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Functional status of the liver during chronic renal failure: an experimental study in the rabbit

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ABSTRACT – Quantitative and qualitative measures of liver function were investigated in rabbits with chronic renal failure (CRF) induced 3 months earlier by surgical reduction of renal mass, and compared with a sham-operated control group. In the CRF group the galactose elimination capacity (GEC) was significantly decreased by 25%, but when related to liver weight the difference was not statistically significant. The clearance of antipyrine was unaffected. The serum activities of alanine aminotransferase, lactate dehydrogenase and alkaline phosphatase were similar in the two groups. The prothrombin index was increased by 20%, and the serum albumin concentration decreased by 9%. By light microscopy no significant morphological changes were found in the livers of the CRF rabbits. The results do not indicate significant changes of the hepatic functional status during moderate chronic renal insufficiency.

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The evidence of altered liver function in patients with chronic renal failure (CRF) consists of increased serum levels of various coagulation factors (1–3) and decreased activity of serum aspartate aminotransferase (4, 5). Rats with CRF have decreased synthesis of albumin (6), and changes in the hepatic drug metabolism during CRF have been described in rats and rabbits as well as in man (7–9). However, CRF is not associated with any clinical syndrome related to changes in liver function (10).

In the present study the effects of CRF on quantitative and qualitative measures of hepatic function as well as liver morphology were investigated in the rabbit to determine whether this experimental model might be useful for studies of the inter-relationships between renal insufficiency and liver function.

Material and methods

Male rabbits of the White Danish Country strain, aged 3–4 months and weighing 3–3.5 kg were used. Chronic renal failure (CRF) was induced in 12 rabbits by a two-step procedure (11). During general anaesthesia with phenobarbital about two-thirds of the surface of the left kidney was cauterized with a heated needle through an abdominal incision. Three weeks later the right kidney was removed. Eight control rabbits were sham-operated twice. All rabbits received 110 g per day of standard rabbit pellets (Boserup®, Faxe, Denmark) to prevent excessive weight gain of the sham-operated controls. Free access to tap water was allowed.

Three months after surgery, renal and hepatic functions were assessed by routine laboratory blood tests.

The glomerular filtration rate was measured as the total plasma clearance of ^{51}Cr-EDTA (11).

The galactose elimination capacity was measured by the single-injection technique. The rabbits were placed in restraining boxes placed on plastic trays to collect voided urine. A catheter was placed in the central artery of one ear for blood sampling and a weighed amount of galactose from a 50% solution (4 mmol per kg body weight) was injected intravenously into the other ear. During the next 3 h, 15–19 timed blood samples were obtained. After the last sample a catheter was inserted to empty the urinary bladder followed by irrigation with 2×10 ml of isotonic saline. The galactose concentrations of the 50% solution and the blood and urine samples were determined enzymatically (12).

The galactose elimination capacity was calculated as $GEC = \frac{A-U}{t_{c=o}+7}$, where A is the amount of galactose injected, U the amount excreted in the urine, $t_{c=o}$ the interception on the time axis of the linear regression of arterial galactose concentration on time, and 7 is a correction for the equilibration of galactose in its volume of distribution (V^D_{gal}) during the elimination (13). The regression analysis included samples obtained from 25 min after the injection until the concentration was below 2 mmol/l, assuming that the injected dose of galactose had equilibrated before 25 min and that the elimination of galactose in rabbits follows kinetics with a low K_m, as it does in pigs (14), with the concentration of galactose declining linearly with time in that interval. V^D_{gal} was calculated as A/C_o, where C_o is the interception of the regression line on the concentration axis.

The clearance of antipyrine was determined simultaneously with the GEC. A weighed amount of a 10% solution of antipyrine (60 mg per kg body weight) was injected into an ear vein and four venous blood samples were drawn from the other ear 3–7 h after the injection. Heparinized plasma samples were analyzed for antipyrine by high pressure liquid chromatography (15). The decrease in plasma concentration of antipyrine was assumed to follow first order kinetics after a distribution phase of 3 h. The clearance of antipyrine was calculated from the linear regression of the log concentration on time as clearance = $k \cdot dose/C_o$, where k is the elimination constant and C_o is the extrapolated concentration at time zero. The volume of distribution V^D is equal to $dose/C_o$.

