〈Regular Article〉

Preventive effect of indoleamine 2,3-dioxygenase 1 inhibition on lipopolysaccharide-induced prostatitis

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ABSTRACT **Introduction and Objectives:** Bacterial infections are the main cause of acute prostatitis and are treated with appropriate antimicrobial therapy. However, approximately 5% of patients continue to have inflammatory symptoms even after receiving antibacterial therapy, leading to refractory conditions. Bacterial prostatitis requires additional therapy, focusing on inflammatory changes. Indoleamine 2,3-dioxygenase 1 (IDO1) catalysis is the first rate-limiting step of tryptophan metabolism. IDO1 is expressed in the prostate and plays a key role in the immune response. As the first step in investigating the relationship between acute prostatitis and IDO1, we investigated the preventive effect of IDO1 inhibition on lipopolysaccharide (LPS)-induced prostatitis using IDO knockout (*Ido1*$^{-/-}$) mice in this study.

Materials and Methods: The study used *Ido1*$^{-/-}$ and wild-type (*Ido1*$^{+/+}$) C57BL/6J male mice aged 10–15 weeks. LPS *Escherichia coli* O26 (100 μg/PBS, 100 μL) was administered transurethrally into the lower urinary tract to create a mouse model of LPS-induced prostatitis. The prostates were removed 1, 3, 5, and 7 days after creating the model mice. Histological, immunohistochemical, and biochemical analyses were used to compare the preventive effect in *Ido1*$^{-/-}$ mice compared with that in Ido1$^{+/+}$ mice.

Results: HE staining showed suppression of ductal destruction following infiltration of inflammatory cells in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice. The enzyme-linked immunosorbent assay (ELISA) method was used for kynurenine pathway analysis, which showed significantly maintained tryptophan levels and decreased L-kynurenine levels in *Ido1*$^{-/-}$ mice compared to *Ido1*$^{+/+}$ mice. The IDO1 assay in *Ido1*$^{+/+}$ mice showed significantly increased levels during all observation periods after creating the model compared with that under normal conditions. Immunofluorescent staining using five types of cytokines and chemokines (IL-2, IL-4, IL-17, CCL2, and CCL3) related to the pathophysiology of acute prostatitis showed decreased

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expression of these cytokines and chemokines in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice. Inflammation-related proteome assays showed decreased levels of IL-1β, IL-4, IL-5, IL-6, IL-17, CCL2, CCL3, CXCL1, CXCL11, and tissue inhibitor of matrix metalloproteinases (TIMP)-1 in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{-/-}$ mice during all observation periods after model creation.

Conclusions: IDO1 is involved in LPS-induced prostatitis through cytokines and chemokines. IDO1 inhibition contributes to the prevention of LPS-induced prostatitis. IDO1 inhibition has the potential to serve as an additional therapy for acute prostatitis.

doi:10.11482/KMJ-E202248141 *(Accepted on October 13, 2022)*

Key words: Indoleamine 2,3-dioxygenase 1, Prostatitis, Prevention, Anti-inflammation, lipopolysaccharide

INTRODUCTION AND OBJECTIVES

Prostatitis is a common syndrome characterized by pain and voiding symptoms. It is often associated with sexual dysfunction[1, 2]. Previous studies showed that 35-50% of men have prostatitis-suspected symptoms during their lifetime, and approximately 10% of men experience prostatitis[1, 2]. Bacterial infection is the main cause of acute prostatitis[2-6]; however, less than 10% of cases have proven bacterial infection[7]. Appropriate antimicrobial therapy is needed for the treatment of bacterial prostatitis, which is effective against commonly associated pathogens such as Gram-negative bacteria[8]. However, approximately 5% of patients continue to experience inflammatory symptoms even after receiving antibacterial therapy, leading to refractory conditions[9]. In addition, general analgesics such as NSAIDs and Cox inhibitors have inferior efficacy and specificity to the prostate and may result in adverse events. For the above reasons, additional therapy focused on inflammatory changes and showing specificity to the prostate is needed for bacterial prostatitis.

Previous reports have shown that T -lymphocytes, macrophages, and cytokines produced by these cells play key roles in prostatitis pathophysiology (Fig. 1)[10, 11]. Infection induces Toll-like receptor (TLRs) expression following epithelial and stromal cell damage[10, 11]. TLRs are a family of transmembrane proteins that recognize pathogen-associated molecular patterns and play key roles in the innate immune system[12]. TLRs are expressed on epithelial cells, lymphocytes, and antigen-presenting cells (mainly dendritic cells and macrophages), and ten functional TLR membranes have been identified in humans[12-14]. TLR ligands include lipoproteins derived from Gram-positive bacteria (TLR1, TLR2, and TLR6), dsRNA (TLR3), LPS derived from gram-negative bacteria (TLR4), flagellin (TLR5), ssRNA derived from viral genomes (TLR7 and TLR8), unmethylated CpG DNA derived from bacterial or viral genomes (TLR9), and orphan receptors in humans (TLR10)[14, 15]. TLR expression activates T-lymphocytes and T-lymphocyte-related cytokines and other cells (Fig. 1)[10, 11]. IFN-γ is produced by Th1 T-cells and activates M1 macrophages (promotion type); IL-4, IL-5, and IL-13 are produced by Th2 T-cells and cause M2 macrophage (suppression type) activation; and IL-17 is produced by Th17 T-cells and induces CCL2 (MCP-1) production (Fig. 1) [10, 11]. TLR expression also activates macrophage-type change from M2 to M1 (Fig. 1)[10, 11]. M1 macrophages produce cytokines including CCL3 (MIP-1α), TNF-α, IL-1β, and IL-6 (Fig. 1)[10, 11]. If this pathway can be suppressed, a new treatment for prostatitis may be identified.

