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Short Communication

Pioglitazone improves visceral sensation and colonic permeability in a rat model of irritable bowel syndrome

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ABSTRACT

Visceral hypersensitivity and impaired gut barrier with minor inflammation are considered to play an important role in the pathophysiology of irritable bowel syndrome (IBS). Since pioglitazone is known to have anti-inflammatory property, we hypothesized that pioglitazone is beneficial for treating IBS. In this study, the effect was tested in rat IBS models such as lipopolysaccharide or repeated water avoidance stress-induced visceral allodynia and increased colonic permeability. Pioglitazone blocked these visceral changes, and GW9662, a peroxisome proliferator-activated receptor gamma (PPAR-γ) antagonist fully reversed the effect by pioglitazone. These results suggest that PPAR-γ activation by pioglitazone may be useful for IBS treatment.

Visceral hypersensitivity and impaired gut barrier function with immune system activation are considered to play an important role in the pathophysiology of irritable bowel syndrome (IBS).[1] There is evidence that increased levels of plasma proinflammatory cytokines and serum lipopolysaccharide (LPS) are observed in some portion of IBS patients, and LPS-cytokine signaling possibly contributes to these visceral functional changes in IBS.[2]

Pioglitazone decreases insulin resistance by the stimulation of peroxisome proliferator-activated receptor gamma (PPAR-γ), and it also displays antinociceptive effect against somatic pain.[3] Incidentally, increased gut permeability was shown to be associated with increased translocation of LPS and contribute to insulin resistance in animal model of diabetes.[4]

In this context, we hypothesized that pioglitazone is beneficial for treating IBS by improving visceral sensation and gut permeability, and attempted to test this hypothesis using two animal IBS models, i.e. LPS or repeated water avoidance stress (WAS)-induced visceral allodynia and increased colonic permeability.[5–7]

We used adult male Sprague Dawley rats weighing approximately 300 g (Charles River Laboratory, Atsugi). Visceral sensation was assessed by abdominal muscle contractions induced by colonic balloon distention (visceromotor response; VMR) using electromyogram (EMG).[5,6] The electrodes for EMG were acutely implanted into the abdominal musculature, and a distention balloon was inserted intra-anally into the colon. The rats were placed in Bollmann cages and the VMR threshold was defined as the distended balloon volume. The percentage change threshold, i.e. the threshold value after treatment divided by the basal threshold value and multiplied by 100, was calculated.

Colonic permeability was determined in vivo as previously described.[7] The colon was ligated at the junction with the cecum, and a catheter was inserted into the proximal colon. Evans blue (1.5%, 1 ml) was instilled into the colon through this catheter. Fifteen min later, the colons were excised, and placed in 2 ml of N,N-dimethylformamide for 12 h. Permeability was calculated by measuring the Evans blue concentration in the supernatant spectrophotometrically.

Water avoidance stress consisted of placing rat on a plastic platform (height, 8 cm; length, 6 cm; width, 6 cm) positioned in the middle of a plastic cage filled with water up to 7 cm of the platform

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height. Control animals were individually placed in the same plastic cage, which was not filled with water (sham stress).[6]

Pioglitazone, fenofibrate (Tokyo Chemical Industry Co., LTD., Tokyo), a PPAR-α agonist and GW9662 (Focus Biomolecules, Plymouth Meeting, PA, USA), a PPAR-γ antagonist were dissolved in dimethyl sulfoxide. LPS obtained from *Escherichia coli* with the serotype 055:B5 (Sigma–Aldrich, St. Louis, MO, USA), N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthesis inhibitor and naloxone hydrochloride (Wako Pure Chemical Industries, Osaka), an opioid receptor antagonist were dissolved in normal saline. The doses of chemicals were determined according to previous studies.[8–10]

Data were expressed as means ± standard error. Multiple comparisons were performed by one-way or two-way analysis of variance followed by Tukey's honestly significant difference test. $p < 0.05$ was considered statistically significant.

For all studies, approval was obtained by the Research and Development and Animal Care Committees at the Asahikawa Medical University (#16191, approved on April 1, 2016).

LPS (1 mg/kg) subcutaneously (sc) reduced the threshold of VMR,[5] and pioglitazone injected thrice at 48 h, 24 h and 30 min before injecting LPS reversed the response by LPS in a dose-dependent manner (Fig. 1A and B). Since pioglitazone at 10 mg/kg fully reversed the response, this dose was employed for the following experiments. LPS increased colonic permeability[7] and the drug also blocked this response (Fig. 1C). Repeated WAS (1 h daily for 3 days) induced visceral allodynia and increased colonic permeability (Fig. 1D),[6,7] and pioglitazone blocked these responses (Fig. 1E and F).

