

# TOP Journal Club

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*Dig Dis Sci. 2005 Oct;50 Suppl 1:S97-S103.*  
**Prevention by rebamipide of acute reflux esophagitis in rats.**

Proinflammatory factors, including neutrophil-derived oxygen free radicals and inflammatory cytokines, have recently been implicated in the pathogenesis of reflux esophagitis. Rebamipide has been widely used as an anti-ulcer agent. The aim of the present study was to assess the protective effect of rebamipide against acute reflux esophagitis in rats. Esophagitis was induced in rats by ligation at the limiting ridge and the lower portion of the duodenum. Vehicle or rebamipide were given as a single dose intraduodenally. Lesion index (LI), thiobarbituric acid-reactive substances (TBA-RS), myeloperoxidase (MPO) activity, mRNA and protein of tumor necrosis factor (TNF)-alpha and cytokine-induced neutrophil chemoattractant (CINC)-1 in the esophageal mucosa were markedly increased; pretreatment with rebamipide, however, significantly reduced both macroscopic and microscopic injuries and increases in inflammatory mediators. The results of this study indicate that rebamipide protects against the occurrence of esophagitis and has highly promising potential as a new therapeutic agent for reflux esophagitis.

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**Rebamipide reduces recurrence of experimental gastric ulcers: role of free radicals and neutrophils.**

Mucosal inflammation is a crucial factor for the recurrence of peptic ulcer. In this study, we examined the effect of rebamipide on neutrophils infiltration, lipid peroxidation, and antioxidative enzyme activities in the recurrence of experimental gastric ulcer. Ulcer recurrence was examined at 60, 100, and 140 days after production of acetic acid-induced gastric ulcers in rats. Gastric neutrophil infiltration, lipid peroxidation, and antioxidative enzyme activities were determined by analyses of myeloperoxidase (MPO) activity, thiobarbituric acid reactive substance (TBARS) levels, and glutathione peroxidase (GSHpx) and superoxide dismutase (SOD) activities in the ulcer region, respectively. The effect of rebamipide, an antigastric-ulcer agent, on ulcer recurrence was

assessed following oral administration at 60 mg/kg/day from day 20. In the control and rebamipide groups, gastric ulcer indices were reduced on day 100 compared with day 60; however, increases were observed on day 140, indicating ulcer recurrence. In the rebamipide group, the ulcer index was smaller than in the control group at each time point and the effect was significant on day 140. Although marked elevation of MPO activities was observed in the control group during the experiment, no significant elevations were seen in the rebamipide group on days 100 and 140. TBARS levels were significantly elevated in the control group on day 140, but not in the rebamipide group. Rebamipide suppressed the decrease in GSHpx activity on day 60. These results suggest that lipid peroxidation of gastric tissue mediated by free radicals from neutrophils is responsible for the recurrence of acetic acid-induced gastric ulcers in rats, and that the elimination of free radicals by rebamipide may contribute to the reduction of severity in ulcer recurrence.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S84-9.*  
**Rebamipide reduces indomethacin-induced gastric injury in mice via down-regulation of ICAM-1 expression.**

Non-steroidal anti-inflammatory drugs (NSAIDs) induced gastric mucosal injury occurs through subsequent events following free radical production derived from activated neutrophils. In this study, we hypothesized that rebamipide, a novel anti-ulcer agent, exerts a protective effect on NSAID-induced gastric injury through its antioxidant properties. The protective effect of rebamipide in a mouse model of indomethacin-induced gastric injury and mechanisms for this effect were investigated. Pre-treatment with rebamipide significantly inhibited indomethacin-induced gastric mucosal injury in mice. Gastric thiobarbituric acid reactive substances (TBARS) levels and myeloperoxidase (MPO) activity substantially increased 3 hr after indomethacin administration. These increases were significantly inhibited by pre-treatment with rebamipide. Furthermore, rebamipide pre-treatment notably decreased intercellular adhesion molecule-1 (ICAM-1) expression that was up-regulated in gastric tissue treated with indomethacin. Therefore, rebamipide may reduce indomethacin-induced gastric mucosal injuries through its antioxidant effect, which inhibits the neutrophil activation step following up-regulation of ICAM-1 expression on endothelial cells.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S76-83.*

