# Response to Urinary Trypsin Inhibitor Therapy in Ulcerative Colitis is Associated With a Decrease in Mast Cell Count in the Colonic Mucosa

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*BACKGROUND:* **Urinary trypsin inhibitor (UTI, ulinastatin ®) inhibits proteinases and has been used for the treatment of ulcerative colitis (UC). We investigated the therapeutic effect of UTI in patients with UC and correlated this effect to mast cell (MC) and macrophages (M) counts in the colonic mucosal wall.**

*DESIGN:* **Patients with UC resistant to corticosteroids (n=16) and normal control subjects (n=10) were included in this study. Biopsy specimens obtained from the sigmoid colon of patients before and after UTI therapy were immunostained with antibodies to tryptase (AA1, MC) and CD68 (M). The number of MC and M in the lamina propria (LP) was determined and expressed per mm² of LP.**

*RESULTS:* **Nine patients with UC responded to UTI treatment. The mean number of MC in the upper part of LP in responders (440±51/mm²) was higher than nonresponders (312±76/mm²) and normal controls (200±47/mm²). MC counts in the lower part of the LP were not different in responders and nonresponders, although the counts in both groups were significantly higher than control. The number of M in the lower part of LP was similar in responders and nonresponders, but were higher than control subjects. M counts in the upper part of LP were similar in both groups of patients and control. Effective treatment with UTI in responders was associated with a significant fall in the number of MC in the upper layer of LP but not in M.**

*CONCLUSION:* **Our results showed that UTI is an effective therapy in steroid-resistant UC. Our results also showed effective therapy with UTI was associated with a reduction in MC counts in the colonic mucosa, suggesting that the control of these cells may mediate, at least in part, the therapeutic effects of UTI in UC.**

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## Introduction

Corticosteroids are effective in bringing about a clinical remission in patients with ulcerative colitis (UC).[1,2] In some cases, however, they are not always effective even when used at a high dose.[3,4] Urinary trypsin inhibitor (UTI) has been used in steroid-resistant UC.[5,6] UTI is a potential inhibitor of proteinases, including neutrophil elastase, trypsin, plasmin, and cathepsin B and H, and has been used for the treatment of experimental and clinical acute pancreatitis, septic shock, and UC.[5-11] However, its pharmacological and pathological effects in UC have not been elucidated.

Mast cells and macrophages are potent source of inflammatory mediators and are widely distributed throughout the gastrointestinal mucosa.[12,13] The function of these two cells seems not to be restricted to inflammatory process. Recent studies have shown that both cell types can also produce regulatory and immunemodulating mediators and cytokines in inflammatory bowel diseases as well as other chronic inflammatory conditions.[14,15] In this regard, the exact role of the mast cells in UC has not been clearly defined. While some investigators have suggested that these cells may influence the course of UC,[16,17] others concluded that there is no relationship between mast cells and UC.[18,19]

In the present study, we examined the effect of UTI in UC resistant to corticosteroids. We also compared mast cell and macrophage counts before and after UTI therapy in patients with UC who responded to UTI and those who failed to respond to UTI.

## Methods

### Patients

The study was carried out between January 1992 and December 1997 in Nagasaki University Hospital. Sixteen patients with UC who were resistant to a standard medical therapy (corticosteroid with or without oral sulfasalazine) were enrolled in the study. We also examined 10 control patients who did not have UC and were not treated with anti-UC medications. The characteristics of these patients are summarized in Table 1. The diagnosis of UC was based on typical symptoms, radiographic and endoscopic findings. To classify patients as treatment-resistant or treatment-sensitive retrospectively, we modified the definition outlined in the Working Term report on medical treatment regimens at accepted therapeutic doses.[2] We defined effective response to therapy as a decrease in disease activity score of 2 or more points. Clinical disease activity of UC was determined by Truelove's criteria.[1] Patients were treated by intravenous injections of 200,000 unit UTI weekly for three months. Total scores were evaluated at wk 2 and 4 wk after commencement of UTI therapy.

### Tissue samples

At least two biopsy specimens from UC patients before and after UTI therapy were obtained from the sigmoid colon from each patient during colonoscopy. The study protocol was approved by the Human Ethics Review Committees of Nagasaki University and a signed consent form was obtained from each subject. The tissue samples were fixed with buffered formalin, embedded in paraffin, and cut into 5 $\mu$ m sections.

