Effects of zinc deficiency on the expression of endothelin-1 in glomeruli of rats with unilateral ureteral obstruction

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Summary

In the present study, we examined the expression of endothelin (ET)-1 and a role of angiotensin II in glomeruli from rats with unilateral ureteral obstruction (UUO) fed a zinc (Zn) deficiency or a standard diet. The expression of ET-1 was substantially potentiated in glomeruli of the obstructed kidney (OK) from rats with UUO fed a Zn deficiency v.s. a standard diet. No expression of ET-1, however, was observed in glomeruli of the contralateral, non-obstructed kidney from rats with UUO fed a Zn deficiency or a standard diet. Prior administration of the angiotensin converting enzyme inhibitor, enalapril markedly decreased the enhanced expression of ET-1 observed in glomeruli of the OK from rats with UUO fed a Zn deficiency diet. Indeed, the renin-angiotensin system (RAS) is known to be activated after the onset of ureteral obstruction. These findings suggest that Zn deficiency intensifies the expression of ET-1 in glomeruli of the OK from rats with UUO by further enhancing the RAS upregulated after the onset of ureteral obstruction.

Introduction

Ureteral obstruction causes a progressive fall in glomerular filtration rate and glomerular capillary plasma flow[1]. These functional changes in glomeruli may be the effect of the renin-angiotensin system (RAS) upregulated after the induction of ureteral obstruction[1]. In several renal diseases, the powerful vasoconstrictors, endothelin (ET)-1 and angiotensin II synergistically act and aggravate renal function, particularly glomerular function[2,3]. The present study was designed to examine if zinc (Zn) deficiency affects the expression of ET-1 and the activity of the RAS which play an important role in the aggravation of renal function using the ureteral obstruction model.

Methods

1. Male Sprague-Dawley rats (Clea Japan Inc., Tokyo, Japan) weighing approximately 200g were pair-fed either a Zn deficiency diet or a standard diet containing 0.02% Zn for 50 days. Dietary compositions such as protein, carbohydrate, fat, minerals and vitamin mixtures were identical except for no addition of Zn in a Zn deficiency diet. A Zn deficiency diet, however, contained 0.5 μg Zn/g diet when examined by the atomic absorption spectrophotometer analysis. The food consumption was monitored daily over the dietary conditioning. The amount of diet ingested was comparable in both groups of rats. The animals were allowed to have distilled water ad libitum during the period of dietary treatment.

2. After dietary treatment,

(1) the quantity (μg/g tissue) of Zn and Cu were determined in duplicate in kidney tissue from rats fed a Zn deficiency (n=6) or a standard (n=6) diet using the atomic absorption spectrophotometer analysis.

(2) rats fed a Zn deficiency (n=4) or a standard (n=4) diet underwent unilateral ureteral obstruction (UUO) under light ether anesthesia as reported previously[4]. Again, another group of rats given a Zn deficiency diet (n=4) intraperitoneally received 15mg/kg of the angiotensin converting enzyme (ACE) inhibitor, enalapril (Merck Sharp & Dome, Rahway, NJ, USA) 24 hours, 12 hours and 1 hour prior to surgery and every 12 hours following surgery for 3 days. Kidneys from each group of rats were harvested 3 days after the induction of UUO and fixed with 4% paraformaldehyde. The kidney sections prepared from all the rats were stained with hematoxylin-eosin. Concomitantly, the immunohistochemical analysis for the expression of ET-1 was carried out using polyclonal antibody against ET-1.

Results

1. The ingestion of a Zn deficiency diet caused a fall in Zn content and a rise in Cu content in kidney tissue. This resulted in a marked elevation in the Cu/Zn ratio in kidney tissue of rats fed a Zn deficiency diet (Table 1).

Table 1 Levels of Zn and Cu and the Cu/Zn ratio in kidney tissue obtained from rats fed a Zn deficiency or a standard diet

Diet	Zn	Cu	Cu/Zn
Zn deficiency	11.14*	15.18*	1.36*
	±0.45	±1.80	±0.15
Standard	15.68	4.92	0.31
	±0.38	±0.40	±0.03

The amounts (μg/g tissue) of Zn and Cu in kidney tissue were determined in duplicate using the atomic absorption spectrophotometer analysis. Data reported are means±S.E. of the values obtained from six rats. Statistical analysis was carried out using unpaired Student's t-test. (*) $P < 0.005$ compared with the standard diet group.