Tryptophan is an essential amino acid, and approximately 95% of tryptophan is degraded by the kynurenine pathway to synthesize intermediate

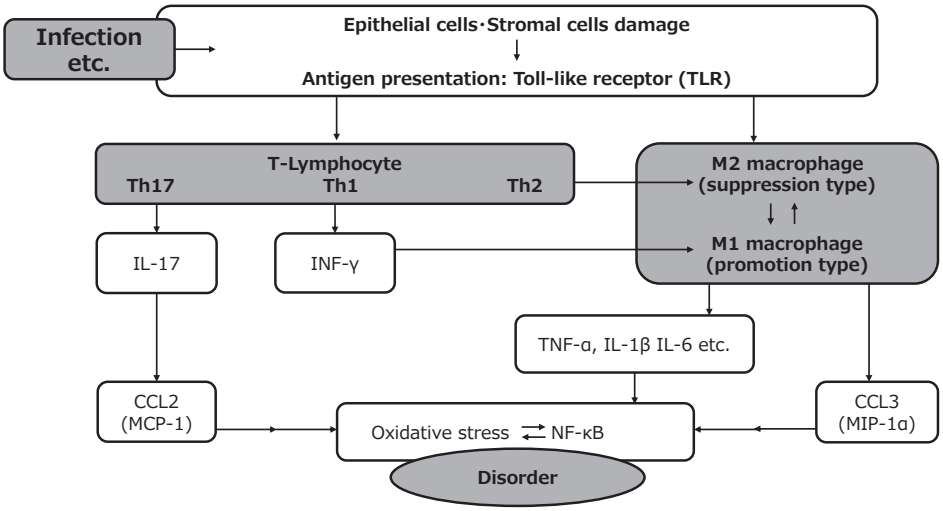

Fig. 1. Pathophysiology of prostatitis
Infection induces Toll-like receptor (TLR) expression following epithelial and stromal cell damage. TLR expression activates T lymphocytes, macrophages, and cytokines, which are produced by these cells, and play key roles in the pathophysiology of prostatitis.

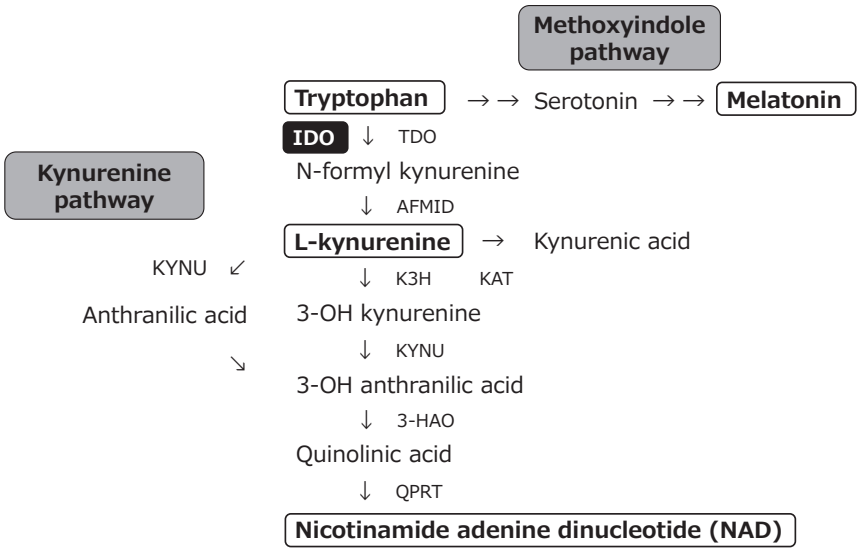

Fig. 2. Tryptophan metabolism
Tryptophan is an essential amino acid, and approximately 95% of tryptophan is degraded by the kynurenine pathway to synthesize intermediate metabolites and nicotinamide adenine dinucleotide. Approximately 1%-5% of tryptophan is degraded by the methoxyindole pathway to synthesize melatonin via serotonin. IDO and tryptophan 2,3-dioxygenase (TDO) are rate-limiting enzymes in the kynurenine pathway. AFMID, arylformamidase; KYNU, kynureninase; K3H, kynurenine 3-hydroxylase; KAT, kynurenine aminotransferase; 3-HAO,3-hydroxyamino oxidase.

metabolites and nicotinamide adenine dinucleotide (Fig. 2)[16–19]. Approximately 1%-5% of tryptophan is degraded by the methoxyindole pathway to synthesize melatonin via serotonin (Fig. 2)[16–19]. Indoleamine 2,3-dioxygenase 1 (IDO1), a monomeric enzyme composed of 403 amino acids

with a molecular weight of approximately 45 kDa, and tryptophan 2,3-dioxygenase (TDO) catalyze the first rate-limiting kynurenine pathway step in tryptophan catabolism (Fig. 2)[16–19]. IDO1 is highly expressed in male reproductive organs such as the prostate and epididymis of mammals[20–22]. IDO1 is also involved in immune response and is induced several times in pathological conditions such as infectious and inflammatory diseases, which activate the immune system[17, 23]. Reports have shown the anti-inflammatory effects of IDO1 on systemic infections[24, 25]. Although the role of IDO1 in the prostate is still unclear, we hypothesized that IDO1 may be involved in inflammatory changes in the prostate and that IDO1 inhibition may suppress inflammatory changes in the prostate. As the first step in investigating the relationship between acute prostatitis and IDO1, we investigated the preventive effect of IDO1 inhibition on lipopolysaccharide (LPS)-induced prostatitis using IDO knockout (*Ido1*$^{-/-}$) mice in this study.

