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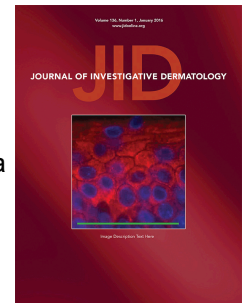
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Journal of Investigative Dermatology

(Letter To The Editor)

**Intrathecal minocycline suppresses itch-related behavior and improves dermatitis  
in a mouse model of atopic dermatitis**

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**Short title:** Therapeutic benefit of minocycline in AD

**Abbreviations:** AD, atopic dermatitis; Dfb, *Dermatophagoides farinae* body; SD, standard deviation; TEWL, transepidermal water loss

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**TO THE EDITOR**

Itch (or pruritus) is a common, sometimes unpleasant sensation that elicits an urge to scratch. Chronic pruritus (defined as itch lasting for > 6 weeks) found in patients with dermatological diseases including atopic dermatitis (AD) is often resistant to conventional treatments, including antihistamines. Such itch is a clinical problem that reduces the quality of life in patients (Warlich et al., 2015). However, the mechanisms underlying chronic and intractable itch have not been clarified.

There is recent accumulating evidence from pain research that spinal microglial activity plays a role in the pathogenesis of neuropathic pain. Neuropathic pain in animal models is ameliorated by intrathecal administration of minocycline, an inhibitor of activated microglia (Tikka and Koistinaho, 2001; McMahon et al., 2005). Meanwhile, the role of spinal microglia in the development of chronic and intractable pruritus, such as AD, has not been fully elucidated. Additionally, the effect of minocycline on pruritus of AD is currently unclear. We therefore investigated the antipruritic effect of minocycline on AD using NC/Nga mice, a model of AD (Tanaka et al., 2012).

Ten-week old male NC/Nga mice (Charles River Laboratories Japan, Yokohama, Japan), were maintained under clean conditions. All animal procedures were approved by the Animal Care and Use Committee of Juntendo University Graduate School of Medicine. We used a *Dermatophagoides farinae* body (Dfb) ointment-induced AD-like mouse model (Dfb-NC/Nga) as described (Yamamoto et al., 2009). Dermatitis was induced by repeated application of Dfb ointment (Biostir, Kobe, Japan) twice a week for 3 weeks (Figure 1a). The severity of skin lesions was graded according to criteria described (Matsuda et al., 1997). After induction, scratching behavior was monitored for 12 hours using a SCLABA<sup>®</sup>-Real system (Noveltec, Kobe, Japan) as described (Tanaka et al., 2012). Under sevoflurane anesthesia, transepidermal water loss (TEWL) was measured using a Tewameter<sup>®</sup> TM210 (Courage & Khazaka Electronic, Cologne,

Germany). Dfb-NC/Nga mice showed significant increases in number of scratching bouts, dermatitis score and TEWL value (Figure S1a-c). Distribution of spinal microglia in the Dfb-NC/Nga mice was examined immunohistochemically with an antibody against Iba1, a microglial marker (Ito et al., 1998). Increased numbers of spinal Iba1-immunoreactive (Iba1<sup>+</sup>) microglia were found in Dfb-NC/Nga mice compared with control mice (Figure S1d -f).

We next examined the effects of minocycline on scratching behavior and dermatitis in Dfb-NC/Nga mice. Minocycline (Sigma-Aldrich, St.Louis, MO) was dissolved in sterile saline, and filtrated using sterile syringe filters. Intrathecal administration of minocycline was performed according to the previously described method (Hylden et al., 1980). Mice with a dermatitis score > 5 were treated by intrathecal administration of sterile saline (vehicle) or minocycline at doses of 5 or 50 µg in 5 µL saline three times a week for 2 weeks (Figure 1a). In the treatment phase, scratching behavior was monitored for 12 hours once a week (Figure 1a). The number of scratching bouts was significantly decreased after treatment for 1 week in 50 µg minocycline-treated mice compared with control mice (Figure 1b). Minocycline was more effective in alleviating scratching behavior after treatment for 2 weeks (Figure 1b). We also monitored locomotion activity in the mice for 12 hours using SCLABA®-Real software (Noveltec) and found no significant difference among the three groups (Figure 1c). The numbers of Iba1<sup>+</sup> microglia in the posterior area of the spinal cord were also significantly decreased after treatment for 2 weeks in 50 µg minocycline-treated mice compared with control mice (Figure 1d and 1e).