**Rebamipide significantly inhibits indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells.**

Nonsteroidal antiinflammatory drugs (NSAIDs) cause complications such as gastrointestinal injury. NSAIDs were recently reported to cause mitochondrial injury: to dissipate the mitochondrial transmembrane potential (MTP), and to induce mitochondrial permeability transition pore (PTP), which liberates cytochrome c. This enzyme generates reactive oxygen species (ROS) thereby triggers caspase cascade and cellular lipid peroxidation, resulting in cellular apoptosis. However, the mechanism of this NSAID-induced MTP's role in cellular apoptosis remains unknown. Rebamipide, an antiulcer drug, is reported to scavenge ROS and to show the protective effects on indomethacin-induced tissue peroxidations. Since cytochrome c and its generation of ROS are involved in indomethacin-induced cellular apoptosis, rebamipide may attenuate mitochondrial damage. The aim of this study was to elucidate whether indomethacin induces both the MTP decrease and cellular apoptosis, and the effect of rebamipide on these phenomena. We examined the effect of rebamipide on 1) MTP change, 2) lipid peroxidation, 3) apoptosis, and 4) caspase activation using gastric mucosal epithelial cell-line treated with indomethacin. With a specially designed fluorescence analyzing microscope system, MTP change, cellular lipid peroxidation, and cellular apoptosis were investigated with the small star, filled following fluorescent dyes, MitoRed, DPPP, and Hoechst 33,258, respectively. Indomethacin treatment decreased MTP but increased both cellular lipid peroxidation and cellular apoptosis via caspase 3 and 9 activation. Rebamipide clearly inhibited these phenomena {in vitro}. We demonstrated that fluorescent dyes such as MitoRed, DPPP, and Hoechst 33,258 are useful indicators for detecting oxidative cellular injuries in living cells. Rebamipide exerts a protective effect on mitochondrial membrane stability in gastric epithelial cells.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S70-5.*

**Gastro-protective agent rebamipide induces cyclooxygenase-2 (COX-2) in gastric epithelial cells.**

Cyclooxygenase-2 (COX-2) is a key enzyme in prostaglandin (PG) synthesis, and COX-2 induction plays an important role in the healing of gastric ulceration. Rebamipide is a gastro-protective agent and

attenuates the activity of neutrophils. A number of reports have shown that rebamipide treatment increases PG production in the gastric mucosa {in vivo}. Although its clinical significance in ulcer healing has been demonstrated, {in vitro} evidence remains to be accumulated. Non-transformed rat gastric mucosal cells (RGM1 cells) were stimulated with rebamipide. RT-PCR and Western blot analysis revealed time and dose-dependent transcriptional and translational stimulation of COX-2. PGE(2) was also produced dose-dependently. However, marked COX-2 induction by rebamipide was transient and lasted less than 24 hr. COX-1 expression was unaltered by rebamipide. Reporter assay results confirmed the stimulation of Cox-2 promoter activity by rebamipide. In conclusion, this study provides {in vitro} evidence that rebamipide transcriptionally induces COX-2 and supports the rationale for its clinical use.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S63-9.*

**Rebamipide reduces delay in gastric ulcer healing in cyclooxygenase-2-deficient mice.**

Rebamipide is an antiulcer drug capable of various actions including the induction of cyclooxygenase-2 (COX-2). In this study, we investigated the effect of rebamipide on gastric ulcer healing in COX-2-deficient mice. Wild-type (N=34) and COX-2-deficient mice (N=28) with gastric ulcers were administered 30 mg/kg of rebamipide or the vehicle. Ulcerous tissues were subjected to measurements of ulcer size, immunohistochemical staining of CD31 (an endothelial cell marker), and mRNA levels. COX-2 deficiency delayed ulcer healing and inhibited angiogenesis and bFGF mRNA expression in the granulation tissue. In wild-type mice, rebamipide accelerated ulcer healing and increased COX-2 mRNA expression. In COX-2-deficient mice, rebamipide prevented delayed ulcer healing and reversed the inhibition in angiogenesis and bFGF mRNA expression. The effect of rebamipide on the enhancement of ulcer healing, angiogenesis, and induction of bFGF expression was more prominent in wild-type mice than in COX-2-deficient mice. In conclusion, rebamipide may accelerate gastric ulcer healing through both COX-2-dependent and COX-2-independent mechanisms.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S56-62.*