MATERIALS AND METHODS

Research approval and animals

This study was approved by the Safety Committee for DNA Experiments (approval number 19-51) and the Animal Research Committee (approval numbers 20-024 and 22-031) of Kawasaki Medical School (Kurashiki, Japan), and was conducted according to the Guidelines for Care and Use of Laboratory Animals of Kawasaki Medical School. The study used *Ido1*$^{-/-}$ (Jackson Laboratory, ME, USA) and wild (*Ido1*$^{+/+}$) type (Jackson Laboratory, ME, USA) of C57BL/6J male mice aged 10-15 weeks. Sedation was achieved by the inhalation of sevoflurane (Pfizer Japan Inc., Tokyo, Japan). LPS *Escherichia Coli* O26 (Wako Pure Chemical Industries, Osaka, Japan) 100 μg/PBS 100 μL was administered transurethrally into the lower urinary tract using a vascular catheter for mice (14213130, Braintree Co. Ltd., Tokyo, Japan) with an outer diameter of 0.4 mm and inner diameter of 0.2 mm to create LPS-induced acute prostatitis model mice. The validity of the LPS-induced acute prostatitis mouse model was determined using histological analysis to confirm infiltration of inflammatory cells in the prostate, and the success rate of model mice creation was 80% (16/20) in a preliminary examination.

Histological analysis of the prostate

Prostate tissues resected from *Ido1*$^{-/-}$ mice at 1, 3, 5, and 7 days after creating model mice were compared with those from *Ido1*$^{+/+}$ mice. The resected prostate tissues were fixed in Carnoy fixative fluid (Wako Pure Chemical, Osaka, Japan) for 3 h. The fixed tissues were dehydrated in an ethanol series and, finally, in absolute ethanol. The dehydrated tissues were embedded in paraffin, cut into 5 μm sections, mounted on slides, and dried for 45 min at 45℃. Tissue slides were dewaxed and hydrated using graded ethanol solutions and water. Histological analysis following hematoxylin-eosin staining was performed using an Olympus BX-53 microscope (Olympus, Tokyo, Japan).

Analysis of the kynurenine pathway in the prostate

Changes in the kynurenine pathway in the prostate were analyzed using a procedure similar to that described above. Cell lysis buffer (Cell Signaling Technology, CA, USA), PMS (1 mM, Cell Signaling Technology), and tissue fragments were removed after centrifugation (15,000 × g, 5 min, 5 ℃) and used to homogenize the excised prostates. IDO1 (Biocompare, CA, USA), tryptophan (Biocompare, CA, USA), and L-kynurenine (Biocompare, CA, USA) assays using the enzyme-linked immunosorbent assay (ELISA) method were performed by setting the measurement wavelength to 540 nm and measurement of absorbance with Varioskan Flash (Thermo Fisher Scientific, MA, USA).

Immunohistochemical analysis of inflammatory changes in the prostate

A procedure similar to that described above was used to analyze the inflammatory changes in the prostate. Immunohistochemical analysis was performed using immunofluorescence staining (ImmPRESS™-AP Reagent, Vector Laboratories, CA, USA). Blocking was performed for 10 min at room temperature with BLOXALL™ blocking solution (Vector Laboratories) after dewaxing and hydration. As shown in Fig. 1, primary antibodies against indicators of Th1 cell-related cytokine: IL2, Th2 cell-related cytokine IL-4, Th17 cell-related cytokine IL-17, T-lymphocytes related cytokine CCL2, and macrophage-related cytokine CCL3 were used, all diluted in 2.5% normal horse serum (Vector Laboratories): anti-IL-2 antibody (ab180780, Abcam plc, Cambridge, UK; diluted 200-fold); IL-4 (NBP1-19772, Novus Biologicals, CO, USA; diluted 200-fold), IL-17 (ab79056, Abcam plc, Cambridge, UK; diluted 200-fold); CCL2 (ab25124, Abcam plc, Cambridge, UK; diluted 200-fold); and CCL3(sc-33203, Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted 100-fold). Rabbit IgG control antibody (Vector Laboratories; diluted 2000-fold) was used as the negative control (NC). The primary antibody reaction was performed overnight at 4 ℃. The secondary antibody reaction was performed using an ImmPRESS™-AP Reagent Anti-Rabbit IgG Polymer Kit (Vector Laboratories) for 30 min at room temperature. The immune substrate was ImmPACT™ Vector Red Alkaline Phosphatase (Vector Laboratories). Counterstaining was performed using 4', 6-diamidino-2-phelylindole (DAPI, Vector Laboratories). After encapsulation using the VectaMount™ Permanent Mounting Medium (Vector Laboratories), immunohistochemical analysis was performed using an Olympus BX-53 microscope (Olympus, Tokyo, Japan).

Biochemical analysis of inflammatory changes in the prostate

A procedure similar to that described above was used to analyze the inflammatory changes in the prostate. A proteome assay using 40 inflammation-related proteome types (Mouse Cytokine Antibody Array Panel A®; R&D Systems, Minneapolis, MN, USA) was performed for inflammatory analysis. The protein concentration in each sample was 300 μg/mL. An image analyzer (Image Quant LAS4000 mini, GE Healthcare Japan, Tokyo, Japan) and image analysis software (Image Quant TL, GE Healthcare Japan) were used to calculate pixel density (PD). The calculated PD was corrected using an endogenous control, while the negative and positive controls were set at PD 0 and 100, respectively.