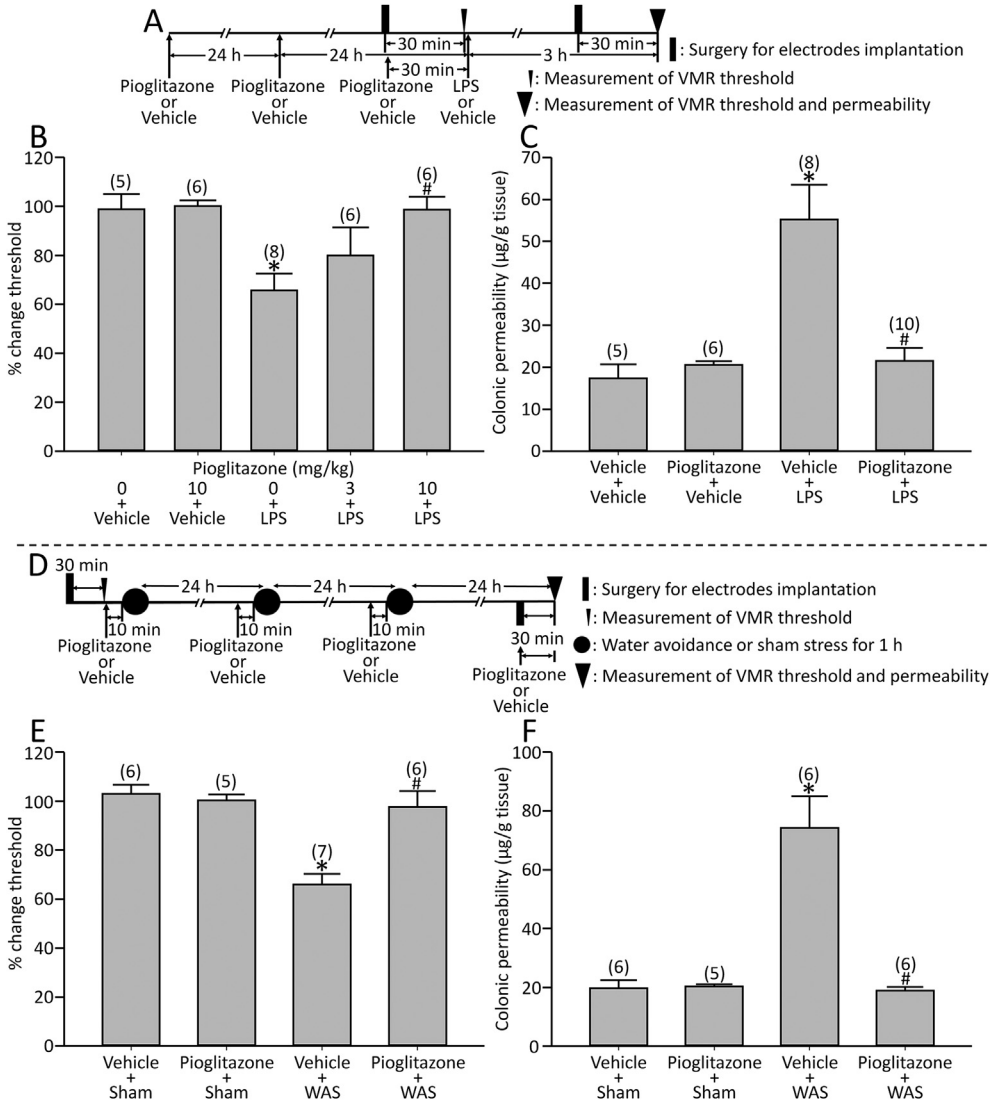

Fig. 1. Effect of pioglitazone on visceral changes in animal IBS models. First the basal threshold of visceromotor response (VMR) was measured. Then, LPS or the vehicle was injected, and the measurement of threshold and colonic permeability were performed at 3 h after the injection. Pioglitazone or the vehicle was injected thrice at 48 h, 24 h and 30 min before injecting LPS (A). LPS induced visceral allodynia, and pioglitazone dose-dependently reversed this response (B). LPS increased colonic permeability, which was blocked by pioglitazone (C). Water avoidance stress (WAS) or sham stress was applied for 1 h for 3 consecutive days. The threshold was measured before the first stress session and at 24 h after undergoing the last session, followed by the measurement of colonic permeability. Pioglitazone or the vehicle was injected 4 times, i.e. at 10 min before each stress session and 30 min before the second measurement of the threshold (D). Pioglitazone also blocked visceral allodynia (E) and increased colonic permeability (F) by repeated WAS. Sham; sham stress. $^*p < 0.05$ vs. vehicle (pioglitazone 0) + vehicle or vehicle + sham, $\#p < 0.05$ vs. vehicle (pioglitazone 0) + LPS or vehicle + WAS by one-way or two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

