

# TOP Journal Club

Vol 9: Number 1 January 2006

## Cilostazol attenuates gray and white matter damage in a rodent model of focal cerebral ischemia.

*Stroke*. 2006 Jan;37(1):223-8

**BACKGROUND AND PURPOSE:** To evaluate whether delayed treatment with the antiplatelet agent cilostazol reduces the volume of infarction in the gray and white matter in a rodent model of permanent focal cerebral ischemia and to explore the mechanism of the neuroprotective effect in vivo. **METHODS:** Cilostazol (30 or 50 mg/kg) or vehicle was administered by gavage 30 minutes and 4 hours after the induction of cerebral ischemia by permanent occlusion of the left middle cerebral artery (MCA). Animals were euthanized 24 hours after MCA occlusion, and the volume of gray matter damage was evaluated by quantitative histopathology. Axonal damage was determined with amyloid precursor protein immunohistochemistry. Dynamic susceptibility contrast MRI was used to assess regional cerebral blood volume (CBV) and cerebral blood flow (CBF). **RESULTS:** Treatment with the higher dose of cilostazol (50 mg/kg) significantly reduced the volume of gray matter damage and axonal damage in the cerebral hemisphere by 45.0% ( $P < 0.02$ ) and 42.4% ( $P < 0.002$ ), respectively, compared with the control group. Relative CBV in the peri-infarct area after MCA occlusion was significantly increased in the cilostazol-treated group (50 mg/kg) compared with the control group ( $P < 0.05$ ). Relative CBF tended to be higher in the cilostazol-treated group compared with the control group. **CONCLUSIONS:** Treatment with cilostazol significantly reduced the gray

and white matter damage associated with permanent focal ischemia. Cilostazol improved CBV and CBF in the peri-infarct area. The major action of cilostazol is to increase perfusion in the ischemic penumbra.

## Effects of cilostazol on long-term clinical outcomes after coronary stenting.]

*Zhonghua Nei Ke Za Zhi*. 2005;44(11):814-7.

**OBJECTIVE:** To evaluate the effects of cilostazol on long-term clinical outcomes in patients underwent coronary stent implantation. **METHODS:** One hundred patients who underwent coronary stenting were randomly assigned to receive cilostazol 200 mg/d for 6 months ( $n = 50$ ) or ticlopidine 500 mg/d for 1 month ( $n = 50$ ). Aspirin 100 mg/d was administered concomitantly with cilostazol or ticlopidine. Angiographic follow-up was carried out at 6 months and clinical follow-up for 3 years after stenting. **RESULTS:** Angiographic restenosis occurred in 5 of 34 patients (14.7%) in cilostazol group and 10 of 37 patients (27.0%) in ticlopidine group ( $P = 0.204$ ). At the end of three-year follow-up, the incidence of major adverse cardiac and cerebral events (MACCE) was greatly reduced in cilostazol group compared with ticlopidine group (16% vs 36%,  $P = 0.023$ ). Changes of Seattle angina questionnaire (SAQ) physical limitation score showed no significant difference between two groups (21.8 +/- 12.3 vs 16.8 +/- 15.9,  $P = 0.086$ ). However, changes the improvement of angina frequency score much more was significant in cilostazol group (22.6 +/- 12.7) compared with that in ticlopidine group (16.1 +/- 13.3,  $P = 0.015$ ). Recurrent angina occurred in 38% of patients in cilostazol group and 54% in ticlopidine group, respectively ( $P = 0.105$ ). Readmission due to cardiac and cerebral vascular diseases was much less in

cilostazol group than that in ticlopidine group (20% vs 40%,  $P = 0.029$ ).  
CONCLUSIONS: Cilostazol treatment significantly reduced MACCE and improved the quality of life of patients in three-year clinical follow-up after coronary stenting.

### **A Preliminary Study on the Effects of Exercising to Maximum Walking Distance on Platelet and Endothelial Function in Patients with Intermittent Claudication.**

*Eur J Vasc Endovasc Surg.* 2005 Dec 14

BACKGROUND: Platelet and endothelial activation has been shown to be increased in patients with intermittent claudication (IC). Recent studies have suggested that exercise may induce further platelet activation. The aims of this study were to investigate the effect of exercising to maximum walking distance on platelet and endothelial function in patients with intermittent claudication who were receiving statin and aspirin therapy compared with age matched healthy controls. METHODS: Platelet aggregation through COX-mediated and thrombin receptor activator peptide (TRAP)-stimulated GPIIb/IIIa pathways was measured by the Ultegra point of care system in 20 patients with IC on aspirin and 20 healthy volunteers before, immediately and 1h after exercising to treadmill maximal walking distance (MWD). Soluble P-selectin, vWF and sICAM were measured using an enzyme linked immuno-sorbent assay technique. RESULTS: Baseline platelet aggregation was significantly reduced in patients with IC compared to volunteers ( $p < 0.05$ ). In patients, exercising to MWD significantly reduced platelet aggregation (COX, median -5% [range -24 to 13%];  $p = 0.02$ ; GPIIIa/IIb, median -13% [range -72 to 33%];  $p = 0.02$ ) immediately post-exercise

which returned to baseline values at 1h. There was no change in the healthy volunteers following the same median duration of exercise. Baseline sP-selectin levels were higher in the patients with IC compared to the healthy volunteers [Median values (interquartile range), 42.72 (33.28-54.24) versus 29.16 (24.40-34.10),  $p = 0.0003$ ] but there were no differences in vWF levels. Both sP-selectin and vWF levels increased significantly in the control and patient group following exercise ( $p < 0.005$ ). sICAM were higher at baseline in the patients with IC but were unchanged following exercise [Median values (interquartile range), 560.9 (405.5-739.4) versus 467.0 (325.7-643.4),  $p < 0.05$ ]. CONCLUSION: This study is the first to show that platelet aggregation is reduced immediately following treadmill exercise to maximum walking distance in patients with IC despite a rise in sP-selectin and vWF, suggesting endothelial activation. The inhibition of platelet aggregation after exercise in subjects on antiplatelet and statin therapy suggests that exercise is unlikely to exacerbate platelet thrombus formation in patients with IC.

### **Prevention by rebamipide of acute reflux esophagitis in rats.**

*Dig Dis Sci.* 2005 Oct;50 Suppl 1:S97-S103.

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.

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

*Dig Dis Sci. 2005 Oct;50 Suppl 1:S90-6.*

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.

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

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

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.

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

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

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.

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

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

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.

<http://www.thai-otsuka.co.th/pxnews/index.html>

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