Vitamin E Ameliorates Renal Fibrosis in Ureteral Obstruction:Role of Maintaining BMP-7 during Epithelial-to-Mesenchymal Transition

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Background: *Epithelial to mesenchymal transition (EMT) is a process which tubular epithelial cells (TEC) undergo a phenotypic conversion to the matrix-producing fibroblasts and myofibroblasts. Phenotypic alteration of TEC was induced by the important cytokine transforming growth factor-β (TGF-β) to development of renal fibrosis. However, bone morphogenetic protein-7 (BMP-7) generally counteracts with TGF-β to maintenance of epithelial phenotype. In the present study, the authors investigated the anti-fibrotic property of vitamin E on unilateral ureteral obstruction (UUO) model mice.*
Material and Method: *UUO or sham-operated mice were randomly assigned to receive vitamin E (alpha tocopherol) or placebo and were sacrificed on days 3, 7 and 14 after operation. Kidney specimens were fixed for pathological study and immunohistochemistry for BMP-7. Protein expression of BMP-7 was determined by western blot analysis. The mRNA expression of BMP-7 and TGF-β1 were measured by real-time RT-PCR.*
Results: *Vitamin E treated UUO mice showed the less severity of renal fibrosis. Tubular atrophy and interstitial fibrosis were significantly attenuated in vitamin E treatment. Immunohistochemistry revealed decreasing of BMP-7 protein expression in cytoplasm of TEC in obstructed kidneys. Moreover, decreasing of BMP-7 protein and downregulation of BMP-7 mRNA in UUO mice were confirmed by western blot and real time RT-PCR. In contrast, vitamin E treatment significantly maintained the expression of BMP-7 protein and mRNA in UUO mice compared with placebo treatment. On the other hand, TGF-β1 mRNA expression showed progressive upregulation in UUO mice on day 3, 7 and 14 compared with sham controls. The expression of TGF-β1 mRNA was significantly lower in all vitamin E treated UUO mice compared with placebo treatment.*
Conclusion*: Vitamin E treatment attenuated the progression of renal fibrosis in obstructed kidney by inhibited the TGF-β1 but maintained the BMP-7 during EMT. Thus, the renoprotective effects of vitamin E could have some therapeutic value to inhibit the progression of renal fibrosis in human.*

Keywords*: Renal fibrosis, Vitamin E, BMP-7, TGF-β1, EMT*

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Chronic kidney disease (CKD) is one of the important public health problems around the world. Many reports demonstrated the rising incidence and prevalence of kidney failure, with poor outcomes and high therapeutic cost[1-3]. CKD is characterized by irreversible and progressive renal fibrosis, which commonly observed tubular atrophy and interstitial fibrosis (TA/IF) in the kidney. One of the main effector cells that contributable to renal fibrosis in CKD is the tubulointerstitial fibroblast. Many studies demonstrated that the relative amounts of the interstitial fibroblasts are originated from the tubular epithelial cells (TEC) through the process of epithelial-to-mesenchymal transition (EMT)[4,5]. During the process

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of EMT, TEC lose their epithelial phenotype but obtain the mesenchymal phenotype, disruption of tubular basement membrane and enhanced cell migration into the interstitial area[6,7]. Transforming growth factor-beta (TGF-β) superfamily has important roles during embryonic development and control kidney homeostasis in the adult. TGF-β1 is proposed to be the major cytokine for inducing EMT. This cytokine regulates a switch in TEC toward a profibrogenic phenotype, which predates onset of interstitial fibrosis[6,8-10]. In contrast, bone morphogenetic protein-7 (BMP-7) is a member of BMP subfamily within the TGF-β superfamily, which acquires the opposite function to TGF-β1. During kidney development, the structures of the nephron from the glomerulus to tubule derive from the metanephric mesenchyme. The mesenchymal cells change their phenotype and produce highly organized epithelial under the control of BMP-7[11,12]. In adult kidney, BMP-7 is expressed in kidney tubules and acts as a survival factor for epithelial position[13]. Interestingly, several studies suggested that BMP-7 might have renoprotective effect on TEC by counteracting with TGF-β1 leading to a repairment of damaged TEC during chronic renal injury[14,15].

