NEPHROTOXICITY OF THE GLOMERULUS: STUDIES WITH ADRIAMYCIN ON ISOLATED GLOMERULI OF THE ATLANTIC HAGFISH MYXINE GLUTINOSA

Sabine Kastner, Lüder M. Fels, Lena Emunds, Hilmar Stolte
Laboratory of Experimental Nephrology, Division of Nephrology, Department of Internal Medicine, Medical School Hannover, FRG

The glomerulus is the segment of the kidney affected by several diseases which may be related to renal metabolic changes. Metabolic changes again can precede kidney damage or be an early marker for a beginning decline in kidney function. Therefore the metabolism of the glomerulus is of importance if the causes and effects of glomerular disease are to be completly understood.

To experimentally induce a glomerular disease and to visualize the different target cells the anticancer drug Adriamycin (ADR) was chosen as a model compound. Several molecular mechanisms are discussed to be responsible for the ADR-nephrotoxicity: 1. Experimental studies support the involvement of ADR-semiquinone radicals generated by ADR-bioreduction. In the presence of oxygen the semiquinones rapidly give rise to reactive oxygen radicals, which are thought to affect cells by lipid peroxidation. The generated oxygen radicals as well as the ADR-semiquinone radicals could further damage membranes, inactivate enzymes and/or interact with macromolecules (SCHEULEN ME et al, Arch. Toxicol. 60: 154, 1987). 2. Other mechanisms depend on the high affinity of ADR to nucleic acids. The drug intercalates into double helical DNA and inhibits polymerases. These interactions have been reported to result in strand breaks, protein-DNA cross links, increased sister chromatid exchange, chromosome aberrations, subsequently leading to inhibition of nuclear processes such as DNA replication, transcription, translation and repair-mechanisms. 3. Other cytotoxic side effects as enzyme inactivation and direct membrane interactions are also discussed (GIANNI L et al, Rev. Biochem. Toxicol. 5: 1, 1983).

To define the mechanisms of functional and metabolic target-selective insults induced by ADR the large glomeruli of the Atlantic hagfish Myxine glutinosa were used as an alternative multicellular in vitro model. One group of animals was pretreated with a single dose of ADR (20 mg/kg b.w.). The toxic effect of ADR is attributed at least partly to the generation of free radicals. These free radicals again might be detoxified with scavengers. Therefore, a second group of animals received a combined treatment of ADR (20 mg/kg b.w.) and the sulfhydryl-donor N-Acetylcysteine (NAC; 450 mg/kg b.w.). - 10 days after treatment functional studies were performed on single isolated perfused glomeruli of Myxine glutinosa (FELS LM et al, Bull. MDIBL 28: 22, 1989). - To study whether the impairment of renal function might be reflected by metabolic alterations we established our model of isolated and in vitro incubated hagfish glomeruli. 10 days after pretreatment the glomeruli were isolated by microdisection and incubated in vitro in a tissue culture with radioactive precursors (^{3}H amino acid mixture TRK 440, ^{3}H uridine, ^{14}C inulin; Amersham Co.). We determined de novo protein synthesis as incorporation of amino acids into TCA-precipitable proteins and de novo RNA synthesis as incorporation of uridine into glomerular RNA (KASTNER S et al, Bull. MDIBL 31: 35, 1992). Glomerular amino acid uptake was defined as the total amount of radio-labeled amino acids accumulated into the glomerulus (free in cytoplasma and incorporated into proteins). To explain possible changes in glomerular amino acid uptake the glomerular inulin space was measured according to the method of SAVIN VJ & TERREROS DA (Kidney Int: 20: 188, 1981). Total glomerular protease activity was quantified with the azocasein test (LANGE J et al, Acta Biol. Med. Ger. 31: 1, 1973) by the release of diazo-amino acids into the supernatant.

