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Original Article

Evaluation of Changes of Kidney Parameteres in New Zealand Rabbits Subsequent to 90 Days Exposure to Uranyl Nitrate in Drinking Water

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Abstract

Background: This study was undertaken to examine the reversibility of renal injury in the male New Zealand white rabbits subsequent to a 90-days exposure to uranyl nitrate (UN) in drinking water. Animals were exposed for 90 days to uranyl nitrate in their drinking water (24 or 500mg/l). Control group were given municipal tap water.

Methods: The indicators of kidney function measured in this study included glucose (marker of tubular) microalbumin(marker of glomerular) and marker for cell toxicity was alkaline phosphatase.

Results: Urinary glucose was found to be significantly different and positively correlated with uranium intake for rabbits. Microalbuminuria was found to be significantly different but this different is in normal range. Increase of alkaline phosphatase at weeks 2, 4, 6 was correlated with uranium intake, but at weeks 8, 10, 13 excretion of alkaline phosphatase was decreased.

Conclusion: These results suggest that chronic ingestion of uranium in drinking water affect kidney function and to the proximal tubule, rather than glomerul.

Keywords: *Uranyl nitrate, New Zealand,Rrabbit, Water consumption*

Introduction

Uranium, the heaviest of the naturally occurring elements, is a metal whose biological effects were described in the literature as early as the 1820s (1, 2). Its nephrotoxic effects are more likely due to its chemical properties rather than its radioactivity, although ingested uranium may have a radiological effect on other tissues of deposition such as bone.

Studies of the toxic effects of uranium intake through various routes were conducted in the 1940s (as part of war effort in the United States) and in postwar experiments. These were carried out largely on laboratory animals (3). Some postwar human studies were conducted with hospital patients at the university of Rochester and Boston Oak Ridge (4, 5).

Two types of biomarker were used in this study: indicators of kidney function and markers for cell toxicity. Kidney function was assessed by glucose and microalbumin. Cell toxicity marker included the alkaline phosphatase enzyme (6-9).

This study was undertaken to examine the reversibility of renal injury in the male New Zealand white rabbits subsequent to a 90 d exposure to uranyl nitrate (UN) in drinking water

Materials and Methods

Study Population Three groups(initial body weight about 1200-1500 g) of male New Zealand white rabbit (obtained from Pasteur Institute) were exposed for 90 d to uranyl nitrate ($UO_2(NO_3)_2.6H_2O$ Merck Cat.N8476) in their drinking water. Exposed groups received drinking water with UN added to concentration of 24 and 500mg/l. most of other researchers were selected high and low doses of uranium compounds for evaluating of their damage to kidney cells and

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comparison between high and low doses, so we selected these doses for our study too. Concurrently the control group was given water without uranium. All animals were acclimated for three weeks prior to start of the study and hosted in stainless steel mesh cages with free access to food and drinking water.

Sample Collection Rabbits were put in metabolic cases and 24-h urine of them was collected six times during the exposure phases. Volume of their urine was recorded and stored in -20 freezer. After six times collection of their urine various analyses such as glucose, alkaline phosphatase and microalbumin were determined. We used Pars Azmon kit for glucose and alkaline phosphatase and Randox kit for microalbumin. All of these tests were duplicated and read by Hitachi analyzer.

Statistical Analysis Data on biomarkers, in exposed (24, 500mg/l) and control groups were checked with nonparametric method. To test the equality of the means in different groups, the Kruskal Wallis test was used (10).

Results

Glucose In all of six times, excretion of glucose increased. There were significant differences in glucosuria between high dose exposure (500 mg/l) and low dose (24 mg/l) and controls groups ($P< 0.05$). Urinary glucose excretion continued in all six times. There were no significant dose- related differences in glucosuria between low dose (24 mg/l) exposed group and control ($P> 0.05$) (Fig 1).

Microalbumin Excretion of microalbumin increased in high exposure group, except weeks 4, 6. There were significant differences in microalbumin between high exposure group (500 mg/l) and low (24 mg/l) or control groups or with both of them ($P< 0.05$). But these differences were in normal range (Fig. 2).

