Decrease of erythrocyte and glomerular membrane negative charges in chronic cadmium poisoning

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ABSTRACT Negative charges on red blood cell membranes were measured by the alcian blue binding test in 11 workers with high exposure to cadmium. Compared with 12 age matched control subjects, cadmium workers showed a significant decrease in red blood cell charge, which on average paralleled both the cadmium body burden and protein excretion. Animal data confirm these observations and also show that the loss of red blood cell charge caused by chronic cadmium poisoning is irreversible and associated with a loss of glomerular negative charges. The present study suggests thus that cadmium can increase the urinary excretion of anionic macromolecules such as albumin by reducing the glomerular polyanion charge.

Fixed negative charges on the glomerular capillary wall (glomerular polyanion) provide an electrostatic barrier to the filtration of anionic macromolecules such as albumin¹ and the loss of glomerular polyanion in clinical or experimental glomerular diseases is usually associated with an enhanced excretion of albumin.^{2,3} Evidence has been presented in two recent studies that the negative charges on the surface of red blood cells (RBC), as determined by the binding of the cationic dye alcian-blue 8GX (AB), might reflect the charge of the glomerular polyanion. Levin *et al* have reported a reduced binding of AB to the RBC of children with minimal change nephrotic syndrome and focal glomerulosclerosis.⁴ Applying the same technique to patients with membranous nephropathy, minimal change nephropathy or IgA nephropathy, Boulton-Jones *et al* concluded that subjects with low RBC charge are more susceptible to the development of heavy proteinuria.⁵ Feehally *et al*, however, failed to confirm this relation between RBC charge and proteinuria in patients with the nephrotic syndrome.⁶ The analytical validity of the AB assay has also been questioned by Sewell and Brenchley⁷ because of the instability of the AB solution and the possible interference of residual albumin. Nevertheless, in the present study the AB binding test has been successfully used to demonstrate a decrease in membrane negative charges in the RBC and glomeruli of people and animals chronically exposed to cadmium (Cd). This loss of negative charges was found to be associated

with a rise in albuminuria, which confirms that the RBC surface charge as assayed by the AB method might be a reliable index of the glomerular polyanion charge.

Methods

HUMAN STUDY

Eleven male workers, aged 34-61 and employed on average for 6.6 years in a secondary non-ferrous smelter, were studied. The concentrations of Cd in the blood and urine greatly exceeded the currently proposed biological limit values⁸ (10 µg Cd/l or 89 nmol/l for Cd in blood and 10 µg Cd/g creatinine or 10 nmol/mmol creatinine for urinary Cd). Thirteen medical and laboratory workers aged 27-45 served as controls.

ANIMAL STUDY

Sprague-Dawley female rats, aged 2 months, were given 100 ppm Cd (as CdCl₂) in deionised drinking water for up to five months; control rats were given deionised drinking water. For the preparation of isolated rat glomeruli and tubules, the animals were anaesthetised with pentobarbital (35 mg/kg) and perfused through the aorta with 200 ml of saline containing procaine (1 g/l). Glomeruli and tubules were then isolated by a graded sieving technique,⁹ washed twice with saline, and resuspended in 1 ml of saline with 1 mmol/l di-isopropylfluorophosphate. They were then homogenised in a glass Potter type homogeniser with a Teflon pestle.

Fig 1 Alcian blue binding to red blood cells of control subjects and Cd exposed workers without or with proteinuria. ^aUrinary albumin > 12 mg/g, creatinine and/or urinary RBP > 300 μg/g creatinine, ^bmean ± SD, ^cgeometric mean (range). *Statistically different from controls; **statistically different from both controls and Cd workers without proteinuria. (One way analysis of variance followed by Tukey's test). 1 mg creatinine/l = 8.84 μmol/l; 1 mg albumin/g creatinine = 0.11 mg/mmol creatinine; 1 μg RBP/g creatinine = 0.113 μg/mmol creatinine; 1 μg Cd/g creatinine = 1 nmol/mmol creatinine; 1 μg Cd/l = 8.9 μmol/l.

CHARGE MEASUREMENT

The binding of AB (alcian blue 8GX, Sigma) to human or rat RBC was measured by the method of Levin *et al.*⁴ The AB solutions were prepared and centrifuged just before use to eliminate possible undissolved dye. RBC were counted with a Technicon autocounter.

The same AB method was applied to measure membrane negative charges in tissues homogenates. Before assay the homogenates were diluted 25 times in phosphate buffered saline. The results were corrected for protein content of the homogenates as determined by the biuret method. To avoid any analytical bias, "controls" and "Cd-exposed" samples were always run simultaneously with the same AB preparation.

The AB binding was determined at AB concentrations ranging from 0.125 to 1 mg/ml (0.085 to 0.77 μmol/ml). For calculating the correlations and also in fig 1, only the values obtained at the AB concentration of 1 mg/ml were used.