Functional studies revealed a significant increase in protein permeability whereas water permeability was significantly decreased after ADR-treatment (Fig. 1). A combined treatment of ADR and NAC protected against the changes in water permeability. The increase in protein permeability caused by ADR however was not prevented by NAC. Protein permeability was still elevated after the combined treatment (FELS LM et al, op. cit.) - Determination of the total contents of the three macromolecules in glomeruli of controls (c) and ADR-treated animals revealed that total DNA was unchanged (c: 0.44±0.02 μg/glomerulus, x±SEM, n=76; ADR: 0.46±0.02, n=99), total RNA was significantly reduced (c: 1.6±0.12 μg/glomerulus, n=121; ADR: 0.86±0.05, n=89) while total protein was slightly elevated (3.56±0.09 μg/glomerulus, n=228; ADR: 3.82±0.1, n=264). After ADR-treatment the de novo protein synthesis of isolated glomeruli was significantly increased in vitro. Total glomerular amino acid uptake into glomeruli of ADR-treated animals was significantly inhibited compared to controls. NAC could not prevent the increase in protein synthesis. The inhibition of total glomerular amino acid uptake in contrast could be prevented by NAC (KASTNER S et al, op. cit.). The reduction of amino acid uptake is not related to a reducting of the glomerular inulin space which was unchanged after ADR-treatment (c: 38.5±15.4 nl/glomerulus, x±SD, n=34; ADR: 34.8±9.1, n=27). RNA-synthesis was significantly inhibited in glomeruli of ADR-treated animals (KASTNER S, op. cit.). The azocasein test revealed a significantly ($p<0.05$) diminished proteolytic activity in glomerular homogenates of ADR-treated animals (c: 4.91±1.49 U/h/mg protein, x±SD, n=5; ADR: 1.71±1.02, n=3) (Fig. 2).

Fig. 1: ADR-effect on protein permeability (measured as sieving coefficient for albumin) and water permeability (measured as hydraulic conductivity) of single isolated perfused glomeruli of Myxine glutinosa. Results are expressed as % of corresponding control values (C).

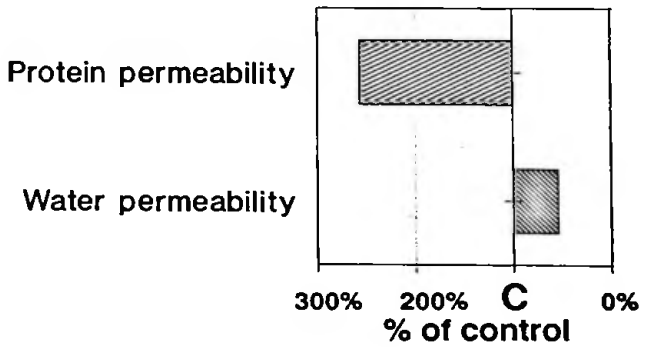
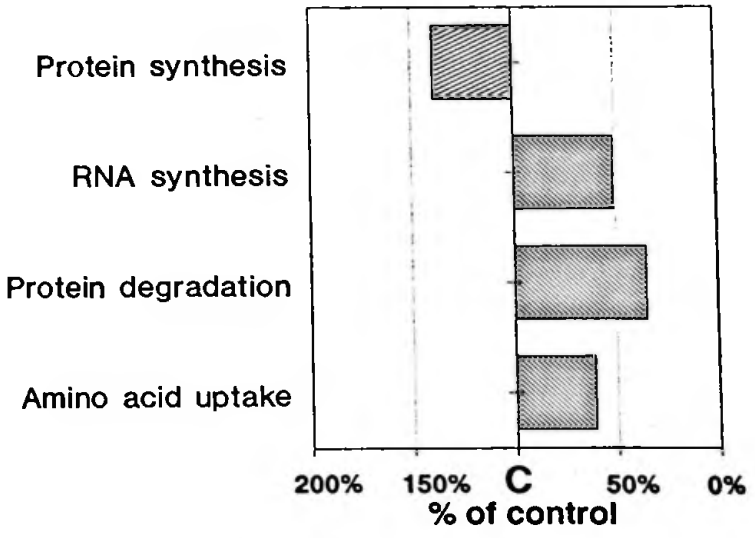


Fig. 2: ADR-effect on in vitro protein synthesis, RNA synthesis, protein degradation and amino acid uptake of isolated glomeruli of ADR-pretreated Myxine glutinosa after 6 h incubation. Results are expressed as % of corresponding control values (C).