### Immunohistochemistry

For immunohistochemical staining of the tissue sample, each section was initially treated for 30 minutes with methanol containing 0.3% $H_2O_2$ to quench endogenous peroxidase activity. Thereafter, non-specific binding sites were blocked by incubation with 10% normal rabbit serum (NRS) for 20 minutes. In the next step, the primary antibody, either mouse monoclonal anti-human mast cell tryptase, AA1, 1:1000 (Dako Co., Tokyo, Japan) or mouse monoclonal anti-human, macrophage, CD68, PG-M1 1:500 (Dako), diluted in PBS, was applied overnight in a sealed humidity chamber. After washing with PBS, the bound antibody was detected by the M-kit (Nichirei, Tokyo), as described previously by Sarin et al.[20]

### Macrophage and mast cell counts

Slides were coded and mast cell and macrophage counts were determined by one investigator (YN) who was blind to the clinical and histological diagnoses. Sections were examined at a high magnification ( $\times$ 400). Mast cells and macrophages located in the lamina propria by dividing two equal parts, upper layer, and lower layer of lamina propria, were counted within the test area of a calibrated ocular grid in ten contiguous non-overlapping fields in each section. We also determined the area fraction of lamina propria by point counting. Mast cell and macrophage counts were expressed as cell numbers/mm$^2$ of lamina propria.

### Statistical analysis

Data were expressed as mean $\pm$ SD. Differences in mast cell and macrophage counts between UC and control were examined for statistical significance using ANOVA and Student's $t$-test. A $p$ value $< 0.05$ denoted the presence of a significant difference.

## Results

### Response to UTI therapy

Clinically, two patients had severe and 14 moderate UC activity before UTI treatment. Seven patients (44%) did not respond to UTI; three of them were ultimately treated by total colectomy. The other nine (64%) patients responded to therapy, particularly in one patient in whom the treatment showed a remarkable effect. There were no serious adverse effects that required discontinuation of therapy. Furthermore, there were no significant differences between responders and non-responders with regard to age, sex, and endoscopic severity of colonic abnormalities or UC disease activity except for the duration of UC (Table 1).

**Table 1.** Characteristics of patients with ulcerative colitis (UC).

|  | Ulcerative colitis | | Control |
|---|---|---|---|
|  | Responders | Non-responders |  |
| Number | 9 | 7 | 10 |
| Age (years)* | 36±18 | 36±19 | 41±1.4 |
| Sex M:F | 6:3 | 5:2 | 7:3 |
| Duration of UC (years)* | 3.0±1.7 | 6.3±9.0¶ | - |
| Endoscopic extent of UC |  |  |  |
|   left side colitis | 2 | 1 | - |
|   pancolitis | 7 | 6 | - |
| Disease activity |  |  |  |
|   moderate | 8 | 6 | - |
|   severe | 1 | 1 | - |
| Treatment |  |  |  |
|   steroids | 8 | 7 | - |
|   sulfasalazine | 9 | 7 | - |

*Data are mean $\pm$ SD, ¶p $< 0.05$ compared to responders

*Mast cell and macrophage counts before UTI therapy*

Immunostaining of the colonic mucosa showed that in control subjects, the number of AA1-positive mast cells in the lower layer of the lamina propria was higher than in the upper layer (Fig. 1A). In contrast, the distribution of mast cells in the lower and upper parts of the lamina propria was similar in patients with UC (Fig. 1B). The number of mast cells in the upper layer of lamina propria in responders was significantly higher than their number in the corresponding region of nonresponders and control subjects (Fig. 2, Table 2). However, there were no significant differences in the number of mast cells present in the lower part of the lamina propria between responders and nonresponders. Mast cell counts in both groups of UC patients was higher than in normal controls (Fig. 2). There were no significant differences in the number of macrophages present in the lower part of the lamina propria between responders and nonresponders, but both counts were higher than in control subjects (Fig. 2). There was no significant difference in macrophage counts in the upper layer of the lamina propria among the three groups (Fig. 2).
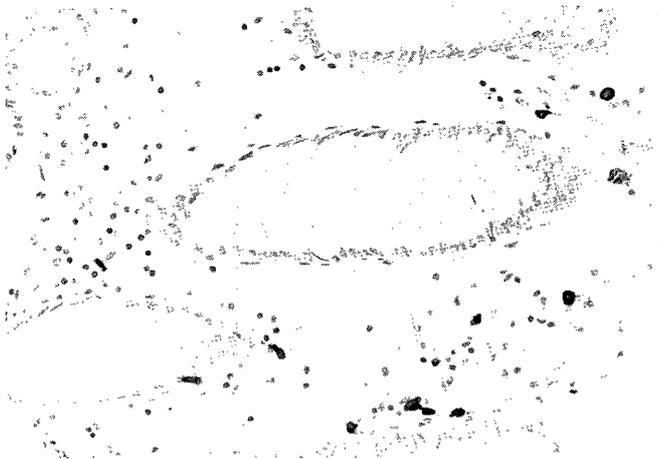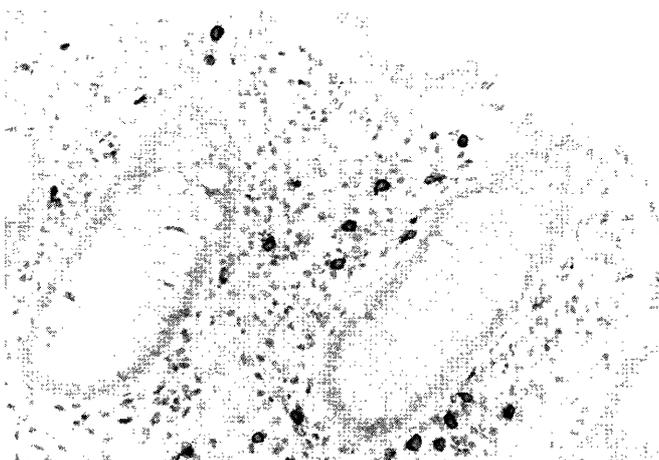


