THE EFFECTS OF ADRIAMYCIN AND CIS-PLATIN ON GLOMERULAR PROTEIN METABOLISM. STUDIES ON THE ATLANTIC HAGFISH MYXINE GLUTINOSA

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It is generally accepted that the cytotoxicity of the anticancer drug Adriamycin (ADR) is caused by enzymatic redox cycling leading to DNA-strand breaks, peroxidation of membrane lipids and enzyme inactivation (Scheulen M.E. et al., Archs. Toxicol. 60: 154, 1987). Other mechanisms as the intercalation into DNA and RNA with subsequent inhibition of replication, transcription and translation are also discussed (Gianni L. et al., Rev. Biochem. Toxicol. 5: 1, 1983).

In previous studies ADR-induced permeablity changes of the glomerular filtration barrier were investigated by applying the model of the single isolated perfused glomerulus of Myxine glutinosa. ADR caused increases in glomerular protein permeability and decreases in glomerular water permeability (Barbey M.-M. et al., Free Rad. Res. Comms. 7: 195, 1989). The studies presented here were aimed to elucidate whether the changes in glomerular function caused by ADR could be explained by concomittant alterations in glomerular metabolism. - ADR is described as a nephrotoxin that primarily targets for the glomerulus. As a negativ control the effects of Cis-diaminedichloroplatinum (II) (cis-platin) were also investigated. Cis-platin is described as a potent tubulotoxic anticancer substance with only minor effects on the glomerulus (Daugaard G. et al., Cancer Chemother. Pharmacol. 21: 163, 1988; Hacke M. et al., Clin. Physiol. Biochem. 1: 17, 1983). Therefore no alterations of glomerular metabolism were expected after application of this compound.

Glomerular metabolism was studied as de novo protein synthesis. The method of an in vitro incubation (tissue culture) of glomeruli isolated from the archinephron of the Atlantic hagfish Myxine glutinosa was applied. Last year this model which had so far only been described for mammalian glomeruli (e.g. Brendel K. et al., J. Pharmacol. Exp. Ther. 187: 342, 1973) was modified for studies on hagfish glomeruli (Kastner S. et al., Bull. MDIBL 29:127, 1990). - The model was also used to investigate whether glomerular RNA synthesis explains alterations in protein metabolism. - Total protein synthesis as studied by the ^{3}H aminoacid incorporation does no elucidate the effects of ADR on individual proteins. But to examine changes in glomerular structure and function it is of main interest to get closer insight into alterations of individual structural proteins of the glomerular filtration barrier (e.g. fibronectin, laminin, collagen IV). Therefore the turnover (synthesis and degradation) of fibronectin (FN) was examined. FN is mainly synthesised by the mesangial cells. As a structural protein of the mesangial matrix it is a major glomerular component.

Myxine was treated with 20 mg/kg b.w. Adriamycin by injection into the caudal blood sinus. 10 days after treatment the glomeruli were isolated by microdissection and incubated as described previously (Kastner S. et al., op. cit.) to determine total de novo protein synthesis by the incorporation of tritiated aminoacids. For cis-platin the experimental design and dosage were the same as for ADR. - De novo RNA-synthesis of glomeruli treated animals was determined by the incorporation of 6-^{3}H uridine. - 20 days after ADR-treatment changes in total FN content in glomerular homogenates were determined semiquantitatively by Western-blotting. All the homogenates were diluted to the same total protein content, separated by SDS-page on a 5-15 % linear

polyacrylamide gradient gel and transferred to a nitrocellulose matrix by the semi-dry technique using a discontinous buffer system.

