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# Protective Effect of Taurine on Indomethacin-induced Gastric Mucosal Injury

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It has been suggested that oxygen-derived free radicals play an important role in the pathophysiology of acute gastric ulceration induced by NSAIDs and ischemia-reperfusion. Taurine is hypothetized to exert its protective effect on NSAIDs-induced gastric injury by its antioxidant properties. Protective effect of taurine on indomethacin-induced gastric mucosal lesion and its protection mechanism were investigated. Intragastric administration of 25 mg/kg of indomethacin induced hemorrhagic lesions on the glandular stomach in rats. Pretreatment with 0.25 or 0.5 g/kg of taurine one day before or for 3 days significantly reduced the gastric lesion formation and inhibited the elevation of lipid peroxide level in gastric mucosa. The luminol-dependent chemiluminescence of rat peritoneal neutrophils increased immediately after treatment of FMLP or indomethacin. Taurine (5-20 mM) inhibited chemiluminescence of neutrophils activated by FMLP. Human neutrophils (polymorphonuclear leukocytes) significantly adhered to the confluent monolayer of human umbilical vein endothelial cells (HUVEC) after coincubation with indomethacin. This neutrophil adhesion induced by indomethacin to HUVEC was prevented by taurine in a dose-dependent manner. These results indicate that the protective effect of taurine against NSAIDs-induced gastric mucosal injury is due to its antioxidant effect, which inhibits lipid peroxidation and neutrophil activation.

**Key Words :** Taurine, Indomethacin, Gastric mucosal injury, Lipid peroxidation, Antioxidant

## INTRODUCTION

It is well documented that nonsteroidal anti-inflammatory drugs (NSAIDs) induced ulceration in the upper gastrointestinal tract (Wallace *et al.*, 1992). Although the underlying mechanisms responsible for this action are still unclear, the ability of these agents to inhibit gastric prostaglandin synthesis is an important contributing factor (Naito *et al.*, 1993). In recent years, a number of studies have highlighted the importance of alterations in mucosal blood flow after NSAIDs administration in the pathogenesis of ulceration seen with several experimental models. It was demonstrated that NSAIDs caused a reduction in gastric mucosal blood flow (Ashey *et al.*, 1985) and further noted that the reduction of mucosal blood flow induced by topical application of aspirin to the rat stomach was preceded by the adherence of white thrombi to vessel walls in the mucosal microcirculation and a reduction in gastric mucosal blood flow (Kitahora *et al.*, 1987). Hemorrhagic lesions subsequently formed in the regions of reduced perfusion. These observation suggested a role for circulating leukocytes in the pathogenesis of NSAIDs-induced ulceration (Wallace *et al.*, 1990).

The activation of neutrophils is accompanied by the release of oxygen derived free radicals. Free radicals have been shown to play a role in various models of gastrointestinal injury such as ischemia-reperfusion injury (Yoshikawa *et al.*, 1989; Takeuchi *et al.*, 1991; Vaananen *et al.*, 1991; Ayene *et al.*, 1992) and some radical scavengers show a protective effect against the mucosal injury induced by active oxygen species resulted from ischemia-reperfusion and NSAIDs treatment (Hirota *et al.*, 1990).

The present study was undertaken to investigate the effect of taurine on indomethacin-induced gastric mucosal injury and mechanism of taurine through its antioxidant effects such as inhibition of lipid peroxidation and neutrophil inactivation.

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## MATERIALS AND METHODS

### Indomethacin-induced gastric mucosal injury

Male Sprague-Dawley rats (180 to 220 g) obtained from Charles River Japan, were fasted 18h before the experiments, but they were allowed free access to water. Gastric mucosal damage was induced by the oral administration of 25 mg/kg of indomethacin (Sigma) suspended in 0.5% carboxymethyl cellulose (CMC) solution with a few drops of Tween 80 in a volume of 0.5 ml/100g of body weight. Taurine (Sigma) was suspended in saline and administered intraperitoneally 1 day before indomethacin treatment in a dose of 0.25 or 0.5 g/kg. An equivalent volume of vehicle was administered to the control rats. A respective treatment was done in a randomized order.

