Review

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New Era of Post Urinary Tract Infection Pain Syndrome

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Urinary tract infection (UTI) in most cases is accompanied by pain. However, in some cases, including asymptomatic bacteriuria (ASB), pain is absent and thus, cannot be characterized. A study with an animal UTI model to quantify pelvic pain showed that *Escherichia coli* (NU14 strains) isolated from urine of patients with acute UTI caused pain, while *E. coli* (83972 strains) isolated from urine of patients with ASB caused no pain. The difference in pain response was not related to bladder colonization or inflammation, but to lipopolysaccharde (LPS) and Toll-like receptor 4, which is an LPS receptor. As the association between interstitial cystitis (IC) and UTI was epidemiologically suggested, an experiment was performed to investigate whether repeated infection with uropathogenic *E. coli* (UPEC) causes chronic pain through central sensitization. The results showed that repeated infection with the wild type UPEC caused temporary pain, while repeated infection with UPEC (SΦ 874 strains) in the absence of O-antigen caused chronic pain. Chronic pain following UTI is related to voiding dysfunction and anxious/depressive behavior. These relationships are mediated by transient receptor potential vanilloid type 1 at the stage of pain development and by C-C chemokine receptor type 2 at the stage of pain maintenance. Based on these findings, temporary *E. coli* infection causes chronic pain, which is one of the characteristics of neuropathic pain. This pattern is similar with the symptoms of IC, supporting the possibility of infection as an etiology of IC.

Keywords: Urinary tract infections; Pain; Interstitial cystitis

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common diseases. Approximately 40-50% of women experience UTI more than once in their lifetime, and a significant number of them are infected repeatedly [1]. Although the causative organisms of UTI vary depending on patients' characteristics, uropathogenic *Escherichia coli* (UPEC) are the leading cause of UTI, accounting for 70-95% of community-acquired UTIs [2]. Patients with UTI often present symptoms as dysuria, frequency, urgency, and pelvic pain; however, about 5% of these patients have no symptoms at all, which is defined as asymptomatic bacteriuria (ASB) [3]. With respect to UTI, an immunological question has been raised as to host-pathogen interaction for a long time, but a clear cause of the disease has not been elucidated to date. Another question concerns the mechanism—and the mediators involved—behind the progression of recurrent UTIs to interstitial cystitis (IC) in some cases, while others are cured without symptoms. Because recurrent uncomplicated cystitis can cause IC, early detection and follow-up of risk factors associated with the

