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Irsogladine : Overview of the Mechanisms of Mucosal Protective and Healing-Promoting Actions in the Gastrointestinal Tract

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Abstract: Irsogladine, a mucosal protective drug, was developed in Japan for the treatment of peptic ulcer disease and acute gastritis. This drug is superior to gefarnate, the same therapeutic category drug, in a randomized, controlled and double-blind clinical study in 1987. The mechanisms of irsogladine's actions are apparently different from those of antisecretory drugs. Irsogladine increases intracellular cyclic adenosine 3',5'-monophosphate content *via* non-selective inhibition of phosphodiesterase isozymes and exhibits gastric cytoprotection partly mediated by endogenous nitric oxide. These effects may account for a variety of actions of irsogladine in the gastrointestinal tract, including facilitation of gap junctional intercellular communication, inhibition of the reduced gastric mucosal blood flow response, suppression of reactive oxygen generation and so on. Since 1984, more than 60 papers have been published to further verify the effects of irsogladine on gap junctional intercellular communication, tight junction, nitric oxide production and neutrophil migration as well as *Helicobacter pylori*-related pathological changes in the stomach as well as the adverse reactions induced in the stomach or the small intestine by various drugs, including nonsteroidal anti-inflammatory drugs, bisphosphonates or selective serotonin re-uptake inhibitors. In this article, we review recent advances in understanding the mechanisms of irsogladine's actions and the most recent data in experimental as well as clinical studies.

Keywords: Irsogladine, anti-ulcer drug, mucosal protection, healing-promoting action, gastric and intestinal lesion.

INTRODUCTION

Irsogladine (2,4-diamino-6-[2,5-dichlorophenyl]-s-triazine maleate) Fig. (**1A**), an anti-ulcer drug widely used in Japan, Korea and China, is known to protect the gastric mucosa by enhancing the mucosal defensive ability through facilitation of gap junctional intracellular communication (GJIC) [1, 2]. This drug is absorbed in the small intestine and distributed in the entire gastrointestinal tract [3]. It has been reported that irsogladine is effective for aphthous stomatitis [4-6], gastric ulcer [7, 8], intestinal mucosal injuries [9-11] and inflammatory bowel disease (IBD) [12, 13].

In this article, we review recent advances in understanding the mechanisms of irsogladine's actions and the most recent data in experimental as well as clinical studies. We discuss the current knowledge on the mechanisms of irsogladine's actions and its efficacy against various gastrointestinal diseases, including *Helicobacter pylori (H. pylori)*-induced gastric ulcer, nonsteroidal anti-inflammatory drug (NSAID)-induced gastric and small intestinal lesions as well as gastric adverse reactions induced by bisphosphonates, selective serotonin re-uptake inhibitors (SSRIs) or anti-thrombotic drugs [7-17].

PHARMACOLOGICAL ACTIONS OF IRSOGLADINE

Inhibition of Phosphodiesterase

Cyclic nucleotides, such as cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP), are degraded into inactive metabolites due to hydrolysis by phosphodiesterase (PDE) [18]. Irsogladine concentration-dependently increased cAMP content in rat glandular stomach but had no effect on the cGMP content. At present, PDE is genetically subdivided into 11 izosymes, five of which, PDE1 to PDE5, have been well characterized pharmacologically; PDE1 is activated by Ca^{2+}/calmodulin and PDE2 by cGMP, yet both catalyze the conversion of cAMP and cGMP into inactive metabolites [18]. By contrast, both PDE3 and PDE4 selectively bind to cAMP as the substrate, while PDE5 catalyzes cGMP's conversion to 5'GMP [18]. The degradation of cAMP by bovine heart PDE was almost completely inhibited by the combined administration of vinpocetine (a PDE1 inhibitor) and cilostamide, (a PDE3 inhibitor), indicating that the degradation is mediated almost exclusively by PDE1 and PDE3 [19]. Irsogladine suppressed cAMP degradation measured in the presence of vinpocetine to almost the same extent as that determined in the presence of cilostamide [19]. It is assumed that irsogladine increases intracellular cAMP content *via* non-selective inhibition of PDE isozymes but not by the activation of adenylate cyclase. Although irsogladine had no effect on cGMP content due to PDE inhibition in the glandular stomach, this drug exhibited gastric protection partly mediated by endogenous nitric oxide (NO), suggesting the possible participation of cGMP in this action [20, 21]. These pharmacological properties might be associated with a variety of actions of irsogladine in the gastrointestinal tract Fig. (**1B**).

Facilitation of Gap Junctional Cellular Communication (GJIC)

The mechanism of the protective action of irsogladine is largely attributable to the facilitation of GJIC in the gastric mucosa through the increase of cAMP production *via* the inhibition of PDE. Gap junctions provide a low-resistance pathway for the exchange of small polar molecules and small peptides (molecular weight < 1200) between adjacent cells [22]. While the channels assembled from connexin (Cx) family members commonly enable the intercellular exchange of small metabolites, second messengers and electrical signals, the diversity of function is attributable to the subset of Cxs that are expressed in certain types [23]. Ueda *et al.* [1] reported that irsogladine dose-dependently increased cell coupling in rabbit gastric epithelial cells Fig. (**2**). Kawasaki *et al.* [24] reported that irsogladine facilitated GJIC in PANC-1 cells expressing Cx43 protein. It was also found that treatment with irsogladine moved the localization of Cx43 immunoreactive spots from the cytoplasm to the boundary regions with neighboring cells, without changing the phosphorylation state of Cx43. In addition, the effects of irsogladine were attenuated by the PKA inhibitor H-89 and the adenylate

