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Original Paper

Urinary Trypsin Inhibitor Attenuates Acute Lung Injury by Improving Endothelial Progenitor Cells Functions

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Key Words
Urinary Trypsin Inhibitor (UTI) • Endothelial progenitor cells (EPCs) • Acute lung injury (ALI) • Akt/eNOS

Abstract
Background: Urinary Trypsin Inhibitor (UTI) is involved in various aspects of tissue repair, regeneration and development. However, the potential role of UTI in protection against acute lung injury (ALI) remains largely unknown. In the present study, we demonstrated that UTI treatment could ameliorate ALI induced by oleic acid (OA) treatment in rabbit model. ***Methods:*** Intravenous application of UTI (10000 U/kg/d) significantly improved the pathologies associated with OA-induced ALI. The lungs were stained with hematoxylin and eosin to scored the lung injury. Peripheral blood mononuclear cells were isolated by density gradient centrifugation with Ficoll-Plaque Plus. The proliferation and ability of tube structure formation of EPCs were observed and the level of phosphorylated Akt protein expression and eNOS protein expression were assayed. ***Results:*** Consistent with pathological scores, UTI treatment significantly reduced wet/dry ratio of OA injured lungs. A quantification of capillary density revealed that UTI treatment led to about 2 fold increase over uninjured control and about 1.5 fold increase over PBS treatment. The capacity for tube formation of EPCs on ECM gel was significantly reduced in the ALI group and recovered with UTI treatment. Quantification of western blot bands was summarized and showed that UTI treatment activates Akt/eNOS signaling. NO production could contribute to the improvement of EPCs function by UTI treatment. ***Conclusions:*** UTI-induced phosphorylation/activation of eNOS and Akt, increases the intracellular level of NO, thereby improving tube formation and proliferation function of EPCs. EPCs function is crucial for re-endothelialization after denuding injuries of arteries.

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Introduction

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pathological changes of ALI are acute inflammatory response and increase of pulmonary microvascular permeability, resulting in influx of protein-rich edema fluid and loss of

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these issues, including anti-inflammation treatments, low tidal volume ventilation and fluid-

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Urinary trypsin Inhibitor (UTI), originally purified from the fresh urine of healthy men, could be used to inhibit the release of a variety of inflammatory factors from neutrophils and

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were initially described and defined as a special type of stem cells by Asahara et al.

had high level of circulating EPCs in their blood within the first day of illness and the number

potential beneficial effects of UTI treatments. The reason for using OA-induced ALI was OA

function, we might observe beneficial effects of UTI following OA injury. It was shown that treatment of UTI significantly improved pathologies of the lung associated with OA application. Specifically, UTI treatment improved vascularization of injured lungs. To test the

Materials and Methods

Animals

Animal model

Co., Ltd., 10000 U/kg/d, intravenous infusion) [19]. The first treatment of UTI was 30 minutes before OA

EPCs Isolation and Cultivation

on culture dishes. Unselected mononuclear cells were plated on fibronectin-coated culture dishes (Biocoat;

Tube-formation Assay

An ECM gel (Sigma) placed on a 96-well culture plate at 37 °C for 1 h to allow solidification after

on the top of the solidified ECM gel in EBM-2 medium supplemented with 0.5% BSA and VEGF (100 ng/mL). Cells were incubated at 37 °C for 12 h. Tube formation was defined as a structure exhibiting a length 4 fold more than its width. The networks of tubes were photographed from six randomly chosen fields

Proliferation Assay

Proliferation of EPCs was determined by direct counting six random high-power microscopic fields

Histopathological examination

The lungs were embedded in paraffin and the sections were stained with hematoxylin and eosin (H&E). Two qualified pathologists, blinded with the treatments, scored the lung injury, according to

Determination of NO Generation

used for the assays. Levels of nitrite and nitrate were measured as described previously. Briefly, nitrate was

Western Blot Analysis

difluoride membranes, then probed with one of the following primary antibodies against t-Akt, t-eNOS,

Statistical analysis

used for statistical analyses. Statistical significance among mean values was evaluated by one-way ANOVA

significant when p value was P<

Results

UTI reduces the pathologies associated with ALI

could significantly improve pathologies associated with ALI, as demonstrated by histological

atelectasis, infiltration of inflammatory cells and total lung injury histology scores. The edema of the lungs following ALI was also quantified by measurement of wet/dry weight

scores, UTI treatment significantly reduced wet/dry ratio of OA injured lungs. Taken together,
in vivo