Rats were killed by exsanguination via the abdominal aorta under inhaled ether anesthesia 6 hours after indomethacin administration. Their stomachs were removed and opened along the greater curvature, and carefully examined macroscopically and microscopically. The extent of gastric mucosal lesions was expressed as the total area of erosions observed with a dissecting microscope (Vaananen *et al.*, 1991).

The levels of thiobarbituric acid (TBA)-reactive substances in gastric mucosal homogenates were measured and used as an index of lipid peroxidation after indomethacin administration. The level of TBA-reactive substances was expressed in terms of nmol of malondialdehyde (Sigma).

### FMLP-elicited chemiluminescence from neutrophils

The rats were injected i.p. with 20 ml of sterile 12% (w/v) sodium caseinate in iso-osmotic (0.9%) NaCl. Twenty hours later, the animals were killed by ether asphyxiation, the peritoneal exudate was collected. After being filtered through three layers of surgical gauze, the exudate was centrifuged at 200×g for 5 min, and the pellet was washed twice with Dulbecco's phosphate-buffered saline (DPBS) (Gibco, Life Technologies N.Y.). The pellet was suspended in 1 ml of iso-osmotic NaCl, 10 ml of distilled water, and 10 ml of 1.8% NaCl to lead to hypotonic lysis of erythrocyte contaminants. The cell suspension was centrifuged and the pellet was resuspended in Hank's balanced salt solution (HBSS) (Sigma) with 1% BSA. The specificity of the cell population was determined by differential counting of smears stained with Wright's stain. Viability of the neutrophils was measured with the trypan blue exclusion technique.

Chemiluminescence was measured by using luminol with microplate scintillation counter, TopCount (Packard, Canberra Co.). Reaction mixtures contained $1.5\times10^6$ granulocytes, 1 μM FMLP (<0.01% DMSO), 0-20 mM taurine, and 0.07 mM luminol (0.05 M Tris HCl, pH 7.4) in 200 μl of HBSS. Luminol was dissolved in 50 mM tris solution and then adjusted to pH 7.4 with 0.1 N HCl. Taurine was diluted with HBSS. Control studies were performed by the addition of saline alone. The results were expressed in terms of the maximal counts per min (Dahlgren, 1991).

### Human neutrophils adherence to HUVEC cells

Human umbilical vein endothelial cells (HUVEC) were plated in Medium 199 (Gibco, Life Technologies N.Y.) supplemented with 10% heat-inactivated fetal calf serum, thymidine (2.4 mg/L), glutamine (230 mg/L), heparin sodium (10 IU/ml), antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.125 μg amphotericin B), and endothelial cell growth factor (80 μg/ml). The cell cultures were incubated at 37°C in a humidified atmosphere with 5% $CO_2$ and expanded by brief trypsinization (0.25% trypsin in PBS containing 0.02% EDTA). And the cells were seeded into gelatin (0.1%) and fibronectin-coated (25 μg/ml) 11 mm, 96-well tissue culture plates and they were used when confluent.

Human neutrophilic polymorphonuclear leukocytes were isolated from the venous blood of healthy adults using Ficoll-plaque. Isolated neutrophils were suspended in PBS and radiolabeled by incubating the cells at $2\times10^7$ cells/ml with 30 μCi $Na^{51}CrO_4$/ml (Du Pont NEN, Massachusetts, USA) neutrophil suspension at 37°C for 60 minutes. The cells were then washed twice with cold PBS at 250 g for 8 minutes to remove unincorporated radioactivity and they were resuspended in plasma-free HBSS. Labelled neutorphils were added to HUVEC monolayers at a neutrophil-to-endothelial cell ratio of 10 : 1 with various concentrations of indomethacin. After coincubation (30 minutes), the amount of added neutrophils that adhered to the HUVEC monolayers was quantitated. After 30 minutes incubation HUVEC were washed to remove the drug, the labelled neutrophils were added to the monolayers, and neutrophil adhesion was assessed (McGregor *et al.*, 1994; Yoshida *et al.*, 1993).