Statistical analysis

Excel Statistics (Microsoft, WA, USA) was used for statistical analysis. Differences between the $Ido1^{-/-}$ mice and the $Ido1^{+/+}$ mice, after creating the model and under normal conditions, were analyzed using the Welch test, with $p < 0.05$ considered to indicate a significant difference. The PD range was defined as 0 (negative control) to 100 (positive control).

RESULTS

IDO1 inhibition suppressed the infiltration of inflammatory cells in the prostate

Histological analysis showed infiltration of inflammatory cells in $Ido1^{+/+}$ mice but not in $Ido1^{-/-}$ mice during all observation periods after creating the model (Fig. 3). Inflammatory cells were mainly observed in interstitial tissues. Destruction of ductal structures was observed in $Ido1^{+/+}$ mice but not in $Ido1^{-/-}$ mice at days 3, 5, and 7 (Fig. 3). Consequently, $Ido1^{-/-}$ mice, but not $Ido1^{+/+}$ mice, showed suppression of ductal destruction following infiltration of inflammatory cells.

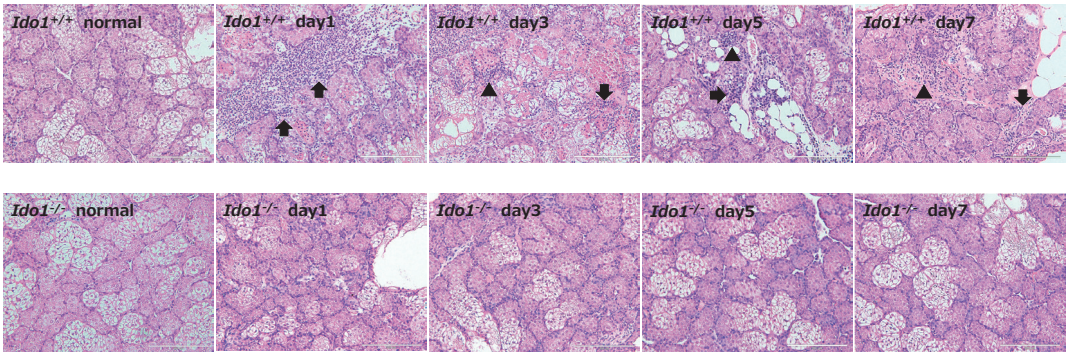

Fig. 3. Histological analysis in LPS-induced prostatitis model
HE staining revealed infiltration of inflammatory cells (arrow) in *Ido1*$^{+/+}$ mice but not in *Ido1*$^{-/-}$ mice during all observation periods after creating the model. Inflammatory cells were mainly observed in the interstitial tissues. Destruction of the ductal structure (arrowhead) was observed in *Ido1*$^{+/+}$ mice but not in *Ido1*$^{-/-}$ mice at days 3, 5, and 7. *Ido1*$^{-/-}$ mice showed suppression of ductal destruction following infiltration of inflammatory cells compared with *Ido1*$^{+/+}$ mice. Scale bar: 200 μm. Magnification: 200×.

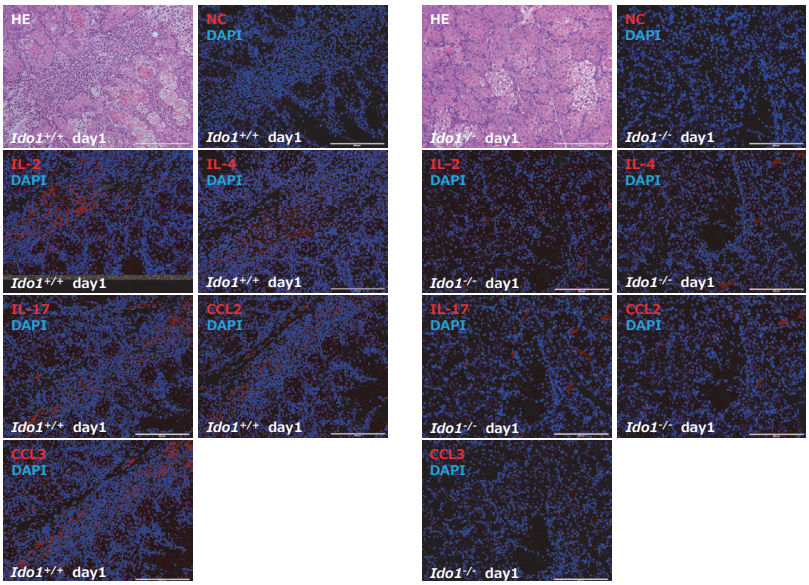

Fig. 4. IDO1 assay in LPS-induced prostatitis model
ELISA in *Ido1*$^{+/+}$ mice showed significantly increased levels of IDO1 at all observation periods after creating the model compared with the normal condition. *Ido1*$^{-/-}$ mice did not show IDO1 expression during all observation periods. Data show the mean and standard error (SE). Differences between model and normal conditions in *Ido1*$^{+/+}$ mice were analyzed by the Welch test. n = 5 for each week.

