Vitamin E Ameliorates Renal Fibrosis by Inhibition of TGF-β/Smad2/3 Signaling Pathway in UUO Mice

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Background: *One striking feature of chronic kidney disease (CKD) is tubular atrophy and interstitial fibrosis (TA/IF). During chronic renal injury, transforming growth factor-beta (TGF-β) is involved in this process causing progression of renal fibrosis. Smad2/3 proteins have been identified to have an important function in the expression of extracellular matrix (ECM) regulation through TGF-β signaling pathway. In the present study, the authors investigated the effect of vitamin E on renal fibrosis in mice model of unilateral ureteral obstruction (UUO).*
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 UUO or sham operation. Kidney specimens were fixed for pathological study and immunohistochemistry for TGF-β1. Protein expression of TGF-β1 and Smad2/3 was determined by western blot analysis. The mRNA expression of TGF-β1 was measured by real-time RT-PCR.*
Results: *Vitamin E treated UUO mice had less severity of renal fibrosis than placebo treatment. TA/IF was significantly attenuated by vitamin E treatment. Immunohistochemistry revealed increasing of TGF-β1 protein expression in the interstitium area of obstructed kidneys. Moreover, increasing of TGF-β1 protein and upregulation of TGF-β1 mRNA in UUO mice were confirmed by western blot and real time RT-PCR. In contrast, vitamin E treatment significantly inhibited the expression of TGF-β1 protein and mRNA in UUO mice compared with placebo treatment. Interestingly, Smad2/3 protein expression became progressive increasing in UUO mice on day 3, 7 and 14 compared with sham controls. The expression of Smad2/3 protein was significantly lower in vitamin E treated UUO mice than placebo treatment in any time points.*
Conclusion*: Vitamin E treatment attenuated the progression of renal fibrosis in obstructed kidneys. The renoprotective effect of vitamin E could be mediated by inhibition of TGF-β/Smad2/3 signaling pathway.*

Keywords*: Vitamin E, TGF-β1, Smad2/3, Renal fibrosis*

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One striking feature of chronic kidney disease (CKD) is tubular atrophy and interstitial fibrosis (TA/IF). Renal fibrosis is characterized by excess accumulation of extracellular matrix (ECM) in the interstitial area. The extensive fibrosis is an important predictor of outcome in CKD patients. Therefore, many investigators have investigated the mechanisms that control the balance between synthesis and degradation of ECM[1]. 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)[2,3]. Transforming growth factor-beta (TGF-β) is a multifunctional cytokine that inhibits the proliferation of many cell types, including epithelial cells, and induces growth arrest and tissue fibrosis[4]. During

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chronic renal injury, TGF-β is involved in a progression of renal fibrosis. Many studies in human and animals demonstrated the important of TGF-β induced ECM accumulation in fibrosis kidney[5]. This cytokine controls a switch during chronic kidney injury toward the pro-fibrotic factor, which promote onset of interstitial fibrosis through activation of Smad signaling pathways[6]. TGF-β initiates its cellular response by binding to its receptor on cell membrane. Activation of the TGF-β type I receptor on TEC induces the phosphorylation of Smad2 and Smad3[7]. After partnering with the common mediator Smad4, these activated Smads translocate into the nucleus[8]. In the nucleus, these Smad proteins operate with various factors to regulate the transcription of target gene by binding to DNA that promote EMT[6]. Therefore, phosphorylation of Smad2/3 proteins have been identified to be an important pro-fibrotic effect regulated through TGF-β signaling pathway[9].

Vitamin E has been subsequently proven as antioxidant which plays an important role in cell survival and protects the integrity of tissues. Vitamin E has recently considered for exerting several effects independent of its antioxidant/radical scavenging ability[10]. Supplement with vitamin E exhibits anti-inflammatory property in both vitro and *in vivo*[11,12]. Vitamin E has been purposed for the prevention and/or treatment of numerous diseases in human[13], due to its antioxidant and anti-inflammatory properties[12,14]. In models of fibrosis, many studies demonstrated that vitamin E supplement down regulated many pro-inflammatory and pro-fibrotic genes expression, especially TGF-β and ameliorated organ injury[15-17]. Vitamin E suppressed some pro-fibrotic cytokines in chronic renal injury model and diminished progression of renal fibrosis[18,19]. Targeting TGF-β in upstream or downstream pathways may be an effective therapeutic strategy against fibrosis.

