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Uranyl Nitrate-Induced Glomerular Basement Membrane Alterations in Rabbits: A Quantitative Analysis

C. Kim McDonald-Taylor, M. Kumar Bhatnagar (deceased), Andy Gilman,[1] Al Yagminas,[1] and Amreek Singh[2]

Department of Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario, [1]Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada and [2]Department of Anatomy and Physiology, Atlantic Veterinary College, Charlottetown, PE, C1A 4P3, Canada

Uranium, a well known nephrotoxin, is water-soluble and forms uranyl ion, UO^{2+}. This principal form of uranium is highly mobile, and is found in aquatic environments. The kidney is a common target organ for toxicity of uranium, and of numerous other substances due to the magnitude of blood flow (20% of the cardiac output) through it, which is greater than that in any other organ. Biokinetics of uranium was studied by La Touche et al. (1987).

Morphologic alterations in the kidney associated with uranium toxicity primarily involve the renal corpuscle and proximal tubule. Pathology in renal glomeruli is manifested by decreased size and density of endothelial fenestrae (Avasthi et al. 1980). Other glomerular epithelial changes include spreading and flattening of podocytes (Kobayasi et al. 1984). Blantz and co-workers (1985) described alterations in the renal corpuscle that were based solely on qualitative observations. With exposure to uranyl nitrate hexahydrate (UN), alterations in the glomerular basement membrane (GBM) cause a reduction in glomerular capillary ultra-filtration coefficient (Stein et al. 1975); these authors also reported alterations in the corpuscle including loss of podocyte foot processes. However, information is scanty on the quantitative assessment of these alterations, and the changes associated with recovery phase following exposure to UN. The purpose of the present study was to determine whether or not the GBM lesions produced by UN administration in drinking water are repaired after UN administration is discontinued.

MATERIALS AND METHODS

The rabbit was selected as the animal of choice because of its high sensitivity to uranium (Foulkes, 1971). Twenty-four, New Zealand white, male weanling rabbits were maintained separately on a 12-hour diurnal cycle and fed *ad libitum*. During an acclimatization period of three weeks it was ensured that

Send reprint requests to Dr. A. Singh at the above address

all the animals were clinically healthy. The rabbits were placed randomly into groups of eight, and had free access to drinking water containing 0, 24 or 600 mg UN/L (BDH Chemicals, Etobicoke, Ontario) for 91 days. Each treatment group was divided into three subgroups defined as no-recovery, 45-day- and 91-day-recovery periods. 'Recovery' involved stopping the administration of UN in drinking water and keeping the animals on 'clean' water (which contained <0.0001 mg uranium/L) for a further 0, 45 or 91 days. All the animals were euthanized with an overdose of sodium pentobarbital.

Nine rabbits for no-recovery, and for 91-day-recovery periods (3 for each treatment, i.e., control, 24 mg UN/L, 600 mg UN/L) and six rabbits for the 45-day-recovery period (3 for each treatment, i.e., control and 600 mg UN/L) were used. Selection of UN dose-levels was based on our range finding studies in rabbits. Conventional electron microscopy techniques in vogue at our laboratories (Singh et al. 1981) were followed. Thin sections from 130 blocks were cut at approximately 70 nm using a diamond knife, and mounted on thin bar copper grids with a tab. The sections were contrasted with uranyl acetate and lead citrate, and examined and photographed in a Jeol 100S electron microscope.

The tab of the grid was placed to the right in the grid holder, and grids were examined in a left to right direction. The first renal corpuscle seen was photographed at 5,000 diameters. One to two micrographs of the corpuscles were taken from sections on one grid, and five grids were examined for each animal resulting in 6-10 micrographs of the corpuscles. Thin sections were also examined for qualitative changes (which will be the topic of a future publication; also, Singh et al. 1985). Electron micrographs used for morphometry were at final magnifications of 13,500 diameters.

