EXTRACELLULAR MATRIX TURNOVER IN GLOMERULI OF MYXINE GLUTINOSA: IN VITRO PHARMACO-TOXICOLOGY STUDIES.

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In the pathogenesis of various glomerulopathies protein accumulation seems to be one of the major features. Both glomerular structural proteins of the extracellular matrix (ECM), i. e. mesangial matrix as well as glomerular basement membrane and deposited plasma proteins are part of the glomerular protein accumulation. Protein accumulation is directly related to the glomerular protein balance depending either on the synthesis and deposition or on the degradation of these proteins. In order to examine the causal relationship between ECM synthesis and/or degradation and glomerular lesions induced by environmental hazards such as heavy metals and therapeutic substances, we applied our multicellular in vitro system of isolated glomeruli of Myxine glutinosa in the experimentally induced Adriamycin (ADR) glomerulopathy. This multicellular in vitro system has the advantage of a low turnover and therefore comes closer to the in vivo situation of glomerular metabolism, providing a valuable tool to study the cascade of pathomechanistic events. It was previously shown that ADR induces an accumulation of total protein into the glomerulus. The postulated pathomechanism is an enhanced total net-protein synthesis caused by a diminished proteolytic degradation while transcription measured by a decreased RNA-synthesis is inhibited (Kastner S. et al., Bull. MDIBL 32: 20, 1993). Total protein synthesis studies, however, do not elucidate the effects of ADR on individual proteins. Therefore, we studied alterations in the metabolism (synthesis and/or degradation) of individual structural extracellular matrix proteins of the glomerular filtration barrier (e.g. fibronectin, laminin, collagen IV and proteoglycans) to explain changes in glomerular structure and function.

On the level of single ECM proteins our studies were focused primarily on fibronectin (FN), occuring almost exclusively in the mesangial matrix. To demonstrate glomerular FN, the proteins in glomerular homogenates were separated by SDS polyacrylamide gradient gel electrophoresis (SDS-PAGE) and transferred to a PVDF matrix by Western-Blotting. FN was visualized with a polyclonal antibody againt purified human plasma FN, cross-reacting as well with Myxine fibronectin. To study possible alterations in fibronectin turnover Myxine glutinosa was treated with 20 mg/kg b.w. ADR and after 10 and 20 days changes in the FN content in glomerular homogenates were determined semiquantitatively by Western-Blotting. Glomerular homogenates were all diluted to the same total protein content to detect an enrichment of FN. For the determination of specific proteolytic activities against FN, FN was labeled with biotin (b) and incubated with glomerular homogenates from controls and ADR-treated animals. Degraded b-FN was separated by SDS-PAGE and transferred to a PVDF membrane by Western-Blotting. The different degradation pattern was visualized with a detection system specific for b-FN. In addition to fibronectin, laminin was demonstrated in glomerular homogenates of untreated animals by the Western-Blot technique as described above using a polyclonal antibody raised against laminin isolated from basement membrane of EHS mouse sarcoma.

Focusing on individual structural proteins of the glomerulus, fibronectin fragments could be immunologically demonstrated in glomerular homogenates by the Western-Blot technique. Most major bands are comigrating with the bands appearing in the FN-standard (Fig. 1). After ADR-treatment the FN-content in glomerular homogenates of ADR-treated animals is elevated compared to controls. Semiquantitatively, protein blotting revealed a higher ratio of fibronectin to total protein in glomerular homogenates of ADR-treated Myxine glutinosa. The fibronectin-band occuring at

58 KD was much stronger in glomerular homogenates of ADR-treated animals as compared to controls (Fig. 2).

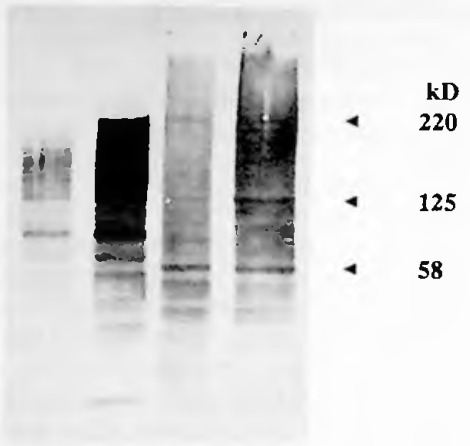


Fig. 1: Western-Blot of glomerular proteins, detected with a polyclonal antibody against human plasma FN; electrophoretic separation in a 5-15 % SDS-PAGE gel. $ST_{1,2}$ = FN-Standards, 1,2 = glomerular homogenates

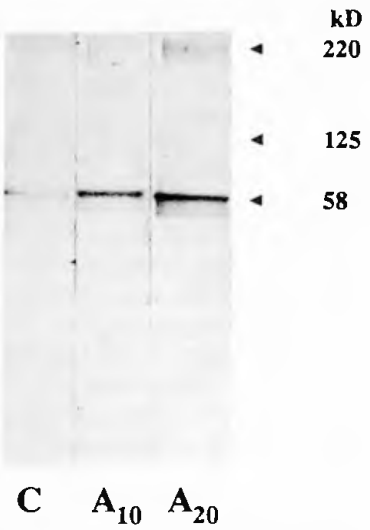


Fig. 2: Semiquantitative Western-Blot of FN in glomerular homogenates of controls and ADR-treated Myxine; electrophoretic separation in a 5-16 % SDS-PAGE gel. C = control, $A_{10,20}$ = Adriamycin-pretreated (10 or 20 days)

In the protease studies, b-FN incubated with glomerular homogenates from ADR-treated animals was, after 12 hours incubation, much less degraded compared to controls; after 24 hours incubation no differences could be detected anymore (Fig. 4). This demonstrates a slower fibronectin degradation after ADR-treatment, indicating that ADR inhibits glomerular proteases. These results suggest that FN is one of the proteins participating in glomerular protein accumulation due to a decreased degradation. Preliminary studies revealed that laminin is also immunologically detectable in the glomeruli of Myxine glutinosa (Fig. 3). Laminin, however, has to be further characterized and studied for possible alterations under toxicological conditions.