UTI treatment increases capillary density of OA injured lungs

To further dissect the beneficial effects of UTI treatment, histologic evaluation of

As shown in Fig. 2, UTI treatment led to a significant enhancement of capillary density as compared to the uninjured group and PBS treatment group as controls. A quantification of

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UTI Increase Proliferation of EPCs

this question, we first tested the effects of UTI on proliferation of EPCs. After isolating EPCs

animals with ALI was significantly improved (P<0.05). In addition, the function of EPCs was

the capacity for tube formation of EPCs on ECM gel was significantly reduced in the ALI

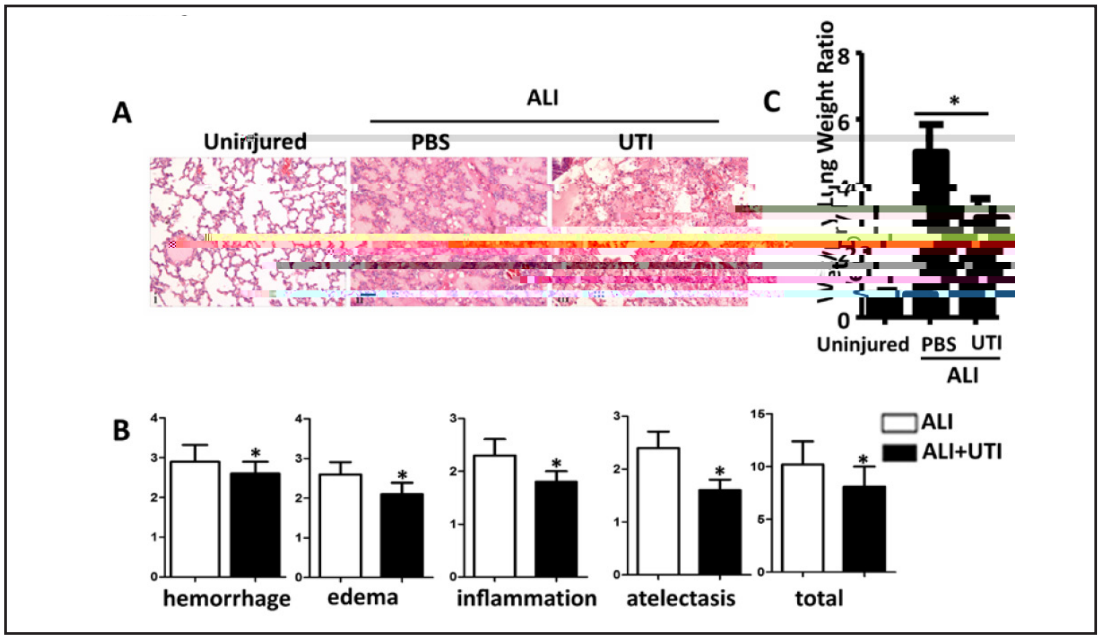
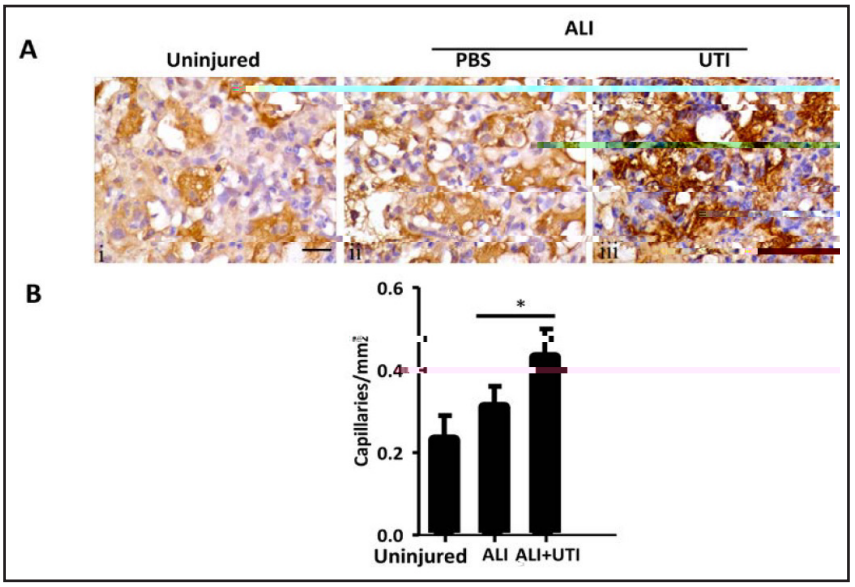