Plasma concentrations of creatinine and protein and the serum activities of alkaline phosphatase, alanine aminotransferase and lactate dehydrogenase were measured by an automatic clinical analyzer (ACA, Dupont Instruments, Wilmington, Delaware, USA). Albumin was determined by the succinate acid buffer method (16), and the prothrombin index as described by Owren & Aas (17).

Statistics

Differences between the two groups were evaluated by the Mann-Whitney rank-sum test. Linear regression was calculated using the method of least squares.

Morphology

When the studies of liver function were completed, the rabbits were killed. Specimens of the livers were fixed in phosphate-buffered 4% formaldehyde (pH 7.0) and processed routinely for light microscopy. Sections were cut at 3 µm and stained with hematoxylin-eosin, orcein, Van Gieson-Alcian and PAS with and without pretreatment with diastase. The slides were evaluated blindly.

Results

The body weight (BW) of the CRF rabbits was significantly decreased, the median value being 82% of the value in the control group (Table 1). The liver weight was 66% of the median value of the controls ($P < 0.05$) but the relative liver weight (g per kg BW) did not differ significantly from the control group. The renal function was significantly reduced, as indicated by the decreased ^{51}Cr-EDTA clearance (26% of the control value) and the three times higher plasma concentration of creatinine. No difference was found in the concentration of total protein, whereas serum albumin and the haematocrit value were both significantly decreased in the CRF rabbits. The median prothrombin index in the CRF group was 20% higher than in the control rabbits ($P < 0.05$). The serum activities of alkaline phosphatase, lactate dehydrogenase and alanine aminotransferase were similar in the two groups.

The clearance of antipyrine was not significantly changed by CRF (Table 1), and no correlation with the degree of renal insufficiency was found. The median values of the distribution volume of antipyrine were also comparable in the two groups, being 0.81 and 0.75 l/kg BW in the CRF and control group, respectively.

The galactose elimination capacity expressed as µmol/kg BW/min was significantly decreased in the CRF group, the median value being 75% of the value in the control group (Fig. 1), and a significant positive linear correlation ($r^2 = 0.56$, $P < 0.001$) with the ^{51}Cr-EDTA clearance was found (Fig. 2). However, when the GEC was calculated in µmol/g liver/min the difference between the two groups was not statistically significant

Table 1

Body weight, liver weight and biochemical values in rabbits after 3 months of chronic renal failure (CRF) and in controls with normal renal function. Values are medians with the range in brackets. Significant differences are indicated as *P < 0.05 and **P < 0.01

	CRF (n = 11)	Controls (n = 8)
Body weight = BW (kg)	**2.73 (2.57–3.23)	3.33 (3.08–3.55)
Liver weight (g)	*60 (56–85)	91 (58–105)
Relative liver weight g/kg BW	23 (20–26)	29 (18–32)
Haematocrit (%)	**37 (32–41)	42 (40–45)
Creatinine (mmol/l)	**0.25 (0.19–1.9)	0.09 (0.08–0.11)
^{51}Cr-EDTA clearance ml/min.kg BW	**1.3 (0.3–2.8)	5.0 (4.3–8.1)
Alanine aminotransferase (u/l)	55 (36–115)	52 (35–74)
Lactate dehydrogenase (u/l)	410 (124–730)	318 (149–403)
Alkaline phosphate (u/l)	63 (35–120)	72 (52–121)
Prothrombin index (%)	*73 (44–153)	61 (37–78)
Albumin (g/l)	**33 (29–35)	37 (34–39)
Galactose elimination capacity (μmol/min·kg BW)	**13.1 (9.7–16.6)	17.4 (14.4–22.6)
Galactose elimination capacity (μmol/min·g liver)	0.56 (0.41–0.75)	0.66 (0.50–1.16)
Antipyrine clearance ml/min·kg BW	5.3 (2.7–9.1)	4.7 (3.3–6.4)
Antipyrine clearance ml/min·g liver	0.23 (0.13–0.43)	0.18 (0.11–0.29)

(Fig. 1). Still, a weak but statistically significant linear correlation ($r^2 = 0.34$, $P < 0.01$) remained between the GEC expressed in these units and the ^{51}Cr-EDTA clearance. The median volume of distribution of galactose was 0.39 l/kg BW in both groups.