IDO1 inhibition suppressed the kynurenine pathway in the prostate

IDO1 assay in *Ido1*$^{+/+}$ mice showed significantly increased IDO1 levels during all observation periods after creating the model compared with that under normal conditions (Fig. 4). *Ido1*$^{-/-}$ mice did not show IDO1 expression throughout the observation period after creating the model (Fig. 4). The tryptophan assay showed significantly increased tryptophan levels in *Ido1*$^{-/-}$ mice compared with

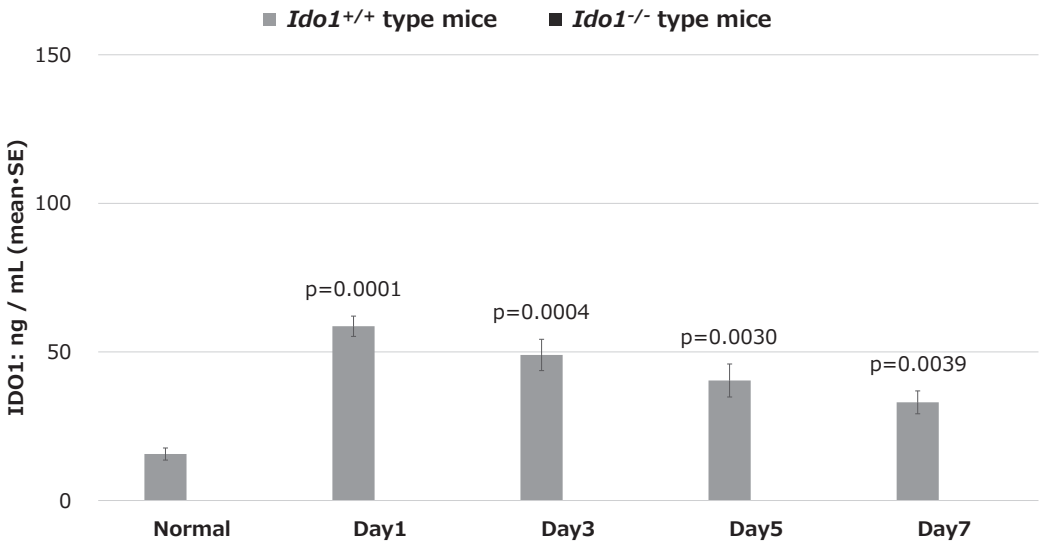

Fig. 5. Tryptophan assay in LPS-induced prostatitis model
ELISA showed maintained levels of tryptophan in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{++-}$ mice at days 1, 3, and 5 after creating the model. Data show the mean and standard error (SE). Differences between *Ido1*$^{-/-}$ mice and *Ido1*$^{+/+}$ mice were analyzed by the Welch test. n = 5 for each week.

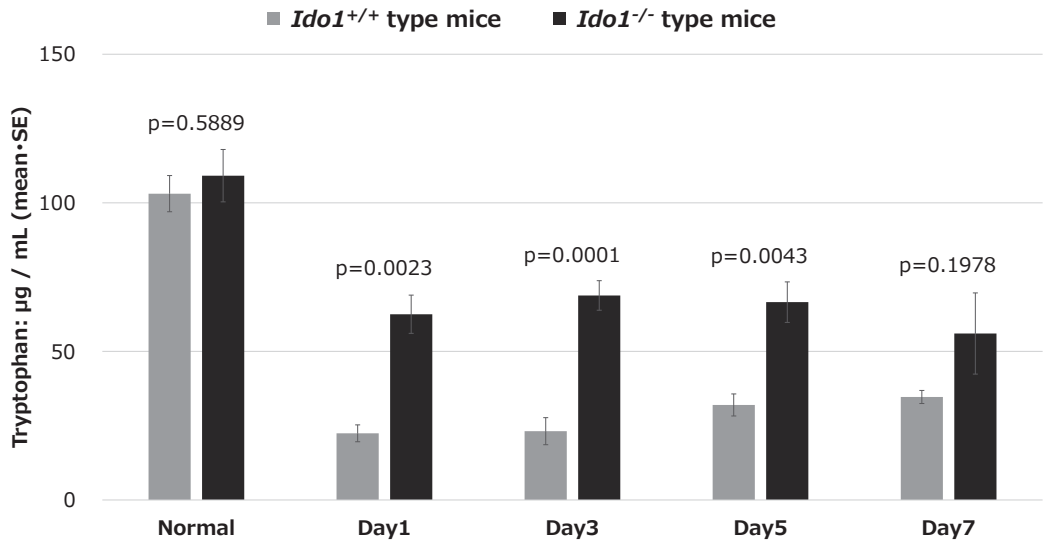

Fig. 6. L-kynurenine assay in LPS-induced prostatitis model
ELISA showed significantly decreased levels of L-kynurenine in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice during all observation periods after creating the model. Data show the mean and standard error (SE). Differences between *Ido1*$^{-/-}$ mice and *Ido1*$^{+/+}$ mice were analyzed by the Welch test. n = 5 for each week.

Ido1$^{-/-}$ mice at days 1, 3, and 5 (Fig. 5). The L-kynurenine assay showed significantly decreased L-kynurenine levels in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{-/-}$ mice during all observation periods after creating the model (Fig. 6).

IDO1 inhibition suppressed inflammation-related cytokines and chemokines in the prostate

Immunofluorescent staining showed decreased expression of five types of cytokines and chemokines (IL-2, IL-4, IL-17, CCL2, and CCL3)