Glomeruli of ADR-treated animals showed significant increases in glomerular protein synthesis (Figure 1). After 12 hours of incubation protein synthesis was significantly higher ($p < 0.001$) in glomeruli isolated from ADR-treated Myxine (1118 ± 425 dpm/μg protein, n=20) as compared to controls (665 ± 240 dpm/μg protein, n=26). With an increasing number of experiments and longer incubation times, our previously reported decreases in protein synthesis caused by ADR could not be verified. An enhanced protein synthesis is well in accordance with our own findings in rat glomeruli (Kastner S. et al., Renal Physiol. Biochem., 14: 48, 1991). - Cis-platin did not alter protein synthesis. There was no significant difference between glomeruli isolated from cis-platin treated Myxine and controls (Figure 2). However, this was not to be expected, because cis-platin treated animals served as a negative control.

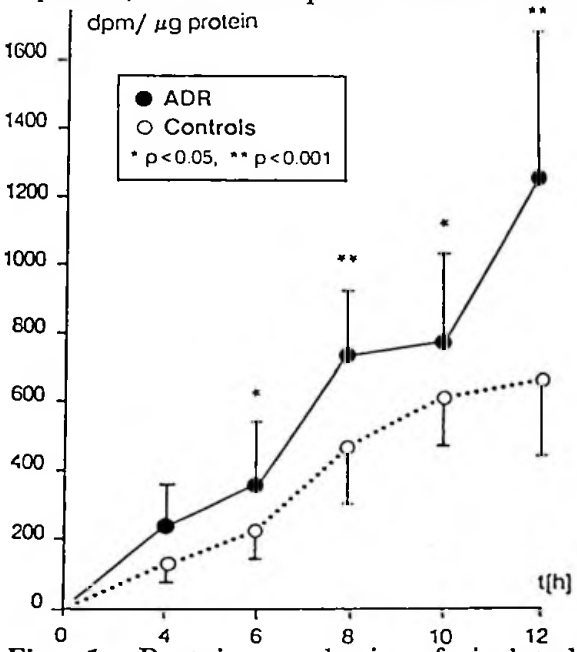

Fig. 1: Protein synthesis of isolated glomeruli of animals treated with Adriamycin (ADR) compared to controls. Results expressed as dpm/μg protein plotted against incubation time. $\bar{x}$ ± SD, statistical significance calculated by Student's t-test

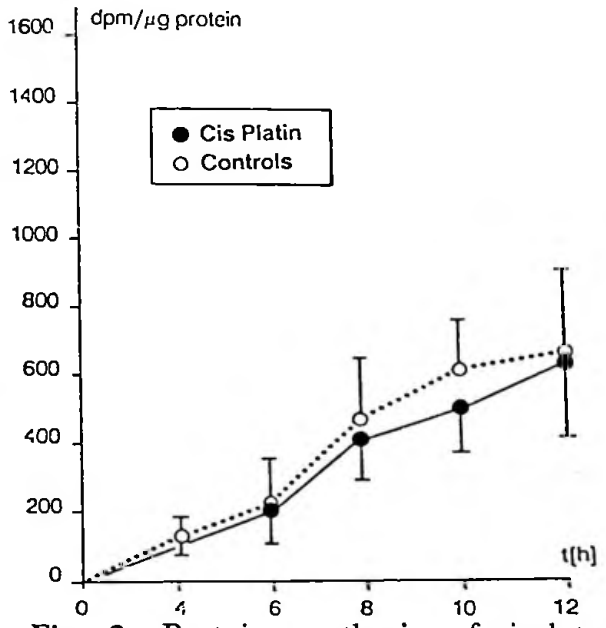

Fig. 2: Protein synthesis of isolated glomeruli of animals treated with Cis-platin (see figure 1).

Altough increases in protein synthesis were observed, no differences in RNA synthesis were found. ^{3}H uridine incorporation of glomeruli from ADR-treated animals did not differ from controls after an 6 or 8 hour incubation (Figure 3). The enhanced protein synthesis of glomeruli of ADR-treated animals could therefore not be explained by an enhanced RNA-synthesis. Hypothetically, a protein synthesis nevertheless increased could as well be due to ADR-effects on RNase activity. This would lead to a longer stability of RNAs, thus causing an increased expression of proteins.