OTHER METHODS

Cd in blood and urine was measured with a Perkin Elmer Zeeman 3030 atomic absorption spectrophotometer. The urinary or serum concentrations of albumin, retinol-binding protein, or β₂-microglobulin in rat or man were determined by latex immunoassay.¹⁰

Results

As shown in fig 1 and in the upper part of fig 2, the AB binding to RBC of workers exposed to Cd was significantly lower than that of the controls ($p < 0.001$, Student's t test). The four workers presenting with an increased urinary excretion of albumin (> 12

Fig 2 Alcian blue binding to red blood cells from Cd exposed workers and to RBC and glomeruli from rats given 100 ppm Cd in drinking water for three months. Dashed lines: controls. Mean $\pm$ SE. * $p < 0.05$ (Student's t test). 1 mg AB = 0.77 μ mol AB.

mg/g creatinine or 1.36 mg/mmol creatinine, $n = 3$) or retinol-binding protein ($> 300 \mu$ g/g creatinine or 34 μ g/mmol creatinine, $n = 3$), or both, have on average a lower RBC charge than workers without proteinuria ($t = 2.07$) (fig 1). These four workers have been exposed to Cd for a longer period and, as reflected by the Cd concentrations in blood and urine, their Cd body burden is accordingly higher. Interestingly, the albumin excretion in the other Cd workers, although in the normal range, is nevertheless significantly higher than that of controls. The progressive loss of RBC charges depicted in fig 1 seems thus to go hand in hand with a progressive rise in the excretion of albumin.

In rats given 100 ppm Cd in drinking water a significant reduction in AB binding to RBC and glomeruli was observed from the third month of treatment (fig 2). AB binding to liver and renal tubule membranes was not affected. The urinary excretion of β_2 -microglobulin was normal but that of albumin was, on average, twice that of the controls ($p < 0.05$). This increase of albuminuria was negatively correlated with the loss of RBC charges ($r = -0.77$, $p < 0.005$, $n = 11$) and also of glomerular charges ($r = -0.51$, $n = 11$) but in the latter case the correlation did not reach the level of significance because of the limited number of observations.

These effects cannot result from a direct neutralisation of membrane negative charges by the Cd^{2+} ion since very high concentrations of Cd fail to inhibit the AB binding to RBC in vitro. In addition, the effect of Cd on RBC charge persists two months after cessation of Cd treatment when the metal concentration in the blood has returned to the normal value.

Discussion

The present study shows that chronic exposure to Cd can result in a loss of the negative charges on the RBC membrane and, as suggested by animal data, in the glomeruli. The lack of effect on the membrane charges on the liver and renal tubules, two important sites of Cd storage, indicates that this phenomenon is restricted to the vascular compartment. The reduction of membrane charges caused by Cd cannot be explained by a simple masking of anionic sites by the Cd^{2+} ion since it cannot be reproduced in vitro. Of special interest is the fact that in both man and rat the loss of RBC charge induced by Cd is associated with a progressive rise in the degree of albuminuria. The causal nature of this association is difficult to establish in workers exposed to Cd who, in most cases, also show an increased urinary excretion of retinol binding protein due to tubular damage. The rise of albuminuria observed in these workers may result partly from an impaired tubular reabsorption as albumin is also extensively reabsorbed by the proximal tubular cells.

In the rat, by contrast, the enhanced excretion of albumin induced by Cd is not accompanied by signs of a tubular defect. It may thus be unequivocally ascribed to an increased glomerular permeability¹¹ that presumably results from a reduced electrostatic repulsion of albumin by the glomerular polyanion as suggested by the negative correlation between RBC charge and albuminuria ($r = -0.77$). The reason why Cd can induce an isolated albuminuria in the rat more frequently than in man is because the rat has a strikingly low threshold for the renal excretion of albumin¹² so that an increase of glomerular permeability leads to a rise of albuminuria at a much earlier stage of Cd intoxication than in man—that is, before the onset of tubular dysfunction.

The absence of a significant correlation between albuminuria and glomerular charge in the rat does not refute our conclusion but may simply mean that the RBC charge is a more reliable index of the glomerular polyanion charge than the total membrane charge measured in the glomerular homogenate.

An interesting feature of the RBC charge determination by the AB method as shown in the present study is its high sensitivity. By contrast with previous studies on patients with advanced renal diseases,^{4,5} a reduction of RBC charge has been found here in Cd exposed subjects presenting no or only subclinical signs of renal injury. This suggests that the RBC charge determination might have, at least under certain conditions such as chronic Cd poisoning, some predictive value with respect to the development of proteinuria. The extent

to which this test may be applied at the individual level, however, remains to be determined.

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