**Rebamipide decreases the susceptibility of gastric mucosa to acid-induced injury in rats by inhibiting neutrophil activation.**

We previously demonstrated that activated neutrophils increased the susceptibility of gastric mucosa to acid-induced injury in rats. As rebamipide, an anti-ulcer agent, inhibits neutrophil activation, we examined whether the rebamipide reduces stress-induced gastric mucosal injury by decreasing susceptibility to acid-induced gastric mucosal injury in rats. Increase in both gastric mucosal permeability and gastric microvascular permeability evaluated by (51)Cr-EDTA clearance and Evans blue leakage, respectively, at 6 hr after Water-Immersion Restraint Stress (WIR) were significantly lower in animals with leukocytopenia than those in controls. Pretreatment with neutrophil elastase (NE) inhibitors, an anti-P-selectin monoclonal antibody (MAb), and rebamipide significantly inhibited these increases at 6 hr after WIR. These treatments also inhibited decrease in gastric mucosal blood flow observed at 6 hr after WIR. Acid-induced exacerbation of gastric mucosal injury in rats at 6 hr after WIR was inhibited by NE inhibitors, anti-P-selectin MAb, and rebamipide. Rebamipide significantly inhibited WIR-induced increase in gastric MPO activity at 8 hr after WIR. Observations in the present study raised a possibility that rebamipide decreases the susceptibility of gastric mucosa to acid-induced injury by inhibiting neutrophil activation.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S3-S11.*

**15th anniversary of rebamipide: looking ahead to the new mechanisms and new applications.**

Rebamipide, a gastro-protective drug, was developed in Japan for the treatment of peptic ulcer disease. It was proven superior to the former same category drug cetraxate in a randomized, controlled, double-blind, comparative clinical study in 1989. Rebamipide's mechanisms of actions are different from anti-secretory drugs; it accelerates and improves the quality of ulcer healing and reduces ulcer recurrence rate. Numerous studies have been conducted to explain the mechanisms responsible for these actions, 37 papers were published by 1998. Major properties of rebamipide include: stimulation of prostaglandin and mucus glycoprotein synthesis, inhibition of reactive oxygen species, inflammatory cytokines and chemokines, and inhibition of neutrophils activation. Since 1998,

107 papers were published, clarifying further effects of rebamipide on cyclooxygenase-2, prostaglandin E receptors, growth factors (i.e., HGF, EGF, and VEGF), heat-shock proteins, nitric oxide, adhesion molecules, neutrophils, and Helicobacter pylori- and NSAID-related pathology. Moreover, inhibitory action of rebamipide on gastric cancer growth has also been shown. In this issue we reviewed recent advances in understanding of rebamipide's mechanism of action and its newest clinical applications.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S124-31.*

**Rebamipide enema is effective for treatment of experimental dextran sulfate sodium induced colitis in rats.**

We investigated therapeutic efficacy of rebamipide using dextran sulfate sodium (DSS) induced colitis model in rats. Three percent DSS solution was given to rats for 9 days. After that, we evaluated the drug efficacy on colitis sustained with continuous drinking of 1% DSS. Twice-daily treatment with 0.3% or 1% rebamipide for 14 days significantly ameliorated the stool abnormality in the colitis model, preferentially suppressed hematochezia. The colonic mucosal lesion, determined by Alcian blue staining on day 24, was significantly reduced by rebamipide enema in a dose-dependent manner. Either rebamipide or 5-aminosalicylic acid (5-ASA) enema treated once daily significantly ameliorated colitis. The minimum effective dose of rebamipide was 0.3% in once-daily treatment, and that of 5-ASA was 10%. In a mechanistic study, the epithelial cell sheet formation of the T84 colon cancer cell was measured as an increase in generation of trans-epithelial electrical resistance in vitro. Rebamipide accelerated the increase, while 5-ASA conversely suppressed it. These results suggest that rebamipide enema is effective for treatment of experimental ulcerative colitis (UC).

