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Selenium deficiency induced damages and altered expressions of metalloproteinases and their inhibitors (MMP1/3, TIMP1/3) in the kidneys of growing rats

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ABSTRACT

Selenium is an essential trace element for the maintenance of structures and functions of kidney. To evaluate the effects of low selenium on the kidneys of growing rats, newborn rats were fed with selenium deficient and normal diets respectively for 109 days. As a result, rats fed with low selenium diets resulted in a decline in the body weight and the concentration of selenium in the kidney, especially the male rats from the low selenium groups. Moreover, the ultrastructure of glomerulus and tubules were damaged in low selenium group: the glomeruli were observed with hyperplasia of mesangial cells, fusion of podocyte foot processes and thickening of basement membrane; and the tubules were observed with vacuolar degenerated epithelial cells, increased edema fluid or protein solution between cells, microvilli edema, increased cell gaps and decreased cell links. Furthermore, the pathological changes in selenium deficient group included the increase of fibers around renal hilum aorta and in the renal collecting duct, and shed of cells in the proximal convoluted tubules. In addition, up-regulated expressions of matrix metalloproteinases (MMP1/3) and down-regulated expressions of their inhibitors (TIMP1/3) at the mRNA and protein levels were also appeared to be relevant to low selenium. The results suggested that low selenium in diet may cause low selenium concentration in the kidney of growing rat and lead to damages of the ultrastructure and extracellular matrix (ECM) of kidney.

1. Introduction

Selenium, an essential trace element, is necessary for normal growth and development of humans and other animals [1]. Selenium is incorporated into vital selenoproteins such as glutathione peroxidase as a part of the active site [2]. There are mounting evidence that suggests the importance of selenium in the development of chronic kidney disease (CKD) [3]. According to World Health Organization (WHO), China is one of the 40 countries designated as low selenium or selenium deficient [4]. Although, there are some areas with high selenium intake in China, the selenium deficient areas account for 72% of the country's total area, its deficiency affects over 70 million people who face the potential adverse health impacts due to selenium deficiency [5].

Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) plays a major role in the maintenance of extracellular matrix (ECM) homeostasis [6]. Constant remodeling of the ECM is necessary for epithelium–mesenchyme interactions in renal development, the determination of nephron number, and for early in-vitro branching morphogenesis [7,8]. Proteins of the MMPs and TIMPs family are involved in the breakdown of extracellular matrix in the disease processes of CKD [9,10]. Altered expression or activity of MMPs have been found in the renal tissue of rats with hydronephrosis [11,12]. MMP modifications were reported to be linked

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to glomerular and tubular alterations [13,14]. However, the relationships of abnormal MMP1/3, TIMP1/3 expressions and glomerular or tubular damages by low selenium were still not clear.

In order to understand the influences of selenium deficiency on the ultrastructure of rat's kidney as well as the expression of MMPs and TIMPs in rat's kidney, in this study, animal experiment was conducted. By using kidneys of normal control rats as comparisons, we investigated the selenium concentrations by atomic fluorescence spectrometry (AFS), the ultrastructure changes of glomerular and tubules by transmission electron microscopy (TEM), the pathological changes by Harris Hematoxylin and Eosin (HE) staining, the expressions of MMP1, MMP3, TIMP1 and TIMP3 at RNA and protein levels by real-time polymerase chain reaction (qPCR) and immunohistochemistry, respectively in the kidneys of growing rats from low selenium group.

2. Materials and methods

2.1. Animal groups and intervention

Twenty-four unweaning Sprague–Dawley rats (SPF class, body weight 30 ± 3 g) (12 male and 12 female) were obtained 18 days after born from the Center of Animals Laboratory in Xi'an Jiaotong University, Xi'an, Shaanxi, PR China. Rats were raised at 22 ± 2 °C with relative humidity of 65 ± 4%, and 12 h light/dark cycle. Before grouping, all rats were quarantined without disease or injury. After quarantine, the rats were divided by a computerized blocking procedure into two groups (every group with 12 rats, 6 male and 6 female). One group was fed with a standard diet (containing 0.18 mg selenium/kg food from Trophic Animal Feed High-tech Co., Jiangsu, China) as control; the other was fed with a low selenium diet (0.02 mg selenium/kg food produced by Trophic Animal Feed High-tech Co., Jiangsu, China) according to AIN-93 M formula. All rats were fed sufficiently with food and deionized water freely accessible at all times for 109 days before sacrifice. Use of animals in this study was in accordance with NIH publication 85-23 "Guide for Care and Use of Laboratory Animals" (NRC, 1996). The experiment was approved by the Animal Ethics Committee, Xi'an Jiaotong University. The work described has been carried out in accordance with EU Directive 2010/63/EU for animal experiments.