Vitamin E, particularly in the form of alpha tocopherol, has been proposed for the prevention or treatment of numerous health problems[16], which is primarily due to its antioxidant and anti-inflammatory properties[17,18]. Supplement with vitamin E exhibited anti-inflammatory activity in both vitro and *in vivo*[18,19]. Vitamin E was demonstrated to suppress pro-fibrotic gene in some chronic renal injury model and diminish progression of renal fibrosis[20]. Many studies showed that vitamin E treatment could decrease the expression of TGF-β1 and ameliorated organ injury, including in pulmonary fibrosis[21], heart fibrosis[22], chronic pancreatitis[23] and other diseases.

In present study, the authors hypothesized that renoprotective effect of vitamin E could be mediated by attenuation of fibrogenesis in the kidney. Therefore, the authors aimed to examine whether vitamin E treatment has inhibitory effects against progression of renal fibrosis in an animal model of complete unilateral ureteral obstruction (UUO). This advantage of vitamin E treatment could become from inhibitory effect against TGF-β1 and protective effect to maintain BMP-7 in the kidney.

Material and Method

Animals Care and Experimental Model

An official ethic committee in Thammasat University approved all experiments on animals. Male ICR mice weighing 25-30 g were obtained from National Laboratory Animal Center (Mahidal University) and allowed to acclimatise for 2 weeks prior to surgery. All mice received tap water and a standard diet and were housed in 12 hr light and 12 hr dark cycle.

All animal experiments were conducted in accord with the Thammasat Animal Experimental Unit Guideline. Mice were anesthetized with pentobarbital sodium at dose of 40-60 mg/kg by intra-peritoneal injections. The abdominal region was shaved and the animals were placed on a heating table to maintain them at constant body temperature at 37 $\pm$ 1°C while under anesthesia. The abdomen was soaked with Betadine, and sterile drapes were applied. A midline abdominal incision was made and both kidneys and ureters were identified. The left ureter was dissected out and ligated with 4.0 silk at two points along its length. The wounds were closed in two layers with 4.0 silk and mice were allowed to recover. Following surgery the animals were returned to the cages, where they had free access to food and water. Mice were divided into the following four experimental groups (total = 48): (1) Sham-operated control group (n = 6): mice were subjected to the surgical procedures described above except for the ureter ligation and received oral placebo. (2) Sham-operated control + vitamin E group (n = 6): these sham-operated mice were received oral vitamin E 250 mg/kgBW. (3) UUO group (n = 18): mice were subjected to the surgical procedures described above and were received oral placebo. (4) UUO + vitamin E group (n = 18): these UUO mice were administered oral vitamin E 250 mg/kgBW. Vitamin E and placebo were administrated every day from the 5 days prior and 14 days after operation. One-third of mice were sacrificed on day 3, one-third on day 7 after UUO or Sham operation and the other on day 14. Kidneys were dissected from mice and sliced from the corona. These sections were fixed in 10% formalin and processed for histology using standard techniques. A small section of the kidney was frozen in liquid nitrogen stored at -70°C for protein measurements by Western blot analysis, while another section was fixed in RNA later Stabilization Solution (Ambion, Inc) for RT-PCR gene expression studies.

Renal histology and immunohistochemistry

Kidneys were dissected from mice and tissue slices were fixed in 10% formalin and processed for histology examination using standard techniques. Formalin tissue was embedded in paraffin and 4

micrometer sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and masson's trichrome. These sections were examined in a blinded fashion by a nephrologist. The percentage of histology changes, including degree of glomerulosclerosis, tubular atrophy and interstitial fibrosis were evaluated under high power magnification (400x) in 5 to 10 consecutive fields and mean percentages of histological change were calculated.

Organs were fixed in 4% paraformaldehyde. Five-micrometer paraffin sections were dewaxed and rehydrated. For antigen retrieval, kidney sections were microwaved for 30 minutes. Endogenous peroxidase was quenched with 3% H_2O_2 for 20 min and non-specific binding blocked with 20% normal goat serum in phosphate-buffered saline (PBS) (pH 7.4). Sections were incubated overnight at 4°C with primary rabbit antibody against BMP-7 (1:500; Abcam: Biomed Diagnostics (Thailand) Co. Ltd) followed by Envision reagent (Dako, Bangkok Thailand) containing anti-rabbit secondary antibody, and finally with 3, 5-diaminobenzidine (DAB) substrate. Negative controls using normal rabbit IgG were also included. Nuclei were counterstained with hematoxylin, and slides were dehydrated and mounted with permount.