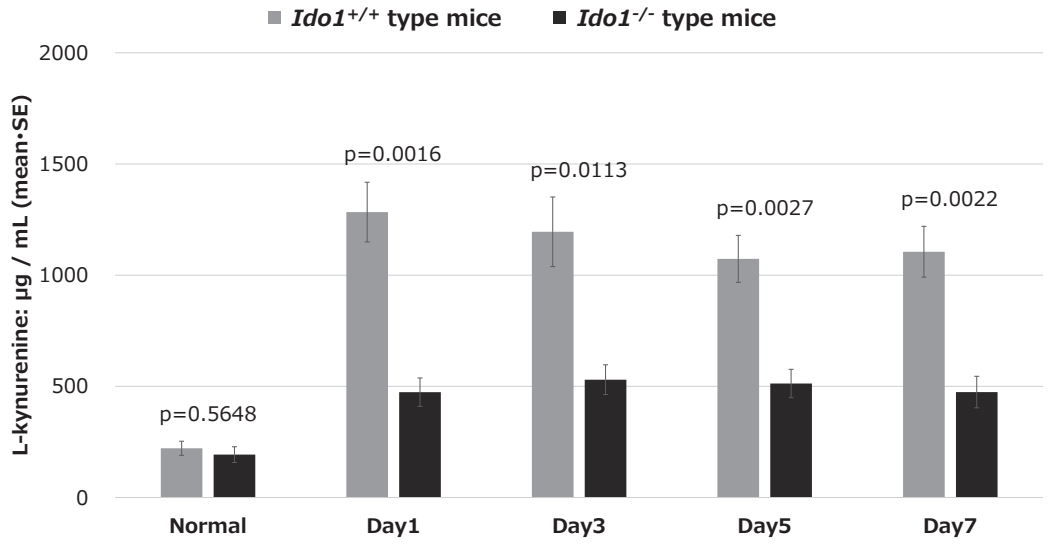

Fig. 7. Immunohistochemical analysis in LPS-induced prostatitis model
Immunofluorescent staining showed decreased expression of IL-2, IL-4, IL-17, CCL2, and CCL3 in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice. The expression of these cytokines in *Ido1*$^{+/+}$ mice was mainly observed in interstitial tissues with infiltration of inflammatory cells. NC, negative control; Rabbit IgG, control antibody. Scale bar: 200 μm. Magnification: 200×.

related to the pathophysiology of acute prostatitis (Fig. 1) in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice (Fig. 7). The expression of these cytokines in *Ido1*+/+ mice was mainly observed in interstitial tissues with infiltration of inflammatory cells (Fig. 7). The inflammation-related proteome assay showed significant differences ($p < 0.05$, Welch test) in the levels of 10 types of cytokines and chemokines (IL-1β, IL-4, IL-5, IL-6, IL-17, CCL2, CCL3 CXCL1, CXCL11, and tissue inhibitor of matrix metalloproteinases (TIMP)-1), including five types of cytokines and chemokines, which were investigated by immunofluorescent staining, in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{+/+}$ mice at all observation periods (Fig. 8). IL-1β, IL-5, IL-17, and CXCL11 levels in *Ido1*$^{+/+}$ mice increased in the early stages, and then leveled off or decreased (Fig. 8). IL-4, IL-6, CCL2, CCL3, CXCL1, and TIPM-1 levels in *Ido1*$^{+/+}$ mice increased over time (Fig. 8). Ten cytokines and chemokines were significantly decreased levels in *Ido1*$^{-/-}$ mice compared to those in *Ido1*$^{+/+}$ mice (Fig. 8).

DISCUSSION

Summary and novelty of this study

Our study is the first to investigate the relationship between acute prostatitis and IDO1. As the first step to establish this relationship, we investigated the preventive effect of IDO1 inhibition in LPS-induced prostatitis using *Ido1*$^{-/-}$ mice. Our results showed a relationship between LPS-induced inflammatory changes and IDO1 expression in the prostate. In addition, our results showed the preventive effect of IDO inhibition on LPS-induced prostatitis. IDO1 inhibition suppressed tissue destruction following infiltration of inflammatory cells and induction of cytokines and chemokines related to the pathophysiology of acute prostatitis before the onset of prostatitis.

Relationship between infectious disease and kynurenine pathway changes

Takikawa[24] reported that IDO1 overinduction in systemic infections activates the kynurenine pathway and leads to tryptophan depletion in

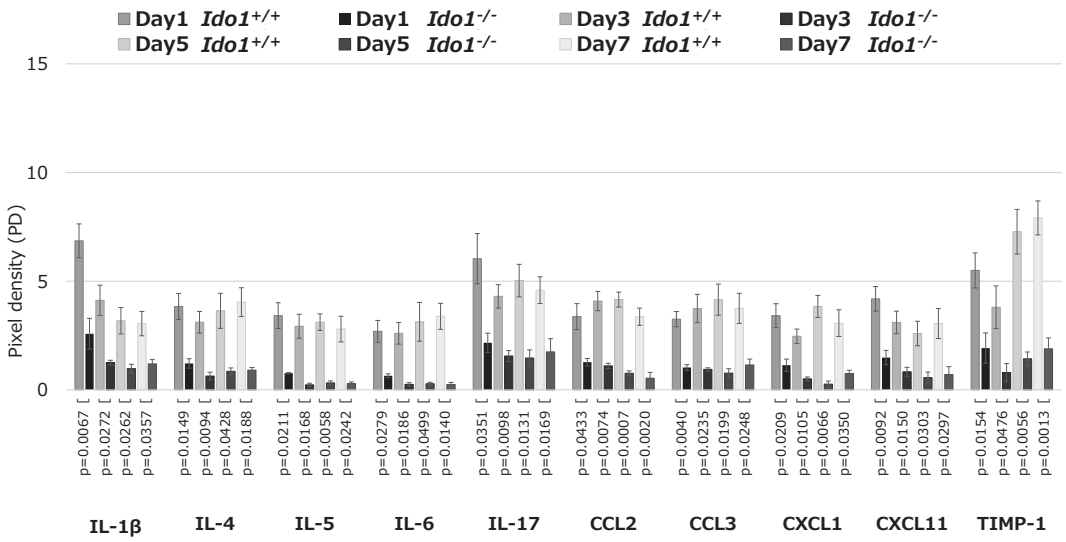

Fig. 8. Inflammatory analysis in LPS-induced prostatitis model
The inflammation-related proteome assay showed significantly decreased levels of IL-1β, IL-4, IL-5, IL-6, IL-17, CCL2, CCL3, CXCL1, CXCL11, and tissue inhibitor of matrix metalloproteinases (TIMP)-1 in *Ido1*$^{-/-}$ mice compared with *Ido1*$^{-/-}$ mice during all observation periods after model creation. The PD range was defined as 0 (negative control) to 100 (positive control). Data are shown as mean with standard error (SE). Differences between the *Ido1*$^{-/-}$ mice and the *Ido1*$^{+/+}$ mice were analyzed by the Welch test, with $p < 0.05$ considered to indicate a significant difference. n = 5 for each week.