Fig. 1.

hemorrhage, atelectasis and inflammatory cell infiltration were observed in OA treatment group, there was less damage in UTI treatment group (H&E, magnification ×100). (B) Lung injury scores in the indicated groups were evaluated by qualified pathologists. *: p<0.05 in UTI-treatment group vs. ALI group. (C) Wet-

wet-to-dry ratio (*P<0.05). Scale bar: 50 μm.

Fig. 2.



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(magnification ×100). Scale bar: 50 μm.

UTI improves functions of EPCs through Akt/eNOS signaling pathway

enhancement of specific phosphorylated (activated) Akt at the serine 473 phosphorylation

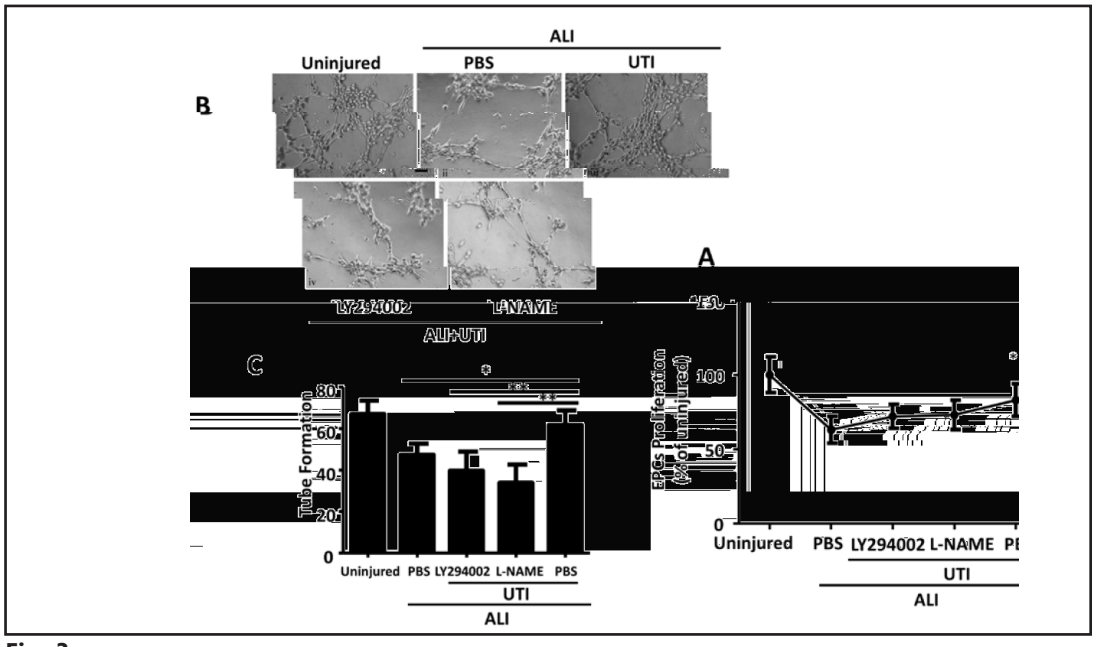


Fig. 3.
for EPCs proliferation activity. Proliferation ability of EPCs was significantly increased in group receiving

0.01). (magnification ×100). Scale bar: 50 μm.

