IN VITRO EVALUATION OF ADRIAMYCIN NEPHROTOXICITY STUDIES ON ISOLATED GLOMERULI OF THE HAGFISH MYXINE GLUTINOSA

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The use of isolated and in vitro incubated glomeruli from mammalian kidneys as a model to monitor glomerular metabolism and to screen the effects of various nephrotoxins is a well established method often described previously (Brendel K. et al., J. Pharmacol. Exp. Ther. 187: 342, 1971; Blau E. et al., J. Lab. Clin. Med. 77: 97, 1971).

Our study was aimed (I) to verify if glomeruli isolated from the archinephron of the Atlantic hagfish Myxine glutinosa offer the same possibilities for metabolic investigations as those isolated from mammalian kidneys and (II) to examine if the anticancer drug Adriamycin causes any alterations in glomerular protein synthesis.

It is generally accepted that the cytotoxicity of Adriamycin is caused by enzymatic redox cycling leading to DNA-strand breaks, peroxidation of membrane lipids and enzyme inactivation. Other mechanisms as the intercalation into DNA and RNA with subsequent inhibition of replication, transcription and translation are also discussed (Scheulen M.E. et al., Archs. Toxicol. 60: 154, 1987). Here, protein synthesis was chosen as a parameter of metabolic activity, quantified by the incorporation of radiolabeled amino acids into cellular constituents of the glomerulus.

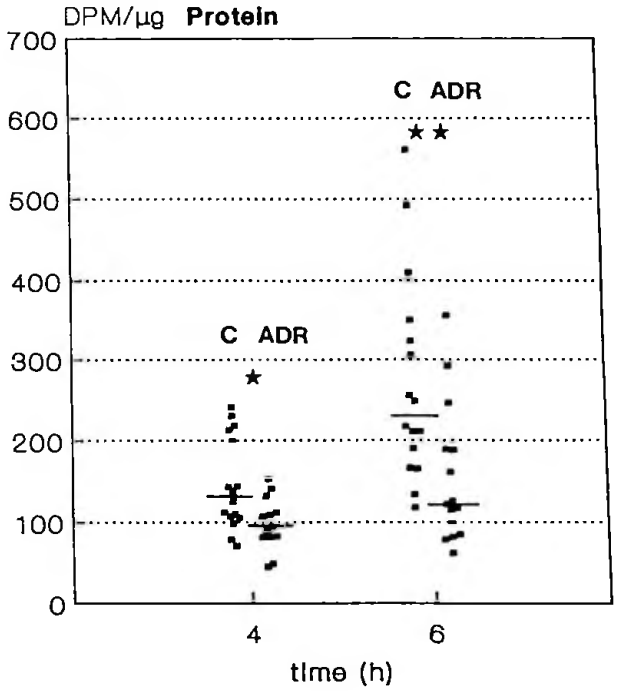

Fig. 1: Amino acid incorporation into isolated glomeruli of Myxine glutinosa expressed as DPM/μg protein plotted against incubation time [h]
Statistical significance calculated by the two sided U-test for independent measurements
* $2\alpha < 0.05$; ** $2\alpha < 0.01$
—— = median value
C = control
ADR = Adriamycin-treated

Ten days after ADR-treatment (20 mg/kg b.w.) the glomeruli were isolated and incubated at 4°C in 1 ml of Myxine-Ringer solution (Riegel J., J. Exp. Biol. 73: 261, 1978) enriched with B-Vitamins, glucose, creatine-phosphate, GTP, ATP and containing 10 μCi/ml of the tritiated amino acid mixture. For comparison the same experiments were performed on control animals. For determing ^{3}H amino acid incorporation the incubations were stopped at 4 and 6 hours by addition of 6 % (w/v) TCA. After washing, the glomerular precipitate was dissolved in 0.5 m NaOH for 12 hours. After addition of 4 ml of scintillation cocktail the incorporated radioactivity was quantified in a ß-scintillation counter. The results were expressed as DPM refering to total

protein content (Bradford M.M., Anal. Biochem. 72: 248, 1976) and total DNA content (Thomas P.S. et al., Anal. Biochem. 89: 35, 1978; modified), respectively.