Dermatitis score was further assessed after each Dfb application in the treatment phase (Figure 1a). In 50 µg minocycline-treated mice, dermatitis score was significantly decreased at 1 week after treatment, with improvement sustained throughout the experiment (Figure 2a and 2b). The TEWL value in dorsal skin lesions in the 50 µg

minocycline-treated mice was also decreased after 2 weeks of treatment (Figure 2c).

We also examined the effects of oral minocycline on the AD-like symptoms shown in Figure S2a. Oral high-dose minocycline improved dermatitis in the Dfb-NC/Nga mice (Figure S2b). The systemic administration showed a tendency to reduce the numbers of scratching bouts (Figure S2c) and spinal Iba1<sup>+</sup> microglia (Figure S2d).

The present study showed that intrathecal minocycline dose- and time-dependently suppressed scratching behavior in Dfb-NC/Nga mice, concomitant with decreased numbers of Iba1<sup>+</sup> microglia. Oral minocycline also had a tendency to suppress scratching behavior and decrease numbers of Iba1<sup>+</sup> microglial (Figure S2). Clinically, minocycline is used as a broad-spectrum tetracycline antibiotic (Garrido-Mesa et al., 2013). In addition to anti-inflammatory and immunomodulatory effects of minocycline (Garrido-Mesa et al., 2013), it inhibits p38 mitogen-activated protein kinase in the microglia and thereby prevents their proliferation (Tikka and Koistinaho, 2001). Minocycline was also shown to reverse microglial reactivity in neuropathic pain models (LeBlanc et al., 2011). A more recent study suggested that spinal reactive astrocytes play a role in itch sensitization of AD model mice (Shiratori-Hayashi et al., 2015). Therefore, although we cannot exclude effects of minocycline on the astrogliosis, the anti-scratching effect of minocycline may be at least partly associated with inhibition of microglial reactivity. This may be supported by a recent study indicating that scratching activates spinal microglia in acute pruritic model mice (Zhang et al., 2015a).

Moreover, removing the claws of NC/Nga mice, thus preventing scratching, successfully inhibited the induction and progression of dermatitis (Hashimoto et al., 2004). We found that intrathecal minocycline improved skin barrier function (Figure 2c) and dermatitis in Dfb-NC/Nga mice (Figure 2b). Dermatitis was also improved by oral high-dose minocycline (Figure S2b). Thus, in addition to the anti-inflammatory effect, an anti-scratching effect by intrathecal minocycline administration may contribute to

improvement of dermatitis through prevention of skin barrier damage.

A recent study also indicated that intrathecal administration of minocycline suppressed scratching behavior in a contact dermatitis (CD) mouse model induced by repeated applications of 2,4-dinitrofluorobenzene (DNFB), although the anti-scratching effect was not sustained after treatment (Zhang et al., 2015b). Thus, given that the pathophysiology and pathological process of AD are different from those of CD induced by haptens, such as DNFB (Kabashima, 2013), the pharmacological efficacy may reflect differences in the degree of microglial activation.

### **Conflict of interest**

The authors declare they have no conflicts of interest.

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### **Supplementary material**

**Supplemental data.** Additional materials and methods.

**Supplementary Figure S1.** Distribution of Iba1<sup>+</sup> microglia in the spinal cord of Dfb-NC/Nga mice.

**Supplementary Figure S2.** Effects of oral minocycline on dermatitis, scratching behavior and spinal Iba1<sup>+</sup> microglia in Dfb-NC/Nga mice.

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### Figure legends

**Figure 1. Effects of intrathecal minocycline on scratching behavior in Dfb-NC/Nga mice.** (a) Experiment schedule. After induction of dermatitis, saline or minocycline was administered intrathecally three times per week for 2 weeks. (b) Scratching bouts were significantly reduced after treatment with 50  $\mu$ g minocycline for 1 and 2 weeks. (c) No significant difference in locomotion activity was observed among the groups. (d) Images of spinal Iba1-immunoreactive (Iba1<sup>+</sup>) microglia (green) in saline- and 50  $\mu$ g minocycline-treated mice. Scale bar; 100  $\mu$ m. (e) The numbers of spinal Iba1<sup>+</sup> microglial significantly decreased after treatment with 50  $\mu$ g minocycline for 2 weeks. Data (mean  $\pm$  SD,  $n = 7-8$  per group) were compared by one-way analysis of variance and Bonferroni's method. \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$  vs. control.