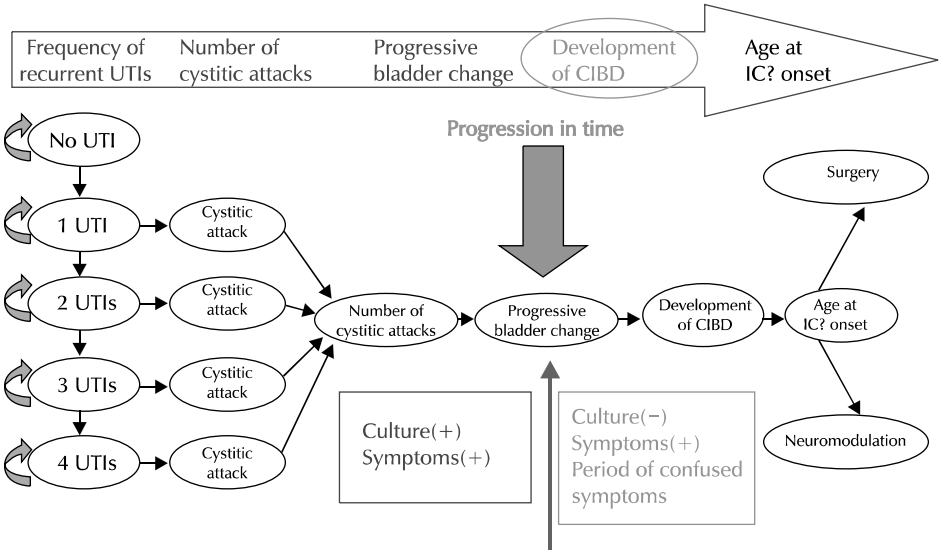

Fig. 1. Diagram of hypothesized mechanism underlying the progression from recurrent cystitis to interstitial cystitis. Adapted from Kim and Kim, *Inflammation and interstitial cystitis. Book of 2nd International Consultation on Interstitial Cystitis*, Japan; 2007. p. 33-7, with permission [4]. UTI: urinary tract infection, CIBD: chronic inflammatory bladder disease, IC: interstitial cystitis.

development of IC are important for the prevention of disease progression (Fig. 1) [4]. Since the clinical perspective of UTI focuses only on pathogens, very few studies have been conducted on its pathophysiological aspect. In addition, little has been known about the development mechanism of voiding symptoms, including frequency, nocturia and urgency, as well as pain, including dysuria, pelvic pain, and suprapubic pain; why the symptoms, if any, persist even after the bacteria were removed with antibiotic treatment; and what factors are involved in these processes. However, recent publication of papers on post-UTI pain will be of help to understand such diseases as bladder pain syndrome/interstitial cystitis (BPS/IC) or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), which have various causes without a clear treatment method [5-7].

MECHANISMS OF PAIN FROM UTI

Post-UTI symptoms are caused mostly by innate immunity. When urinary tract pathogens are combined with Toll-like receptor 4 (TLR4) of the epithelial cells by p-fimbriae or type 1 fimbriae, which are the pathogenicity island, the release of cytokine is activated through cell signaling in the cells, and the processes of sterilization and apoptosis take place by the secreted substances [8,9]. Mulvey et al. [10] found that UPEC with type 1 pili can invade the epithelial cells covered by the surface of the bladder and replicate. The invasion into the epithelial cells of the bladder induces a host immune response, causing exfoliation and death on the surface of the epithelial cells [11,12]. The mechanism behind the exfoliation of the infected bladder cells is similar to that of apoptosis, which becomes an effective host defense strategy [13]. Contrastingly, pathogens establish a temporary defense environment upon the invasion through replication and reawakening, making consecutive invasions into the lower epithelial cells with persistent presence [10]. The establishment of such storage for pathogens in the urinary track is helpful in explaining the chronic pattern of UTI [2,10]. In 2004, Justice et al. [14] reported that UPEC invades the urothelium and forms intracellular bacterial communities (IBCs) in the cytoplasm of host cells, making it possible to avoid the influence of phagocytic cells and antibiotics, which can be an important cause of recurrent cystitis. However, treatments such as antibiotics kill most UPECs, leaving only a small number of UPECs alive. This is called quiescent intracellular reservoirs and causes latent infection for a few months as opposed to IBCs, which is established on a temporary basis after infection [15]. Bacterial cystitis characteristically causes the symptoms of frequency, urgency, dysuria and suprapubic pain [16]. While most patients show rapid improvement with antibiotic treatment, some patients continue to experience pain in the bladder despite having clean urine. However, to date, the mechanism of pain development by an infection remains unknown. There is a report that inflammation causes pain, but infection-related pain has rarely been studied; and in

a very few that we are aware of, only animal models have been used [5,17,18]. This study reviewed these studies on animal models to better understand the mechanism of post-UTI pain.

