Influence of processing temperature on devitalization and inflammatory cell reaction in high hydrostatic pressure treatment of allogenic bone tissue

Henrike Loeffler¹, Christopher Pohl², Annett Künler¹, Rainer Bader¹, Janine Waletzko-Hellwig¹
¹Research Laboratory of Biomechanics and Implant Technology, Rostock University Medical Center, Rostock, Germany, ²Department of General Surgery, Visceral, Thoracic and Vascular Surgery, University Medical Center Greifswald, Germany

Disclosures: All Authors (N)

INTRODUCTION: High hydrostatic pressure (HHP), widely used for food decontamination, has also gained interest for medical application in recent years. It was shown that HHP can successfully destroy mammalian cells while maintaining the extracellular matrix and biomechanical properties of the tissue. However, the processes of devitalization are not understood in detail yet, but are of great importance for processed allogenic bone tissue after implantation. Cell death mechanisms in bone can react apoptotic (HHP 200 - 300 MPa) or necrotic (HHP > 300 MPa) to HHP, whereas necrosis-related reactions led to the release of proinflammatory cytokines that would promote rejection processes in the recipient. To avoid this, applied HHP must be chosen carefully, with anti-inflamatory cell deaths being preferred. In addition to devitalization, microbial decontamination of the tissues should also be achieved in order to circumvent matrix-damaging sterilization protocols. In our own preliminary work, promising results for successful decontamination could already be obtained at HHP >350 MPa and temperatures of 10 °C. However, it is currently unknown how low temperatures and HHP may affect human trabecular bone. In order to determine the HHP protocol which promotes decontamination, but also causes anti-inflammatory reactions in case of cell death within the bone graft, the objective of our present study was to characterize the reaction of human trabecular bone to different magnitude of high hydrostatic pressures applied at various temperatures.

METHODS: Human trabecular bone samples were gained from patients who underwent total hip replacement and signed a declaration of consent before surgery. The use of the material for research purposes was approved by the local ethics committee (ethics approval number A2010-10). The femoral heads were cut into cancellous blocks with a sample size of 5 x 5 x 5 mm. Samples were collected in cryogenic tubes which were filled with 0.9 % saline solution and closed air bubble free. Pressures of 150 MPa, 250 MPa, 350 MPa, 450 MPa and 600 MPa were applied at 10 °C, 20 °C and 30 °C for 20 min. Afterwards, specimens were incubated at 4 °C or 37 °C for 1 h in 0.9 % saline solution. A control group which was treated like the other specimens with exception of HHP application was accompanied. All samples were prepared for RNA isolation and gene expression analysis was performed with regard to caspase-1, caspase-3, caspase-8, caspase-9 and MLKL.

RESULTS SECTION: Gene expression analysis showed that specimens reacted with various cell deaths to pressure applications and in dependence of the application temperature and subsequent incubation temperature. Samples treated with HHP at 10 °C, only slight caspase-8 expression was observed, whereas caspase-9 was significantly overexpressed at pressures of 250 MPa and higher compared to the control. HHP application of 20 °C promoted the overexpression of caspase-8 (from pressures of 250 MPa on), but only with a downstream incubation at 37 °C. In contrast, the expression of caspase-9 was reduced to the control level. A further reduction of caspase-9 expression below the control level was shown for HHP applications at 30 °C. However, the expression of caspase-8 was highest here. The previously described trend of increased expression of genes upon downstream incubation at 37 °C was again evident considering caspase-1 and MLKL expression. Increased caspase-1 expression was primarily observed at HHP applications of 350 MPa and 600 MPa at 10 °C and 350 MPa at 30 °C. In contrast, MLKL overexpression was mainly observed at a treatment von 250 MPa and 350 MPa at 20 °C or at 600 MPa and 30 °C.

DISCUSSION: Our data show that HHP treatment causes different types of cell death depending not only on the magnitude of hydrostatic pressure but also on the temperature during and after HHP application. Both, extrinsic (caspase-8) or intrinsic (caspase-9) apoptotic signal cascades can be induced which are temperature related. This allows different assumptions: first, caspases could have different activity optima, caspase-9 could be prominently active at low temperatures, caspase-8 at higher temperatures. Second, when specimens are HHP treated at 30 °C, the temperature plays a secondary role and the reaction of the cells is mainly caused due to the HHP treatment and is not the results of a combination of high pressures and low temperatures. Besides apoptotic pathways, also hints for the signaling cascade of pyroptotic (caspase-1) and necroptotic (MLKL) origin could be observed. Those were mainly observed at application temperatures of 10 °C, but with a subsequent incubation of 37 °C. This indicates that these signaling cascades need a physiological temperature to develop. Although the presented data provide hints for the different reactions, further studies should also focus on histological analysis with which specific protein expression for cell death could be analyzed in the complete sample. Since it has already been shown in vitro that the level of HHP is associated with enhanced resorption processes, the influence of the modified parameters for HHP treatment on the osteoclastic differentiation and their resorption behavior should be addressed in further studies.

SIGNIFICANCE/CLINICAL RELEVANCE: In this study we present experimental data concerning cellular responses of human bone tissue related cells to HHP application at various temperatures during and after treatment. With HHP it is possible to induce anti-inflammatory cell death in the treated tissue, which are essential to avoid graft rejection in the recipient after transplanting HHP processed tissue. HHP protocols which promote anti-inflammatory devitalization and simultaneous microbial decontamination of bone allografts should be established in the future.

ACKNOWLEDGEMENTS: We thank Daniel Wolter (Department of Maxillofacial and Plastic Surgery, Rostock University Medical Center, Germany) for the preparation of bone tissue samples.