Therefore, the present study was designed to test the hypothesis that unilateral ureteral obstruction (UUO) leads to TA/IF, consequently development of renal fibrosis. Treatment with vitamin E could attenuate renal fibrosis by anti-fibrotic effect through the inhibiting of TGF-β/Smad2/3 signaling pathway.

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 (Mahidol 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 a 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 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 others 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 RNAlater 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 TGF-β1 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), 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 rabbit polyclonal antibody to TGF-β1 (Santa Cruz Biotechnology; 1:1,000 dilution) and rabbit polyclonal antibody to Smad2/3 (Santa Cruz Biotechnology; 1:1,000 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, Roch 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 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 2 x QuantiTech Probe mastermix (Qiagen, Chatworth, CA, USA), 0.5 ml of 20mM forward primer, 0.5 ml of 20 mM reverse primer, 0.2 ml of 20mM probe and 6.8 ml of RNase-free water. Following the addition of 2 ml of cDNA template, PCR amplification was performed using

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

Gene	Sequence
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'

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 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

In the present study, the renoprotective effect of vitamin E on renal fibrosis was demonstrated in a mice model of UUO. From PAS staining, the UUO animals exhibited 10% of TA at day 3, 19% at day 7 and progressed to 45% at day 14 after UUO compared with the sham control (Fig. 1A). However, the severity of TA was significantly lower in obstructed kidneys with vitamin E treatment, 4% at day 3, 7% at day 7 and 19% at day 14 compared with the placebo treatment ($p < 0.05$) (Fig. 1A). Masson's trichrome stained kidney sections demonstrated a significantly increased of collagen deposit in interstitial area for 10% since day 3, 20% at day 7 and growth to 59% at 14 days after undergoing UUO in placebo treatment (Fig. 1B and Fig. 2B-2D) compared with the sham control. In contrast, treatment with vitamin E significantly suppressed the changes of collagen deposit in UUO mice to only 4% at day 3, 12% at day 7 and 26% at day 14 compared with the placebo treatment (Fig. 1B and Fig. 2F-2H) ($p < 0.05$).

Vitamin E treatment attenuated increased TGF-β1 in UUO kidneys. The author investigated the effect of vitamin E could prevent renal fibrosis. The changes in the protein and mRNA levels of TGF-β1 in the obstructed kidneys at day 3, day 7 and day 14 after UUO were observed. By immunohistochemistry, TGF-β1 revealed no labeling in sham kidneys (Fig. 3A), whereas the recognition of TGF-β1 was strongly detection at the interstitial area of placebo treated obstructed kidneys since days 3 after UUO (Fig. 3B) and further increased staining were demonstrated at days 7 and days 14 (Fig. 3C and 3D). No staining of TGF-β1 in sham with vitamin E treatment similar to sham kidney (Fig. 3E). On the other hand, UUO mice treated with vitamin E was associated with decreased TGF-β1 staining during the time course of obstruction compared with the placebo treatment (Fig. 3F-3H) ($p < 0.05$).

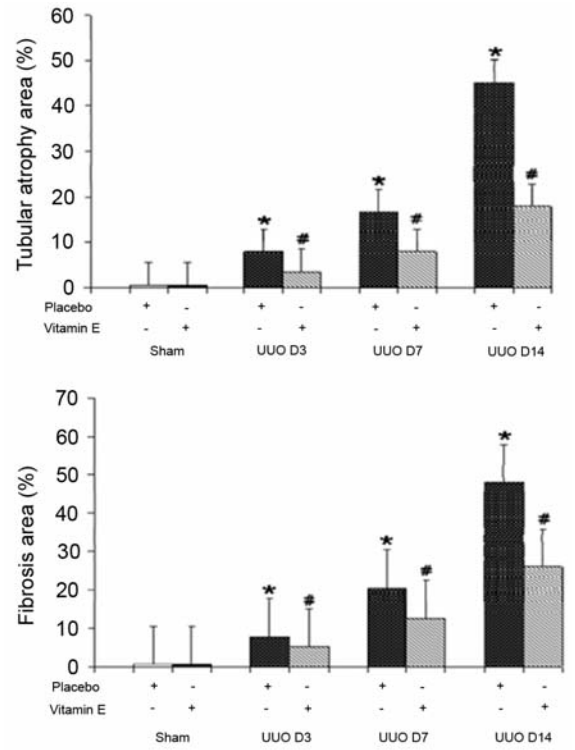

Fig. 1 The percentage of histopathology changes, including degree of tubular atrophy (1A) and interstitial fibrosis (1B) in UUO mice. Values are means ± SD Significant difference *$p < 0.05$ compared with sham group; #$p < 0.05$ compared with UUO group

