FREE RADICAL FORMATION AND ADRIAMYCIN NEPHROTOXICITY. STUDIES IN THE HAGFISH (MYXINE GLUTINOSA).

Fels, Lüder-Meinert; Barbey, Mark-Michael;
Soose, Mechthild; Stolte, Hilmar
Department of Nephrology, Medical School Hannover , FRG

The antineoplastic compound adriamycin (ADR, doxurubicin hydrochloride) is a widely used anthracycline antibiotic, but its use is restricted by a variety of severe toxic effects (Lefrak et al, Cancer 32: 302,1973). The early toxic effect of ADR is suggested to be related to oxygen free radicals, generated through ADR bioreduction (Myers C.E. Anthracyclines. In: Pharmacological principles of cancer treatment. B.Chabner (ed.), Sunders, 1982; Mimnnaugh et al, Biochem Pharmac 35: 4327, 1986). ADR can lead to acute renal failure by selectively damaging the glomerulus. A single dose of 5 mg/kg b.w ADR caused increases in protein permeability and a reduction of GFR in rats (Soose et al., Clin. Physiol. Biochem. 6:310, 1988).

The animal model of the hagfish Myxine glutinosa was used in this study to gain further insight into the first mechanisms of action of ADR at the level of the whole animal and at the level of single glomeruli.

12 hours after injection of ADR (7.5 mg/kg b.w.) into the caudal blood sinus a predominant accumulation in the liver, kidney and heart could be observed. Plasma levels of malondialdehyde, reported as a final product of lipid peroxidation (Tanizawa et al., Chem Pharm Bull 31 : 1714, 1983) were found to increase significantly after application of ADR (fig. 1).

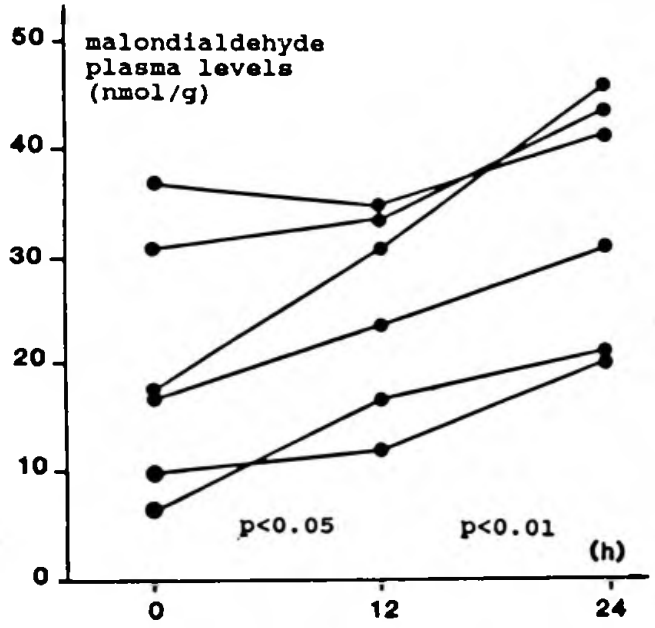

Fig. 1 Increases of plasma levels of malondialdehyde as a product of lipid peroxidation 12 and 24 hours after injection of 20 mg/kg b.w. ADR in hagfish.

Glutathione is a major protectant against autooxidation of cellular components (Ross, Pharmac Ther 37: 231, 1988). Glutathione (GSH) concentrations were measured (Yang et al., Toxicol 45: 25, 1987) in different tissues of Myxine since it has been shown that low endogenous GSH pools render cells more susceptible to free radical attack (Olson et al., Life Sci 29: 13993, 1981). Glutathione concentrations in Myxine were comparatively low; with 1.58 ± 0.6 µmol/g wet weight the kidney showed concentrations four times lower than measured in rats with the same assay.

To study the effects of ADR-uptake into the kidney and the effects of a generation of malondialdehyde in Myxine, the model of the isolated perfused single glomerulus of Myxine (Elger, Stolte, Bull MDIBL 24: 56, 1984) was used. The parameters measured were permeability changes of the glomerular barrier for water and proteins. Single glomeruli were perfused in vitro with a Ringers solution containing 0.75 g/100 ml albumin. The ultrafiltrate was analysed for protein (Rocket-Immunoelectrophoresis according to Laurell, Anal Biochem 15: 45, 1966). Measurements of filtration rate, of filtration pressure and direct morphometric measurements of the glomerular capillary surface allowed a determination of the k-value, that is the hydraulic conductivity of the individual glomeruli.

ADR (20 mg/kg b.w.) resulted in decreases of water permeability (k), whereas the sieving coefficient (φ) for protein increased (fig.2). There were no indications that ADR causes a reduced renal perfusate flow by affecting the renal vasculature. It is therefore assumed that ADR directly leads to changes of the glomerular barrier.