Morphology

On inspection the livers as well as their cut surfaces appeared normal. Light microscopy revealed no major abnormalities induced by CRF. The general structure of the livers was preserved. A slight portal inflammatory infiltrate consisting of lymphocytes, histiocytes and eosinophils was present in three of the CRF rabbits (Fig. 3). Similar lesions were found in one of the control rabbits. The hepatocytes appeared normal in most specimens but slight microvesicular steatosis was found

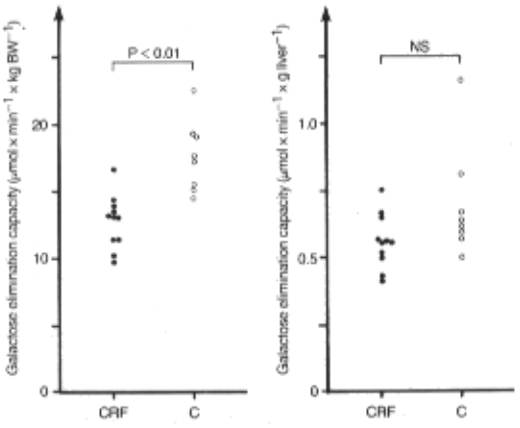

Fig. 1. Galactose elimination capacity in rabbits with chronic renal failure (CRF) and controls with normal renal function (C). A statistically significant difference is present only when GEC is expressed in μmol/kg BW/min.

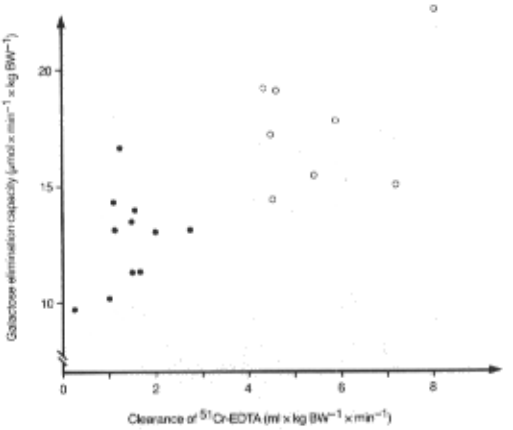

Fig. 2. A positive linear correlation is shown between the glomerular filtration rate (clearance of ^{51}Cr-EDTA) and the galactose elimination capacity ($r^2 = 0.56$, $P < 0.001$).

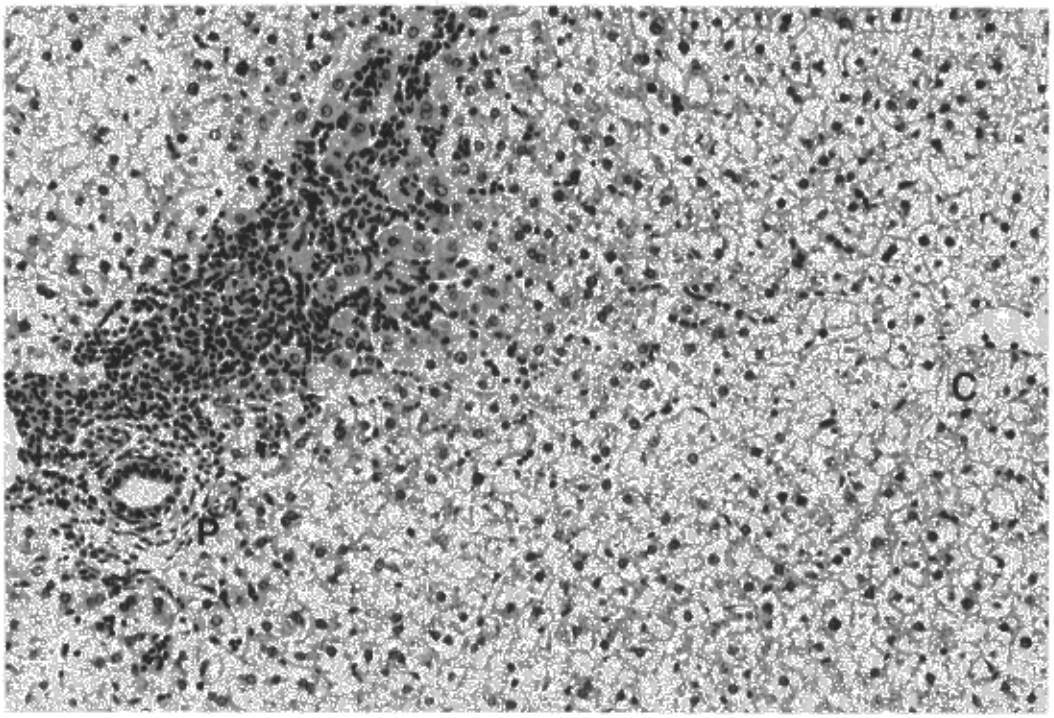

Fig. 3. Liver tissue from an uraemic rabbit. A dense inflammatory infiltrate mainly composed of mononuclear cells is seen in the portal tract (P) with "spill-over" into the adjacent parenchyma, but no piecemeal necroses are present. The centrilobular area (C) is unaffected. HE × 240 (primary magnification × 50).

in three CRF rabbits. There were no changes in the bile ducts, the sinusoids or the Kupffer cells.