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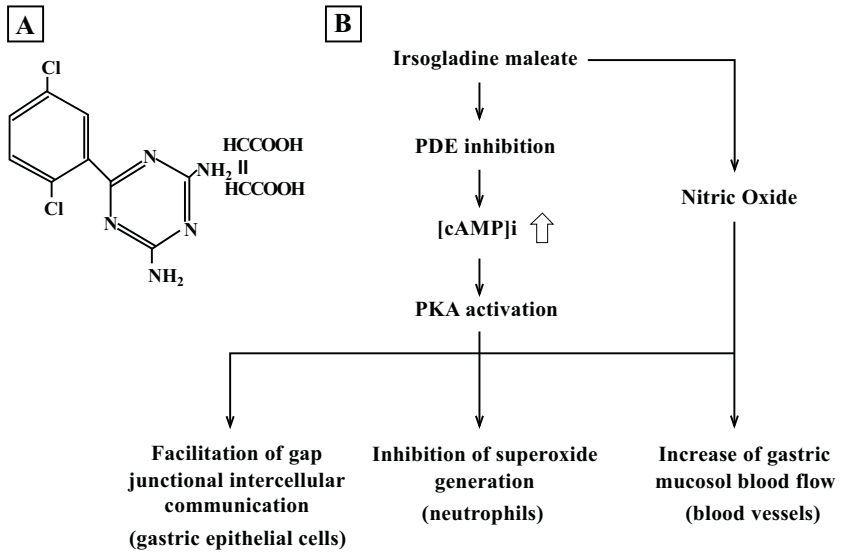

Fig. (1). Chemical structure of irsogladine **(A)** and the possible mechanisms of actions of this drug **(B)**.

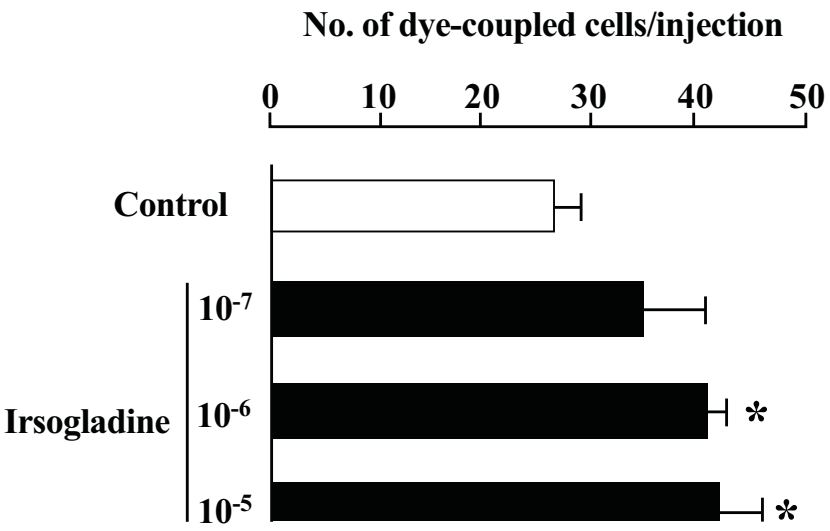

Fig. (2). Effect of irsogladine on GJIC of cultured rabbit gastric epithelial cells. A fluorescence indicator, Lucifer yellow CH, was microinjected into a cell 1 min after the addition of irsogladine (10^{-7}-10^{-5} M) or the vehicle. Three minutes after the microinjection, the extent of dye transfer was recorded with a video system under fluorescence microscopy. The capacity for dye coupling between the cells was assayed by a count of the Lucifer yellow-fluorescent cells per microinjection. Each column with a bar represents the mean ± SE of nine microinjections. *Significant difference from the vehicle (Control) at $P<0.05$. (data adopted after modification from ref. 1)

cyclase inhibitor SQ22536 [24]. Thus, these results suggest that irsogladine up-regulates GJIC *via* regulation of the PKA pathway.

Recently, several studies have suggested that Cxs can induce and maintain tight junctions in both GJIC-dependent and -independent manners in epithelial cells [25-29]. The mechanism of action of Cxs is unclear, but the facilitation of GJIC is known to promote the translation of a protein that is a component of tight junctions [23]. Tight junctions are intercellular junctions adjacent to the apical end of the lateral membrane surface. They have two functions as a barrier (or gate) and fence. The barrier function is relevant to edema [25], jaundice [26], diarrhea [27], and blood-borne metastasis [28]. Morita *et al.* [29] reported that the facilitation by irsogladine of GJIC suppressed the increase in permeability through the up-regulation of caludin-4, the component protein of tight junctions. It is still unclear what types of molecules in GJIC are responsible for tight junctions. Further investigation is certainly needed to clarify the molecular mechanism for the regulation of tight junctions.

Inhibition of Superoxide Generation

Superoxide production by neutrophils plays a role in the pathogenesis of gastric mucosal lesions induced by various ulcerogenic agents and *H. pylori* infection [30, 31]. Kyoi *et al.* [32] reported that irsogladine suppressed the production of superoxide *via* cAMP formation in the isolated human neutrophils. The levels of cAMP in human neutrophils were elevated by rolipram, a selective inhibitor of PDE4, but not by inhibitors of PDE1, PDE2 and PDE3. Irsogladine also increased cAMP formation in neutrophils in a concentration- dependent manner. A non-selective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX), alone significantly increased cAMP content, but irsogladine was unable to further increase cAMP content in the presence of IBMX. Irsogladine concentration-depen-

dently inhibited the production of superoxide by various stimuli, including formyl-methionyl-leucyl- phenylalanine, opsonized zymosan, guanosine 5'-[gamma-thio] triphosphate, A23187 and phorbol 12-myristate 13-acetate [32]. These effects of irsogladine were mimicked by rolipram and IBMX as well as dibutyryl cAMP. The inhibitory effects of irsogladine and rolipram on superoxide production were reversed by a protein kinase A inhibitor H-89 [32]. These results indicate that irsogladine inhibits superoxide production in human neutrophils by the increase of cAMP content through the inhibition of PDE4, contributing to the antiulcer effects of irsogladine against gastric mucosal lesions in response to oxidative stress.