## RESULTS

### Effect of taurine on indomethacin-induced gastric mucosal injury

Multiple erosions and bleeding were present in the stomach 6 hours after administration. The total area of gastric erosions was significantly larger in the indomethacin-treared rats than the normal rats (Fig. 1, 2). Taurine as well as rebamipide, mucus protecting agent, significantly inhibited the formation of gastric lesions. Histologic examination revealed that the indomethacin-induced lesions in the control rats penetrated deeply into mucosa. Hemorrhagic necrosis and diffused necrosis of villous ridges were present, and

villous tip of the mucosa was completely destroyed. Treatment with taurine of 500 mg/kg reduced the depth and severity of the lesions (Fig. 3). Mild to moderate villous atropy was noted and severe nectrotic changes of mucus ridges were not seen.

The lipid peroxide level in the antral mucosa of the untreated rats was 85.1±5.2 nmoles/mg of wet weight. TBA-reactive substances significantly in-

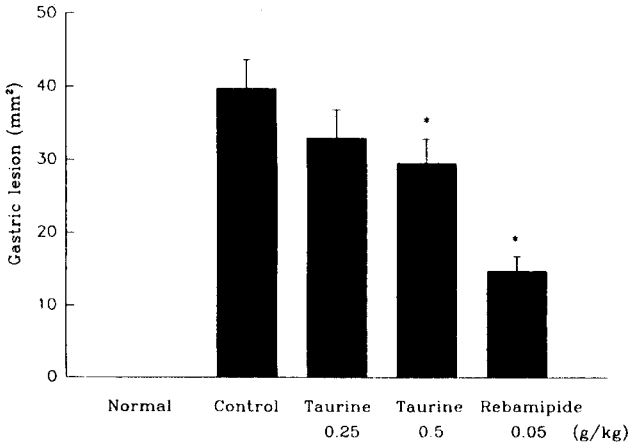


**Fig. 1.** Effect of taurine on the formation of gastric mucosal lesion 6 hours after oral administration of indomethacin, 25 mg/kg to rats. Each value indicates mean±SE of 3 seperate experiments (n=10). *p<0.05

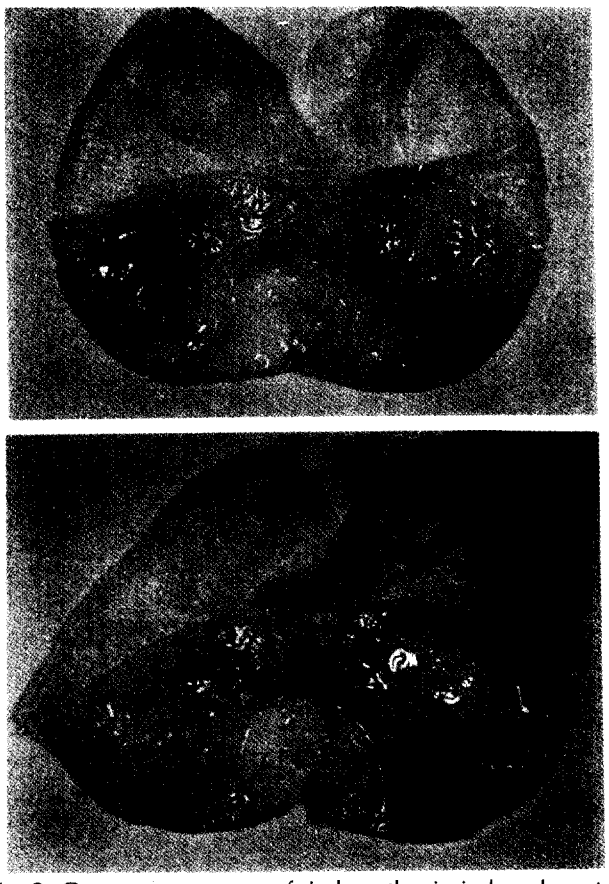

**Fig. 2.** Gross appearance of indomethacin-induced gastric mucosal injury in rats. Hemorrhagic erosions were observed linearly along the mucosal foldings in control rats (above) 6 hours after indomethacin administarion (25 mg/kg). Injury was inhibited by the treatment with taurine (below) in a dose of 0.5 g/kg.