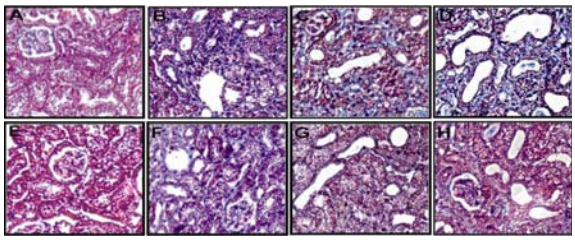

Fig. 2 Treatment with vitamin E inhibited progression of renal fibrosis in UUO mice. Masson's trichrome staining for assessing renal interstitial fibrosis in UUO mice. (A) Sham-operated control. (B) The obstructed kidneys showed progressive tubular atrophy and interstitial fibrosis at day 3, (C) day 7, and (D) day 14 after UUO compared with the sham group which was apparently ameliorated by vitamin E treatment (F through H)

Quantity assessment by western blot analysis demonstrated progressive increased TGF-β1 protein expression in the obstructed kidneys since day 3, 7 through 14 compared with the sham kidneys (Fig. 4). In contrast, TGF-β1 protein level revealed significantly decreased in the vitamin E treatment obstructed kidneys, compared with placebo treated group in any time course (Fig. 4) ($p < 0.05$). By the way, sequence of TGF-β1 mRNA upregulation was demonstrated in obstructed kidneys since 3 day after UUO and progressive elevation in day 7 to day 14. However, treatment with vitamin E in UUO mice become significantly suppressed TGF-β1 mRNA expression during match time courses compared with placebo treatment (Fig. 5) ($p < 0.05$).

Vitamin E treatment attenuated increased expression of Smad 2/3 in UUO kidneys. The authors examined the effect of vitamin E treatment in UUO mice on in TGF-β/pro-fibrosis Smad2/3 signaling pathway. Western blot analysis demonstrated the progressive increased Smad2/3 protein levels in the obstructed kidneys with placebo treatment compared with sham kidneys (Fig. 6). In contrast, UUO mice with vitamin E treatment showed significantly inhibited the increasing of the Smad2/3 protein compared with the placebo treated UUO mice (Fig. 6) ($p < 0.05$).

Discussion

In the present study, the renoprotective effect of vitamin E was demonstrated in a mice model of UUO. Renal fibrosis is a final outcome of chronic renal diseases that leads to an end-stage renal disease[20]. TGF-β has been regarded as the most important cytokine in the progression of the interstitial fibrosis. TGF-β is upregulated in UUO model, which is associated with TA/IF and increased synthesis of ECM[21]. Many evidences establish a critical role of TGF-β signaling in the development of renal fibrosis. Smad proteins mainly have been induced by TGF-β in fibrotic kidneys especially Smad2/3. Stimulation by TGF-β, transmembrane type II TGF-β receptor forms tight complexes with the type I receptor, leading to phosphorylation and activation of Smad2 and Smad3[22]. Phosphorylated Smads collaborate with the common mediator Smad 4 and translocation into the nucleus, where they control the transcription of TGF-β/Smad2/3 pro-fibrotic genes. Accumulation of ECM in the kidney is the consequent from stimulation of TGF-β/Smad 2/3 signaling pathway. Although, any specific interventions that inhibit the progression of CKD are unavailable from any reviews. Inhibiting over expression of TGF-β and Smad2/3 may be the best therapeutic options which potentially retard renal

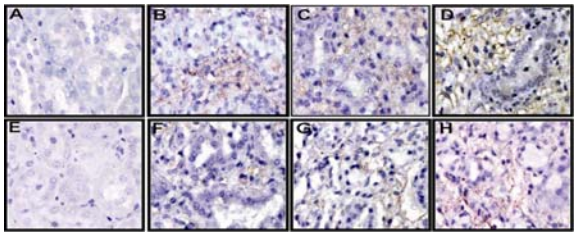

Fig. 3 TGF-β1 protein expression by immunohistochemical staining. (A) In sham-operated control kidneys, rare TGF-β1 labelling was detected. (B) In UUO, advance increased TGF-β labelling was demonstrated in the interstitium area in the obstructed kidneys compared with the sham at day 3, (C) day 7, and (D) day 14. (E) In sham + vitamin E kidneys, no TGF-β1 staining was seen similar to the sham group. (F) In contrast, decreased of TGF-β1 expression was observed in UUO mice with vitamin E treatment at day 3, (G) day 7 and (H) day 14 compared with placebo treatment groups