1. Lipopolysaccharide

As an endotoxin, the major component of the membrane of gram-negative bacteria, lipopolysaccharide (LPS), is well known as a biological mediator. Biological effects of LPS include relaxation of the vascular smooth muscle, the activation of macrophages and monocytes, and the activation of endothelial adhesion molecules of the endothelial cells [19]. In addition, LPS is widely used in the induction of the experiment models of organ-specific bacterial inflammatory diseases. In a study to investigate whether LPS is associated with UTI pain by injecting LPS refined with NU14 (cystitis strain) and 83972 (ASB strain) into the bladder of mice, interestingly, the mouse injected with 83972 LPS shows no change for 4 days, while the one with NU14 LPS manifests a rapidly increasing pain level after 1 hour, reaching the highest pain level after 4 hours. Moreover, there was no difference in the level of urine neutrophil myeloperoxidase (MPO) after 6 hours between the mouse with NU14 LPS and that with 83972 LPS injection. This result indicates that NU14 LPS induces pain independent of inflammation [17]. Several studies showed that LPS is a major mediator of bladder inflammatory response through interaction with TLR4 [20-23]. While the cystitis model induced by injecting *E. coli* 1677 into the bladder showed an increased thermal sensitivity, the mouse (C3H/HeJ) lacking TLR4 showed no such increase, [5] and the pain response was significantly low when NU14 LPS is injected in the mouse lacking TLR4 [17]. It is, therefore, thought that LPS induces pain through TLR4.

2. Mast Cell

The mast cell is a cell involved in allergy, chronic inflammation, angiogenesis, and wound healing, and it mediates chronic inflammatory responses by releasing pro-inflammatory mediators. This cell releases histamine and causes pain, vasodilatation, and fibrosis of the tissue by damaging the nerve fibers. The mast cell in IC is present mostly in the detrusor muscle. It is suggested that when the mast cell count is >20 cells/mm^2, the diagnosis of IC has a 88% of specificity and 95% of sensitivity [24]. In a neurogenic cystitis model induced by pseudorabies virus, Rudick et al. [25] reported that the mast cell is a related factor for bladder pain. Moreover, another study suggested that the mast cell plays a crucial role in the defense of bacterial infection [26]. Studies on antibacterial peptides are also actively underway; it is known that alpha, beta 2 defensin, and cathelicidin activate the mast cell, which, therefore, require further studies for their roles in IC [27]. In an experiment with animals infected with NU14, however, a mouse lacking the mast cell ($Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$) shows a similar pain response with a wild-type mouse, indicating that the mast cell is independent of the occurrence of UTI pain. The contradictory role of the mast cell in the pelvic pain model is caused by the pain inducing mechanism. Pain in neurogenic cystitis depends on the histamine released from the mast cell and is transduced by histamine receptors 1 and 2. In contrast, pelvic pain in the UTI model induced with LPS is transduced by TLR4. Therefore, it seems that the contradictory role of the mast cell in the cystitis model is attributable to the initiating insult that start from the central nerve system in neurogenic cystitis and the peripheral nerve system in UTI [17].

3. Inflammation, Bacterial Colonization

Inflammation is a type of defense response to stimulation, damage, and infection. In addition, it shows the signs of redness, swelling, heat, pain, and loss of function. Redness and heat are due to the increased blood flow, swelling is due to increased vascular permeability, and pain is due to the activation and sensitization of the primary afferent nerve fiber. Inflammatory mediators (bradykinin, platelet-activating factor, prostaglandins, leukotrienes, amines, purines, cytokines, and chemokines) act on specific targets (e.g., microvascular system), cause local secretion of other mediators from leukocyte (e.g., mast cell, basophil), and draw other leukocytes (e.g., neutrophil) to the sites of inflammation. Through this inflammatory process, the damaged tissues are removed and damaged sites are recovered. Chronic inflammation refers to a prolonged state of inflammation and can be caused when acute inflammation is not cured, or when there is repeated occurrence of acute inflammation, or when the response to stimulation or damage appears in a gradual manner. In a UTI model, MPO is quantified as a marker of inflammation [28]. Rudick et al. [17] performed an animal experiment to examine

the correlation between inflammation or bacterial colonization and pain. The results showed that there is no difference in the MPO level between 83972-infected mice and NU14-infected mice for both 6 and 24 hours after the infection. In addition, no correlation was found between pain response and the MPO level both 6 and 24 hours after the infection. Furthermore, bacterial colonization was measured 24 hours and 14 days after the infection and was significantly higher in NU14-infected mice, although no correlation with pain response was found. Therefore, inflammation and bacterial colonization do not have correlation with pain.