LPS-induced sepsis model mice. Overinduction of IDO1 promotes catabolism and the return of tryptophan levels to the normal range as a protective response[24]. The promotion of catabolism changes colloid osmotic pressure and leads to critical conditions in vivo[24]. Jung *et al*. reported that IDO1 inhibition using *Ido1*$^{-/-}$ mice and 1-methyl-D-tryptophan treatment (IDO1 inhibitor) suppressed proinflammatory cytokines, such as TNF-α, IL-6, and IL-12, and reduced septic shock deaths in LPS-induced sepsis model mice[25]. It is assumed that there is a relationship between IDO1 induction and inflammatory changes from the above reports[24, 25], and our previous study showed a relationship between LPS-induced inflammatory changes and IDO1 in local organs such as the prostate and epididymis[26]. However, the mechanism by which IDO exacerbates inflammatory changes remains unclear. Our results suggest that a decrease in tryptophan and / or an increase in its metabolites may be involved in these mechanisms. Additional studies on tryptophan replacement or metabolite inhibition are required to clarify these mechanisms.

Anti-inflammatory mechanism of IDO1 inhibition in LPS-induced prostatitis

Reports have shown that IDO1 regulates mRNA expression through activin A, a member of the TGF-β family[27, 28]. IDO1, which is regulated by activin A, activates TLR expression in immune cells[29]. LPS-induced IDO1 activates TLR4 and TLR9 expression[30], and TLR4 is expressed in the prostate[31]. Damage to stromal and epithelial cells caused by prostatitis induces TLR expression in the prostate[10, 11]. Reports suggest that IDO1 may be involved in LPS-induced prostatitis mediated by TLR[10–11, 27–31] —TLR expression activates T lymphocytes, macrophages, and related cytokines and chemokines[10, 11]. Our histological analysis showed that destruction of the ductal structure

following infiltration of inflammatory cells in *Ido1*$^{+/+}$ mice mainly originated from the interstitial tissues. This damage may induce TLR expression and activate T-lymphocytes, macrophages, and related cytokines and chemokines. Our results also showed a suppressive effect on tissue destruction following infiltration of inflammatory cells and decreased levels of cytokines and chemokines in *Ido1*$^{-/-}$ mice. These results suggest that IDO inhibition suppresses inflammatory changes in the upstream pathway (including TLR expression level) in prostatitis, and has the potential to be an add-on treatment when antibiotics do not show sufficient anti-inflammatory effects in clinical practice.

Limitations of the study

Although our study showed a preventive effect of IDO1 inhibition in LPS-induced prostatitis, the therapeutic effect of IDO1 inhibition has not been demonstrated. Additional research is needed to investigate the therapeutic effects of IDO1 inhibition using IDO1 inhibitors. In addition, our study showed the possibility of IDO1 inhibition that did not lead to refractory conditions during our observation periods; however, long-term changes have not been confirmed. Additional research is needed to investigate prostatic changes over a longer period.

CONCLUSIONS

IDO1 is involved in LPS-induced prostatitis via cytokines and chemokines. IDO1 inhibition contributed to the prevention of LPS-induced prostatitis. IDO1 inhibition has the potential to serve as an additional therapy for acute prostatitis.

ACKNOWLEDGMENTS

This study was supported by the Kawasaki Foundation for Medical Science, Medical Welfare, and Research funds. We would like to thank Editage (www.editage.com) for English language editing.

CONFLICT OF INTEREST

None.

REFERENCES

1) Krieger JN, Lee SW, Jeon J, cheah PY. Liong ML. Riley DE: Epidemiology of prostatitis. Int J Antimicrob Agents. 2008; 31: S85-S90. doi: 10.1016/j-ijantimicag.2007.08.028.

2) Lipsky BA, Byren I, Hoey CT: Treatment of bacterial prostatitis. Clin Infect Dis. 2010; 50: 1641-1652. doi: 10.1086/652861.

3) Krieger JN, Ross SO, Limaye AP, Riley DE: Inconsistent localization of gram-positive bacteria to prostate-specific specimens from patients with chronic prostatitis. Urology. 2005; 66: 721-725. doi: 10.1016/j.urology.2005.04.065.

4) Naber KG: Management of bacterial prostatitis: what's new?. BJU Int. 2008; 101: 7-10. doi: 10.1111/j.1464-410x.2008.07495.x.

5) Naber KG, Roscher K, Botto H, Schaefer V: Oral levofloxacin 500 mg once daily in the treatment of chronic bacterial prostatitis. Int J Antimicrob Agents. 2008; 32: 145-153. doi: 10.1016/j.ijantmicag.2008.03.014.

6) Nickel JC, Xiang J: Clinical significance of nontraditional bacterial uropathogens in the management of chronic prostatitis. J Urol. 2008; 179: 1391-1395. doi: 10.1016/j.juro.2007.11.081.

