regarding their role during the course of bone regeneration remains scarce.

As bone regeneration occurs in the Masquelet-therapy and the treatment is both highly standardized and regularly monitored, this treatment has become valuable in studying biological processes occurring during bone regeneration. In addition, analysis of serum cytokine expression patterns was established as a valid method in the evaluation of biological processes occurring during fracture healing and bone regeneration [13–15]. Therefore, in the current study we sought to determine if serum concentrations of MMPs correlate with bone regeneration occurring during the course of the Masquelet-therapy and to identify if MMPs may serve as early biomarkers reflecting successful bone regeneration and tissue remodeling.

Material and methods

Study design

The current study was designed as a prospective clinical observer study. We enrolled 207 patients between March 2012 and March 2014 who received surgical treatment of non-unions subsequent to long bone fractures in the Department of Orthopedics and Trauma Surgery of the University Hospital Heidelberg. In order to minimize possible confounders strict inclusion and exclusion criteria were applied and ultimately study patients were matched. Inclusion criteria were failed bone healing after diaphyseal fractures of the tibia or femur, the age between 18 and 80 years and a written declaration of consent. Exclusion criteria were the inability to a written consent, chronic diseases, chronic intake of immunosuppressive medication, renal failure, hepatic insufficiency, malignomas, collagenosis and chronic inflammatory bowel disease. Patients who underwent revision surgery or additional surgical therapy during the study period were excluded from our study. The study was conducted in accordance with the declaration of Helsinki. Furthermore, inclusion of patients started subsequent to the approval by the local ethics committee of the Ruprechts-Karls-University of Heidelberg (S-636/2011).

Induced membrane technique (Masquelet-therapy)

The Masquelet-therapy induces bone regeneration via a two-stage procedure (step I and step II) [13]. The initial surgical treatment consists of debridement of the non-union, avital bone and avital surrounding soft tissue leaving a defect site. In the same procedure, the emerging defect size is filled with polymethyl methacrylate (PMMA) that induces the vascularized Masquelet membrane [5]. In addition, during the first step tissue samples are extracted and subsequently microbiologically examined in order to guarantee asepsis. Once asepsis is achieved the spacer will remain 6 weeks *in situ* to ensure a fully-grown Masquelet membrane [16]. In a consecutive second step the spacer will be removed while leaving the membrane unimpaired and the cavity is filled with autologous bone marrow. Thus, providing a beneficial local microenvironment that results in bone regeneration by an osteoinductive, osteoconductive and osteogenic stimulus [5]. Additionally, to provide a further stimulus for bone regeneration growth factors are added; in particular bone morphogenic protein 7 was added in each of the included patients (3.3 mg BMP-7) [6,7,16,17].

Patient demographics

A total of 207 patients who received surgical treatment of non-unions subsequent to long bone fractures in the Department of Orthopedics and Trauma Surgery of the University Hospital Heidelberg were enrolled in the current study. 72 of these 207 study participants were treated with the Masquelet-therapy and completed the required follow up examination. Out of these 72 patients 7 patients had to be excluded because they received BMP-2 instead of BMP-7, another 5 were excluded due to a necessary repetition of step I because of local infections and 11 patients were excluded because the existence of two non-unions simultaneously. Patients that failed to show consolidation within 6 months of the second step of the Masquelet-therapy were assigned to the Non-Responder group, whereas patients that showed proper consolidation within 6 months of the second step of the Masquelet-therapy were assigned to the Responder group. In particular, 16 of the remaining 49 patients were Responder and 33 Non-Responder to the therapy.

Matching

We compared two groups of patients:

1 Responder to the induced membrane technique
2 Non-Responder to the induced membrane technique

Patients were matched on the basis of three criteria: age, sex and BMI. If more than one match was found for a non-union patient, than the patient with the most similar type of fracture was chosen. According to the matching criteria two groups (n = 10) could be formed of the above mentioned total study patients. (Table 1)

Responder

A total of 6 male and 4 female patients with an average age of 50.8 ± 13.05 years were included into the Responder group (n = 10). Patient demographic showed that 7 patients suffered from a tibial non-union whereas 3 patients suffered from a femoral non-union, 1 patients had diabetes, 5 patients were non-smoker, 3 patients smoker and 2 patients former smoker [18]. The average BMI was 27.92 with a range of 19.84–38.6 and patients were previously treated with a mean of 2.5 surgeries.

Non-responder

A total of 6 male and 4 female patients with an average age of 50.7 ± 10.4 years were included into the Non-Responder group (n = 10). Patient demographic showed that 7 patients suffered from a femoral non-union whereas 3 patients suffered from a tibial non-union, 2 patients had diabetes, 5 patients were non-smoker and 5 patients were smoker [18]. The average BMI was 32.24 with a range of 23.57–39.54 and patients were previously treated with a mean of 3.8 surgeries.

Follow up and outcome

Follow up consisted of both clinical and radiological examination by two independent experienced trauma surgeons. Time

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