Several pathomechanisms are leading to glomerular diseases summarized in Fig. 4: free radicals, DNA-interactions and other cytotoxic side effects as the altered activity of proteolytic enzymes. It is concluded that free radicals cause membrane dysfunction, leading metabolically to a decreased amino acid uptake and functionally to a decreased water permeability. It is further postulated that the increased protein synthesis, not preventable by the radical scavenger NAC, is a net-effect of disturbed synthesis and or degradation processes occuring at the same time. The decreased RNA-content is the direct result of an inhibited trancription, possibly leading to a reduced translation, which again can be compensated by a decreased degradation of proteins. The increase in net-protein synthesis leads to an increase in total protein content of the glomerulus. The accumulated protein could be material of the mesangial matrix or of the glomerular basement membrane causing functional alterations of the glomerular filtration barrier for proteins.

It is further proposed from this study that different final barriers are responsible for water and protein permeability. ADR impairs the function of these barriers via different mechanisms. The glomerular epithelium is supposed to be a target of ADR-derived radicals, thereby leading to a decrease in water permeability. The increased protein permeability could be explained by a sieving defect of the glomerular basement membrane, independent of radical formation.

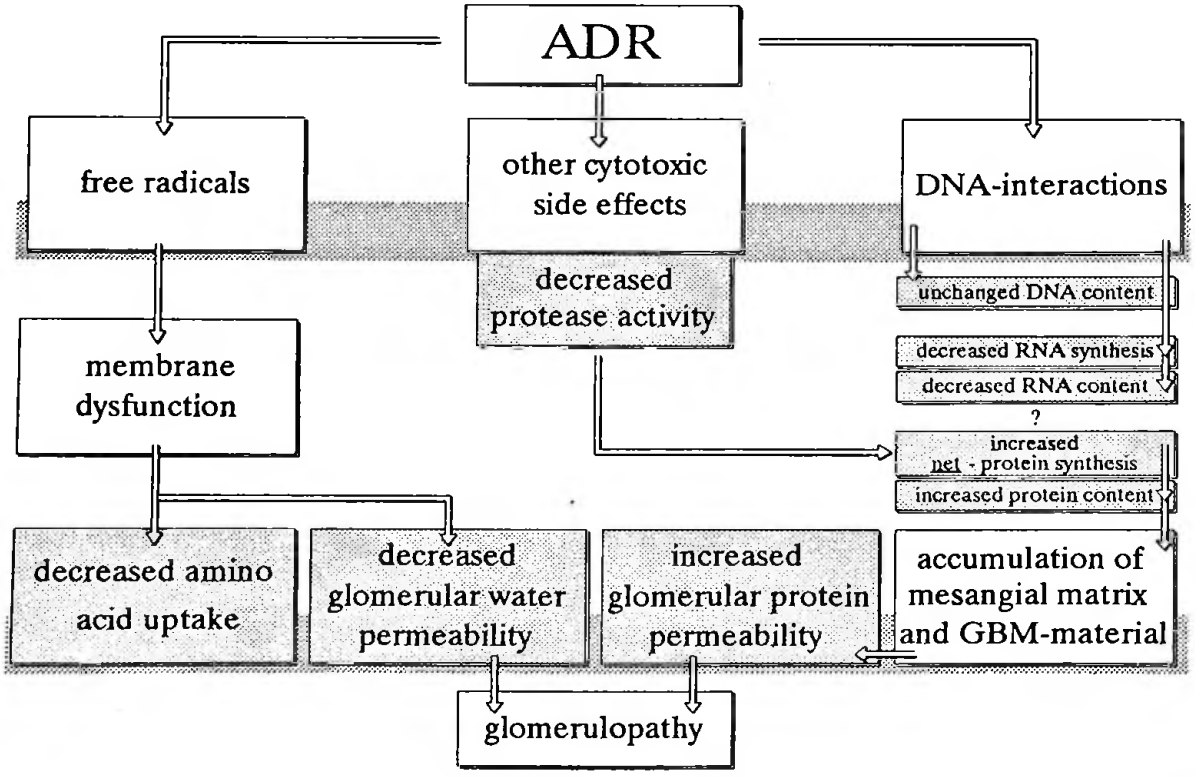


Fig. 3: Postulated cascade of pathologic events contributing to the development of a glomerulopathy
(results of this study)

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