Thickness of GBM was measured on electron micrographs using digital callipers (Mitutoyo Digimatic, Mississauga, Ontario) following the method of Osterby (1971; 1975). Measurements were taken at approximately 35 mm increments resulting in an average of 34 measurements per micrograph (range 15 - 51), using 20-30 micrographs per treatment group, and 6-10 micrographs per animal. The three control groups comprising nine animals had a total of 905, 956, and 746 micrographs for the GBM measurements for the no-recovery, 45-day- and 91-day-recovery periods, respectively. The two 24 mg UN/L groups had a total of 817, and 846 micrographs for the measurements for the no-recovery and 91-day-recovery periods, respectively. The three 600 mg UN/L groups had a total of 602, 855, and 748 micrographs for the measurements for the no-recovery, 45-day- and 91-day-recovery periods, respectively. Thickness was measured from basal plasma membrane of endothelium to apical plasma membrane of foot processes as illustrated in Figure 1. The means and standard errors of the thickness measurements were calculated using the means and general linear model procedures of Statistical Analysis System. A requirement for the calculations to be accurate, is the necessity to have a large number of measurements (Osterby 1971).

RESULTS AND DISCUSSION

Ultrastructure of renal corpuscles from animals of the control groups was apparently normal (Fig. 1). Animals from the 24 mg UN/L group had thicker GBM that exhibited budding, and the animals in the 600 mg UN/L had, relatively, the thickest GBM, which also showed budding (Fig. 2). Means of the thickness measurements (± SEM) in animals of the control groups were 80.00 nm ± 1.48, 79.26 ± 0.74, and 80.00 nm ± 0.74 for the three recovery periods; these values were not significantly different ($p < 0.05$) (Fig. 3A). The basement membrane in the 24 mg UN/L (± SEM) for 91-day-recovery period was significantly thicker ($p < 0.05$) at 102.96 nm ± 0.74 than that in the no-recovery period at 96.30 ± 1.48 (Fig. 3A). The mean (± SEM) of the thickness measurements of the corpuscles from the no-recovery period in the 600 mg UN/L group was 108.89 ± 3.70, and for the 45-day and 91-day-recovery periods was 108.89 ± 0.74 and 117.04 nm ± 0.74, respectively. The mean of the GBM thickness measurements for the no-recovery period was significantly less than that of the corpuscles from the 91-day-recovery period, but not of those from the 45-day recovery period ($p < 0.05$). The thickness in the 45-day recovery period was significantly less ($p < 0.05$) than that in the 91-day-recovery period (Fig. 3A).

For a comparison among the groups, the rabbits from the no-recovery period treated with 24 or 600 mg UN/L had significantly thicker ($p < 0.05$) GBM than that in the animals from the control groups. The GBM thickness in the treated animals from the 24 mg UN/L group was significantly different ($p < 0.05$) from that in the animals of the 600 mg UN/L group (Fig. 3B). The basement membrane in the 45-day-recovery 600 mg UN/L treated rabbits was significantly thicker ($p < 0.05$) than that in the animals of the control group (Fig. 3B). The mean of thickness measurements in animals of the 91-day recovery control group was significantly less ($p < 0.05$) than that in both the 24 or 600 mg UN/L treated rabbits. Animals in the 600 mg UN/L group had a significantly thicker ($p < 0.05$) GBM than that in the 24 mg UN/L group animals (Fig. 3B).

In the present study, thickness of the GBM increased in rabbits as dose of UN increased for no-recovery, 45- and 91-day-recovery periods. As well, the thickness increased as the recovery period increased for both 24 or 600 mg UN/L treatment groups. It is known that lamina densa of GBM thickens with age (Steffes et al. 1983). Thus, the thickening observed in our study may represent an acceleration of the aging process, or the normal progression of aging is accompanied by deposition of immune complex in the longer recovery periods (Steffes et al. 1983; Hayashida et al. 1986).

Kanwar and Farquhar (1979) reported that anionic sites in the GBM were composed of polygonal particles with fine filaments radiating from their tips.