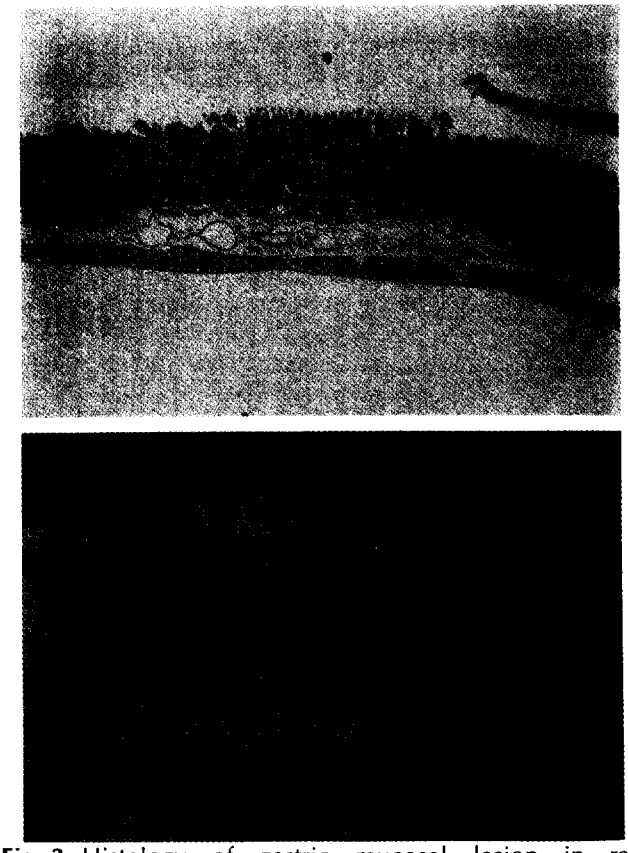

**Fig. 3.** Histology of gastric mucosal lesion in rats (hematoxylin and erosin ×40) Above: control, below: taurine-treated rat.

**Table I.** Effect of taurine on the lipid peroxide concentration in the antral mucosa. The gastric lesion was induced by intragastric administration of indomethacin, 25 mg/kg to rats. The mucosa was seperated from the antrum of stomach 6 hours after administration of indomethacin. Lipid peroxide level is shown as the amount of malondialdehyde (MDA) determined by TBA method. Data repersents mean±S.E.

| Treatment | TBA reactive materials | |
|---|---|---|
| | (nmoles /g wet weight) | % of inhibition |
| Normal | 85.1±5.2 | |
| Indomethacin | | |
| +vehicle | 127±15.6 | |
| +taurine, 250 mg/kg | 106±9.32 | 50.1 |
| +taurine, 500 mg/kg | 100±18.2 | 64.4 |
| + rebamipide 50 mg/kg | 87.1±14.3 | 95.5 |

creased 6 hours after indomethacin administration compared to the value in normal rats. Taurine, 250 or 500 mg/kg significantly inhibited the increase in TBA-reactive substances (Table I). But taurine had no inhibitory effect on NADPH-dependent microsomal lipid peroxidation in the rat liver.

### Effect of taurine on FMLP-elicited chemiluminescence from neutrophils

When rat peritoneal neutrophils were exposed to FMLP a chemiluminescence response was obtained and the peak appeared at around 1 min after addition of FMLP. Indomethacin, like FMLP, induced luminol-dependent chemiluminescence immediately after treatment (Fig. 4). Taurine at 5-20 mM dose-dependently inhibited chemiluminescence of neutrophils activated by FMLP and inhibition concentration of 50% ($IC_{50}$) was 5.52 mg/ml (Fig. 5). Reactive oxygen species such as hypochlorous acid (HOCl), which was produced from neutrophils activated by FMLP, has been proposed as being directly in the luminol-dependent chemiluminescence. Taurine is the HOCl scavenger and oxygen radical (Cantin, 1994; Schuller-levis *et al*, 1994) and it should thus inhibit the chemiluminescence activity dependent on reactive oxygen species. Taurine had no effect on neutrophil viability in this study.

### Effect of taurine on adherence of human neutrophils to HUVEC cells

Human neutrophils adhered to confluent monolayer of HUVEC. The basal adhesion (in the absence of drugs) of the neutrophils to the monolayers ranged between 14.6-24.8% adherence.