Effect on Gastric Mucosal Blood Flow

Sato *et al*. reported that irsogladine prevented the reduction of gastric mucosal blood flow induced by diclofenac, a NSAID, in dogs [33]. Kyoi *et al*. [34] showed that irsogladine ameliorated monochloramine (NH_2Cl)-induced decrease in gastric mucosal blood flow, which was reversed by pretreatment with N^G-nitro-L-arginine methylester (L-NAME), an inhibitor of NO synthase. Furthermore, irsogladine restored the NH_2Cl-induced decrease in cGMP production in the stomach, and this effect was also attenuated by pretreatment with L-NAME, suggesting the involvement of NO in the recovery of cGMP by irsogladine. It is assumed that irsogladine ameliorates abnormality in gastric mucosal blood flow mediated by endogenous NO and cGMP production. Indeed, Yamamoto *et al*. [20] reported that irsogladine dose-dependently prevented the formation of gastric mucosal lesions induced by NH_2Cl. This effect was significantly mitigated by L-NAME but not by aminoguanidine, a relatively selective inhibitor of the inducible NO synthase, indomethacin or chemical ablation of capsaicin-sensitive afferent neurons. These results suggest that NO derived from constitutive NO synthase, but not prostaglandin (PG) or sensory neurons, is involved in the cytoprotective action of irsogladine, including the beneficial influence on gastric mucosal blood flow.

MUCOSAL PROTECTIVE ACTION OF IRSOGLADINE THROUGHOUT GASTROINTESTINAL TRACT

Aaphthous Stomatitis

Aphthous stomatitis is the most common oral mucosal lesion in patients. It is often a recurrent and periodic lesion that causes clinically significant morbidity. Although several factors have been suggested, the etiology of recurrent aphthous stomatitis is unknown. Various treatment modalities are used, but no therapy is definitive. Hara *et al*. reported that the administration of irsogladine heals oral aphtha more rapidly in patients with relapsing aphthous stomatitis than spontaneous healing [4]. In addition, Yoshida *et al*. [5] also showed that irsogladine effectively prevented the development of methotrexate-induced aphthous stomatitis in patients with rheumatoid arthritis. Nanke *et al*. [6] demonstrated that irsogladine reduced aphthous stomatitis/oral ulcers in patients with Behcet disease (BD) Fig. (**3**).

Hara *et al*. [4] reported that Cx26 and Cx31 exist in the oral mucosa; however, in their studies no difference was found between patients with and without aphthous stomatitis. Likewise, no significant difference in Cx expression in the oral mucosa was found between patients and the control, yet evidence showing the existence of gap junctions in the oral mucosa of patients led us to expect the therapeutic effectiveness of drugs which modify the function of gap junctions in patients with aphthous stomatitis.

2. Gastric ulcer in clinical trials

Higuchi *et al*. [35] reported that to cure gastric ulcers, the therapeutic effect of *H. pylori* eradication alone was insufficient and further treatment following eradication therapy is necessary in Japanese patients, unlike Caucasian patients. Moreover, since the incidence of eradication therapy has recently decreased due to the development of resistance to clarithromycin, another drug should be taken after the eradication regimen until eradication is confirmed. Hiraishi *et al*. [7] reported that treatment with irsogladine following *H. pylori* eradication therapy significantly accelerated gastric ulcer healing compared to placebo Fig. (**4**). Moreover, Murakami *et al*. [8] also clearly showed that after eradication therapy, the healing rate of irsogladine and famotidine was similar and that irsogladine was more effective than famotidine in patients with drinking habits Fig. (**5A**) or smoking habits Fig. (**5B**). Notably, ethanol is known to increase intracellular Ca^{2+} concentration and reduce intracellular communication [36, 37]. A previous study showed that irsogladine inhibits an increase in intracellular Ca^{2+} concentration as well as a decrease in the activation of intracellular communication, and these effects may not be affected by alcohol consumption [2]. It is also reported that one of the pathogenic mechanisms of ethanol-induced

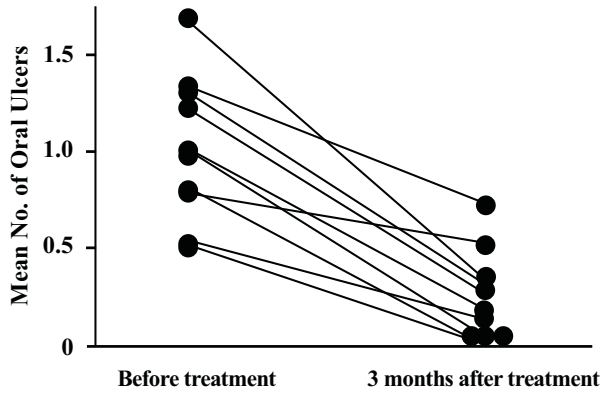

Fig. (3). Effect of irsogladine on the mean number of oral cavity ulcers (aphthous stomatitis lesions) in patients with Behcet's disease. Irsogladine (2-4 mg/day) was administered p.o. to 10 Behcet's disease patients. Efficacy was evaluated on the basis of the macroscopic findings of aphthous lesions. The number of aphthous lesions was counted 3 times before and after the administration of irsogladine. Note that significant difference was found in the mean number between "Before treatment" and "3 months after treatment" at P<0.0003. (data adopted after modification from ref. 6)