Protein extraction

Briefly, 40 mg of kidney (wet weight) was homogenized in 240 µl of 40 mM Tris-HCl (pH 7.6) buffer containing 0.1% Nodinet P-40, 0.05% sodium deoxycholate, 0.01% SDS, 150 mM NaCl and 10 mM 2-mercaptoethanol. Homogenates were treated with 60 µg/ml of PMSF and centrifuged in a pre-chilled rotor at 15,000 xg for 15 min. Supernatants were stored at -70°C. Protein content was measured using a BCA™ Protein Assay Kit (PIERCE, IL, USA).

Western blotting

Protein samples were electrophoresed on 12% SDS-PAGE mini-gels and wet-transferred (Bio-Rad, ON, Canada) onto nitrocellulose membranes. Membranes were treated with blocking solution followed by an overnight incubation at 4°C with rabbit polyclonal antibody to BMP-7 (Abcam: Biomed Diagnostics (Thailand) Co. Ltd); 1:500 dilution) in 5% BSA-TTBS. The secondary antibody (PIERCE, IL, USA) was diluted to 1:100,000 in 5% BSA-TTBS and membranes treated for 1 hour at room temperature. Signals were visualized by chemiluminescent detection according to the manufacturers' instructions (PIERCE, IL, USA). Signals were quantified using GeneGnome Syngene Bio Imagine and GeneSnap image acquisition software (Syngene, MD, USA).

Real time polymerase chain reaction (RT-PCR)

Total RNA were extracted using the RNeasy mini kit (Qiagen, Chatworth, CA, USA) according to the manufacturers' instructions. High-quality RNA was eluted in 35 µl RNase-free water. An aliquot of each RNA preparation was used to determine total RNA quality and concentration, measured at 260 nm (OD_{260}). Pure RNA possessed an OD_{260}/OD_{280} ratio of 1.6-1.9. Total RNA (0.25 µg) was reverse-transcribed to cDNA by Taqman™ Reverse Transcriptase Reagent (Applied Biosystems, Roche Molecular Biochemical, NJ, USA) using random primers using the following cycling conditions: 25°C, 10 min; 48°C, 30 min; 95°C, 5 min. The mRNA levels of TGF-β1, BMP-7 and hypoxanthine phosphoribosyltransferase (HPRT) were measured using a ABI PRISM 7700 Sequence Detection System (SDS version 1.6; PE Applied Biosystems). The primers and probe used were as follows (Table 1). Each PCR was assembled in 20 ml volumes consisting of 10 ml of 2xQuantiTech Probe master mix (Qiagen, Chatworth, CA, USA), 0.5 ml of 20 mM forward primer, 0.5 ml of 20 mM reverse primer, 0.2 ml of 20 mM probe and 6.8 ml of RNase-free water. Following the addition of 2 ml of cDNA template, PCR amplification was performed using an initial denaturation step. Real-time PCR results were automatically recorded by ABI PRISM 7700 Sequence Detection System (SDS version 1.6; PE Applied Biosystems) and analyzed by relative quantification using the comparative Ct method. Ratios for TGF-β1/HPRT and BMP-7/HPRT mRNA were calculated for each sample and expressed as the mean ± SD.

Statistical analyses

Data were expressed as mean ± SD. Statistical analyses were carried out using the SPSS software (version 15.0). Statistically significant differences among groups were calculated by ANOVA Bonferroni and Mann-Whitney tests using the least significant difference method. Statistical significances were defined as $p < 0.05$.