7) Mody L, Krein SL, Saint S, *et al.*: A targeted infection prevention intervention in nursing home residents with indwelling devices: a randomized clinical trial. JAMA Intern Med. 2015; 175: 714-723. doi: 10.1001/jamainternmed.2015.132.

8) Kwan ACF, Beahm NP: Fosfomycin for bacterial prostatitis: a review. Int J Antimicrob Agents. 2020; 56: 106106. doi: 10.1016/j.ijantimicag.2020.106106.

9) Benelli A, Hossain H, Pilatz A, *et al.*: Prostatitis and its management. European Urology Supplements. 2017; 16: 132-137.

10) Paulis G: Inflammatory mechanisms and oxidative stress in prostatitis: the possible role of antioxidant therapy. Res Rep Urol. 2018; 10: 75-87. doi: 10.2147/RPU.S170400.

11) Nishishita N, Ohira S, Tone S, *et al.*: Anti-inflammatory effect of tadalafil, a phosphodiesterase 5 (PDE5) inhibitor, in autoimmune prostatitis. Kawasaki Med J.

2021; 47: 193-201.

12) Gambara G, De Cesaris P, De Nunzio C, Ziparo E, Tubaro A, Filippini A, Riccioli A: Toll-like receptors in prostate infection and cancer between bench and bedside. J Cell Mol Med. 2013; 17: 713-722. doi: 10.1111/jcmm.12055.

13) Janeway CA Jr, Medzhitov R: Innate immune recognition. Annu Rev Immunol. 2002; 20: 197-216. doi: 10.1146/annurev.immunol.20.083001.084359.

14) Takeda K, Kaisho T, Akira S: Toll-like receptors. Annu Rev Immunol. 2003; 21: 335-376. doi: 10.1146/annurev.immunol.21.120601.141126.

15) Chuang T, Ulevitch RJ: Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. Biochim Biophys Acta. 2001; 1518: 157-161. doi: 10.1016/s0167-478(00)00289-x.

16) Shimizu T, Nomiyama S, Hirata F, Hayaishi O: Indoleamine 2,3-dioxygenase. Purification and some properties. J Biol Chem. 1978; 253: 4700-4706.

17) Takikawa O, Yoshida R, Kido R, Hayaishi O: Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. J Biol Chem. 1986; 261: 3648-3653.

18) Werner ER, Bitterlich G, Fuchs D, Hausen A, Reibnegger G, Szabo G, Dierich MP, Wachter H: Human macrophages degrade tryptophan upon induction by interferon-gamma. Life Sci. 1987; 41: 273-280. doi: 10.1016/0024-3205(87)90149-4.

19) Taylor MW, Feng GS: Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J. 1991; 5: 2516-2522.

20) National Library Medicine; National Center for Biotechnology Information. Ido1 indoleamine 2,3-dioxygenase 1 [*Homo sapiens* (human)]. Gene ID: 3620, updated on 7-Aug-2022. Bethesda, MD 20894, USA. https://www.ncbi.nlm.nih.gov/gene/3620.

21) National Library Medicine; National Center for Biotechnology Information. Ido1 indoleamine 2,3-dioxygenase 1 [*Mus musculus* (house mouse)]. Gene ID: 15930, updated on 12-Jul-2022. Bethesda, MD 20894, USA. https://www.ncbi.nlm.nih.gov/gene/15930.

22) Jrad-Lamine A, Henry-Berger J, Gourbeyre P, *et al.*: Deficient tryptophan catabolism along the kynurenine pathway reveals that the epididymis is in a unique tolerogenic state. J Biol Chem. 2011; 286: 8030-8042. doi: 10.1074/jbc.M110.172114.

23) Yoshida R, Hayaishi O: Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. Proc Natl Acad Sci U S A. 1978; 75: 3998-4000. doi: 10.1073/pnas.75.8.3998.

24) Takikawa O: Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism. Biochem Biophys Res Commun. 2005; 338: 12-19. doi: 10.1016/j.bbrc.2005.09.032.

25) Jung ID, Lee MG, Chang JH, *et al.*: Blockade of indoleamine 2,3-dioxygenase protects mice against lipopolysaccharide-induced endotoxin shock. J Immunol. 2009; 182: 3146-3154. doi: 10.4049/jimmunol.0803104.

26) Ohira S: The role of indoleamine 2,3-dioxygenase (IDO) in epididymitis. Kawasaki Medical Journal. 2018; 44: 1-10. doi: 10.11482/KMJ-T44(1)1.

27) Winnall WR, Wu H, Sarraj MA,Rogers PA, de Kretser DM, Girling JE. Hedger MP: Expression patterns of activin, inhibin and follistatin variants in the adult male mouse reproductive tract suggest important roles in the epididymis and vas deferens. Reprod Fertil Dev. 2013; 25: 570-580. doi: 10.1071/RD11287.

28) Pallotta MT, Orabona C, Volpi C, *et al.*: Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. Nat Immunol. 2011; 12: 870-878. doi: 10.1038/ni.2077.

29) Hedger MP: Immunophysiology and pathology of inflammation in the testis and epididymis. J Androl. 2011; 32: 625-640. doi: 10.2164/jandrol.111.012989.

30) Salazar F, Awuah D, Negm OH, Shakib F, Ghaemmaghami AM: The role of indoleamine 2,3-dioxygenase-aryl hydrocarbon receptor pathway in the TLR4-induced tolerogenic phenotype in human DCs. Sci Rep. 2017; 7: 43337. doi: 10.1038/srep43337.

31) Ou T, Lilly M, Jiang W: The Pathologic Role of Toll-Like Receptor 4 in Prostate Cancer. Front Immunol. 2018; 9:1188. doi: 10.3389/fimmu.2018.01188.