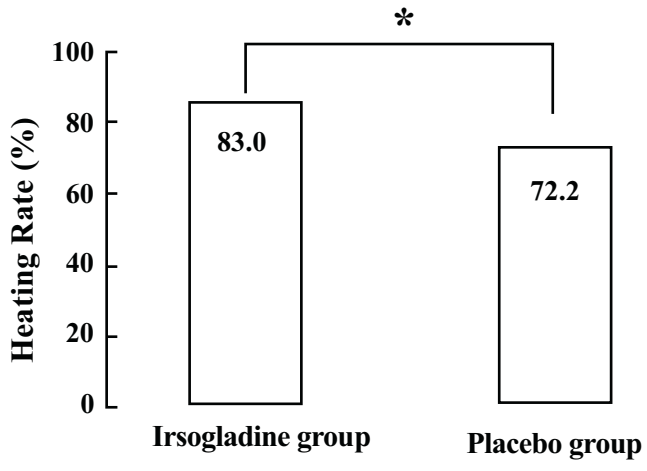

Fig. (4). Effect of irsogladine on gastric ulcer healing in patients. Three hundred and twenty-two patients with a single *H. pylori*-positive gastric ulcer were subjected to eradication treatment, then assigned randomly to a treatment group (irsogladine: 4 mg/day; N = 150) or a control group (placebo; N = 161). The rates of gastric ulcer healing were compared after 7 weeks of treatment (the final number of patients was 141 for the "Irsogladine group" and 151 for the "placebo group"). *Significant difference from "Placebo group" at $P = 0.0276$. (data adopted after modification from ref. 7)

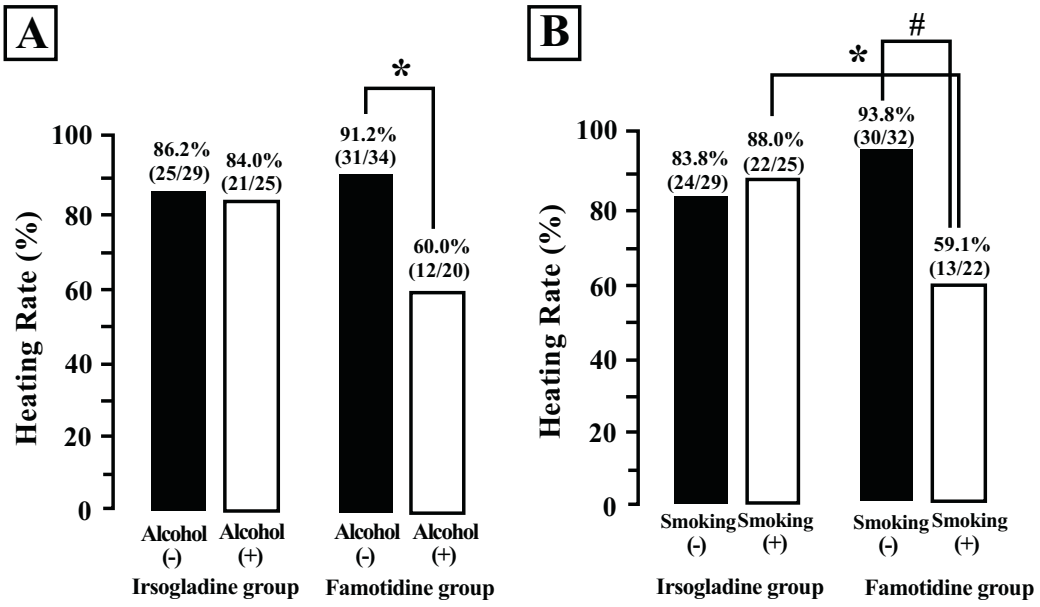

Fig. (5). Effect of irsogladine on gastric ulcer healing in patients with *H. pylori* infection, with or without alcohol drinking or smoking. One hundred nineteen gastric ulcer patients with *H. pylori* infection were randomized to treatment with irsogladine (4 mg/day; N = 60) or famotidine (40 mg/day; N = 59) following 1-week *H. pylori* eradication therapy. After treatment for 4 weeks, gastric ulcer healing was assessed. **A:** Gastric ulcer healing in patients with or without alcohol drinking. Irsogladine group/Alcohol (-) (N = 29), Irsogladine group/ Alcohol (+) (N = 25); Famotidine group/Alcohol (-) (N = 34), Famotidine group/Alcohol (+) (N = 20). *Significant difference at $P = 0.0119$; Famotidine group/Alcohol (-) v.s. Famotidine group/Alcohol (+). **B:** Gastric ulcer healing in patients with or without smoking. Irsogladine group/Smoking (-) (N = 29), Irsogladine group/Smoking (+) (N = 25); Famotidine group/ Smoking (-) (N = 32), Famotidine group/Smoking (+) (N = 22). *Significant difference between Irsogladine group/Smoking (+) and Famotidine group/Smoking (+) at P=0.0023; #Significant difference between Famotidine group/Smoking (+) and Famotidine group/Smoking (-) at P=0.0041. (data adopted after modification from ref. 8)

gastric injury is a decrease in the gastric mucosal contact angle with ethanol [38]. Irsogladine inhibited the decrease in the mucosal contact angle, resulting in the prevention of mucosal injury [38]. Many studies have suggested that a decrease in gastric mucosal blood flow is an important element in the pathogenesis of gastric ulcers, particularly decreased the mucosal blood flow in association with smoking [39]. Since irsogladine improves gastric mucosal blood flow, most likely *via* the enhancement of cAMP and/or NO production, it is assumed that the decreased blood flow in smokers may be ameliorated by this drug.