Results

Vitamin E protects against renal fibrosis in mice UUO model. The authors assess the renoprotective effect of vitamin E on renal fibrosis. The kidneys of mice subjected to sham or UUO operation and treated with either placebo or vitamin E were examined for histopathology. From PAS staining, the obstructed

kidney exhibited significantly increased TA since days 3, days 7 through days 14 compared with the sham control (Table 2). However, TA was decreased with vitamin E treatment in any time course of UUO kidneys compared with the placebo treatment (Table 2) ($p < 0.05$). Masson's trichrome stained kidney sections demonstrated a progressive increasing of collagen deposit in interstitial area since 3, 7 and 14 days after undergoing UUO in placebo treatment (Fig. 1B-1D, Table 2). In contrast, treatment with vitamin E suppressed collagen deposit in UUO mice any time course of UUO compared with the placebo treatment (Fig. 1F-1H, Table 2) ($p < 0.05$).

Vitamin E treatment could maintain the expression of BMP-7 in UUO kidneys. In sham kidneys, we demonstrated the staining of BMP-7 (Fig. 2A) in the cytoplasm of TEC, whereas the labeling of BMP-7 was decreased in cytoplasm of TEC particularly in dilated and atrophic tubules of the placebo treated UUO kidneys since day 3 after UUO (Fig. 2B) and progressive loss until day 14 (Fig. 2C and 2D). Staining of BMP-7 in sham with vitamin E treatment appeared similar to sham kidneys (Fig. 2E). Whereas, vitamin E treatment in mice with UUO demonstrated the significantly preserved the cytoplasm of TEC staining intensity of BMP-7 in the obstructed kidneys in any time courses (Fig. 2F through 2H). Moreover, we examined the effects of vitamin E treatment in UUO mice on protein and mRNA expression in BMP-7. From western blot analysis, the authors demonstrated the progressive decreasing of BMP-7 protein level in the obstructed kidneys with placebo treatment since day

Table 1. Sequences of real-time PCR primers and probes

Gene	Sequence
BMP-7	
Forward	5'-TGGATGGGCAGAGCATCAA-3'
Reverse	5'-CTTGGAG CGATTCTGGCTG-3'
Probe	5'-FAM-ATTGGACGGCATGGACCCCAGA-TAMRA-3'
TGF-β1	
Forward	5'-GGCTACCATGCCAACCAGCCTGGTGTACTCA-3'
Reverse	5'-CCGGGTTGTGTTGGTTGTAGA-3'
Probe	5'-FAM-CACACAGTACAGCAAGGTCCTTGCCCT-TAMRA-3'
HPRT	
Forward	5'-TGACACTGGTAAAACAATGCAAACT-3'
Reverse	5'-AACAAAGTCTGGCCTGTATCCAA-3'
Probe	5'-FAM-TTCACCAGCAAGCTTGCAACCTTAACC-TAMRA-3'

Table 2. The percentage of histopathology changes, such as degree of glomerulosclerosis, tubular atrophy and interstitial fibrosis in UUO mice

	Sham		UUO					
	Day 0		Day 3		Day 7		Day 14	
	placebo	vitamin E	placebo	vitamin E	placebo	vitamin E	placebo	vitamin E
Glomerular Sclerosis (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.6 ± 0.4	0.5 ± 0.5	1.5 ± 0.8	1.4 ± 0.8
Tubular Atrophy (%)	0.4 ± 0.5	0.5 ± 0.4	9.6 ± 1.8*	3.8 ± 1.2**	18.6 ± 3.8*	7.4 ± 1.6**	44.6 ± 4.7*	18.6 ± 5.8**
Interstitial Fibrosis (%)	0.4 ± 0.5	0.5 ± 0.4	9.8 ± 1.9*	4.2 ± 0.8**	20.2 ± 3.2*	11.6 ± 1.9**	58.6 ± 6.8*	25.8 ± 8.4**

Values are means ± SD
Significant difference *$p < 0.05$ compared to sham group; **$P < 0.05$ compared to UUO group

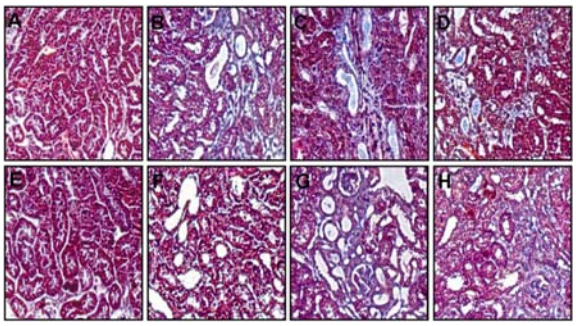

Fig. 1 Treatment with vitamin E inhibited a progression of renal fibrosis in UUO mice. Masson's trichrome staining for assessing renal interstitial fibrosis in UUO mice. (A) sham-operated control. (B) The obstructed kidneys showed progressive tubular atrophy and interstitial fibrosis at day 3, (C) day 7 and (D) day 14 after UUO compared with the sham group which was apparently ameliorated by vitamin E treatment (F-H)