Discussion

The method used to induce CRF was successful in the sense that it resulted in significant and rather stable degrees of renal insufficiency without any mortality. The CRF rabbits, however, did not thrive well and despite the food restriction, a significant difference in body and liver weight remained between the two groups. The relative liver weight, however, was comparable to that of the control group. The possible influence of these factors for the results of the liver function tests remains uncertain.

Several of the variables used to evaluate liver function in the present study were changed in the CRF rabbits in contrast to a previous study of rabbits with a lesser degree of renal insufficiency (18).

The galactose elimination capacity (GEC), which reflects cytosolic hepatocytic function, is believed to be a measure of functional liver mass (13, 19). In the CRF rabbits this parameter was significantly reduced when related to body weight, but the GEC per g liver weight was not significantly reduced ($P_{2\alpha} = 0.10$). As a proof of altered hepatocytic function these findings are therefore not conclusive. If a reduction in hepatocytic function during CRF is accepted as a biological fact, the explanation remains to be elucidated. No studies of GEC in uraemic patients have been published.

The microsomal oxidative drug-metabolizing capacity as measured by the antipyrine clearance was not affected in the CRF group of rabbits, in contrast to the decrease observed in uraemic patients (8). In uraemic patients dose adjustments of many drugs have been recommended, even when hepatic metabolism is the main route of elimination (20). Both induction and inhibition of

liver enzymes have been described, and changes in the drug protein binding properties of uraemic plasma may be important too (21). In rats and rabbits with CRF, decreased hepatic metabolism of aminopyrine and dicoumarol has been found (22, 9). The decreased drug-metabolizing capacity of the livers of uraemic rats has been related to a concomitant decrease in the hepatic content of cytochrome P-450 (7), but the inhibition by urea of the metabolism of hexobarbital and antipyrine in a liver homogenate indicates a direct effect of urea as well (23).

The serum concentration of total protein in the CRF rabbits was unchanged, whereas serum albumin was significantly decreased. In uraemic patients serum protein and albumin are often decreased, but this could be due to renal losses and dietary protein restrictions. In uraemic rats, decreased synthesis of both total protein and albumin has been demonstrated (6). The prothrombin index, which is an indicator of the microsomal hepatic capacity of protein synthesis (24), however, was significantly increased in the CRF rabbits. In uraemic patients the prothrombin index as well as other coagulation factors are increased, but the mechanism is not known although decreased renal degradation may be a contributing factor (1–3).

Several enzyme activities in serum are commonly measured to evaluate liver function. In the present study no significant changes were found in the activities of alanine aminotransferase, lactate dehydrogenase or alkaline phosphatase in the CRF rabbits. In uraemic patients the glutamine oxalacetic acid transaminase is decreased (4, 25). Since the transaminase activity increases in patients after haemodialysis and also after *in vitro* dialysis of serum, an inhibitory substance, which accumulates during CRF, is probably responsible (26). Due to the frequency of renal osteodystrophy in uraemic patients, the serum activity of alkaline phosphatases is an unreliable measure of liver function unless isoenzymes are separated (27).

The effect of CRF on liver morphology has not been studied systematically in man. In uraemic rats, light microscopy has not revealed any changes, whereas examination by electron microscopy has shown disorganization of hepatocytic mitochondria as the main abnormality (22). It is difficult to imagine major effects on liver function caused by the scattered periportal cellular infiltrations observed in our CRF rabbits, especially since similar slight lesions could be demonstrated in one of the control rabbits as well.

In summary, no significant changes in the morphology of the liver were found in the present study of rabbits with moderate renal insufficiency. Several biochemical measures of liver function were abnormal, but the quantitative functional tests gave no conclusive evidence of an altered functional status of the liver. However, CRF rabbits may still be a useful model for future investigations of liver function during chronic renal failure.

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