As shown in fig. 1, the isolated glomeruli are in vitro capable of carrying out metabolic processes, maintaining viability for at least 6 hours. This could be observed in both groups, control glomeruli and those isolated from ADR-pretreated animals. Further, it could be shown that glomerular metabolism is markedly influenced by the administration of Adriamycin. The activity of amino acid incorporation significantly decreased in glomeruli isolated from the archinephron of pretreated fish; the slope from 4 to 6 hours was less pronounced after ADR-treatment in comparison with control data.

For further insight the protein/DNA ratio was determined. Fig. 2b shows a lower protein/DNA ratio in glomeruli of animals treated with ADR in contrast to control animals (fig. 2a). This is in accordance with a reduced protein synthesis (see fig. 1).

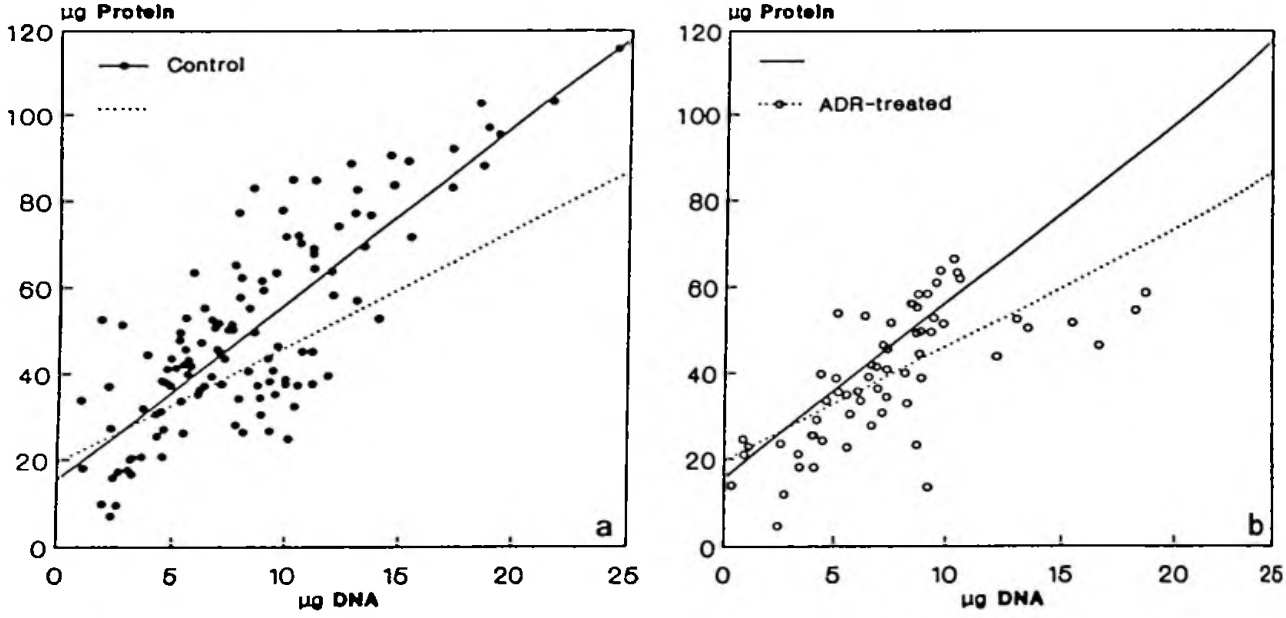

Fig. 2: Relationship between total protein content [μg] and total DNA content [μg] in isolated glomeruli of Myxine glutinosa

In summary it could be shown that isolated and in vitro incubated glomeruli of Myxine glutinosa are a valuable tool to study glomerular metabolism and to quantify the effects of nephrotoxic substances. Further our results demonstrate that the anticancer drug Adriamycin lowers the incorporation of amino acids into glomerular cell constituents, indicating an inhibition of glomerular protein synthesis.

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