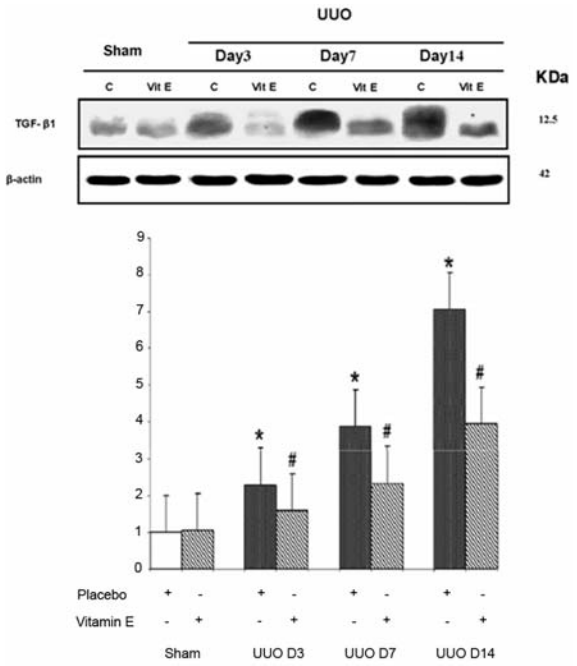

Fig. 4 Western blot analysis for TGF-β1 protein expression in UUO mice. TGF-β1 protein expression was significantly increased in UUO mice on day 3, day 7 and day 14 compared with sham or sham + vitamin E. Treatment with vitamin E decreased TGF-β1 level in any time course. *$p < 0.05$ vs. sham group; #$p < 0.05$ vs. UUO group

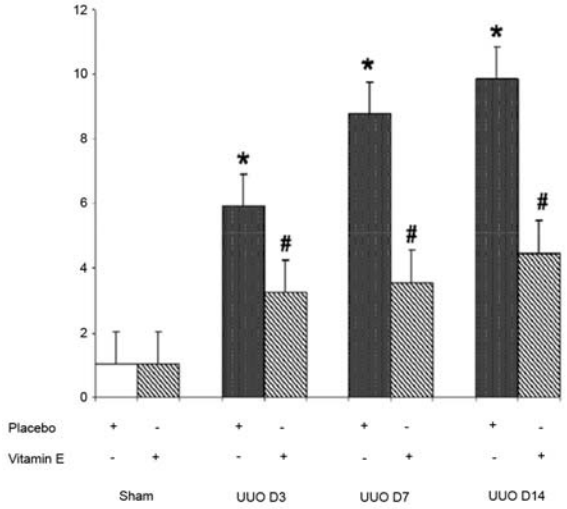

Fig. 5 Real time RT-PCR for TGF-β1 mRNA expression in UUO mice. TGF-β1 mRNA expression was progressively upregulated in UUO mice on day 3, day 7 and day 14 compared with sham and sham + vitamin E. TGF-β1 mRNA expression was significantly downregulated in vitamin E treated groups compared with placebo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