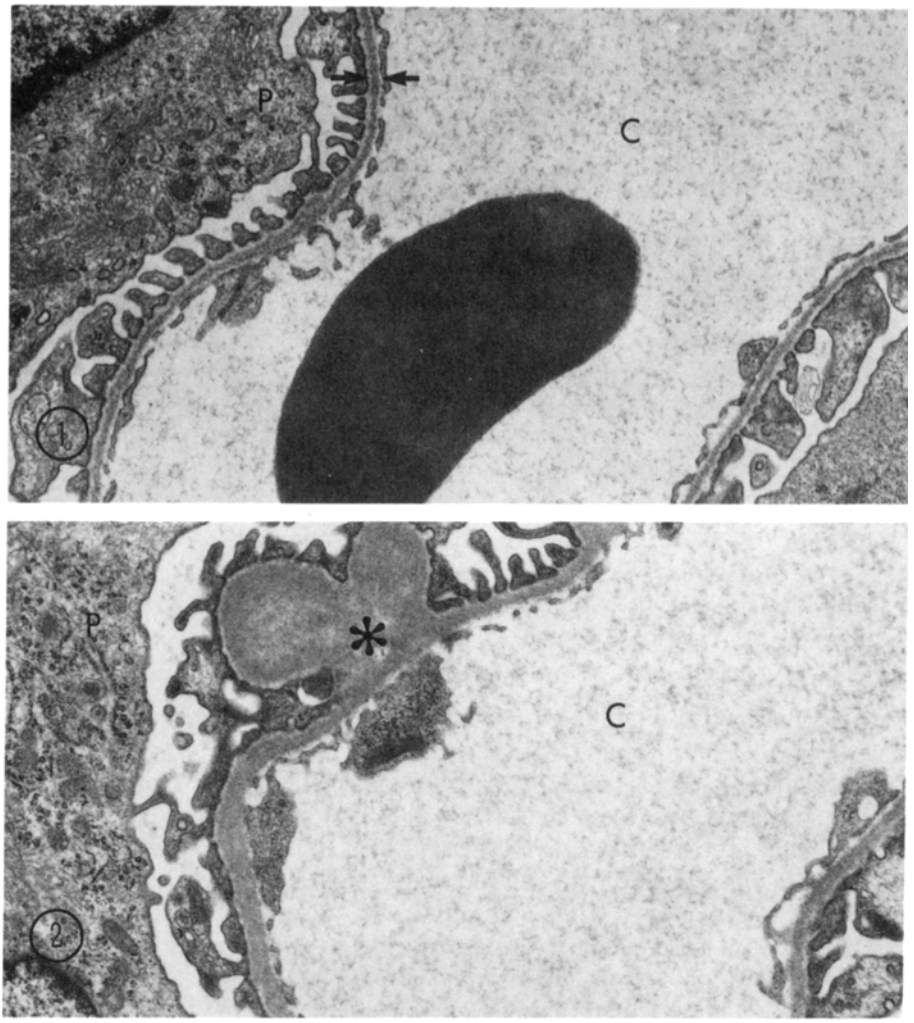
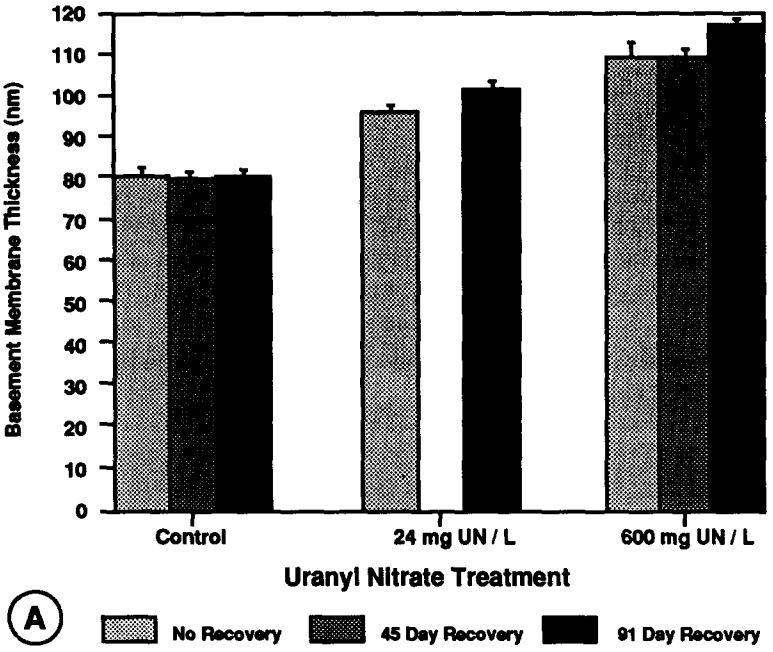