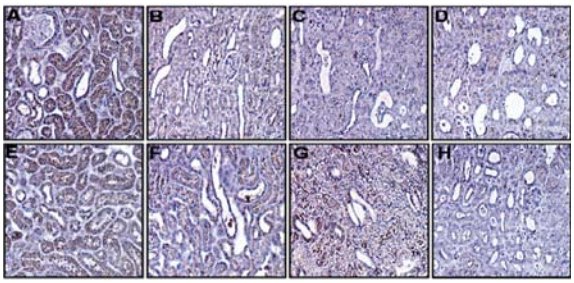

Fig. 2 BMP-7 protein expression by immunohistochemical staining. (A) In sham-operated control kidneys, BMP-7 was detected in the cytoplasm of TEC, whereas the labeling of BMP-7 was decreased in cytoplasm of TEC particularly in dilated and atrophic tubules of the UUO kidneys compared with the sham at (B) day 3, (C) day 7, and (D) day 14. (E) In sham + vitamin E kidneys, BMP-7 staining was seen similar to the sham group. (F) In contrast, staining of BMP-7 was stronger in UUO mice with vitamin E treatment at day 3, (G) day 7, and (H) day 14 compared to placebo treatment groups

3, 7 through 14 compared with sham kidneys (Fig. 3) ($p < 0.05$). In contrast, UUO mice with vitamin E treatment showed significantly delayed the declining of BMP-7 protein, (Fig. 3) compared with the placebo treated UUO mice in matched time courses ($p < 0.05$). Consistent with the immunohistochemistry and western blot analysis, real time RT-PCR revealed the downregulation of BMP-7 mRNA expression which significantly progressive changed in any time course during

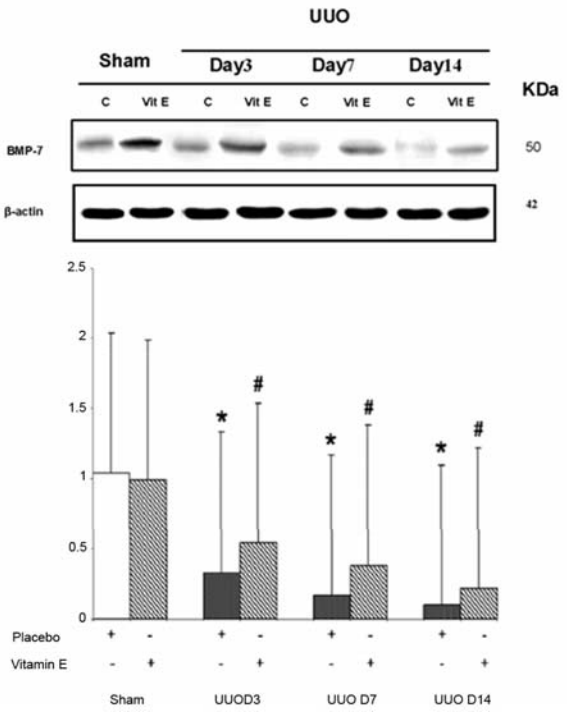

Fig. 3 Western blot analysis for BMP-7 protein expression in UUO mice. BMP-7 protein expression was significantly decreased in UUO mice on day 3, day 7 and day 14 compared with sham or sham + vitamin E. Treatment with vitamin E resulted in a maintain the levels of BMP-7 in any time course. *$p < 0.05$ vs. sham group; #$p < 0.05$ vs. UUO group

obstructive processes (Fig. 4) ($p < 0.05$). On the other hand, treatment with vitamin E in UUO mice showed significantly maintained the downregulation of BMP-7 mRNA expression, compared with placebo treatment UUO mice (Fig. 4) ($p < 0.05$).