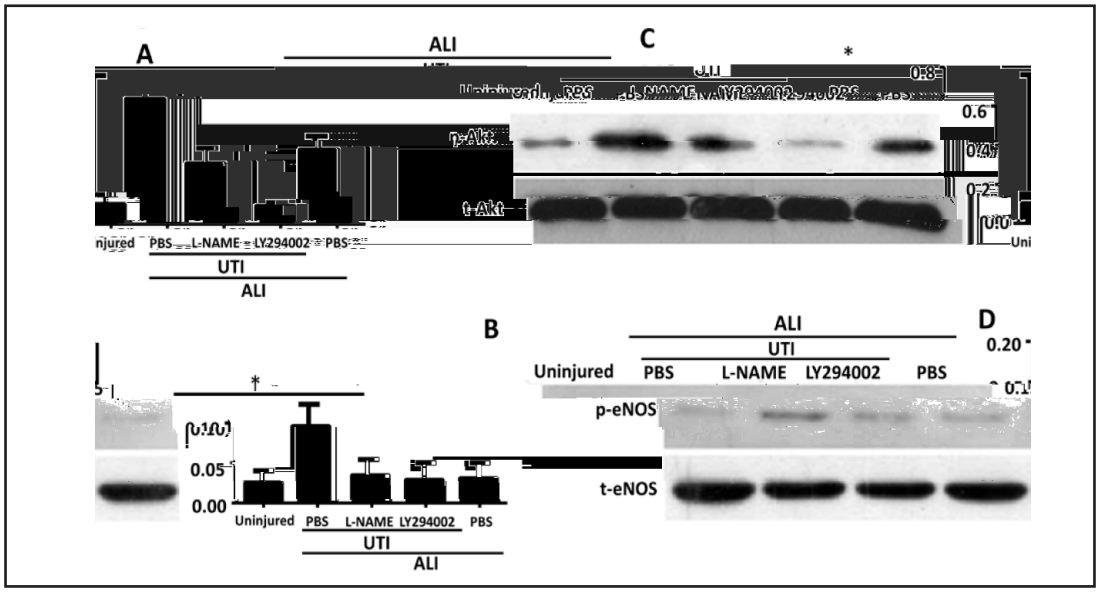
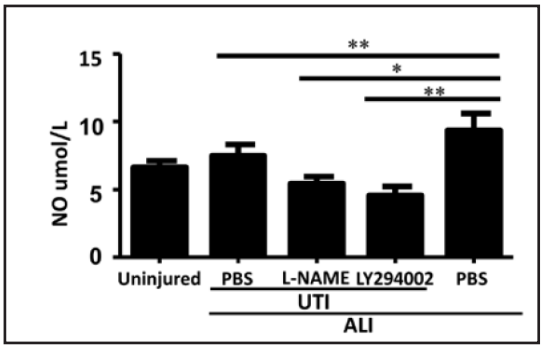


Fig. 4.
and eNOS (B) phosphorylation without influence t-AKT. There was more significant phosphorylation in ALI

phosphorylation. (C, D) Quantification of western blot bands was summarized. Data were represented by mean ± SEM. (n=3 independent experiment, *: p<0.05).

its phosphorylation at Ser1177. As shown in Fig. 4B, significant increase of eNOS

Fig. 5.



To further confirm the beneficial effects of UTI are through Akt/eNOS signaling

proliferation and tube formation of EPCs were significantly blocked by treatments of

Discussion

through enhancing angiogenesis and other mechanisms.However, the potential beneficial

efficacy of UTI treatment. With this established animal model, we found that infusion of UTI significantly reduced edema, inflammation, atelectasis and area of hemorrhage in the

linking beneficial effect of UTI on the vasculogenesis of injured lungs and proliferation activities of EPCs. Our finding may partly explain the mechanisms underlying UTI mediated

significantly elevated in patients with ALI/ARDS. Similarly, at-risk patients who subsequently

in a significantly increased endothelial permeability, capillarity damage and hemorrhage.

significantly lower in animals receiving UTI treatment. Consistent with previous reports, our histologic examinations also revealed significantly reduced edema, infiltration of inflammatory cells, atelectasis and area of hemorrhage in the lung sections of UTI-treated

in vivo

contribute greatly to neovascularization, we investigated the beneficial effects of UTI on ALI as a result of improved EPCs function. Firstly, significant differences were noted in the tube formation in UTI treated group. We demonstrated significant improved effects of UTI on the

is a significant regulator of EPCs. To minimize the possibility that our observations wer

and proliferation function of EPCs. The therapeutic benefits of UTI could be attributable, at

Disclosure Statement

The authors declare no conflict of interest of this work.

Acknowledgement

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