**Figure 2. Effects of minocycline on dermatitis and transepidermal water loss (TEWL) in Dfb-NC/Nga mice.** (a) Representative images of lesional skin in Dfb-NC/Nga mice before and after two weeks of treatment with minocycline. (b) In the 50  $\mu$ g minocycline-treated mice, dermatitis score was significantly decreased after 1 week of treatment, with improvement sustained for 2 weeks ( $P < 0.01$ ). (c) The TEWL value in the dorsal skin lesions significantly improved after 2 weeks of treatment in 50  $\mu$ g minocycline-treated mice. Data (mean  $\pm$ SD,  $n = 7-8$  per group) were compared by one-way analysis of variance and Bonferroni's method. \* $P < 0.05$ , \*\* $P < 0.01$  vs. control.

Figure 1. Torigoe et al.

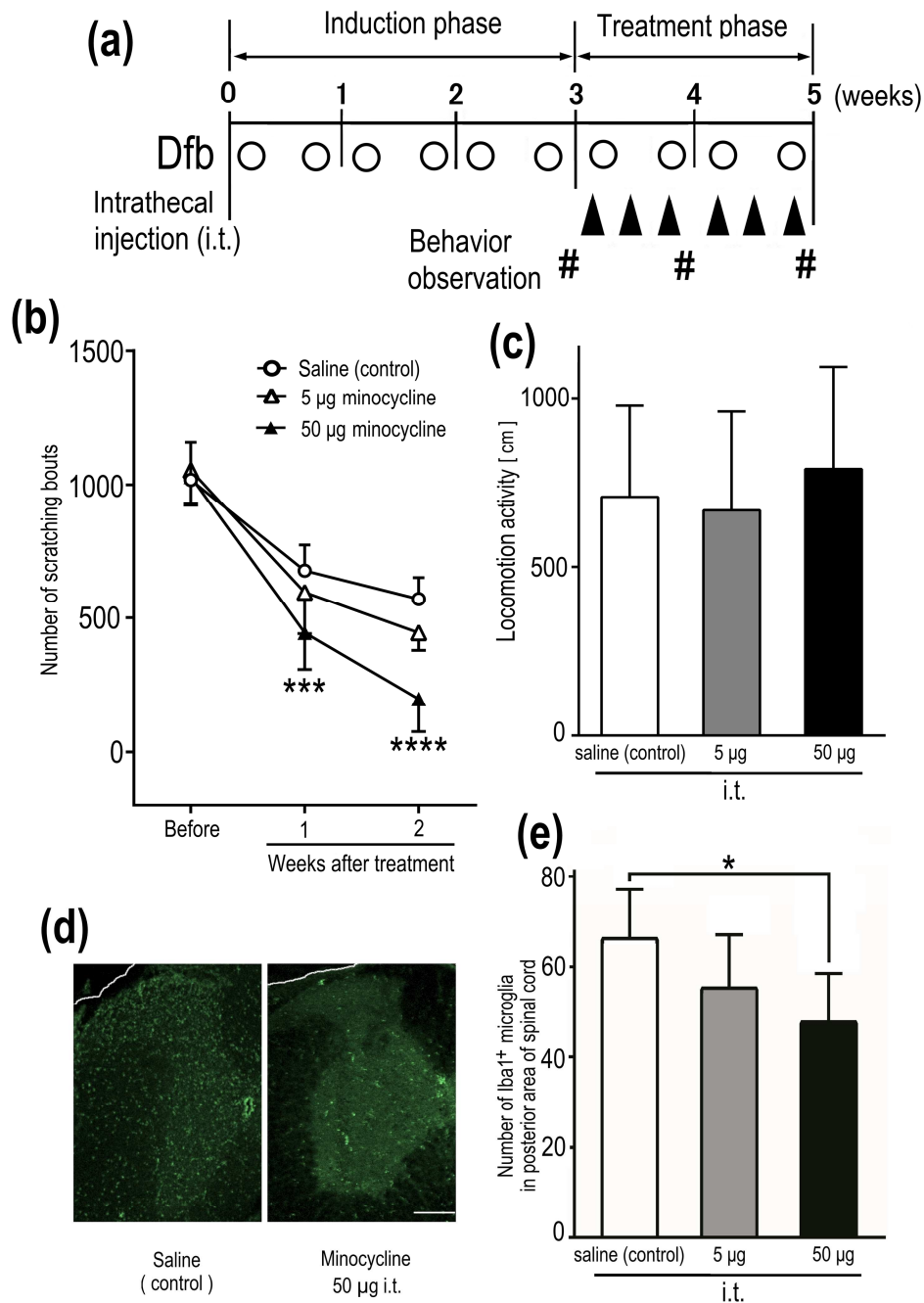


Figure 2. Torigoe et al.

