EARLY INDICATORS OF LEAD NEPHROPATHY

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INTRODUCTION

Detection of early lead nephropathy in industrial workers has been difficult, particularly as albuminuria is absent until late stages of the disease (1). Changes in serum creatinine and blood urea nitrogen (BUN) have proved to be relatively insensitive indices of renal impairment in individual workers, although epidemiological studies in large numbers of workers have indicated a statistically significant increase in both parameters with length of exposure to lead, even when corrected for age (2). Furthermore, Wedeen (1) demonstrated that lead workers with normal serum creatinine and BUN values may have subtle renal impairment, as indicated by a reduction in glomerular filtration rate (GFR) and abnormal renal biopsies.

It was the purpose of the present study to determine in an animal model of lead toxicity whether selected urinary enzymes and/or immuno-assayable brush border antigen might serve as early indicators of lead nephrotoxicity. Several urinary enzymes, derived from renal tubular cytoplasm, or proximal tubular brush border, have been proposed as markers of tubular injury. Of these substances, the lysosomal enzyme, N-acetyl-ß-D-glucosaminidase (NAG), has been successfully employed in several clinical situations (e.g., acute renal failure, renal transplant rejection) (3). Glutathione-S-transferase (GST), a component of ligandin also appears in the urine following tubular injury, but selectively reflects chemical injury rather than ischaemic injury (4). Renal shedding of proximal tubular brush border antigen (BBA) measured by specific monoclonal antibodies in an ELISA assay, has already proved to be a useful marker of tubular injury in groups of subjects exposed to or treated with cadmium, cisplatin, chromium, mercury, and hydrocarbons (5).

The animal model of lead nephrotoxicity has been based on prior studies by Goyer et al (6,7), in which animals were given 1% lead acetate in drinking water. The principal modifications were a reduction in lead concentration to 0.5% and the use of a semi-purified diet, shown to enhance the sensitivity of the rat to lead intoxication (8). At different time intervals after exposure, urinary excretion of NAG, GST and BBA in experimental animals and pair-fed controls were correlated with whole blood, plasma, and urinary lead values, true serum creatinine chromogen, iothalamate clearance (as an index of GFR) and renal pathology.
METHODS

Animals: Male Sprague-Dawley rats were fed a semi-purified diet (ICN Pharmaceuticals, Ohio) and given 0.5% lead acetate in their drinking water beginning at eight weeks of age. Experimental animals were pair-fed with their counterpart controls. Four groups of animals (n = 11 in each group) were studied: I) one month control (C1); II) one month experimental (EC1); III) three month control (C3); IV) three month experimental (EC3).

Pathology: Kidneys were excised, blot dried, weighed and 1-2 mm slices were dissected out and fixed for electron and light microscopy. Electron microscopy staining was performed with uranyl acetate and lead citrate; light microscopy sections cut thin (3um) and stained with hematoxylin and eosin.

Determination of Glomerular Filtration Rate: GFR was assessed indirectly by measurement of serum true creatinine chromagen according to the method of Polar and Metcoff (9). A more precise measure of GFR was afforded by employing the single injection iothalamate clearance technique (10). Clearances are expressed as ml/min/lOO g body weight.

Urine Collection and Analysis: Urine samples were collected in chilled containers and aliquots removed and frozen until required. Urinary creatinine and NAG activity were measured colourimetrically (11, 12) and GST was determined by the method of Feinfeld et al (4). Enzyme activity for all procedures was expressed per g creatinine. Appearance of brush border membranes in urine was determined by ELISA as described by Mutti et al (13).

Ultrafiltration of Plasma Plasma samples were filtered through Amicon membranes with molecular weight exclusion of 50 K daltons. Filtrates were collected in plastic tubes and their lead contents were determined.

Determination of Lead in Blood and Urine: Lead determinations were performed in duplicate on whole blood, urine, and plasma, using an atomic absorption spectrophotometer (Perkin Elmer, Model #305, with graphite furnace).

Statistical Analysis: Comparisons between experimental and control groups were performed by unpaired t-test. Correlations were calculated by method of least squares.

RESULTS

Pathology EC1 kidneys showed no differences from control animals except for the appearance of some nuclear inclusion bodies in both the proximal convoluted tubules and pars recta. EC3 kidneys demonstrated enlargement of proximal convoluted tubules with nucleomegaly (Fig. 1). Nuclear inclusion bodies were wide-spread. Tubules were irregularly dilated with spotty and patchy atrophy. Glomeruli appeared relatively normal with the exception of mesangial thickening in some glomeruli of EC3 animals.

When compared to control rats (Fig. 1C), proximal tubules of EC1 kidneys revealed nucleomegaly, with nuclei containing dense small frequently lobulated inclusion bodies exhibiting peripherally radiating spikes (Fig. 1A). There were some dilated cisterns of endoplasmic reticulum and increased lysosomes, with associated crowding of mitochondria away from the base of the cell toward the nucleus. Mitochondria were rounded and showed irregular and angular arrangement of cristae. Occasional cells