3. Gastrointestinal Lesions Caused by Various Drugs

a) NSAIDs: NSAIDs are the most commonly prescribed drugs for the treatment of arthritis and inflammation; however, they frequently cause gastrointestinal complications such as erosions and ulcers. The pathophysiology of these complications is largely accounted for by the suppression of PG production due to inhibition of cyclooxygenase (COX) activity. Capsule endoscopy and double balloon endoscopy, advanced modalities that now allow for full investigation of the entire small intestine, have revealed that NSAIDs can cause a variety of abnormalities in the small intestine. Conventional NSAIDs induce small intestinal injuries in over 50% of patients [40, 41]. Proton pump inhibitor (PPI) is the standard treatment for the prevention of NSAID-induced upper gastrointestinal mucosal injuries; however, several studies showed that PPI is ineffective in preventing NSAID-induced enteropathy in experimental animals and humans [41, 42]. Recently, Wallace *et al.* [43] reported that PPIs exacerbate NSAID-induced intestinal damage at least in part because of significant shifts in enteric microbial populations. They showed that omeprazole treatment did not result in mucosal injury or inflammation induced by naproxen or celecoxib; however, there were marked shifts in the numbers and types of enteric bacteria, including a significant reduction of jejunal *Actinobacteria* and *Bifidobacteria* spp. It has also been suggested that PPI can affect infection, fracture and trace element absorption [44]. Accordingly, there seem to be great benefits from a health-economic point of view, if a drug exerts protective effects by actions other than acid inhibition for the treatment of NSAID-induced enteropathy.

Interestingly, irsogladine exhibited a potent protection against indomethacin-induced small intestinal lesions, together with the suppression of various inflammatory responses [9] Fig. (**6**). Functional mechanism for NSAID-induced intestinal lesions has been studied extensively, and up to date, the following functional changes are known to be involved in the pathogenic mechanism of these lesions, including an intestinal hypermotility, a decrease of mucus secretion, an increase of iNOS, and mucosal invasion of enterobacteria [45]. Irsogladine prevented enterobacterial invasion as well as the expression of iNOS mRNA in the mucosa following indomethacin treatment, the major pathogenic event in the ulcerogenic response [9]. The mechanism how bacteria invades in the mucosa remains unknown, yet previous studies suggest that the decrease of mucus secretion may contribute to this process after indomethacin treatment [46-48]. Since irsogladine increases PAS-positive materials in the intestine, probably by stimulating mucus secretion [9], it is assumed that this drug protects the small intestine against NSAID-induced injury, at least partly, mediated by stimulation of mucus secretion.

Kuramoto *et al.* [11] reported that irsogladine suppressed small intestinal lesions in response to diclofenac for 2 weeks in healthy volunteers compared with omeprazole. This study also demonstrated that the efficacy of irsogladine against NSAID-induced lesions from the esophagus to the duodenum was similar to that of omeprazole. In general, the pathogenesis of esophageal lesions is closely associated with gastric acid secretion, inasmuch as antisecretary drugs are very effective against this disease. In contrast, it is reported that gastric acid destroys tight junctions in the esophageal epithelium and such destructions may cause esophageal lesions

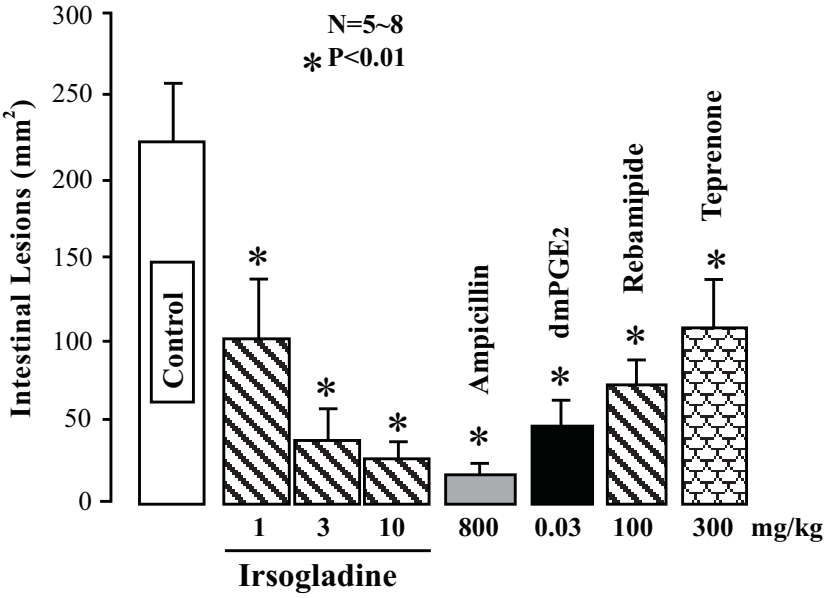

Fig. (6). Effects of irsogladine, rebamipide, teprenone, $dmPGE_2$ and ampicillin on indomethacin-induced small intestinal lesions in rats. The animals were given indomethacin (10 mg/kg, s.c.) and killed 24 h later. Irsogladine (1-10 mg/kg), rebamipide (100 mg/kg), teprenone (300 mg/kg), or $dmPGE_2$ (0.03 mg/kg) was given p.o. twice, 30 min before and 6 h after the administration of indomethacin, while ampicillin (800 mg/kg) was given s.c. twice, 18 and 0.5 h before indomethacin. Data are presented as the mean ± SE of 5-8 rats. *Significant difference from control at $P < 0.01$. (data adopted after modification from ref. 9)