Fig. 1A



Fig. 1B

**Fig. 1.** Representative sections of the colonic mucosa stained for AA1. *A*: Note the high number of AA1-positive cells in the lower part of the lamina propria than in the upper part in the mucosa of the normal colon. *B*: The distribution of AA1-positive cells in ulcerative colitis in the lower part is similar to that in the upper part of the lamina propria. Magnification ×400.



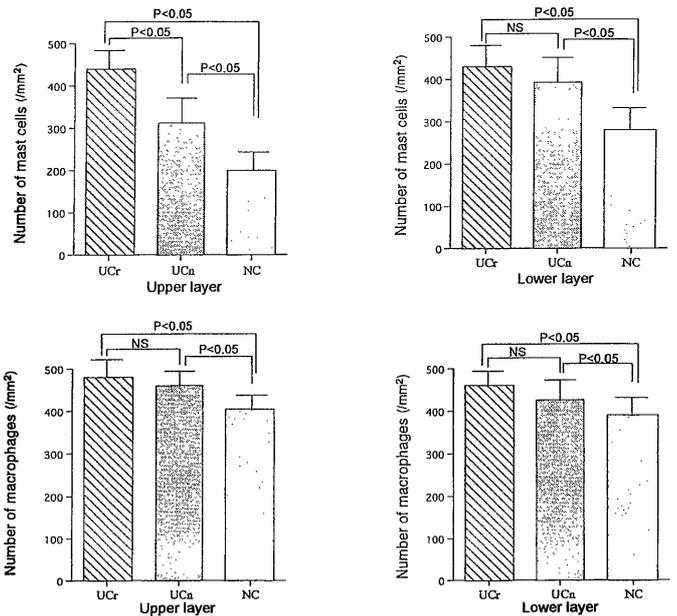**Fig. 2.** The number of mast cells and macrophages in lamina propria

**Table 2.** The number of mast cells & macrophages in the colonic mucosa before UTI therapy

| | Mast cells (/mm$^2$) | | Macrophages (/mm$^2$) | |
|---|---|---|---|---|
| | upper | lower | upper | lower |
| Ulcerative colitis | | | | |
| Treatment sensitive (n=5) | 440±51* | 429±64 | 481±60 | 460±40 |
| Treatment resistant (n=5) | 312±76 | 392±66 | 460±34 | 424±67 |
| Normal control(n=10) | 200±47 | 280±61 | 406±31 | 260±52 |

*$P < 0.05$, compared with the treatment resistant patients.
Data are mean ± SD.

*Mast cell and macrophage counts after UTI therapy*

We also examined biopsies obtained after UTI therapy in four responders and compared mast cell and macrophage counts to those before therapy. UTI treatment resulted in a significant reduction in the number of mast cells in the upper layer of the lamina propria from 423±40 to 299±86 cell/mm$^2$ (p < 0.05). While UTI therapy also decreased the number of mast cells

in the lower layer, the change was not statistically significant (Fig. 3, Table 3). UTI treatment did not produce a significant change in macrophage counts in both layers of the lamina propria (Fig. 3).
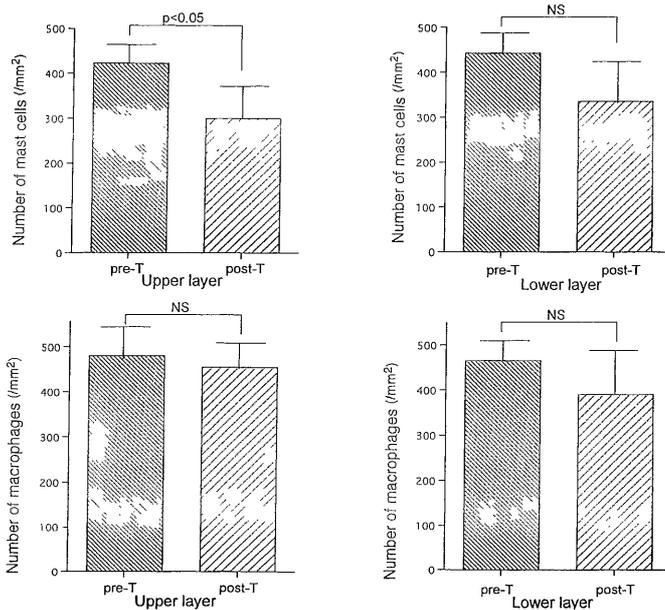