Alkaline phosphatase: At weeks 2, 4, 6 excretion of alkaline phosphatase (AP) in high dose (500mg UN/l) increased. There were significant differences in AP between high dose exposure (500 mg/l) and low dose exposure(24 mg/l) or control groups($P< 0.05$), but at weeks 8,10,13 there was no significant differences in AP between high exposure group and low or control groups ($P> 0.05$). In these weeks the amount of AP decreased in high exposure group (Fig. 3).

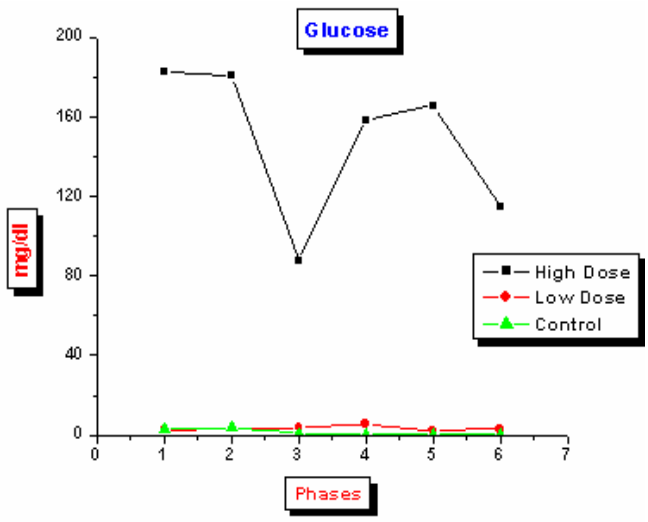

Fig. 1: Variation of urinary glucose with uranium intake in six times

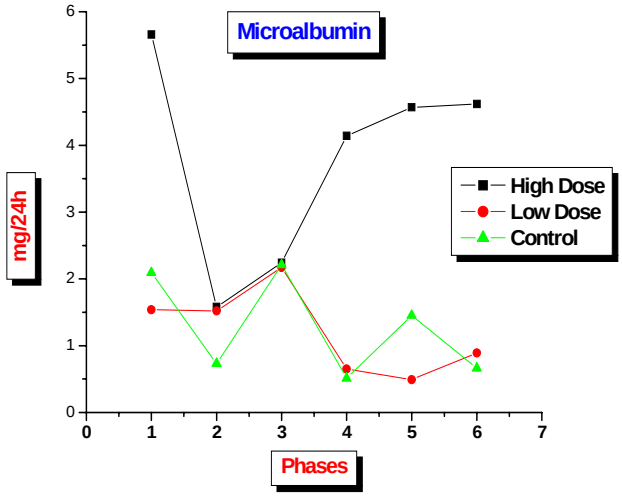

Fig. 2: Varaiation of urinary microalbumin in these six times

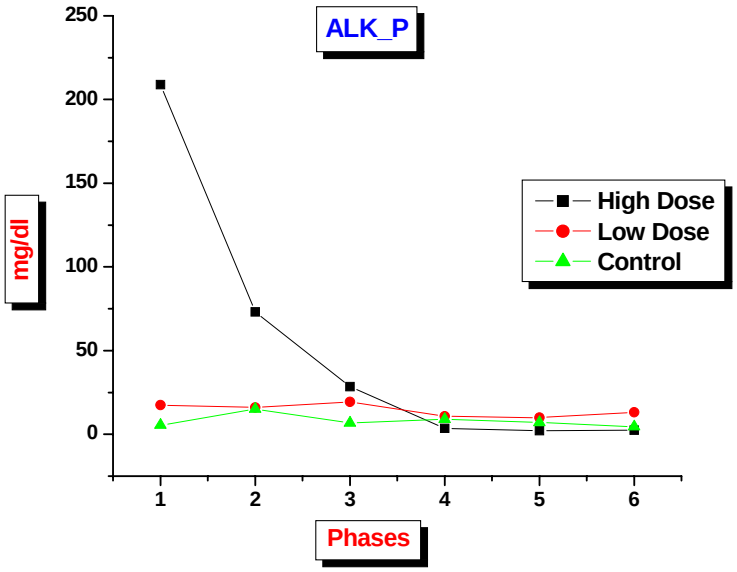

Fig. 3: Variation of urinary excretion of alkaline phosphatase in three groups

Discussion

Early studies on biological effects of uranium indicated that uranium salts given by mouth present a hazard as a feeble poison, but facilitating death after intravenous injection (11). The oral administration of uranium in the form of sulfate (300 mg) or nitrate (900 mg) did not demonstrate any immediate symptoms, whereas 4 g of uranyl nitrate produced emesis in dogs (11). Thirty years after Leconte reported the unique effects of uranium acetate and nitrate on the uropoietic system. In our study consistence increase of glucosuria were shown in high exposure group ($P<$ 0.05) but in low exposure and control groups it was in normal range (Fig. 1).

Our results about glucosuria was associated with other researchers that reported glucosuria in their studies such as; uranium salts consistently produced anuria, oliguria and glucosuria in dogs with lethal dose of 0.6-1 g in rabbits (11). In a study conducted by Diamond rats injected with uranyl fluoride solution, glucose was found to be the most sensitive of the biochemical indicators of renal injury used, exhibiting a 150-fold elevation in treated rats over controls(9). In other studies the early changes observed in glucose during exposure period of the 600 mg/l animals (12, 13). In the study of Zamora with chronic ingestion of uranium in drinking water, glucosuria was found to be significantly different and positively correlated with uranium intake for males, females (6). Glucosuria typically was associated with acute uranyl nephrotoxicity (14).