[49, 50]. Thus, it might be useful in clinical conditions if irsogladine ameriolates the deterioration of barrier functions induced by noxious stimuli through the regulation of tight junctions. In this trial, the efficacy of irsogladine in the stomach and duodenum was not different from that of omeprazole. Sato *et al*. [33] reported that irsogladine alleviates the reduction of gastric mucosal blood flow induced by diclofenac, most likely *via* the enhancement of cAMP and/or NO production [19-21]. Hence, these functions may contribute to the preventive effect of irsogladine against NSAID-induced gastrointestinal ulcers.

b) Antithrombotic drug: Low-dose aspirin (LDA) is frequently prescribed in Japan, mostly because of the increasing elderly population. Recent studies showed that mucosal breaks caused by taking LDA occurred not only in the upper gastrointestinal tract [51] but also in the lower GI tract [52]; however, preventive therapy for aspirin-induced intestinal lesions has not been fully investigated. We devised a new method to induce mucosal lesions in the rat small intestine by intraduodenal administration of LDA [53]. It was found that irsogladine dose-dependently and significantly prevented small intestinal lesions in response to LDA, although the mechanism of this action remains unknown [54].

On the other hand, the risk of upper gastrointestinal bleeding is known to increase by the concomitant use of antiplatelet drugs clopidogrel and LDA [55, 56]. Many studies have reported the effect of antisecretory drugs on bleeding in the upper GI tract associated with low-dose ASA and clopidogrel [57, 58]. We examined the effect of clopydgrel, a P_2Y_{12} receptor antagonist, on gastric bleeding induced by luminal perfusion with LDA in rats, with co-perfusion of exogenous HCl or under stimulation of endogenous acid secretion, and investigated the prophylactic effect of irsogladine on gastric bleeding under such conditions. It was found that the ulcerogenic and bleeding responses to acidified LDA were aggravated by pretreatment with clopidogrel in both cases, and irsogladine dose-dependently reduced the severity of gastric damage and bleeding caused by such dual antiplatelet therapy when exogenous HCl was co-perfused or when acid secretion was stimulated by histamine [16, 17] Fig. (**7**). Although PPIs are frequently used to prevent gastrointestinal bleeding [59], some studies recommend not adding a PPI to dual therapy without formal indications [60, 61]. Clopidogrel is a prodrug and requires several biotransformational steps, mediated mainly by cytochrome P-450 isoenzymes, to generate an active metabolite. The isoenzyme CYP2C19 seems to be one of the determinants of the pharmacodynamic response to clopidogrel and is also involved in the metabolism of PPIs, such as omeprazole [60, 61]. It is assumed that PPIs reduce the biological action of clopidogrel, probably through competitive metabolic effects on CYP2C19. Irsogladine, which is not likely to interfere with the biotransformation of clopidogrel, can be used as a prophylactic for preventing gastric bleeding during LDA- clopidogrel therapy.

c) SSRI: Recent studies suggested a risk of adverse gastric reaction in patients from concomitant use of SSRIs with NSAIDs [62, 63]. We confirmed in rats that paroxetine, a SSRI, markedly aggravated indomethacin-induced antral damage in refed rats, mostly changing superficial/non-hemorrhagic lesions to deep/ hemorrhagic lesions [15] Fig. (**8**). This effect of paroxetine was reproduced by exogenous 5-HT and abrogated by ondansetrone, a $5HT_3$ antagonist, suggesting the involvement of endogenous 5-HT/5-HT_3 receptors in the pathogenic mechanism. Furthermore, we found that these antral lesions were suppressed by antisecretory and mucosal protective drugs as well as anti-oxidative agents [15]. It is assumed that SSRIs aggravate NSAID-induced antral damage, probably *via* the activation of 5-HT_3 receptors, and the mechanism of aggravation may involve the corrosive action of acid/pepsin as well as a weakening of the antioxidative system. Certainly, irsogladine significantly prevented the aggravation by paroxetine of indomethacin-induced antral lesions. The combined administration of indomethacin and paroxetine had no effect on gastric secretion but significantly decreased mucosal superoxide dismutase (SOD) activity as well as GSH content, confirming the involvement of oxidative stress in the pathogenesis. It is assumed that the protective effect of irsogradine against the gastric adverse reaction of SSRI may be associated, at least partly, with enhancement of the anti-oxidative system, such as the inhibition of superoxide production.

d) Bisphosphonate: Recent studies showed that alendronate, a nitrogen-containing bisphosphonate, produced gastric lesions and worsened gastric lesions in response to NSAIDs [64, 65]. Alendro-

