

## Summary

Asymmetric Dimethyl-L-arginine (ADMA) is an endogenous inhibitor of the NO-synthase. ADMA originates from the degradation of methylated proteins. ADMA is eliminated either by renal excretion or enzymatic degradation by DDAH. Plasma-ADMA-concentrations are elevated in diseases linked to endothelial dysfunction. The endothelial dysfunction is characterised by a disproportion between NO availability and NO demand.

So far, the determination of ADMA has been done by HPLC. This method is time consuming and therefore not optimal for the measurement of ADMA in clinical trials. In the last three years, several other methods have been developed, namely LC-tandem MS, GC-tandem MS and GC-MS. They are easier to perform and faster, but the equipment is expensive and not available in many laboratories. The aim of this work was to develop a simple assay for the determination of ADMA by an immunological method.

For this purpose a monoclonal antibody and a polyclonal antiserum were developed. To create the monoclonal antibody four Balb/c-mice were immunized with an ADMA-KLH-conjugate. After successful immunization, the mice were sacrificed and their spleen removed. The B-lymphocytes were isolated from the spleens and were fused with myeloma cells from the same genetic background. From these fusions several hybridoma cell lines were established. The hybridomas were screened for anti-ADMA antibody secreting cells. In a simplified test for cross reactivity, the hybridomas were tested in order to find out whether the secreted antibodies bind more strongly to an ADMA-BSA-conjugate than to a SDMA-BSA-conjugate. At this step the hybridoma 142D6 was isolated and recloned. The ELISA was developed with the cell culture supernatant of the clone 142D6-4E8.

In parallel a polyclonal antiserum against ADMA was raised from rabbits by DLD Diagnostika, Hamburg. DLD developed an ELISA from the antiserum which was validated by us. To validate the ELISA, we determined the variation, the precision and the accuracy. The validation was done for samples from human, mouse and rat. After the validation we compared the ELISA to LC-tandem MS and GC-MS. For the comparison with LC-tandem MS, we measured the ADMA-concentrations in 29 human subjects by LC-tandem MS and by ELISA. The results were compared by linear regression. For the comparison of the ELISA and GC-MS, we measured the ADMA-concentrations in 15 cell culture supernatants by ELISA and GC-MS. The mean of the results determined by each method were compared by t-test, because the range of ADMA concentrations was too low for a comparison by linear

regression. The result was that the ADMA-concentrations determined by the ELISA were comparable to those determined by LC-tandem MS and GC-MS.

The validated ELISA on the basis of the polyclonal antiserum was used to measure the ADMA concentrations in the plasma of patients who participated in a clinical trial. 213 patients with acute myocardial infarction received randomly either captopril or losartan for a follow-up period of three years (median 27 months). The plasma ADMA concentrations were measured at the beginning and at the end of the follow-up. The endpoints of the trial were death, reinfarction, stroke and resuscitation. The analysis of the data showed that the plasma ADMA concentration is a prognostic factor for cardiovascular events, independent from other cardiovascular risk factors. Furthermore, we were able to show that therapy with either captopril or losartan has no effect on the plasma ADMA concentration.