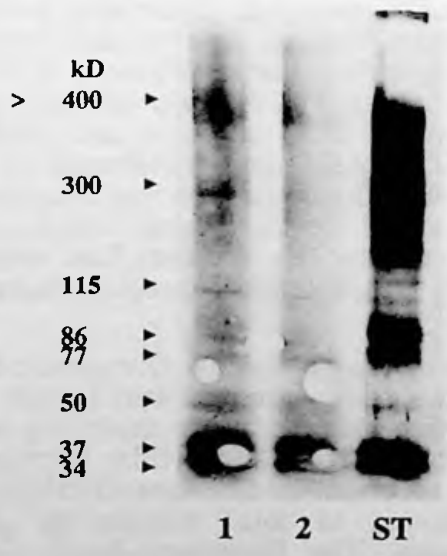
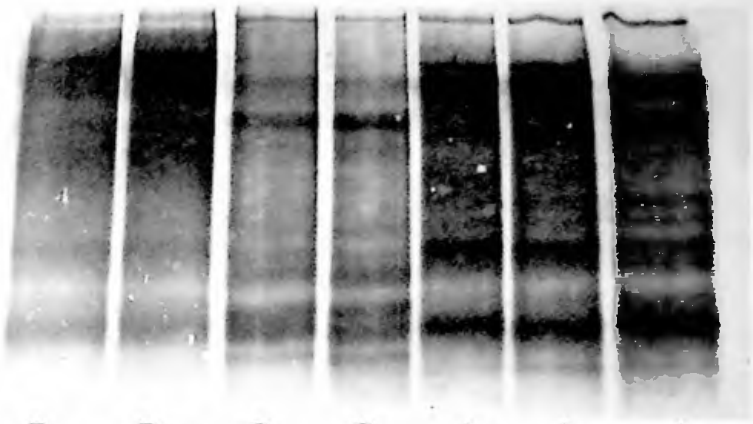


Fig. 3: Western-Blots of glomerular proteins, detected with a polyclonal antibody against laminin; electrophoretic separation in a 4-10 % SDS-PAGE gel. 1,2 = glomerular homogenates, ST = Laminin Standard

a



b

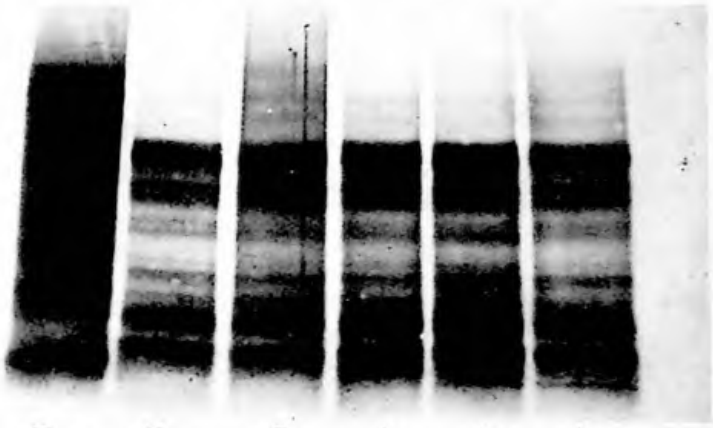


Fig. 4: Western-Blot of the degradation pattern of b-FN after 12 hours (**a**) and 24 hours (**b**) incubation with glomerular homogenates of controls and ADR-treated animals, respectively. R = blank = Myxine Ringer solution, $C_{1,2}$ = controls, A_{1-3} = ADR-treated

Extracellular matrix proteins like fibronectin and laminin are detectable in the glomeruli of the primitive vertebrate Myxine glutinosa, indicating that ECM proteins are conservative proteins occuring early in phylogentic development of the glomerulus. As previously shown in the multicellular in vitro model of isolated glomeruli of Myxine glutinosa, the model compound ADR affects the balanced relationship between synthesis (reduced RNA-synthesis) and degradation (inhibited proteolytic activity) of glomerular proteins. Finally total net-protein synthesis is increased, resulting in an accumulation of total protein in the glomerulus (Kastner S. et al., Fig. 3, op. cit.). The accumulated protein could be either material of the mesangial matrix or of the glomerular basement membrane causing functional alterations of the glomerular filtration barrier for proteins, resulting in an impairment of kidney function (Border W.A., Kidney Int. 34: 419, 1988). This year we focused on alterations in the metabolism of individual extracellular matrix proteins during glomerulopathy, again using ADR to experimentally induce a glomerular lesion. The results confirmed a disturbed balance of ECM synthesis and degradation. ADR causes a net-accumulation of the ECM protein fibronectin, due to an inhibition of fibronectin-degrading proteases. It remains, however, open whether the increased net-FN synthesis is due to an effect on the translational or on the transcriptional level. To answer this question, the fibronectin mRNA level has still to be quantified.

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