Sawsan Mohammed
Dr. med.

Development and evaluation of a nanobody/intrabody-based capture method for protection against bacterial Shiga toxin

Fach: Kinderheilkunde und Deutsches Krebsforschungszentrums (DKFZ) Heidelberg

Doktorvater: Herr Prof. Dr. med. Burkhard Tönshoff

Hemolytic uremic syndrome is a clinical condition characterized by triad of hemolytic anemia, thrombocytopenia and acute renal failure. Hemolytic uremic syndrome is the most common cause of acute renal failure in infants and young children. According to etiology, it can be classified into two forms, an atypical or non-diarrhea-associated and the more common typical or diarrhea-positive form. The latter is most often due to infection with Shiga toxin-producing *Escherichia coli* and *Shigella*. The main pathogenic mediator Shiga toxin infiltrates host cells *via* retrograde transport into the cytosol.

The currently available treatments to manage this condition are not specific and mostly symptomatic. Several past attempts to neutralize the toxin by peptide binders or antibodies have performed badly in clinical trials, most likely due to the rapid clearance of the toxin from the circulation and cellular uptake, prior to the onset of systemic symptoms. Recently, a novel class of specific binders based on single-domain antibodies, so called nanobodies, was raised in Llama against Shiga toxin and were shown to protect Shiga toxin-exposed mice. In this study, it was investigated whether this new binder class may be employed to target Shiga toxin after cell entry to boost clinical applicability.

Two published nanobody clones with high binding affinity to the B subunit of Shiga toxin 2a were selected to investigate their effect against the toxin.

To test the extracellular effect of anti-Shiga toxin nanobodies, purified nanobodies were added to the medium of Shiga toxin-sensitive human cells. Cell viability was evaluated utilizing a colorimetric resazurin assay after 24 h. The addition of purified anti-Shiga toxin nanobody to the medium of toxin sensitive cells did not alter the toxicity of Shiga toxin at the chosen molar ratios compared to the control cells.

To investigate the intracellular effects, nanobody sequences were cloned in-frame into a backbone vector carrying an endoplasmatic reticulum-localization signal and green fluorescent

protein. Shiga toxin-sensitive human cells were transfected with the vector, Shiga toxin added after 24 h and cell viability determined at different time points by resazurin assay.

The intracellular endoplasmatic reticulum -localized expression of the selected anti-Shiga toxin nanobodies, but also endoplasmatic reticulum-targeted vector without nanobody insert protected Shiga toxin-sensitive cells compared to the wild type, whereas control plasmids did not show significant differences to wild type. While inconclusive with regards to the protective potential of intracellular anti-Shiga toxin nanobodies, a novel and significant finding of this study is the observed intrinsic, highly protective effect of the endoplasmatic reticulum-targeting sequence (SEKDEL/KDEL).