We showed no association between increased uranium through drinking water and glomeral injury (urinary albumin). In our study microalbuminuria in high exposure group showed a statistically significant difference in normal range in comparison with low exposure and control groups (Fig 2). Our results are consistent with previous finding, suggesting that uranium effects kidney tubular function (6,15) but Kurttio showed an association between increased uranium exposure through drinking water and tubular function, but not between uranium exposure and glomerul injury (i.e., creatinine clearance and urinary albumin) (16).

It is known that high dosing with uranium can lead to structural changes in the brush border membrane of the proximal tubules (17). Loss of microvilli has been observed as early as 1h after injection of 10mgU/kg into rats (17). Alkaline

phosphatase is present on the membrane of the brush border of proximal tubule cells (8). An increased activity of ALP has been observed in urine soon after acute exposure to uranium (18). We showed these effects and rapid regeneration of tubular system with ALP. Destruction of tubular system increases of excretion of ALP, as we showed this effect at weeks 2, 4, 6 in high dose (500 mg/l) group. With repeated doses of uranium exposure, damaged tubular system had a rapid regeneration, with appearance of large nuclei, meiotic activity, replacement and resistance to uranium (19) as we showed at weeks 8,10,13 that there were no significant differences in ALP between high exposure group and low or control groups ($P > 0.05$) (Fig. 3). This resistance was associated with atypical cells of regenerated tubular epithelium (19).

Experimental models and pathoanatomy and clinical studies on uranium toxic effects on renal system, demonstrated the tubular alterations, with the functional pattern of glucosuria, excretion of ALP without glomerular alterations. In cases of non lethal poisoning damaged tubular epithelium rapidly regenerated, with subsequent tolerance to large doses of uranium (20). Regenerated epithelium was of a metaplastic histologic type, different the normal epithelium and the postulated tolerance mechanism was the inability of uranium compounds to interact with renal tubular cells (20). However, if the process of repair has not been completed and if damaged tubular epithelium was repaired by non-damaged tubular cells, there was no resistance to the subsequent uranium- induced poisoning, but the exact mechanism of this resistance has not been totally clarified (11,20).

Our study suggests that chronic ingestion of uranium in drinking water affect kidney function and to the proximal tubule, rather than glomerul.

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The authors declare that they have no Conflict of Interests.

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