MECHANISTIC STUDIES ON THE INFLUENCE OF ADRIAMYCIN ON AMINO ACID UPTAKE AND AMINO ACID INCORPORATION INTO ISOLATED GLOMERULI OF THE ATLANTIC HAGFISH MYXINE GLUTINOSA

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Previous metabolic studies on isolated and in vitro incubated glomeruli of the Atlantic hagfish Myxine glutinosa showed that Adriamycin (ADR) increases the incorporation of amino acids into glomerular proteins. This might be due to alterations in different pathways:

- 1. uptake of amino acids into the glomerulus
- 2. RNA-synthesis
- 3. proteolytic degradation

It is generally accepted that the cytotoxicity of the anticancer drug Adriamycin 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). Therefore we also focused on the involvement of free radicals. 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).

Myxine was treated with 20 mg/kg b.w. ADR by injection into the caudal blood sinus. A second group of animals was treated with 20 mg/kg b.w. ADR + 450 mg/kg b.w. of the sulfhydryl-donor N-Acetylcysteine (NAC); a third group was treated with NAC alone. 10 days after treatment the glomeruli were isolated by microdissection and incubated with ^{3}H amino acids as described previously (Kastner, S. et al., Bull. MDIBL 29: 127, 1990). After incubation the total uptake of ^{3}H amino acids into the glomerulus and the incorporation of ^{3}H amino acids into glomerular TCA-precipitable proteins were determined. Glomerular de novo RNA-synthesis was quantified by the incorporation of 6-^{3}H uridine into RNA of glomeruli from controls and ADR-treated animals (Kastner, S. et al., Bull. MDIBL 30: 120, 1991).

The results showed that the amino acid uptake into glomeruli of ADR-treated animals is significantly inhibited compared to controls. Studies on glomeruli isolated from animals which received combined treatment of ADR and the radical scavenger N-Acetylcysteine revealed that NAC could prevent the inhibition of amino acid uptake after ADR-treatment (Fig. 1). 12 hours after incubation there is no significant difference in amino acid uptake between the control group (13942 ± 685 DPM/glomerulus, n=10) the NAC group (14190 ± 2074, n=10) and the ADR+NAC group (16045 ± 806, n=11). The amino acid uptake into glomeruli of ADR-treated animals is decreased to 7649 ± 1010 DPM/glomerulus (n=10). - In contrast the stimulation of amino acid incorporation into glomerular TCA-precipitable proteins could not be prevented by the radical scavenger N-Acetylcysteine. 12 hours after incubation the amino acid incorporation is not significantly different between the ADR group (7353 ± 926 DPM/glomerulus, n=27) and the ADR+NAC group (6633 ± 866, n=20) (Fig. 2). - RNA-synthesis quantified by the incorporation of 6-^{3}H uridine into glomerular RNA is significantly decreased after 8 hours incubation in glomeruli of ADR-treated animals (206 ± 22 DPM/glomerulus, n=26) compared to controls (438 ± 60, n= 45) (Fig. 3).

As already suggested these studies indicate that Adriamycin acts via different pathomechanisms: - 1. ADR reduced the amino acid uptake into glomerular cells. This effect is preventable by the radical scavenger N-Acetylcysteine. Therefore oxidative stress on membrane components seems to be a reasonable explanation for the inhibited

amino acid uptake. Amino acid incorporation into glomerular proteins is increased. This increase is not preventable by the radical scavenger NAC. This effect is best explained by metabolic disturbances in protein synthesis and/or protein degradation. - 2. The RNA-studies showed after 8 hours incubation a significant inhibition of RNA-synthesis in glomeruli isolated from ADR-treated animals. The enhanced amino acid incorporation of glomeruli from ADR-treated animals could therefore not be explained by an enhanced RNA-synthesis. Hypothetically, increased protein synthesis could as well be due to ADR-effects on RNase activity. This could lead to a longer stability of RNAs, thus causing an increased expression of proteins. - 3. Another explanation for the accumulation of ^{3}H amino acids in glomerular proteins might be a decreased degradation caused by an inhibition of proteolytic enzymes. It has been frequently reported that ADR interferes with proteolytic activities either directly or mediated by reactive oxygen species. Therefore it remains to be further elucidated if the accumulation of ^{3}H amino acids in glomerular proteins is due to an enhanced protein synthesis or a decreased proteolytic degradation.

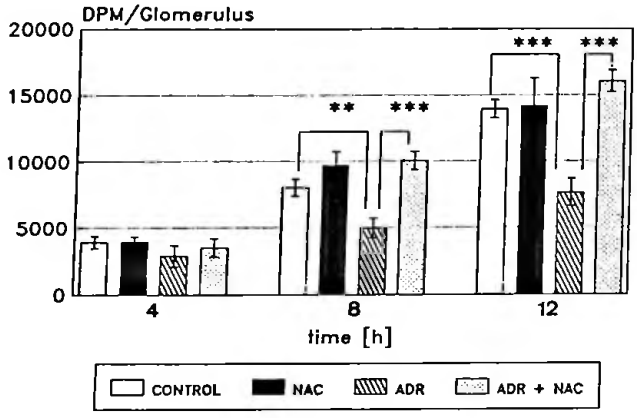

Fig. 1: Amino acid **uptake** into the glomerulus, results expressed as DPM/glomerulus ± S.E.M., statistical significance calculated by Students t-test
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = not significant

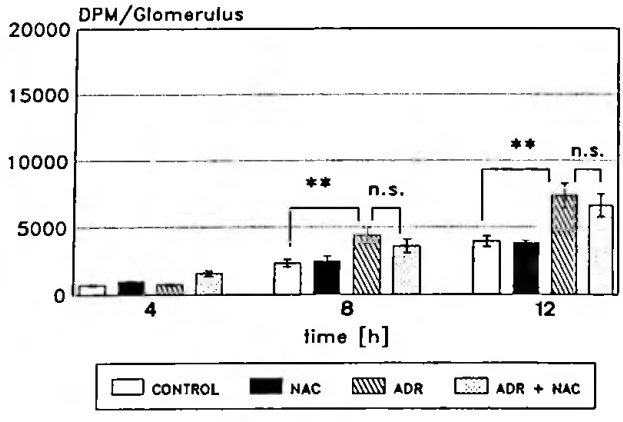

Fig. 2: Amino acid **incorporation** into glomerular TCA-precipitable proteins (see Fig. 1)

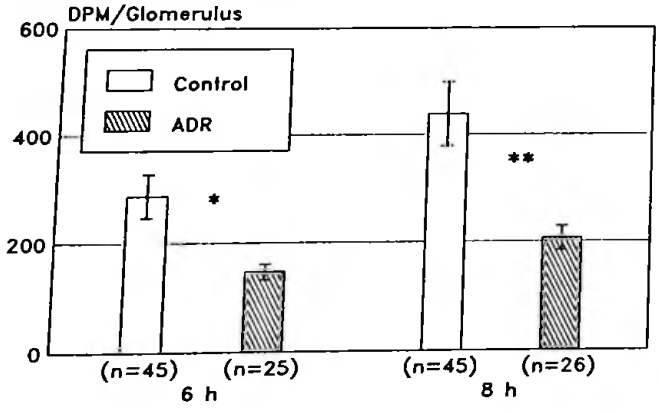

Fig. 3: Glomerular RNA synthesis, results expressed as DPM/glomerulus ± S.E.M. (see Fig. 1)

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