4. Type 1 Pilus

It is proven that type 1 pilus was expressed in many types of clinically identified UPECs and that the removal of FimH in the pilus can inhibit the permeability of *E. coli*. Therefore, this indicates that UPEC requires FimH for microbial penetration or the resultant colonization [29,30]. The most significant difference between NU14 strains and 83972 strains is the presence of type 1 pili. Accordingly, an experiment was performed using strains lacking FimH to investigate whether type 1 pili are involved in UTI pain. The results showed that pain was induced both in NU14 (*fimH*$^+$) and NU14-1 (*fimH*$^-$), but not in 83972, which was endowed with the functional type 1 pilus expression. This indicates that type 1 pilus is not involved in UTI pain [17].

5. O-Antigen

LPS is comprised of three major constituents: the acylated lipid A component embedded in the outer membrane, a core oligosaccharide, and O-antigen—a complex carbohydrate structure. Lipid A is a major mediator of inflammation by means of interactions with TLR4. Lipid A is released after the death of bacterial cells and acts as a potent toxin. It releases inflammation mediators, including prostaglandin, cytokine, and interferon, by activating leukocytes and activates complement [31]. However, the role of O-antigen in bacterial pathogenesis is not fully understood to date [32]. Rudick et al. [18] found that the UPEC mutant (*waaL*), which has a deficiency in O-antigen biosynthesis, induces chronic pain that persists even after the death of bacteria, and that the chronic pain is dependent on TLR4, regardless of inflammation, like acute pain. This result supports the fact that chronic pain is associated with infection.

6. Peripheral and Central Mechanism

Both peripheral sensory stimulation and central nervous system (CNS) sensitization are involved in chronic pain [33]. Callsen-Cencic and Mense [34] suggested that the hyper-reactivity of voiding reflex in the inflammatory response is associated with the activated synthesis of substance P, calcitonin gene-related peptide (CGRP), and galanin with nitric oxide synthase (NOS) in the afferent nerve fiber from the bladder that enters the superior lumbar and sacral spinal cord. It is thought that bladder inflammation not only induces bladder hyper-reactivity, but it also is known to induce pain by increasing the sensitivity of the afferent nerve, in which a lot of mediators are involved. Known to integrate the nociceptive stimuli in sensory neurons, transient receptor potential vanilloid type 1 (TRPV1) receptor senses heat, triggers inflammation, and is involved in various pain syndromes [35-39]. There has been a report showing that LPS increases the expression and sensitivity of TRPV1 in trigeminal sensory neurons by activating TLR4 [40]. A vanilloid receptor agonist, resiniferatoxin (RTX), anesthetizes pain sensation by desensitizing the sensory nerve in the bladder by releasing neurotransmitters, such as substance P and CGRP, in the distal part of the sensory nerve. In a randomized placebo-controlled group study on 18 patients with

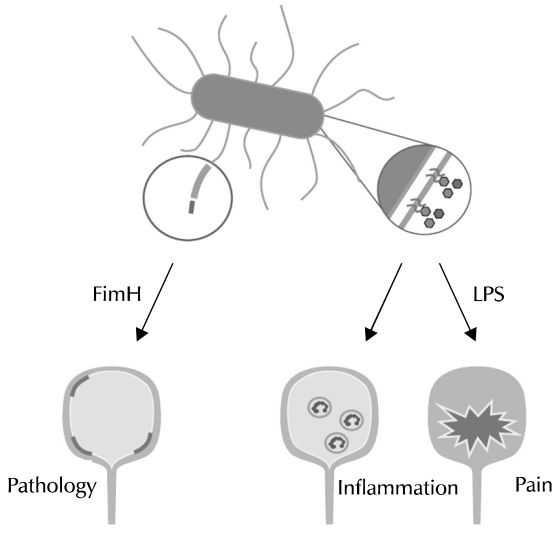

Fig. 2. Lipopolysaccharde (LPS) mediates urinary tract infection pain, independent of inflammation. Adapted from the article of Rosen and Klumpp, Int J Urol 2014;21 Suppl 1:26-32, with permission [42].