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S119-23.*

**Rebamipide enemas-new effective treatment for patients with corticosteroid dependent or resistant ulcerative colitis.**

In this study we investigated the effect of rebamipide enema in patients with steroid-resistant and/or dependent ulcerative colitis. Rebamipide enemas were administered twice daily for a 12-week period; this treatment was further continued longer in patients who requested this. Disease activity index as reflecting the clinical condition and endoscopic index with histological grading were determined before and after the treatment

period. Nine of 11 (81.8%) patients on 12-week treatment with rebamipide approved and were classified as colitis in remission. Moreover, seven of 11 patients requested long-term medication, the longest medication term being 80 weeks. These results medicated that rebamipide enemas may be effective in patients with steroid-resistant and/or dependent ulcerative colitis.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S113-8.*  
**Direct autoradiographic evidence that rebamipide interacts with neutrophils in dextran sulfate sodium induced colitis in rats.**

To determine the effects of rebamipide during the early stages of colitis development, colitis was induced in rats by oral administration of dextran sulfate sodium for 3 or 7 days. The target sites of (3)H-rebamipide were examined by intra-aortic infusion of the radiolabeled compound followed by autoradiography. (3)H-rebamipide was localized in goblet cells in the colon of the control rat, whereas it accumulated in the cytoplasm of mesenchymal cells in dextran sulfate sodium treated rats, localized predominantly to polymorphonuclear leucocytes.

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S104-12.*  
**Rebamipide, a gastro-protective drug, inhibits indomethacin-induced apoptosis in cultured rat gastric mucosal cells: association with the inhibition of growth arrest and DNA damage-induced 45 alpha expression.**

Rebamipide, a gastromucosal protective drug, suppresses indomethacin-induced gastropathy in humans and rodents. Effects of rebamipide on gene expression in indomethacin-treated gastric mucosal cells (RGM1) were investigated using high-density oligonucleotide arrays. Indomethacin induced apoptosis in RGM1 cells in a dose-dependent manner. Rebamipide pretreatment significantly reduced indomethacin-induced apoptosis. We used gene expression profiling on high-density oligonucleotide probe arrays to characterize the transcriptional response of RGM1 cells to indomethacin treatment for 6 hr. Of the 8,799 probes examined, 717 (8.1%) were induced (400 probes) or repressed (317 probes) at least 1.5-fold. Among the 158 genes that were induced by indomethacin at least 2.0-fold, four genes that were down-regulated by rebamipide at least 2.0-fold are listed: growth arrest and DNA-damage-inducible 45 alpha (GADD 45 alpha), golgi SNAP receptor complex member 1, iodothyronine deiodinases, and

transcription factor 8. Real time-PCR confirmed GADD 45 alpha expression and its inhibition by rebamipide. Inhibition of apoptosis-related genes is possibly important for the cytoprotective effect of rebamipide against indomethacin-induced gastric mucosal cell injury

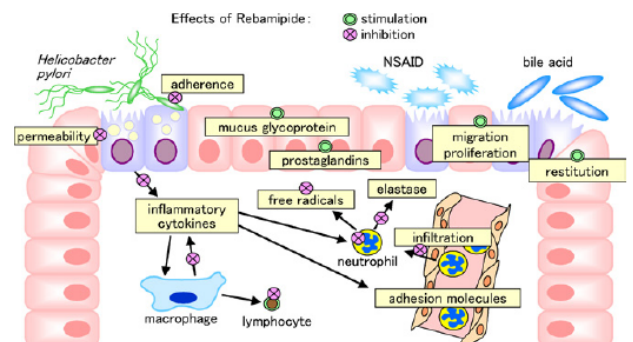


Fig 4. Proposed mechanisms of mucosal protective, ulcer healing, and anti-inflammatory actions of rebamipide. Modified from a previous review article by Arakawa *et al.* in the Dig Dis Sci Supplement in 1998 (Ref 2).

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