**Fig. 3.** The number of mast cells and macrophages in lamina propria

**Table 3.** The number of mast cells & macrophages in the colonic mucosa after UTI therapy

|  | Mast cells (/mm$^2$) | | Macrophages (/mm$^2$) | |
| --- | --- | --- | --- | --- |
|  | upper | lower | upper | lower |
| Treatment sensitive Ulcerative colitis |  |  |  |  |
| Treatment sensitive | 423±40* | 442± 66 | 481±69 | 465± 46 |
| Treatment resistant | 299±86 | 335±113 | 455±53 | 390±102 |

*P < 0.05, compared with the post treatment patients.
Data are mean ± SD.

## Discussion

UTI is a unique trypsin inhibitor purified from human urine.[21,22] It exerts a membrane stabilization action on leukocytes and macrophages and protects against free radical-induced lipid peroxidation.[11,23] Mast cells and macrophages are known to produce several inflammatory mediators such as histamine, prostaglandin, and cytokines.[13-15] Minocha et al.[19] and Higa et al.[24] suggested that mast cells are not crucial for the development of experimental colitis in mice. Compared to the normal pattern, the number of mast cells in the intestinal mucosa of patients with UC has been reported to be higher in some studies,[25,26] similar,[17,20] or lower in

others.[16,18] These discrepancies may be due to degranulation of mast cells in the colonic mucosa; since such cells do not stain by mast cell stains.[15] However, the presence of high mast cell counts in UC have been reported in more recent studies that employed immunostaining for tryptase.[16,17]

Glucocorticoids are the most effective agents for the treatment of severe UC, but the exact mechanism of action of these potent drugs remains unknown.[27] In this regard, Shimada et al.[4] reported recently that nonresponders to glucocorticoid therapy show a significant increase both in the number of glucocorticoid binding sites and in apparent dissociation contact compared with responders.[4] However, UTI does not bind to glucocorticoid receptors, even when the number of these receptors is increased.[28]

Mast cells are thought to have a protective effect in the mucosa by enhancing epithelial repair.[29] Furthermore, the mast cell protease, tryptase, enhances interleukin (IL)-8 release.[29,30] Increased number of mast cells in colonic wall may protect the mucosa by limiting the spread of inflammation.[17] Goldsmith and coworkers[27] reported a significant reduction in mast cell counts in patients with inflammatory bowel disease (IBD) treated with corticosteroids compared with control patients and patients with IBD who were not treated with corticosteroids. Our data also demonstrated that UTI significantly reduced mast cell population, particularly those in the upper layer of the lamina propria (Fig. 3).

A low molecular weight serine protease inhibitor, named trypstatin, which has a high degree of sequence homology with UTI, markedly inhibits tryptase in rat mast cell.[31] King et al.[16] reported a protective effect for mast cells in patients with UC and that 69% of patients in remission have high mast cell counts in the colonic mucosa.[16] Trypstatin has a high degree of sequence homology with human and bovine inter-alpha-trypsin inhibitor.[32,33] Mast cells are often seen degranulated in areas of active disease, suggesting that the inflammatory mediators released from these cells contribute to the pathophysiology of these disorders.[33] Accumulation of mast cells at the visible line of demarcation between normal and abnormal mucosa suggests that these cells may play a critical role in either accelerating the process of inflammation or in suppressing continued extension of the disease.[17]

Resistance to steroid therapy in patients with UC is probably due to several factors. These include long duration of disease activity and presence of perinuclear antineutrophil cytoplasmic antibodies (pANCA).[34] Sandborn et al.[35] postulated that the increased frequency of pANCA in treatment-resistant left-sided UC

may reflect a possible association between these anti-bodies and relative resistance to medical therapy in patients with UC.

In conclusion, our data showed that UTI can be effective in steroid-resistant patients with UC. To our knowledge, this is the first study reporting a correlation between UTI therapy and colonic mast cell counts. Our data suggest that the number of mast cells in the upper part of the lamina propria in patients with UC may be a useful marker for a positive response to UTI therapy. Further studies are necessary to investigate the interaction between UTI and inflammatory cells in the colonic mucosa.

## Acknowledgment

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