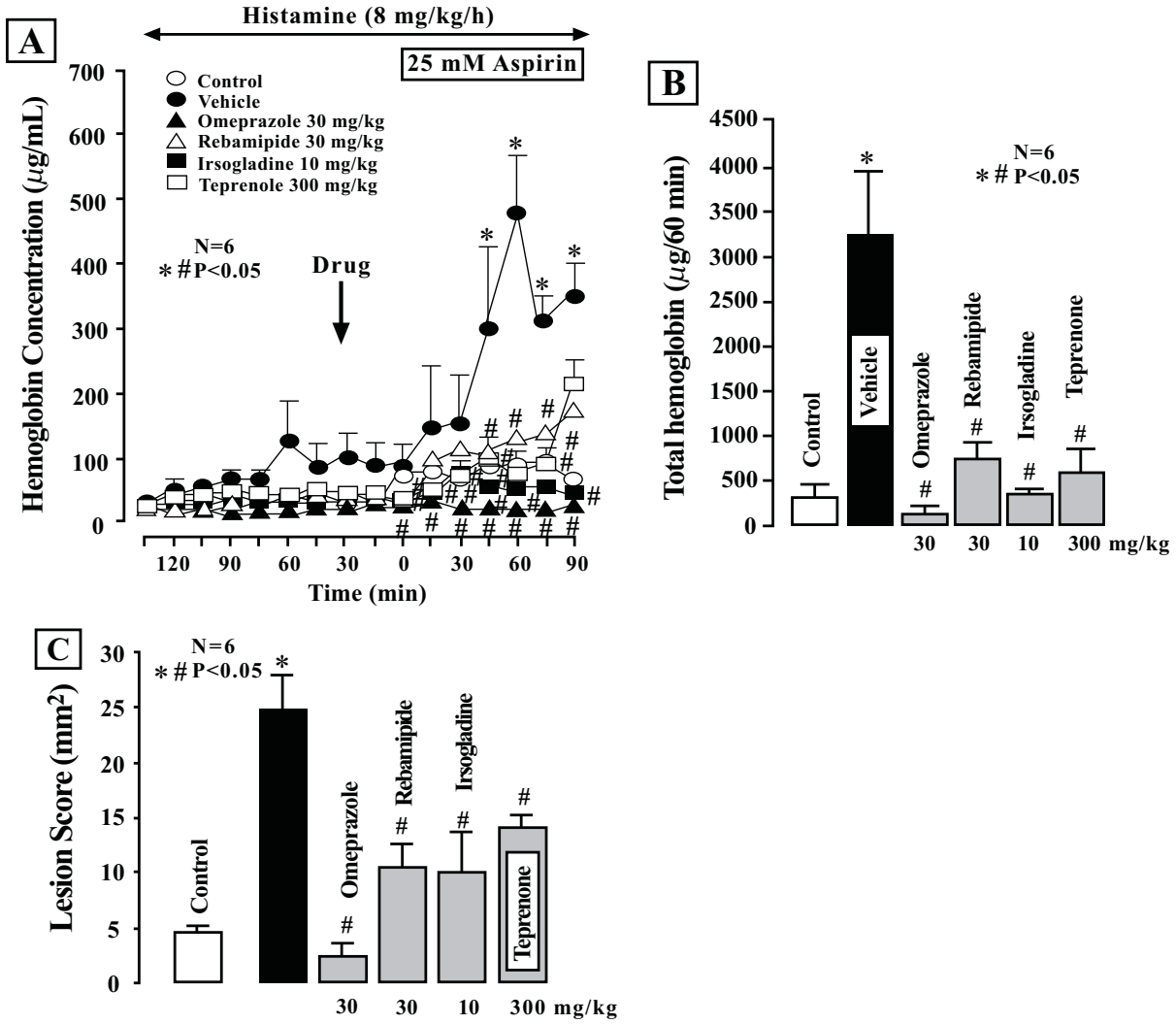

Fig. (7). Effect of various anti-ulcer drugs on gastric bleeding and hemorrhagic lesions induced by luminal perfusion of LDA in the presence of clopidogrel under histamine-stimulated acid secretion in urethane-anesthetized rats. Acid secretion was stimulated by the i.v. infusion of histamine (8 mg/kg/h) for 210 min. LDA (25 mM) was perfused in the stomach for the last 90 min of the experimental period. Clopidogrel (30 mg/kg) was given p.o. 24 h before LDA perfusion. Irsogladine (10 mg/kg), rebamipide (30 mg/kg), teprenone (300 mg/kg) or omeprazole (30 mg/kg) was given i.d. 30 min before LDA. **Fig. A** shows the time-course of change in the hemoglobin concentration in the luminal perfusate, and the datas are presented as the mean ± SE of values determined every 15 min from 6 rats. **Fig. B** shows total hemoglobin output in the perfusate for the last 90 min, while **Fig. C** shows hemorrhagic lesions, and these data are presented as the mean ± SE of 6 rats. Significant difference at $P < 0.05$; * from control (without clopidogrel); # from vehicle. (data adopted after modification from ref. 17)

nate did not acutely produce gross damage in the stomach of fasted rats, yet caused ulcers in the antrum with severe edema and inflammation after refeeding for 3 days; the damage was covered with a white cap, mainly composed of inflammatory cells and fibrin-like substances [14]. In addition, alendronate increased microvascular permeability in the antral mucosa, and the amount of dye extravasated in the mucosa was correlated with the severity of damage. Irsogladine was effective against alendronate-induced antral ulcers and inhibited both the development of ulceration and the increase of vascular permeability in the antrum [66] "Fig. (**9**)". The impairment of the anti-oxidative system may account partly for the pathogenic mechanism of these lesions, although neither acid secretion nor endogenous PGs contributed to the development of these lesions. Concurrent use of PPI was associated with a dose-dependent loss of protection against hip fracture with alendronate in elderly patients [67]. Higher calcium intake has been associated with decreased bone loss, while lower calcium absorption has been associated with increased fracture risk [68, 69]. Accordingly, it would be great benefits if a drug, like irsogladine, exerted a protective effect against bisphosphonate-induced gastric lesions by actions other than acid inhibition.

Inflammatory Bowel Disease

Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, has become an important health problem. Nakagawa *et al*. [12] found that irsogladine potently suppressed inflammatory bowel disease in IL-10-deficient mice through the inhibition of certain types of cytokines. Suzuki *et al.* [13] reported that intrarectal administration of irsogladine inhibited fibrosis in dextran sulfate sodium-induced colitis by a direct or indirect effect on profibrogenic factors or fibroblasts. The effect of irsogladine on intestinal fibrosis should be further investigated.