Vitamin E treatment inhibited TGF-β1 gene expression in UUO kidneys. Next, the authors also assessed expression level of TGF-β1 mRNA, the most important cytokine which induced renal fibrosis. TGF-β1 mRNA expression was upregulated significantly 5.89 fold at day 3 after undergoing UUO. In addition, upregulation of TGF-β1 mRNA was 8.78 fold at day 7, and 9.86 fold at day 14 in obstructed kidneys. In contrast, treatment with vitamin E in UUO mice become significantly inhibited the upregulation of TGF-β1 mRNA expression compared with placebo treated UUO mice (Fig. 5) ($p < 0.05$).

Discussion

CKD is becoming a major public health

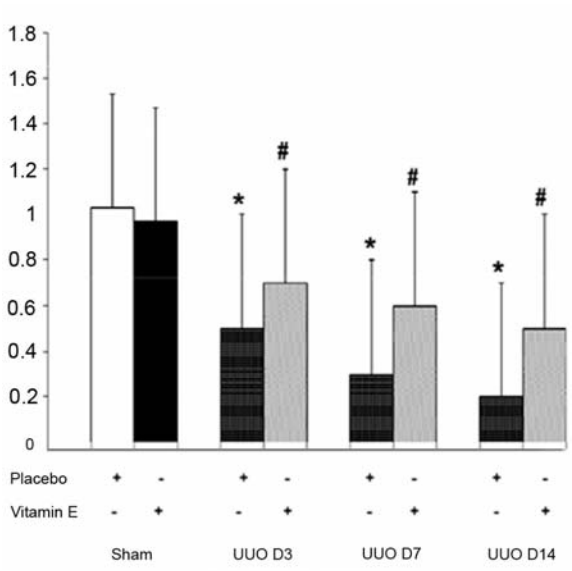

Fig. 4 Real time RT-PCR for BMP-7 mRNA expression in UUO mice. BMP-7 mRNA expression was markedly downregulated in UUO mice on day 3, day 7 and day 14 compared with sham and sham + vitamin E. Treatment with vitamin E in obstructed kidneys showed significantly higher level of BMP-7 than placebo treatment groups. *$p < 0.05$ vs. sham group; #$p < 0.05$ vs. UUO group

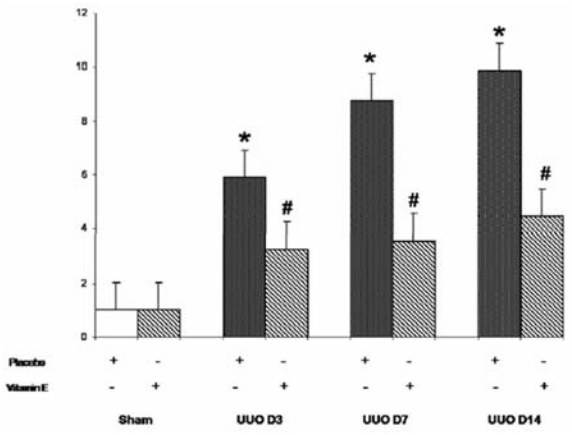

Fig. 5 Real time RT-PCR for TGF-β1 mRNA expression in UUO mice. TGF-β1 mRNA expression was markedly progressive upregulated in UUO mice on day 3, day 7 and day 14 compared with sham and sham + vitamin E and significantly downregulated in vitamin E treated groups compared with placebo treatment groups. *$p < 0.05$ vs. sham group; #$p < 0.05$ vs. UUO group.