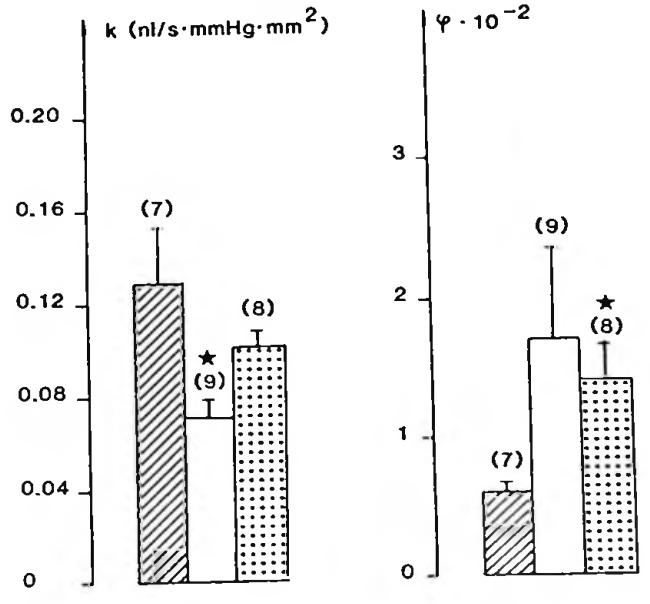

Fig. 2 Changes of hydraulic conductivity (k) and sieving coefficient for albumin (φ) ten days after application of 20 mg ADR/kg b.w. Controls: hatched bars. ADR-treated: white bars. ADR plus N-acetylcysteine: dotted bars. x ±S.E.M., *p < 0.005

As sulhydryl donors have been effective in preventing cells from oxidative damage by free radicals (Olson et al., Ibid.), the protective effect of N-acetyl cysteine (NAC) was studied. Combined treatment of ADR and NAC (given o.55 before and after and 4 hours after ADR-application, total dose 450 mg/kg b.w.) lessened the changes of protein and water permeability caused by ADR (fig.2).

Formation of free radicals during metabolism of ADR is dependent on the presence of oxygen (Scheulen et al., Arch Toxicol 60: 154, 1987). Animals were therefore exposed to artificial atmospheres of either 20 % O_2 /80% N_2 or 80% O_2 /20% N_2 for 48 hours. After further 8 days under normal O_2 - conditions, a significant decrease of the water permeability (k) was measured in the group exposed to 80 O_2 . No correlation was found between oxygen pressure applied and protein permeability (tab. 1). This oxidative component of ADR-induced cytotoxicity has also been reported for cardiac myocyte cultures, where lipid peroxidation could only detected when the cells were exposed to ADR and hyperoxia (Julicher et al, Res Com Chem Path Pharm 47: 35, 1985).

Tab. 3 Influence of ADR (5 mg/kg b.w.) under different oxygen pressures on hydraulic conductivity (k) and sieving coefficient for albumin (φ) of the isolated perfused single glomerulus of Myxine glutinosa

	n	k . 10^{-2} (nl/s mmHg mm)	φ . 10^{-2}
controls (20 or 80 % O_2)	7	5.9 ± 1.7	1.3 ± 0.2
ADR 20 % O_2	8	4.6 ± 3.2	8.1 ± 9.6 *
ADR 80 % O_2	7	3.3 ± 2.6	6.9 ± 6.7 *

x ±S.D., + $p < 0.05$ against controls

The increased protein permeability of the ADR-impaired single glomerulus could be explained with a sieving defect of the glomerular basement membrane. Former studies of ADR nephrosis in rats indicated that the functional charge barrier remained intact and proteinuria derived from a size selective defect of the GBM (Weening et al., Kidney Int 24: 152, 1983).

The glomerular epithelium is considered the main barrier for water. ADR-induced lesions could occur here, thereby leading to an altered permeability for water. In rodent kidneys ADR-induced losses of foot processes and replacement of epithelial cytoplasm have already been shown (Fajardo et al., Lab Invest 43:242, 1980).

It is concluded that the early nephrotoxic effect of ADR is mediated by free radical formation. Oxidative stress on membrane compounds seems to reduce the water permeability of the glomerular barrier, while the protein permeability increases. Free radicals could also lead to DNA-strand-breaks as indicated by Scheulen et al. (Ibid.), thus altering the metabolism and/or synthesis of glomerular components. This would be in accordance with a proliferation of mesangial matrix in ADR nephrosis (Fajardo et al., Ibid.), possibly reflected by an impaired turnover of the extracellular matrix protein fibronectin in ADR-treated rats (Soose, unpublished data).

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