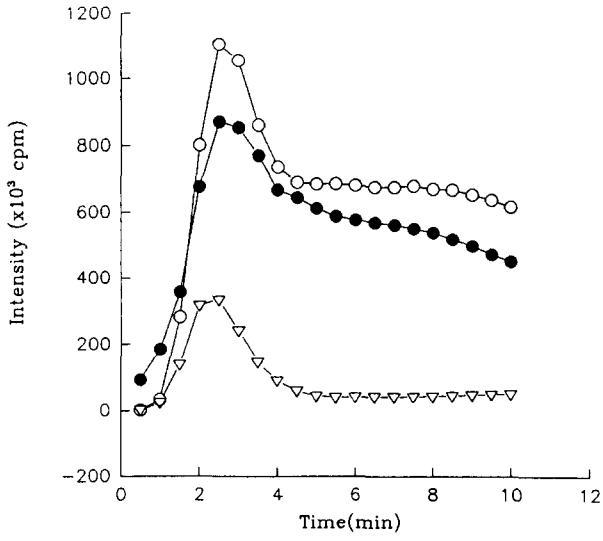


**Fig. 4.** Luminol-dependent chemiluminescence of rat peritoneal neutrophils induced by 10 uM FMLP (○), 0.5 mM indomethacin (●) and HBSS (▽).

As shown in Fig. 6, indomethacin induced a dose-dependent increase in neutrophil adherence to HUVEC monolayers. The effect of taurine on the neutrophil adherence to the monolayer is seen in Fig. 7. Taurine significantly inhibited neutrophil adhesion induced by indomethacin. The inhibition was below basal adhesion.

It was demonstrated that indomethacin caused the alteration of the arachidonic acid metabolism pathway and it was led to production of leukotriens and

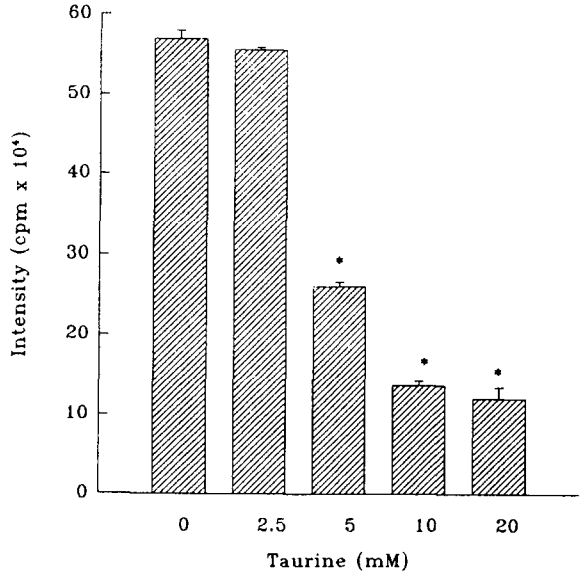


**Fig. 5.** Effect of taurine on luminol-dependent chemiluminescence in FMLP-stimulated neutrophils. *p<0.05 compared with HBSS

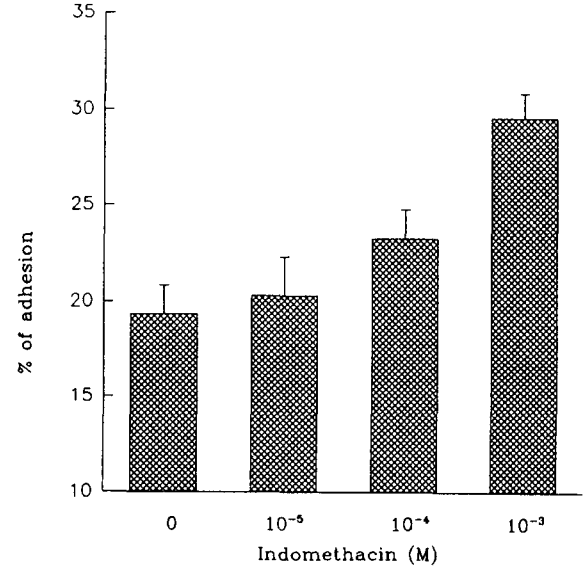


**Fig. 6.** Stimulation of neutrophil adhesion to HUVEC monolayer by indomethacin. $^{51}Cr$ labelled neutrophils were added to HUVEC monolayer in the presence of various concentrations of indomethacin.