problem worldwide. The current burden of disease might due to a change of the underlying pathogenicity of CKD. EMT and apoptosis of TEC beside tubulointerstitial infiltration of inflammatory cells are well known characteristic of chronic kidney injury as demonstrated in UUO model[4,24]. Most striking feature observed after UUO is the development of TA/IF in the progression of renal diseases[25]. Importantly, a large proportion of the interstitial fibroblasts are known to be originated from the TEC through the process of EMT in the progression to the renal fibrosis. Many studies *in vivo* and *vitro* demonstrated that TGF-β1 is the most powerful cytokine which induced EMT[8,9]. In contrast, BMP-7 has the opposite interaction and counteract with TGF-β1 to support TEC function and architecture[14,26]. Even though, any specific therapies that inhibit the progression of CKD are unavailable from any revisions. Inhibiting EMT of TEC could be the best therapeutic option which potentially retarded renal fibrosis. The present study demonstrated that anti-inflammatory capacity of vitamin E was able to suppress TGF-β1 expression different to maintain BMP-7 level in the obstructed kidneys. Inhibiting TGF-β1 and preserving BMP-7 were the renoprotective effect of vitamin E that delays the progression of TA/IF in UUO mice. Fibrogenesis amelioration by blockade EMT could be mediated by inhibition of the TGF-β1 but maintain BMP-7 counter balance. These findings suggested that treatment with vitamin E could be applied to inhibit the development of renal fibrosis by attenuating EMT from the imbalance of TGF-β1/BMP-7 signalling pathway.

In present study, the authors' used the UUO model in mice to induce TA/IF that develop progression of renal fibrosis. The authors demonstrated that UUO induced an over expression of TGF-β1 gene in the obstructed kidney. TGF-β is known to be a major cytokine that regulate EMT, so the increasing of TGF-β1 in the obstructed kidneys could be involved in the loss of the epithelial phenotype and the achievement of the mesenchymal phenotype. TGF-β is a multifunctional cytokine that control various cellular processes, such as proliferation, apoptosis, growth arrest, and renal fibrosis through EMT[10]. Many studies have demonstrated that TGF-β promotes renal fibrosis through EMT and counteracts with BMP-7[14,26]. During kidney development, BMP-7 plays the important role to promote MET inducing nephron formation. In addition, BMP-7 was demonstrated to maintain the epithelial state of TEC[27]. In present study, BMP-7 was downregulated during UUO in any time course. This effect could reduce the protective mechanism of TEC by loss BMP-7 and TEC change to be the mesenchymal

cells consequently. These data represented the protective effect of BMP-7 as an anti-fibrosis during chronic kidney injury and could be suppressed by the potent inflammatory cytokine TGF-β.

Vitamin E has been proposed for the prevention or treatment of numerous health problems due to its antioxidant and anti-inflammatory properties[17]. In present study, the vitamin E treatment effectively reduced TA/IF in UUO mice. In addition, treatment with vitamin E can suppress the upregulation of TGF-β during time course of UUO similar to many studies during acute and chronic inflammation[20,28]. The benefit of vitamin E treatment including inhibition of TGF-β inducing EMT was demonstrated in the present study. In contrast, treatment with vitamin E can preserve BMP-7 in obstructed kidneys. Maintaining of BMP-7 by vitamin E could be the renoprotective effect by supporting function and phenotype of TEC leading to ameliorate EMT. Fibrogenesis prevention by vitamin E presented similar as BMP-7 treatment in chronic renal injury[29].

In conclusion, vitamin E treatment protected the kidney from chronic injury by modifying the notion of TGF-β and BMP-7 signals regulating TEC during EMT. Administration of vitamin E could be an effective treatment to preserve renal histology and renal function in UUO model. Thus, the renoprotective effects of vitamin E could have some therapeutic values in inhibiting the progression of renal fibrosis in human.

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Potential conflicts of interest

None.

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วิตามินอีชนิดแอลฟาชะลอการเกิดพังผืดในไตที่เกิดจากการอุดกั้นของทางเดินปัสสาวะ: บทบาทของการคงอยู่ของ BMP-7 ระหว่างการเปลี่ยนของเซลล์เยื่อบุผิวเป็นเซลล์เนื้อเยื่อยึดต่อ