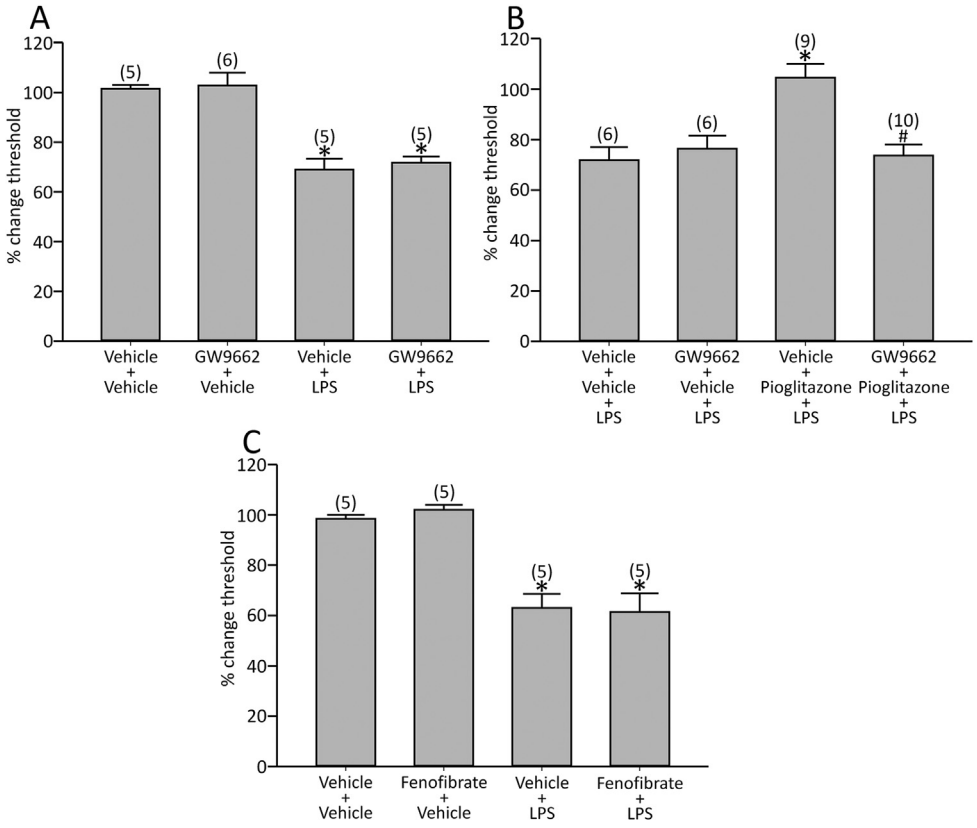

Fig. 2. GW9662 injected thrice at 48 h, 24 h and 30 min before injecting LPS did not modify the response induced by LPS (A). The drug injected with pioglitazone fully reversed the antinociceptive effect by pioglitazone on LPS-induced visceral allodynia (B). Meanwhile, fenofibrate injected thrice did not alter the response by LPS (C). $^{*}p < 0.05$ vs. vehicle + vehicle or vehicle + vehicle + LPS, $\#p < 0.05$ vs. vehicle + pioglitazone + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

GW9662 (3 mg/kg sc) did not modify the threshold change by vehicle or LPS (Fig. 2A). The drug injected together with pioglitazone completely reversed the antinociceptive effect by pioglitazone in LPS model (Fig. 2B). Fenofibrate (20 mg/kg sc) did not modify the response by LPS (Fig. 2C), indicating that stimulation of PPAR-α did not display visceral antinociception against LPS.

Additionally, L-NAME (10 mg/kg intraperitoneally) or naloxone (1 mg/kg sc) neither altered the response by vehicle or LPS nor the effect by pioglitazone (Fig. 3A–D). Pioglitazone, GW9662, fenofibrate, L-NAME or naloxone per se did not change the basal threshold of VMR (data were not shown).

The information regarding the effect of pioglitazone on gut function has been scarce. Only one study reported that pioglitazone inhibited visceral hypersensitivity induced by intracolonic instillation of acetic acid in rats.[8] This animal model is considered to simulate post-infectious IBS, which is a minor subset of IBS patients.

Water avoidance stress is a conventional psychological stress protocol. Since accumulation of continuous life stress causes the exacerbation of symptoms in majority of IBS patients, repeated WAS is thought to be one of the common animal IBS models.[11] Visceral allodynia and increased colonic permeability induced by repeated WAS were mediated via interleukin (IL)-1, IL-6,[6] and toll-like receptor 4,[7] and LPS injection also induced similar visceral changes through common pathways to repeated WAS.[5,7] We disclosed that pioglitazone blocked not only visceral allodynia but also increased colonic permeability in these animal IBS models, which was for the first time.

