

## LOW PROTEIN DIET PREVENTS GLOMERULAR DAMAGE IN ADRIAMYCIN-TREATED RATS

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### Summary

The effect of two isocaloric diets with different protein content (6% and 20% protein) on urinary protein excretion, renal function and glomerular morphology was investigated in a model of glomerular damage induced in rats by adriamycin which closely mimics human minimal change nephropathy. Unlike animals fed the standard diet, animals fed the low protein diet were protected from the development of glomerular damage.

### Introduction

Evidence is now emerging which indicates that dietary manipulation influences experimental [1-4] and human renal diseases [5]. Protein restriction reduces glomerular injury in rats with nephrotoxic serum nephritis [1] and protects NZB x NZW mice from the development of immune nephritis [2]. Proteinuria is also decreased in rats with reduction of renal mass by a limited protein intake [4]. It has been suggested that in this model low protein intake is beneficial because the diet limits the haemodynamic changes occurring in surviving nephrons [6]. We have investigated the effect of a low protein diet on proteinuria, renal function and glomerular morphology in the model of adriamycin-induced glomerulopathy in rats [7].

Adriamycin induces glomerular damage in rats characterized by persistent proteinuria which develops fully 13-15 days after a single intravenous injection of 5mg/kg of the drug. Electronmicroscopy shows alterations of glomerular visceral epithelial cells with foot process fusion. The clinical and morphological expression of the disease resembles minimal change nephropathy in humans.

### Materials and methods

*Experimental design* Sprague-Dawley (CD-COBS) male rats (Charles River Italia, S.p.A., Calco, Italy) weighing 185-200g at the start of the experiment

were used. Nephrotic syndrome was induced by a single intravenous injection of 5mg/kg adriamycin (Atriblastina, Farmitalia Carlo Erba, Milan, Italy) as previously described [7]. Control rats received the solvent alone.

Rats were divided into six groups: Group 1 (n=10) received adriamycin and were fed a standard diet (Altromin-Rieper, Vandoies, Italy) containing 20 per cent protein. Group 2 (n=10) were fed a low protein diet containing six per cent protein, starting seven days before adriamycin and continuing throughout the experimental period. Group 3 (n=5) were fed a low protein diet containing six per cent protein, starting the day after adriamycin. Group 4 (n=10) served as control and were fed a standard diet. Groups 5 and 6 were used to study kidney distribution of adriamycin as described below.

*Diets* The two diets were isocaloric (3300Kcal/kg) and differed in their protein content, six per cent of weight for the low protein diet and 20 per cent for the standard diet. Other calories were supplied by corn starch, saccharose, cellulose and soy oil. Mineral and vitamin supplements were equal in the two diets, in particular the diet phosphorus content was the same. All rats had free access to water.

*Renal distribution of adriamycin* Two groups of rats were used: Group 5 (n=15) were fed the standard diet and group 6 (n=15) were fed the low protein diet, starting seven days before the experiment. Three animals per point of each group were killed 5, 15, 30, 120 and 360 minutes after adriamycin (5mg/kg intravenously). Kidneys were removed and immediately frozen at  $-20^{\circ}\text{C}$  until analysis. Kidney adriamycin was quantified by high performance liquid chromatography as previously described [8].

*Renal clearance studies* Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined by inulin and p-aminohippuric acid (PAH) clearance methods. Rats were anaesthetized by intraperitoneal injection of Inactin (16mg/100g of body weight), placed on a constant temperature table and tracheotomized; 2.5ml of a solution containing 5% inulin (E Merck, Darmstadt, FRG) and 0.2% PAH (Sigma Chemical Co, St Louis, Missouri, USA) in saline solution, was injected as a priming dose, followed by constant infusion. A 60 minute period of constant infusion was allowed before initiation of clearances. One hundred microlitre blood samples were obtained from the femoral artery at the mid-point of each clearance period. Urine was collected via a polyethylene tube inserted in the bladder. Inulin and PAH concentrations in plasma and urine were determined by using colorimetric assay modified for microlitre samples. During the experiments, arterial blood pressure was monitored. Each experiment involved three clearance periods each lasting 40 minutes.

*Histological studies* Renal tissue specimens were obtained from kidney biopsies. Light microscopy and electronmicroscopy studies were performed according to the method previously described [7].

*Other investigations* Total serum proteins were measured according to the

method of Lowry, with bovine serum albumin as standard. Urines were collected using metabolic cages over a 24-hour period and proteinuria was determined by the sulphosalicylic acid method.

Results were analysed by unpaired Student's 't' test and Duncan's multiple range test.

## Results

*Proteinuria and morphological studies* Adriamycin treated rats on the standard diet, group 1, developed glomerular disease. Three weeks after adriamycin injection, the animals were heavily proteinuric ( $398 \pm 112$  mg/day) and the abnormal proteinuria persisted throughout the experimental period. Table I

TABLE I

Group	n	Serum protein (g/dl)	Urinary protein (mg/day)	GFR (ml/min/100g)	RPF (ml/min/100g)	FF (%)
1	10	6.7 $\pm$ 1	607 $\pm$ 136	0.76 $\pm$ 0.24	2.14 $\pm$ 0.37	36.0 $\pm$ 13.7
2	10	6.5 $\pm$ 0.8	8 $\pm$ 3	0.95 $\pm$ 0.21	3.82 $\pm$ 0.75	26.0 $\pm$ 9.1
3	5	6.4 $\pm$ 0.9	7 $\pm$ 4	—	—	—
4	10	7.0 $\pm$ 0.5	8 $\pm$ 5	0.86 $\pm$ 0.16	2.57 $\pm$ 0.43	34.0 $\pm$ 6.1

Results are expressed as mean  $\pm$  SD

Statistical analysis is reported in the text (Results section)

shows the urinary protein values at day 28 after adriamycin (groups 2, 3, 4 =  $p < 0.001$  – Duncan's multiple range test – compared to group 1). Light microscopy showed only a swelling of podocytes, whereas by electronmicroscopy glomerular visceral epithelial cells displayed very pronounced changes with foot process fusion, cytoplasmic blebs, protein droplets and villous transformation.