SUMMARY AND FUTURE PROSPECTS

Irsogladine was developed in Japan as a mucosal protective drug and used for the treatment of peptic ulcer disease and acute

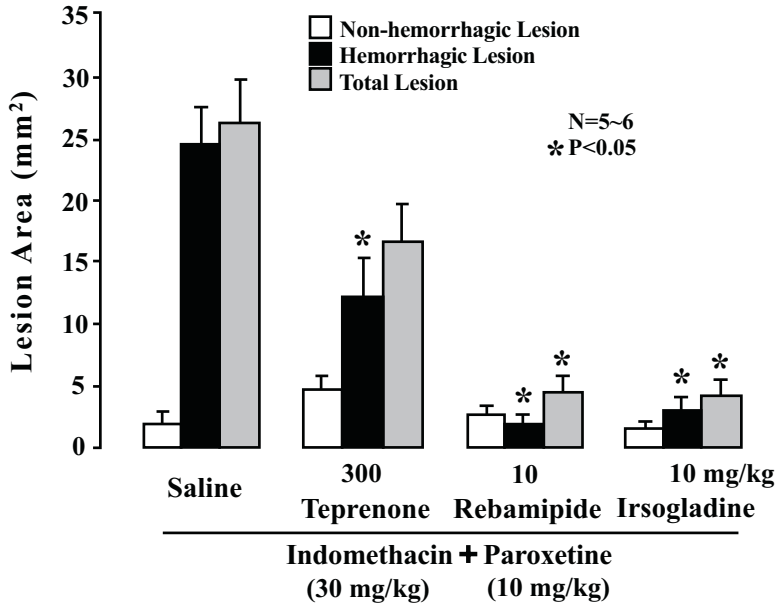

Fig. (8). Effects of irsogladine, rebamipide, and teprenone on the development of antral lesions induced in rats by the combined administration of indomethacin and paroxetine. Animals fasted for 24 h were refed for 1 h, then administered indomethacin (30 mg/kg, s.c.). Paroxetine (10 mg/kg) was given p.o. 30 min before the administration of indomethacin. Irsogladine (10 mg/kg), rebamipide (10 mg/kg) or teprenone (300 mg/kg) was administered p.o. 1 h before indomethacin. Data are presented as the mean ± S.E. of 5-6 rats. *Significant difference from saline at $P < 0.05$. (data adopted after modification from ref. 15)

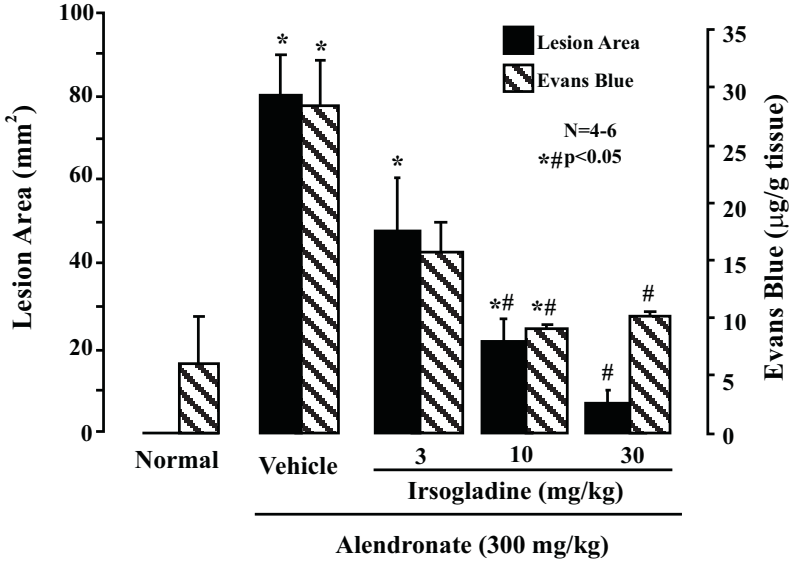

Fig. (9). The effect of irsogladine on antral lesion induced by alendronate in the rat stomach. Animals fasted for 24 hr were given alendronate (300 mg/kg, p.o.), followed by refeeding, and killed 3 days later. To determine microvascular permeability, 1 ml of 1% Evans blue was injected intravenously 30 min before killing. Irsogladine (3-30 mg/kg) was given p.o. 30 min before and 10 hr after alendronate on the first day and twice daily for 2 days thereafter. Figures show the lesion scores and the extravasated amount of Evans blue, and the data are presented as the means ± SE of 4-6 rats. Significant difference at $P < 0.05$, *from normal, #from vehicle. Data are presented as the mean ± SE of 4-6 rats. Significant difference at $P < 0.05$, *from normal, #from vehicle. (data adopted after modification from ref. 66)

gastritis. Up to date, several studies showed the positive effect of irsogladine in patients with aphthous stomatitis, gastric ulcers, intestinal mucosal injuries and inflammatory bowel disease [4-13]. Although the detailed mechanisms of the irsogladine's actions remain unknown, they are different from those of antisecretory drugs and are thought to involve the facilitation of GJIC, inhibition of decreased gastric mucosal blood flow, suppression of reactive oxygen generation and so on. Recently, this drug has been demonstrated to be effective against the gastric adverse reactions induced in rats by NSAIDs, bisphosphonates, SSRIs and anti-thrombotic drugs. Irsogladine is a promising drug that can be used as a prophylactic against the adverse effects of various drugs in the gastrointestinal tract [9, 10, 14-17]. Further studies are required to understand the detailed mechanisms of irsogladine's actions and efficacy against various diseases in the gastrointestinal tract.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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