Figure 1. Electronmicrograph of portion of a renal corpuscle from a rabbit of a control group to depict normal thickness of the basement membrane. Arrows point to an area used for thickness measurement. C = Capillary lumen; P = Podocyte. x 15,500.
Figure 2. Micrograph of a region similar to that in the Figure 1, but from an animal of the no-recovery 600 mg UN/L group. Note the thickened membrane, and its budding (*). C = Capillary lumen; P = Podocyte. x 15,500.

In addition to connecting filaments to one another, these tips connect adjoining endothelial and epithelial plasma membranes in the laminae interna and externa. Such negative charges are essential for maintenance of normal glomerular filtration as well as function in attachment of the cells to a GBM.



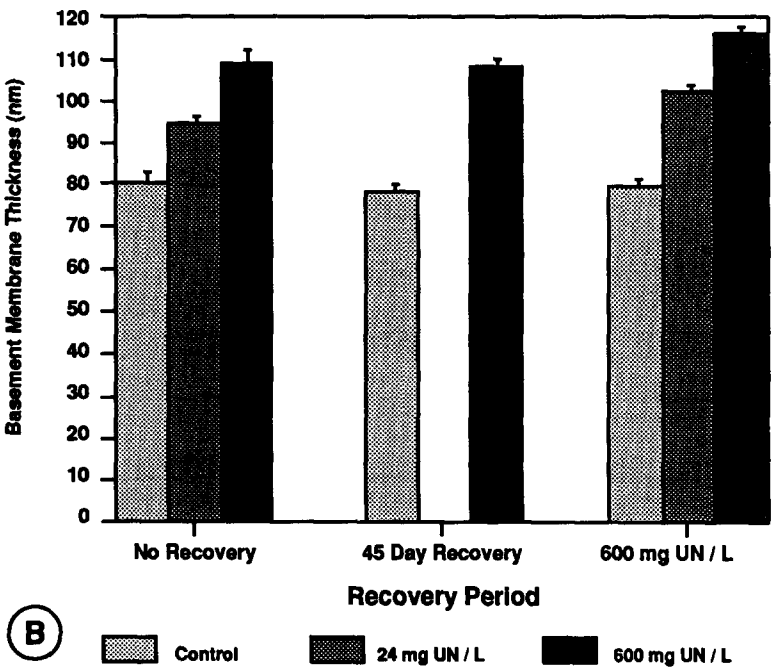


Figure 3. Measurements of glomerular basement membrane thickness and their comparisons between (A) recovery periods within the treatments, and (B) treatments within the recovery periods.

Polycations cause glomerular alterations that suggest loss of membrane integrity and cellular rigidity by interacting with anionic sites (Seiler et al. 1975). Permeability characteristics of glomerular filtration barrier may be modified by such changes leading to thickening and other alterations of its basement membrane.

The GBM thickening noted in the present study may result from alterations in the mesangium which would be unable to maintain a constant turnover, and a normal thickness of the GBM. Because kidney is a storage area for uranium, alterations causing the GBM thickening could continue over time or be severe enough to cause permanent damage. This may account for augmentation in the membrane thickness noted in the present study with the increased recovery period.

Budding of the GBM occurred mostly in the high UN dose groups. Such protuberances may be similar to those of immune complex depositions which were described in guinea pigs immunized against insulin, in a strain of mice with spontaneous immune complex nephritis, and in diabetic mouse mutants (Wehner et al. 1973).

It may be concluded that the UN has dose-related, renotoxic effects in New Zealand white rabbits exposed subchronically to concentrations of 24 or 600 mg UN/L. The ultrastructural alterations were persistent over the 45-day- or 91-day-recovery periods, likely due to the ability of the kidney to store uranium. As well, the time for the repair and regeneration of the renal corpuscle cells is directly proportional to the severity of the initial dose.

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