Focusing on individual structural proteins of the glomerulus, it could be shown that the fibronectin content is elevated in glomeruli of ADR-treated animals. Semiquantitatively, protein blotting revealed a higher ratio of fibronectin to total protein

in glomerular homogenates of ADR-treated Myxine. The fibronectin-band occuring at 50 KD was much stronger in glomerular homogenates of ADR-treated animals as compared to controls (Figure 4).

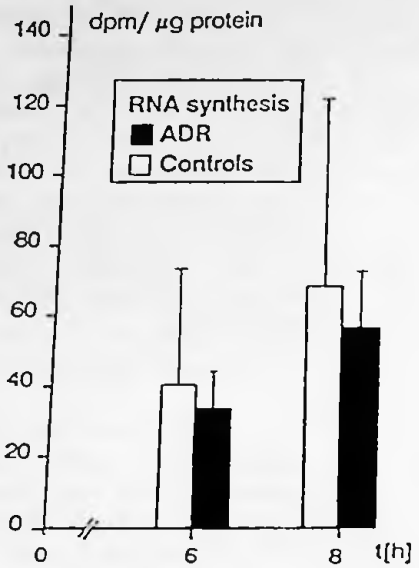

Fig. 3: RNA synthesis of isolated glomeruli of ADR-treated Myxine compared to controls. Results expressed as dpm/µg protein

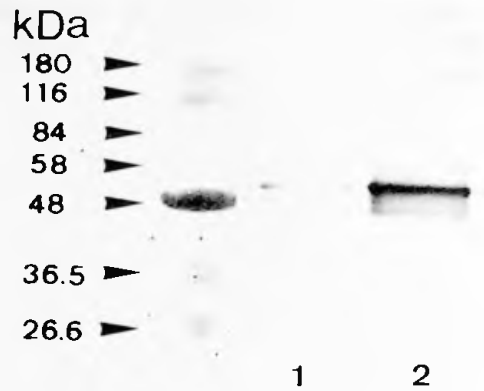

Fig. 4: Characterization of FN in homogenates from glomeruli of Myxine treated with ADR and of controls. Samples were diluted in sample buffer to same total protein concentrations, separated by SDS-page and analysed by Western-blotting.
Lane **1**: ADR-treated, 20 days
Lane **2**: control

The results show that ADR directly interferes with the glomerular metabolism. It leads to an enhanced protein synthesis and an enrichment of the extracellular matrix protein fibronectin in the glomerulus. As the mesangium is mainly involved in the turnover of fibronectin, this is in accordance with structural alterations decribed elsewhere. It was shown that a proliferation of the mesangium and the mesangial matrix and a thickening of the glomerular basement membrane occur in ADR nephrosis (Fajardo L.F. et al., Lab. Invest. 43: 24, 1980; O'Donell M.P. et al., J. Lab. Clin. Med. 106: 62, 1985). The alterations in glomerular permeability caused by ADR might therefore be related to the described impairment of glomerular protein metabolism .

Due to the marked morphological, functional and biochemical heterogeneity of the kidney chemically induced lesions are often restricted to discrete target sites. ADR is a model compound that selectively injures the glomerulus, whereas cis-platin primarily targets for the tubulus. The finding that ADR enhances protein synthesis, whereas cis-platin shows no effect, indicates that our model offers the same possibilities for studying specific target cell toxicity of nephrotoxic compounds as higher vertebrates.

As shown here in the model of isolated glomeruli of Myxine glutinosa in the cascade of nephrotoxic events finally leading to an impairment of kidney function, metabolic alterations can precede functional changes. Therefore, extrapolated on higher vertebrates or man, changes in glomerular protein synthesis or glomerular protein pattern (like changes in the glomerular content of the extracellular matrix protein fibronectin found in our study) may allow an early diagnosis of an altered kidney function.

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