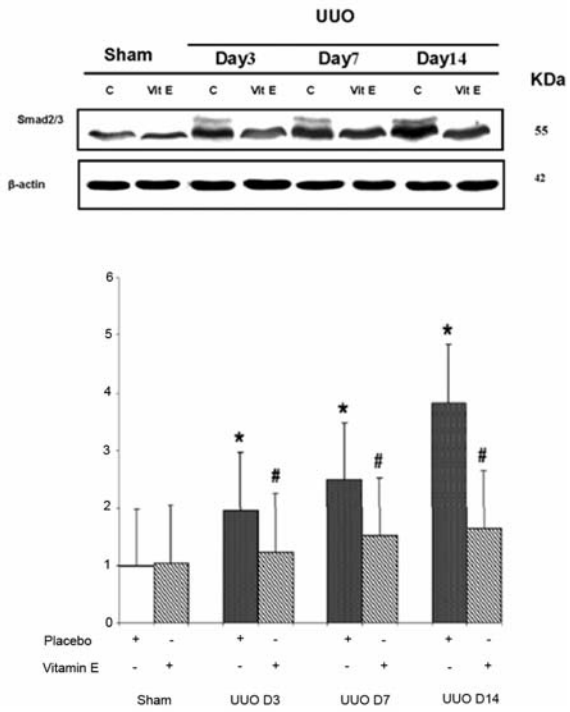

Fig. 6 Western blot analysis for Smad2/3 protein expression in UUO mice. Significantly progressive increased Smad2/3 protein expression was demonstrated in UUO mice on day 3, day 7 and day 14 compared with sham and sham + vitamin E. Treatment with vitamin E resulted in a decrease in the level of Smad2/3 protein compared with placebo treatment groups. *p < 0.05 vs. sham group; #p < 0.05 vs. UUO group

fibrosis. The present study, the authors demonstrated that anti-inflammatory effect of vitamin E could suppress TGF-β1 and Smad2/3 expression in the obstructed kidneys. These results showed the renoprotective effect of vitamin E to attenuate the progression of TA/IF in UUO mice. Fibrogenesis inhibition with vitamin E was demonstrated by deceasing of TGF-β1 and Smad2/3 that represent profibrotic mediators. Ameliorate fibrosis could be mediated by attenuation of the TGF-β1 induced pro-fibrosis Smad 2/3 signaling pathway. The authors' findings suggest that treatment with vitamin E could be applied to attenuate the development of renal fibrosis by TGF-β/Smad2/3 signal inhibition.

In the 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 increase of TGF-β1 protein and gene expression in the obstructed kidney as shown in the interstitial area by immunohistochemistry staining. Moreover, the authors found that the increasing of Smad2/3 protein was similar to TGF-β1 expression in the fibrotic kidney. Many studies have demonstrated that TGF-β promotes renal fibrosis by activation of pro-fibrosis Smad2/3 signaling pathway also[23-25]. Thus, upregulation of TGF-β induced pro-fibrosis Smad2/3 expression in UUO could be the major factor induce renal fibrosis. These data support the notion that stimulation of TGF-β through Smad2/3 signal is regulated by the dynamic processes in the fibrogenesis kidney.

Vitamin E has several protective effects independent of its antioxidant ability[10]. Supplement with vitamin E revealed anti-inflammatory property in both vitro and *in vivo*[11,12]. Whereas, some of the recent studies indicate that vitamin E was demonstrated to suppress some pro-fibrotic cytokines in chronic renal injury model and diminish progression of renal fibrosis[18,19]. In the present study, the vitamin E treatment effectively reduced TA/IF in obstructed kidney. Moreover, treatment with vitamin E can reduce the TGF-β1 protein and mRNA expression meaning inhibit the inflammatory process during chronic kidney injury. This renoprotective effect similar to earlier study that vitamin E can suppress the overexpression

TGF-β1 during acute and chronic injury[18,26]. In addition, treatment with vitamin E can suppress the increasing of Smad2/3 protein during any time course of UUO. From previous studies, Smad3 is central to the pathogenesis of interstitial fibrosis during kidney injury[7,27]. Smad3 knockout mice are protected from renal interstitial fibrosis and showed collagen accumulation reduction in the kidneys after UUO[23]. In addition, primary TEC from the Smad3 null mice were resistant to induction of fibrogenesis regulatory genes during EMT[28]. Blockade of Smad signal by hepatocyte growth factor and bone morphogenic protein-7 is also mechanistically related to the attenuate renal fibrosis in EMT[29,30]. Comparable with these data, the authors' results confirm the benefit of vitamin E treatment can inhibit the process of TGF-β/Smad2/3 induced fibrogenesis in the kidney.

In conclusion, vitamin E therapy could significantly suppress the exacerbation of TGF-β/Smad2/3 signal in chronic kidney injury and attenuate the development of TA/IF in UUO mice. This present study provided a motion for future investigators to explore the therapeutic potential of vitamin E to prevent renal fibrosis in CKD patients.

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Potential conflicts of interest

None.