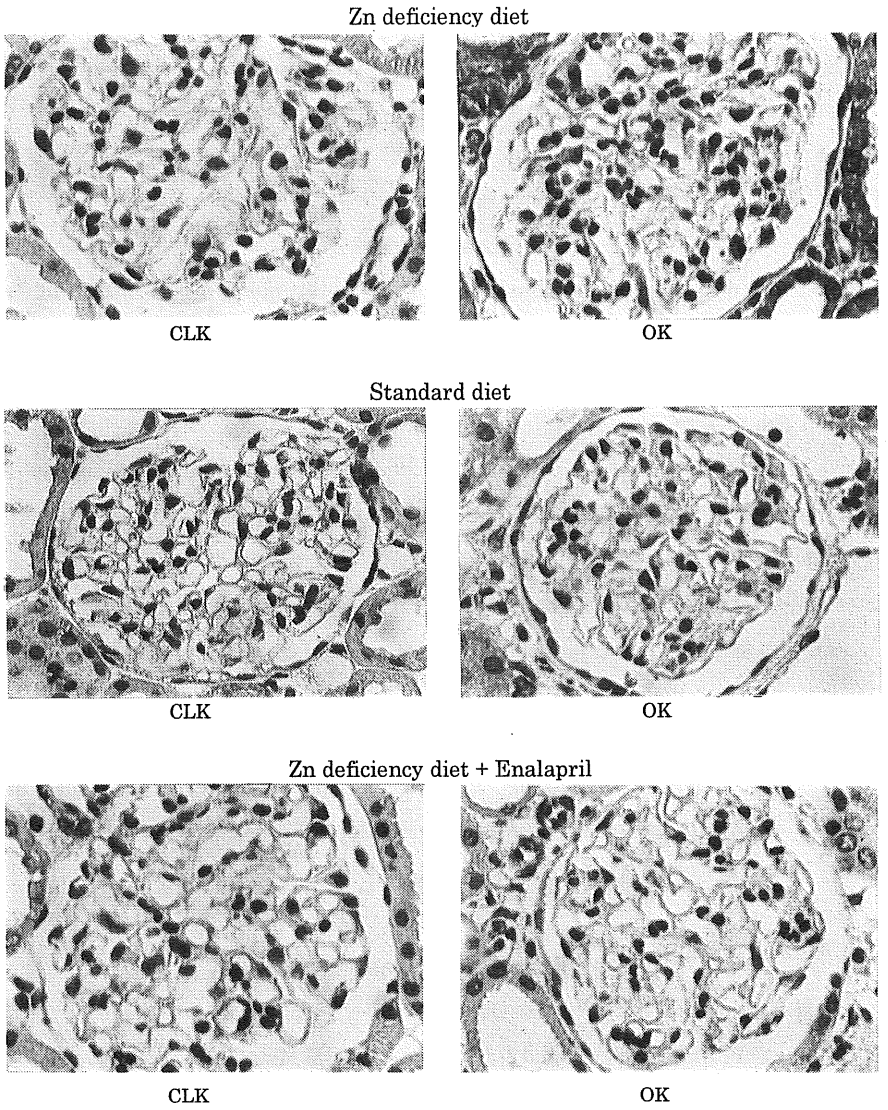

Figure 1 Photomicrographs of immunohistochemical studies for endothelin-1, Glomeruli from the contralateral, non-obstructed kidney (CLK) and the obstructed kidney (OK) are presented. Microscopic magnification: ×400.

2. There were no significant histopathological findings in glomeruli of the contralateral, non-obstructed kidney (CLK) and the obstructed kidney (OK) from rats with UUO fed a Zn deficiency or a standard diet (not shown).

3. In glomeruli of the OK from rats with UUO, the expression of ET-1 was observed in mesangial cells, endothelial cells and epithelial cells. No significant expression of ET-1, however, was seen in glomeruli of the CLK from rats with UUO (Figure 1).

4. The expression of ET-1 was substantially increased in glomeruli of the OK from rats with UUO fed a Zn deficiency v.s. a standard diet. Prior administration of the ACE inhibitor, enalapril reduced the increased expression of ET-1 in glomeruli of the OK from rats with UUO fed a Zn deficiency diet (Figure 1).

Discussion

1. The ingestion of a diet depleted Zn augments intestinal absorption of Cu^{2+} instead of Zn^{2+} [5]. This increased absorption of Cu^{2+} brings the replacement of Zn with Cu in tissue characterized by an elevation in the Cu/Zn ratio[5]. Thus, our data indicate that kidneys of rats fed a Zn deficiency diet are under the condition deficient in Zn.

2. It is well known that the RAS is activated shortly after the onset of ureteral obstruction[1]. In the present study, the expression of ET-1 was greater in glomeruli of the OK from rats with UUO fed a Zn deficiency diet than in those of the OK from rats with UUO fed a standard diet. However, blockade of endogenous angiotensin II with the ACE inhibitor, enalapril markedly decreased the expression of ET-1 observed in glomeruli of the OK from rats with UUO fed a Zn deficiency diet. These observations suggest that Zn deficiency augments the expression of ET-1 in glomeruli of the OK from rats with UUO by further enhancing the RAS activated after the induction of ureteral obstruction.

3. There is the possibility that Zn deficiency aggravates renal function, especially glomerular function in rats with UUO by further increasing the vasoconstrictors, ET-1 and angiotensin II upregulated after the induction of ureteral obstruction.

References

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