2.2. Collection of kidney samples

Before the sacrifice of rats, the weight of every rat was obtained. Then after the sacrifice of rats, kidney samples were harvested for further experiments. Samples from every rat were cut into pieces on ice, which were collected into 1.5 ml sterile non-enzymatic eppendorf tubes (transferred to liquid nitrogen and then to −80 °C freezer), 1.5 ml eppendorf tubes with 2.5% glutaraldehyde fix solution, and 50 ml 4% paraformaldehyde in PBS.

2.3. Selenium concentration detection

The concentrations of selenium in kidney samples (n = 12 from each group) were determined by atomic fluorescence spectrometry (AFS-2202E, Beijing Kchuang Haiguang Instrument Co., Ltd., China). Standard selenium solutions were used to calibrate the results. The standard curve linear equations of IF (fluorescence intensity) and C (concentration of selenium) was obtained as $IF = 68.944 \times C - 0.610$, correlation coefficient $r = 0.9999$, the sample was analyzed in triplets. We quantified the samples according to the following procedure: an accurately weighed sample (less than 0.05 g) was placed in 15 ml tube with 2 ml mixed acid solution (HNO_3:$HClO_4$ = 4:1), the sample was digested overnight at room temperature and later between the temperature of 170 °C and 190 °C when all the tissue was dissolved in the acid solution, until thick white smoke appeared, the solution was digested to slightly dry, and then 2 ml 1:1 HCl were added, when the thick white smoke appeared again, the mixed solution was digested to slightly dry, then 20% HCl were added to volume to 15 ml, the solution was placed overnight at room temperature and finally analyzed by AFS following the manufactory's measurement instructions of AFS-2202E.

Table 1
The selenium concentrations in the rat's kidneys from low selenium and control groups.

	Selenium concentrations (μg/g)				Mean (μg/g)		SD (μg/g)		P^a
Group	ZM	ZF	SM	SF	*Z*	*S*	*Z*	*S*	value
	1.58	1.31	0.54	0.81	1.29	0.72	0.19	0.12	<0.001
	1.12	1.14	0.51	0.84					
	1.34	1.19	0.55	0.77					
	1.32	1.36	0.61	0.93					
	1.25	1.27	0.57	0.82					
	1.33	1.31	0.52	1.12					
Mean (μg/g)	1.32	1.26	0.55	0.88					
SD (μg/g)	0.15	0.08	0.04	0.13					
P^b value	0.412		0.001						

Z: control group; *S*: low selenium group; ZM: male rats in control group; ZF: female rats in control group; SM: male rats in low selenium group; SF: female rats in low selenium group.

[a] P value for se-deficient vs. control rats by t test.

[b] P value for female groups vs. male groups by t test in both Z and S groups.

2.4. Ultrastructure observed by TEM

Transmission electron microscopy (TEM, H-7650, Hitachi, Japan) was used to investigate the ultrastructure of the samples (n = 4 from each group). Samples were fixed in 2.5% glutaraldehyde solution, washed by 0.1 M PBS for 30 min, fixed by 1% osmium tetroxide solution for 2 h, washed by 0.1 M PBS for 10 min, dehydrated by ethanol, replaced by propylene oxide for 10 min, embedded in epoxy resin. Then 1–2 μm ultrathin sections were cut and stained by methylene blue with an optical microscope for location, the sections were sliced by ultramicrotome (Sweden LKB-V) to obtain ultrathin slices with 50–70 nm. The slices were stained with uranyl acetate and lead citrate and finally observed by TEM.

2.5. RNA extraction and qPCR

Total RNAs were extracted using RNAfast 200 kit (Fastagen, Shanghai, China). The quality and concentration of RNA were assayed by a NanoDrop spectrophotometer (Thermo Scientific, USA), and then subjected to reverse transcription using RevertAidTM First Strand cDNA Synthesis kit (Fermentas, MBI, Vilnius, Lithuania) by eppendorf gradient type mastercycler (Eppendorf, Hamburg, Germany). Reverse transcription products were used for quantitative real-time PCR analysis (n = 4 from each group) performed with iQTM5 Real-time PCR Detection Systems device (Bio-Rad, Philadelphia, PA, USA) using BioEasy SYBR Green I Real Time PCR Kit (Bioer, Hangzhou, China) with oligonucleotide pairs specific for rats MMP1, MMP3, TIMP1, TIMP3 and GAPDH with the following cycling conditions: 95 °C for 10 min, 95 °C for 15 s, 60 °C for 60 s with 45 cycles, followed by a melting curve analysis. The forward and reverse primer pairs designed to generate a 110-bp fragment of MMP1, a 161-bp fragment of MMP3, a 85-bp fragment of TIMP1, a 149-bp fragment of TIMP3, and a 138-bp fragment of GAPDH (an internal control) are presented in Table 1. The results of relative gene expression data were analyzed using Real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method [15].