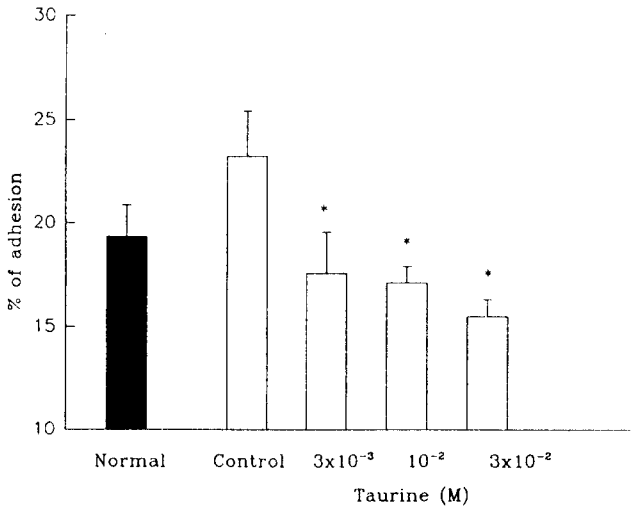


**Fig. 7.** Inhibition of indomethacin-stimulated neutrophil adhesion to HUVEC monolayer by taurine. $^{51}Cr$ labelled neutrophils were stimulated by indomethacin (100 uM) and added to HUVEC monolayer in the presence of various concentrations of taurine.

then it should increase the adherence of neutrophils to endothelial cells (Wallace *et al*, 1992; Gimbrone *et al*, 1984). Taurine reduced reactive oxygen species and LTB4 levels by manipulation of the MPO-$H_2O_2$-halide system, and indomethacin-induced neutrophil adhesion to endothelial cells was reduced.

## DISCUSSION

Taurine was found to inhibit the increase in gastric mucosal lesions induced by indomethacin. It significantly inhibited the increase in TBA-reactive substances in the gastric mucosa 6 hours after indomethacin administration. Many studies have focused on the role of lipid peroxidation mediated by oxygen radicals in the development of gastric mucosal injury (Yoshikawa *et al.*, 1989; Takeuchi *et al.*, 1991; Vaananen *et al.*, 1991; Ayene *et al.*, 1992). It was found that the gastric mucosal injury and the levels of TBA-reactive substances in the gastric mucosa were significantly inhibited by pretreatment with superoxide dismutase, a scavenger of superoxide, and by dimethylsulfoxide, a hydroxy radical, indicating that the lipid peroxidation induced oxygen radicals play an important role in the pathogenesis of indomethacin induced gastric mucosal damage as well as in gastric injury produced by ischemia-reperfusion (Yoshikawa *et al.*, 1989) ). But our study showed that taurine itself did not act as superoxide radical scavenger. The exact mechanism of the *in vivo* pharmacological effect of taurine remains to be determined.

In the present study taurine inhibited the neutrophil activation and the neutrophil adherence to endothelial cells. The source of active oxygen species in the indomethacin induced gastric lesion model is known to be neutrophils which were adhered to endothelial cells and activated (Wallace *et al.*, 1992). Depletion of circulating neutrophils, by treatment with antineutrophil serum or methotrexate, significantly reduce the damage induced by indomethacin (Wallace *et al.*, 1990). Prevention of leukocyte adherence to the vascular endothelium through pretreatment with monoclonal antibody directed against the common subunit of the granulocyte adherence glycoprotein (CD11/CD18) resulted in significant reduction in the extent of indomethacin-induced gastric ulceration (Gimbrone *et al.*, 1984; Vaananen *et al.*, 1991).

Many evidences suggest that taurine has antioxidant effect *in vivo* and *in vitro* (Aruoma *et al.*, 1988; Green *et al.*, 1991). Taurine has been found to scavenge hypochlorous acid generated by polymorphonuclear leukocytes (Cantin, 1994) and it inhibits lipid peroxidation of various membranes, such as rat mesangial cells and rod outer segments (Pasantes-Morales *et al.*, 1984, 1985; Trachtman *et al.*, 1993). Also taurine protects against pulmonary fibrosis induced by chemicals and ozone (Banks *et al.*, 1990; Giri *et al.*, 1992; Wang *et al.*, 1985). These findings suggest that taurine act as an antioxidant *in vivo*, inhibiting lipid peroxidation and neutrophil activation and that this action may be partially responsible for the mucus protecting effect.

## ACKNOWLEDGMENT

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