We also found that the antinociceptive effect by pioglitazone was reversed by GW9662, but fenofibrate did not alter the response by LPS suggesting that the effect of pioglitazone is PPAR-γ dependent, and PPAR-γ signaling may be specific for mediating the antinociception against LPS-induced visceral allodynia.

PPAR-γ ligands attenuate the inflammatory response in sepsis through regulation of the NF-κB.[12] PPAR-γ activation by pioglitazone decreased expression of proinflammatory cytokine genes, and also reduced LPS-induced increased expression of IL-1β and IL-6 in human monocytes.[13] These lines of evidence suggest that the effect of pioglitazone may result from inhibition of cytokine signaling via PPAR-γ.

Previous study showed that the antinociceptive effect of pioglitazone was mediated via NO pathway.[8] Moreover, opioid signaling is known to be involved with altered visceral sensation induced by stress.[14] However, neither NO nor opioid signaling mediated the effect of pioglitazone in the current study. Further studies are needed to explore the precise mechanisms of pioglitazone on visceral changes.

In summary, we showed that pioglitazone blocked visceral allodynia and increased colonic permeability in animal IBS models. Since increased gut permeability may also play a crucial role in the pathophysiology of diabetes,[4] our results suggest that improved gut barrier may be one of the mechanisms of decreasing insulin resistance by pioglitazone.

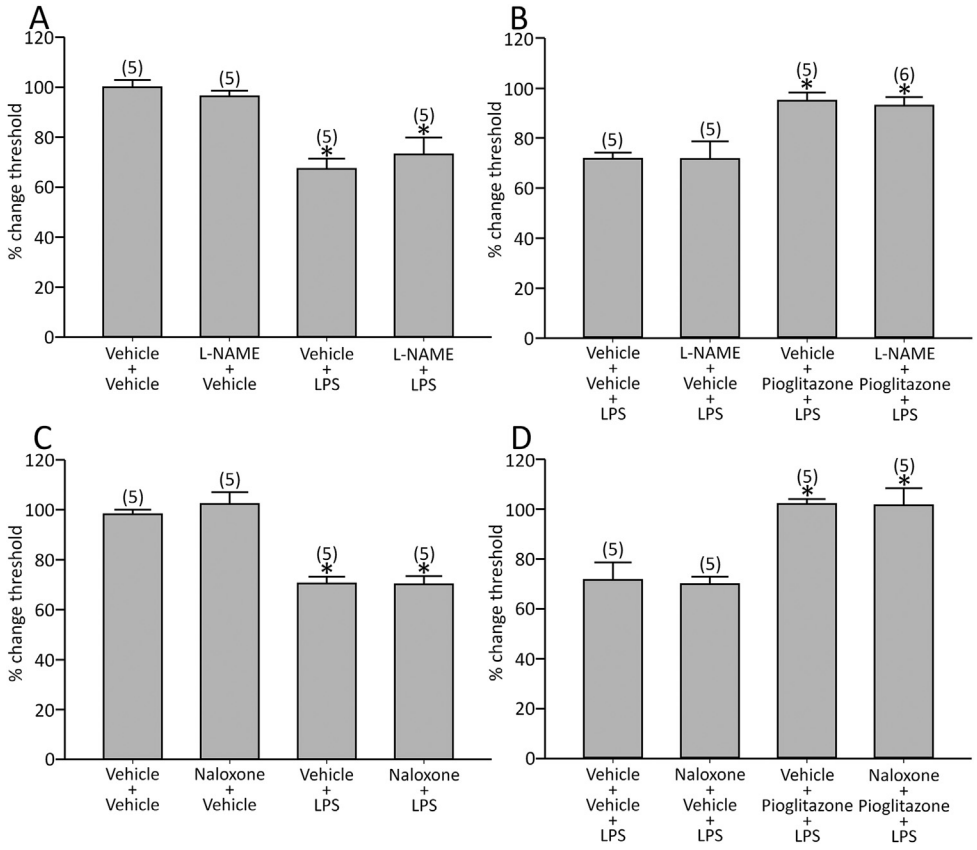

Fig. 3. L-NAME injected thrice at 48 h, 24 h and 30 min before injecting LPS did not modify the response by LPS (A), and it did not change the effect by pioglitazone (B). Additionally, naloxone injected thrice neither altered the response by LPS (C) nor the effect by pioglitazone (D). *$p < 0.05$ vs. vehicle + vehicle or vehicle + vehicle + LPS by two-way analysis of variance followed by Tukey's honestly significant difference test. Each column represents the mean ± standard error. The number of rats examined is shown in parentheses.

Conflict of interest

The authors declare that they have no conflict of interest.

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