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ภูมิหลัง: *การเปลี่ยนของเซลล์เยื่อบุผิวเป็นเนื้อเยื่อยึดต่อเป็นกลไกที่เซลล์เยื่อบุผิวของท่อไตเกิดการเปลี่ยนแปลงลักษณะทางกายภาพกลายเป็นเซลล์ที่เป็นเนื้อเส้นใยที่สามารถผลิตเนื้อเยื่อเสริมสร้างได้หรือเซลล์เนื้อเยื่อยึดต่อ การเปลี่ยนแปลงลักษณะทางกายภาพของเซลล์เยื่อบุผิวนี้ถูกเหนี่ยวนำโดย cytokine ที่สำคัญคือ transforming growth factor-beta (TGF-β) ทำให้มีการเกิดพังผืดขึ้นในไต อย่างไรก็ตาม bone morphogenetic protein-7 (BMP-7) สามารถลดผลกระทบจาก TGF-β เพื่อดำรงรักษาไว้ซึ่งลักษณะทางกายภาพของเซลล์เยื่อบุผิว ในการศึกษานี้ผู้วิจัยได้ทำการการทดสอบ ถึงคุณสมบัติในการป้องกันการเกิดพังผืดของวิตามินอีในหนู ที่ได้รับการเหนี่ยวนำให้เกิดการอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียว*

วัสดุและวิธีการ: *หนูที่ได้รับการเหนี่ยวนำให้เกิดการอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียว หรือหนูที่ถูกเหนี่ยวนำโดยการทำหัตรการหลอกถูกสุ่มจำแนกออกเป็น 2 กลุ่ม คือ กลุ่มที่ได้รับวิตามินอี หรือยาหลอกและถูกพิสูจน์ทราบในวันที่ 3 วันที่ 7 และวันที่ 14 หลังจากการทำหัตถการ ชิ้นเนื้อไตได้ถูกจัดเตรียมเพื่อการศึกษาทางพยาธิวิทยาและการตรวจย้อมทาง immunohistochemistry สำหรับโปรตีน BMP-7 ผู้วิจัยได้ทำการตรวจวัดปริมาณของโปรตีน BMP-7 โดยวิธี western blot และตรวจวัดปริมาณของยีน BMP-7 และ TGF-β1 โดยวิธี real time RT-PCR*

ผลการศึกษา: *ไตของหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะที่ได้รับวิตามินอีเกิดความรุนแรง ของการเกิดพังผืดในไตน้อยกว่ากลุ่มที่ได้รับยาหลอก การลีบเล็กของเซลล์เยื่อบุผิวท่อไต และการเกิดพังผืดในไตลดลงอย่างมีนัยสำคัญทางสถิติในหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะกลุ่มที่ได้รับวิตามินอี จากการย้อมทาง immunohistochemistry แสดงให้เห็นถึงการลดลงของโปรตีน BMP-7 ในไซโตพลาสซึมของเซลล์เยื่อบุผิวท่อไตที่เกิดการอุดกั้นของทางเดินปัสสาวะ นอกจากนี้การลดลงของโปรตีนและยีน BMP-7 ในไตหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะถูกยืนยันโดยการตรวจด้วยวิธี western blot และ real time RT-PCR ในทางตรงกันข้ามการรักษาด้วยวิตามินอีสามารถดำรงรักษาไว้ซึ่งระดับของโปรตีนและยีน BMP-7 ได้อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับ หนูกลุ่มที่ได้รับยาหลอก นอกจากนี้การปรากฏของยีน TGF-β1 พบว่ามีการเพิ่มขึ้นอย่างต่อเนื่องในไตหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะตั้งแต่วันที่ 3 จนถึงวันที่ 14 หลังการทำหัตถการเมื่อเปรียบเทียบกับกลุ่มควบคุม ส่วนการปรากฏของยีน TGF-β1 พบว่ามีระดับต่ำกว่าอย่างมีนัยสำคัญทางสถิติในหนูกลุ่มที่ได้รับวิตามินอี เปรียบเทียบกับหนูกลุ่มที่ได้รับยาหลอก*

สรุป: *การรักษาด้วยวิตามินอีชะลอการเกิดพังผืดในไตของหนูที่ได้รับการเหนี่ยวนำ ให้เกิดการอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียวโดยการยับยั้งสัญญาน TGF-β1 แต่ดำรงรักษาไว้ซึ่ง BMP-7 ระหว่างการเปลี่ยนของเซลล์เยื่อบุผิวไปเป็นเซลล์เนื้อเยื่อยึดต่อ การรักษาด้วยวิตามินอีอาจนำไปใช้ในการพัฒนาการป้องกัน และรักษาการเกิดพังผืดในไตของมนุษย์ต่อไป*