Rats fed the low protein diet, groups 2 and 3, did not develop glomerular damage. Protein excretion was within the normal range ( $8 \pm 5$  mg/day) throughout the experimental period. No alterations in the kidney specimens were found by light microscopy, and glomerular and tubular ultrastructure appeared normal in all specimens examined by electronmicroscopy.

*Renal distribution of adriamycin* Kidney distribution of adriamycin, measured at different times after injection in rats on the standard or the low protein diet, was similar in the two groups of rats (Figure 1).

*Renal clearance studies* In animals of groups 1 and 2, GFR as measured by inulin clearance, was the same as in the control group (Table I). RPF, as measured by PAH clearance was increased in group 2 compared to controls ( $p < 0.01$ , Duncan's multiple range test). The filtration fraction (FF) calculated from GFR and RPF was not significantly different in the three groups.

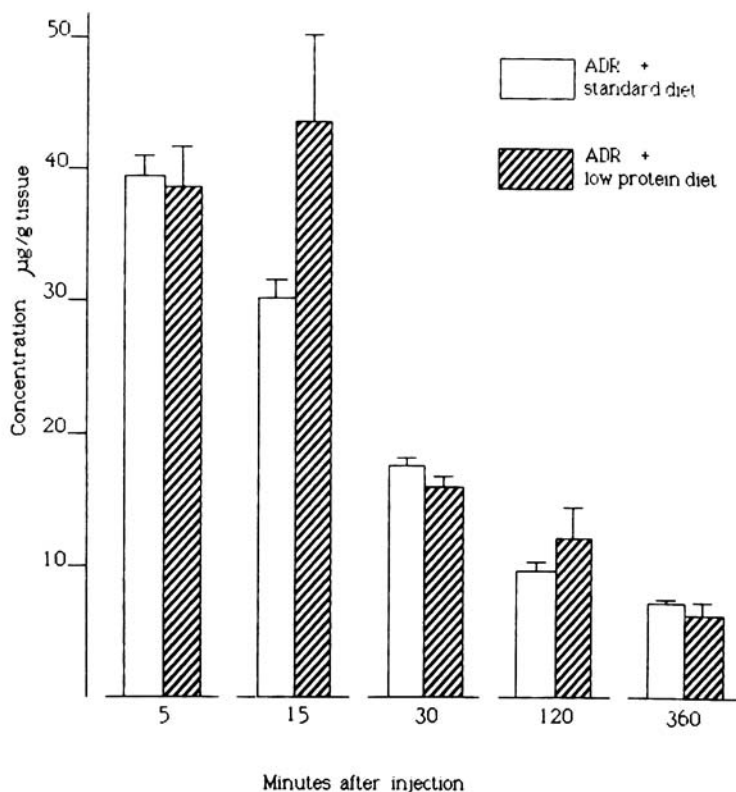


Figure 1. Kidney levels of adriamycin at different times after drug injection in rats fed the standard or the low protein diet

*Other investigations* Serum proteins measured at the end of the experimental period in the three groups of adriamycin treated rats were comparable to the values found in the control group (Table I).

## Discussion

Our results demonstrate that dietary restriction protects animals from developing glomerular damage induced by adriamycin. Animals fed a low protein diet had negligible proteinuria in comparison to rats fed a standard diet. The protective effect of the low protein diet does not result from a different exposure of the kidneys to adriamycin. This evidence derives by direct measurements of the drug concentration in renal tissue and by the finding that rats starting the low protein regimen the day after adriamycin had the same degree of protection as animals starting the diet one week before adriamycin injection. The mechanism by which the low protein diet protects against proteinuria cannot be established on the basis of the results of the present study. GFR and RPF were not significantly modified in nephrotic rats receiving the standard diet compared to

control animals. On the contrary, the low protein regimen induced a significant elevation in RPF compared to the standard diet, but had no influence on GFR. It has been recognized that glomerular filtration of proteins is governed by the permeability properties of the glomerular membrane and depends also on haemodynamic conditions through the convective and diffusive transport of macromolecules across the glomerular capillary wall [9]. Since dietary manipulation in our experimental conditions was associated with changes in RPF we wondered whether these changes could be responsible for the anti-proteinuric effect of protein restriction. The increase in RPF without change either in GFR and in afferent arteriole plasma protein concentration would result in a decreased protein concentration in the efferent arteriole. Using a mass balance equation of protein flux across the glomerular network, we estimated that the efferent arteriole protein should decrease, with a low protein diet, from 10.4 to 8.8g/dl. The protein transport across glomerular membrane is linearly dependent on plasma protein concentration in glomerular capillaries acting on the diffusion and convection of macromolecules across the membrane. A decreased plasma protein concentration in rats on a low protein diet, induced by the higher value of RPF, could explain a reduction in protein excretion; however, our data indicate a dramatic decrease in proteinuria that is unlikely to be justified by the slight decrease in plasma protein concentration along the glomerular capillary bed. The observed haemodynamic changes can only offer a partial explanation for the protective effects of the low protein diet that can be better explained by preservation of the glomerular membrane selectivity properties.

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