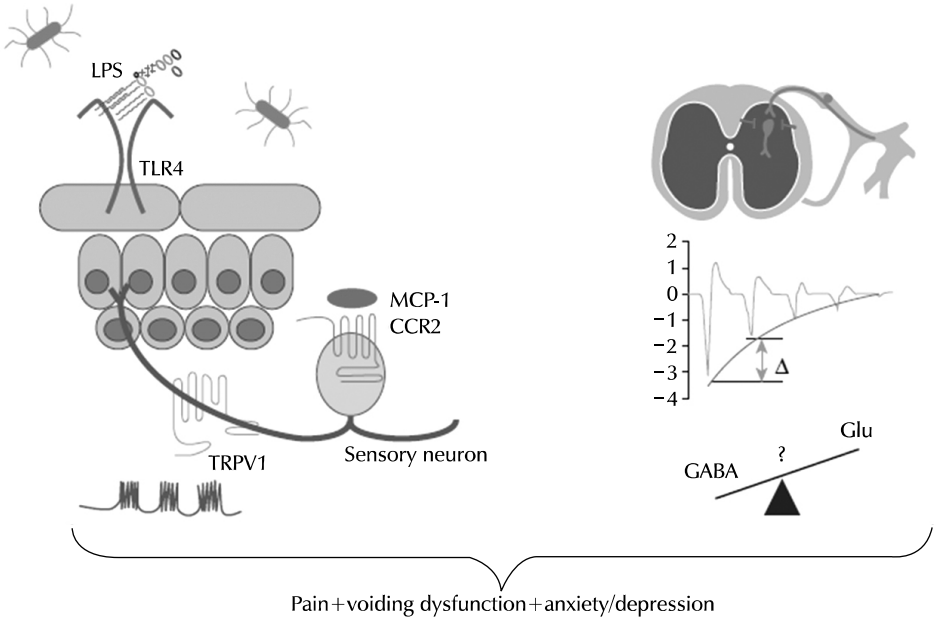

Fig. 3. Post-urinary tract infection chronic pain is mediated by multiple receptors. Adapted from the article of Rosen and Klumpp, Int J Urol 2014;21 Suppl 1: 26-32, with permission [42]. LPS: lipopolysaccharide, TLR4: Toll-like receptor 4, MCP-1: monocyte chemotactic protein 1, CCR2: C-C chemokine receptor type 2, TRPV1: transient receptor potential vanilloid type 1, GABA: gamma-aminobutyric acid.

hypersensitive disorder, Lazzeri et al. [41] reported that urinary frequency decreased from 12.4 times to 7.1 times, nocturia decreased from 3.7 times to 1.7 times, and pain level significantly decreased after 1 month of treatment in the group with RTX injection in the bladder compared with the placebo group. It is reported that C-C chemokine receptor type 2 (CCR2) antagonist reduces post-UTI chronic pain, and that the increase of monocyte chemotactic protein 1 is associated with CP/CPPS. Therefore, CCR2 antagonist can be effective for post-UTI chronic pain [6,7]. The spinal cords of NU14-infected mice showed a diminished-response characteristic of the inhibitory control that were not significantly different from that of controls. In contrast, the spinal cords of *waaL*- and SΦ874-infected mice showed a significantly less inhibition, and these deficits in the inhibited responses were observed at multiple stimulus intensities [18]. These findings demonstrate CNS hyperexcitability in models of post-UTI chronic pain consistent with central sensitization.

CONCLUSIONS

There are studies using animal models on the mechanism of pain response to *E. coli* infection in UTI. It is thought that pain is mediated by LPS and LPS receptor, as well as TLR4 and the subsequent afferent responses, including TRPV1 and CCR2 regardless of inflammation (Fig. 2, 3) [42]. Studies on the mechanism of post-UTI pain syndrome will be valuable to understand and treat BPS/IC and CP/CPPS, which are characterized by chronic pelvic pain.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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