Examination of the Correlation between Hypervascularity and Physeal Bone Bridge Formation

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Introduction

The formation of bone bridges is reported to be initiated by physeal lesions caused by a trauma or intra-operatively by implants advanced through the growth plate in order to stabilize fractures [1, 2]. Bone bridges, usually reversible, can cause full or partial premature physeal closure due to incomplete decomposition and therefore lead to full or partial growth arrest [3]. The aim of this study was the observation of bone bridge formation and the investigation of hypervascularity as one hypothesized underlying mechanism for bone bridge formation in a living animal model.

Materials and Methods

In the course of the study, 5 Sprague-Dawley rats (4 weeks old, weighing 100g at the beginning of the study) were subjected to unilateral growth plate lesion using a standardized drill procedure causing a transphyseal lesion of 1.2mm diameter of the proximal tibial physis. Imaging of the morphology and investigation of physeal vascularisation using dynamic contrast enhanced (DCE) MRI were performed on a clinical 3T scanner with 38mT/m gradient strength using 18mm surface coils (Rapid Biomedical, Germany).

Measurements were performed on days 1, 3, 7, 14, 28, 42 and 82 post-operation administrating double-dose (0.2mmol/kg) injections of Gadovist for DCE-MRI. Morphological scans were 3D FLASH sequences (256x256 matrix, TE 7ms, TR 100ms, resolution 0.19mmx0.19mmx0.7mm, FA 15). DCE-MRI was performed using a PD-weighted 3D FLASH sequence (256x256 matrix, TE 2.95ms, TR 100ms, resolution 0.19mmx0.19mmx0.7mm, FA 5) as reference scan and a T1-weighted 3D FLASH sequence (256x256 matrix, TE 2.95ms, TR 8.09ms, resolution 0.19mmx0.19mmx0.7mm, FA 30) with a temporal resolution of 13.32s for bolus-enhanced measurements.

Vascular properties of the lesioned tissue were investigated in vivo by applying a reference region model (RRM) accounting for transcytolemmal water exchange [4] to the acquired DCE data. Additionally, factors related to vascular growth and oxygenation were determined by performing real-time polymerase chain reaction (PCR) analyses of tissue taken from a different cohort of animals subjected to the same treatment at the beginning of the study. The RRM provided estimates of the pharmacokinetic parameters of the observed tissue while PCR analyses yielded expression rates of genes related to vascularity.

Results and Discussion

Imaging of the morphology (**Fig. 1**) unequivocally documented the formation of bone bridges. On day 1 the lesion can clearly be detected. On day 7 post-operation the growth plate lesion has already been closed by cartilaginous tissue. While on day 28 only first indications of discontinuances in the growth plate are observed images acquired on day 42 show a replacement of cartilage by bony tissue causing a disruption in the growth plate (**Fig. 1d**).

Bone bridge formation can also easily be detected in the results of the RRM fits as bone bridges resemble well-perfused periosteum and therefore produce an essential increase in transfer coefficient (K^{trans}) values in the observed area (**Fig. 2**) on day 42. While the determination of bone bridge formation forms an important basis, the main scope of this study focused on the interval prior to bone bridge formation. Estimates of the pharmacokinetic parameters interestingly showed no signs of increased vascularity on day 1 post-operation. On day 3 a slight increase of vascularity could be measured, however, a remarkable rise of K^{trans} values could not be observed before day 7 post-operation where the transfer coefficient reached values in the range of $0.2min^{-1}$.

Results of PCR analyses (**Fig. 3**) provided additional information which eased the interpretation of the kinetic parameters. Expression of hypoxia-inducible factor 1a (Hif1a) showed a slight upward trend from day 1 on with maximum levels on day 3 (**Fig. 3a**). The trend of expression of the vascular endothelial growth factor VEGFa did not show an increase before day 7 but then yielded an expression of up to 9 times higher compared to expression rates before operation (**Fig. 3b**). It is important to note that values of both, Hif1a and VEGFa epressions have returned to normal values on day 14 post-operation which means that the increased values of K^{trans} on day 14 are due to first formation of bone bridges.

Investigations showed that the application of an RRM was especially useful for this type of study as typically no adequate arterial input function can be measured.

In conclusion, it can be stated that hypervascularity is associated with the formation of bone bridges after transphyseal lesion which is confirmed by DCE and PCR measurements. The actual formation process was clearly documented by morphological imaging as well as DCE measurements and by combining PCR and DCE results it could additionally be shown that first formations already take place 2 weeks after physeal trauma while the maximum bone bridge density is not reached before 6 weeks.

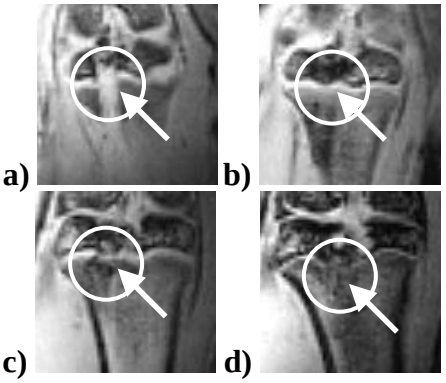

Fig. 1: Images of the tibial growth plate on days a) 1 b) 7 c) 28 and d) 42. While in a) the lesion is clearly detectable the growth plate is already closed on day 7. In c) first discontinuances can be seen and on day 42 the growth plate is disrupted again.

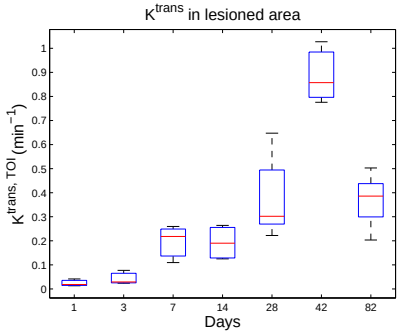

Fig. 2: Results of the RRM fits. A first slight increase in K^{trans} can be seen on day 3 post-operation. On day 7 a remarkable increase can be detected whose decline overlaps with formation of first bone bridges.

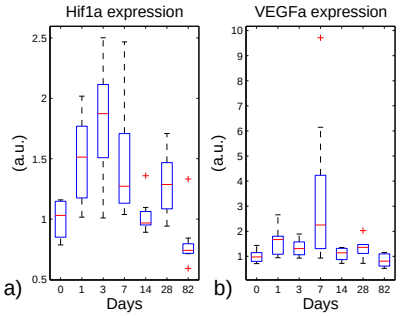

Fig. 3: Results of the PCR analysis: a) Hif1a and b) VEGFa expressions. Hif1a expressions show a slight maximum on day 3 while VEGFa expression shows essentially increased values on day 7.

Acknowlegdements

This work is supported by SFB F3209-N18.

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