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วิตามินอีชนิดแอลฟาชะลอการเกิดพังผืดในไตโดยการยับยั้งเส้นทางของสัญญาณ TGF-β/Smad2/3 ในหนูที่ถูกเหนี่ยวนำให้เกิดการอุดกั้นของทางเดินปัสสาวะ

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ภูมิหลัง: *การลีบลงของเซลล์เยื่อบุผิวของท่อไตและการเกิดพังผืดในไตเป็นหนึ่งในลักษณะที่สำคัญของโรคไตเรื้อรัง โดย transforming growth factor-beta (TGF-β) มีส่วนร่วมในกระบวนการของการเกิดพังผืดในไตอย่างต่อเนื่องนี้ โปรตีน Smad2/3 ได้รับการพิสูจน์ว่ามีหน้าที่ที่สำคัญในการควบคุมการสะสมของ extracellular matrix โดยผ่านเส้นทางสัญญาณของ TGF-β การศึกษานี้ผู้นิพนธ์ได้ทำการทดสอบคุณสมบัติในการป้องกันการเกิดพังผืดของวิตามินอีในหนูที่ได้รับการเหนี่ยวนำให้เกิดการอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียว*

วัสดุและวิธีการ: *หนูที่ได้รับการเหนี่ยวนำให้เกิดการอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียว หรือหนูที่ถูกเหนี่ยวนำโดยการทำหัตถการหลอกถูกสุ่มจำแนกออกเป็น 2 กลุ่ม คือ กลุ่มที่ได้รับวิตามินอี หรือยาหลอก และได้ถูกพิสูจน์ทราบในวันที่ 3 วันที่ 7 และวันที่ 14 หลังจากการทำหัตถการ ต่อจากนั้นชิ้นเนื้อไตได้ถูกจัดเตรียมเพื่อการศึกษาทางพยาธิวิทยาและการตรวจย้อมทาง immunohistochemistry สำหรับโปรตีน TGF-β1 ผู้นิพนธ์ได้ทำการตรวจวัดปริมาณของโปรตีน TGF-β1 และ Smad2/3 โดยวิธี Western blot และตรวจวัดปริมาณของจีน TGF-β1 ด้วยวิธี real time RT-PCR*

ผลการศึกษา: *ไตของหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะที่ได้รับวิตามินอี เกิดความรุนแรงของการเกิดพังผืดในไตน้อยกว่ากลุ่มที่ได้รับยาหลอก การลีบเล็กของเซลล์เยื่อบุผิวท่อไต และการเกิดพังผืดในไตลดลงอย่างมีนัยสำคัญทางสถิติในหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะกลุ่มที่ได้รับวิตามินอี จากการย้อมทาง immunohistochemistry พบการเพิ่มขึ้นของโปรตีน TGF-β1 ในบริเวณช่องว่างระหว่างท่อไตของไตหนู ที่เกิดการอุดกั้นของทางเดินปัสสาวะ นอกจากนี้การเพิ่มขึ้นของโปรตีนและจีน TGF-β1 ในไตหนูที่เกิดการอุดกั้นของทางเดินปัสสาวะถูกยืนยันโดยการตรวจด้วยวิธี Western blot และ real time RT-PCR ในทางตรงกันข้ามการรักษาด้วยวิตามินอีสามารถยับยั้งการเพิ่มขึ้นของโปรตีนและจีน TGF-β1 ได้อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูกลุ่มที่ได้รับยาหลอก นอกจากนี้ปริมาณของโปรตีน Smad2/3 มีการเพิ่มขึ้นอย่างต่อเนื่องในไตหนู ที่เกิดการอุดกั้นของทางเดินปัสสาวะตั้งแต่วันที่ 3 จนถึงวันที่ 14 หลังการทำหัตถการเมื่อเปรียบเทียบกับกลุ่มควบคุม ส่วนการปรากฏของโปรตีน Smad2/3 พบว่ามีระดับต่ำกว่าอย่างมีนัยสำคัญทางสถิติในหนู กลุ่มที่ได้รับวิตามินอีเปรียบเทียบกับหนูกลุ่มที่ได้รับยาหลอกในทุกช่วงเวลา*

สรุป: *การรักษาด้วยวิตามินอีชะลอการเกิดพังผืดในไตของหนูที่ได้รับการเหนี่ยวนำให้เกิด การอุดกั้นของทางเดินปัสสาวะเพียงข้างเดียว แสดงให้เห็นว่าผลของการป้องกันไตโดยวิตามินอีนี้ เกิดจากการยับยั้งเส้นทางสัญญาณของ TGF-β และ Smad2/3*