Time-Course Study of Gastric Damages in Rats by Anti-Inflammatory Drugs Using a Gastroscope and Its Quantification

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Abstract—Time-course studies on gastric damages in rats caused by nonsteroidal anti-inflammatory drugs (NSAIDs) were performed using a gastroscope, and the readings were quantified to obtain the Congestion-Hemorrhage Index (CHI) for evaluating the potencies of the damaging properties of NSAID. The correlation between CHI and Ulcer Index (UI), the quantified value obtained by the conventional methods, was highly significant at 6 and 24 hr after forced oral administration of NSAID. The peak CHIs of aspirin (300 mg/kg), indomethacin (60 mg/kg), mefenamic acid (300 mg/kg) and fenoprofen calcium (300 mg/kg) appeared approximately 24 hr after a single forced oral administration of drugs. Thus, it was suggested that an observation at 24 hr in addition to one at 6 to 7 hr might be necessary for the examination of damaged gastric mucosa. Under the present experimental conditions, fenoprofen calcium caused the greatest damages on gastric mucosa among the four NSAIDs. Mefenamic acid showed the least damaging potency on gastric mucosa, having a smaller CHI than that of aspirin. Indomethacin possessed a stronger damaging property than aspirin.

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, indomethacin, possess excellent analgesic, antipyretic and anti-inflammatory effects (1-4) and are widely accepted in daily practice (4, 5). However, as to their deleterious effects, these drugs also induce erosion and ulcers on gastrointestinal mucosa (4, 6-9).

The common characteristic of these drugs is their ability to inhibit cyclooxygenase, which results in the inhibition of prostaglandin (PG) synthesis (10-12). As a cytoprotection factor, PG plays an important role in the prevention of ulceration. NSAIDs also readily cause gastrointestinal damage in experimental animals, thus one can use NSAIDs to induce experimental ulceration for the purpose of studying of the etiology of gastrointestinal ulceration (7, 8, 13-18). However, only a few reports have dealt with the detailed time-course of changes in the gastric mucosa after

drug administration such as the initiation and progression of erosion and ulcer, repair and finally the disappearance of erosion and ulcer. Anderson (19) reported the incidence of animals with gastric damage and the mean number of mucosal erosions at 15, 30, 60, 120 and 180 min, and 24 and 36 hr after the administration of ball-milled aspirin at 100 or 500 mg/kg to fasted guinea pigs. They demonstrated that the majority of lesions were established within 1 to 2 hr of dosing, and there was little subsequent change in the stomachs at 3 hr; furthermore, there was no hemorrhage or scarring at 24 or 36 hr. Seegars et al. (20) reported the erosive activity of aspirin (250 mg/kg) in male rats at 0.5, 1.5, 4, 8 and 17 hr after administration; they demonstrated that small erosion-foci (<2 mm) appeared in the glandular mucosa within 1.5 hr after aspirin treatment, and the maximal erosion-response was reached after

8 hr.

The experimental ulcer caused by NSAID using animals had been applied extensively in the preclinical examinations for the screening of drugs for the treatment of peptic ulcer. In such cases, most of the reports have evaluated the effectiveness by use of the ulcer index (UI) obtained at less than 7 hr after the administration of NSAID (13, 21, 22). Studies to determine if determining the index at less than 7 hr is suitable for these experiments has also not been made.

The present investigation was employed a gastroscope to observe the changes of gastric mucosa during a 5 day period beginning from immediately after the administration of NSAID to complete healing of the ulcer. The degree of damage determined by gastroscopic observation was quantified to obtain a reliable method for the evaluation of drugs. A comparison between the present new evaluation and the conventional method was also done by obtaining CHIs and UIs at 6 hr and 24 hr after administration of NSAIDs such as aspirin, indomethacin, mefenamic acid and fenoprofen calcium.

Materials and Methods

Animals: Slc Sprague-Dawley strain male rats, 5 weeks old, were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals. They were kept at a room temperature of $23 \pm 1^\circ\text{C}$, humidity of $60 \pm 10\%$, and lighting for 12 hr a day (7:00–19:00). Rats were maintained on commercial rat chow (MF, Oriental Yeast Co., Japan) and purified water (distilled water) ad libitum for the duration of the study. Before the experiments, the rats were starved for 24 hr but allowed free access to water.

Drugs: Aspirin (Lot No. F121) was purchased from Tsukishima Pharmaceutical Co., and indomethacin (Lot No. PEP7180) was purchased from Wako Pure Chemical Industries, Ltd. Mefenamic acid (Lot No. M-51129) and fenoprofen calcium were gifts from Meiji Seika Kaisha, Ltd. and Yamanouchi Pharmaceutical Co., Ltd., respectively. These drugs were suspended into 1% carboxymethyl cellulose (CMC) and given by stomach sonde. The doses of aspirin, indomethacin, mefenamic acid and fenoprofen calcium were

300, 60, 300 and 300 mg/kg, respectively.

A gastroscope: Endoscope (FBS-3.5TH, 3.5 mm ϕ , Machida, Japan) equipped with an angle system, gas and water inlets, a light source (RM-300T, Machida, Japan), and a camera (Kouwa Scopecamera SQ-16, Kouwa, Japan) was used. An arthroscope (No. 54736, 1.8 mm ϕ , Machida, Japan) equipped with a light source (RH-150II, Machida, Japan) was used for the insertion of esophagus cannula.

Observation of gastric mucosa through the gastroscope: Twenty-five rats were starved for 24 hr, but allowed free access to water, and then divided into 5 groups (5 rats/group). Groups 1 to 4 were orally given aspirin, indomethacin, mefenamic acid and fenoprofen calcium, respectively. The fifth group was a control and given the same volume (0.5 ml) of CMC solution. Rats were anesthetized with a single i.v. administration through the jugular vein of 20 mg/kg of pentobarbital sodium (Nembutal, Abbott) 30 min after forced oral administration of NSAID, and then they were fixed supine on a CFK operating table. Using the arthroscope, a cannula made of a polyethylene tube (1.5 mm ϕ) was inserted into the esophagus. The fiberscope, lubricated with lidocaine jelly (Xylocaine, Fujisawa), was then inserted approx. 12 cm until the top reached to the gastropyloric region. The changes of damaged gastric mucosa, congestion and hemorrhage were observed at 0.5, 1, 3, 6, 9, 12, 18, 24, 36, 48, 72, 96 and 120 hr after administration of the NSAID. In order to evaluate the degree of change the damages were evaluated by the following point system: point redness by congestion, 1; point hemorrhage, 2; linear redness, 2; minor linear hemorrhage, 3; mild linear hemorrhage, 4; severe linear hemorrhage, 5; and the total score/stomach was termed the Congestion-Hemorrhage Index (CHI).

Determination of ulcer index (UI) by the conventional method: Rats were treated in the same manner as above and then anesthetized with ether at 6 and 24 hr after the administration of NSAID. Subsequently, their stomachs were extracted. After treatment with buffered formalin for 20 min, the stomach was incised along the greater

curvature, and then the entire area was examined for the presence of mucosal lesions. The length (mm) of each necrotic lesion was measured, summed per stomach, and the value was used as the UI (23).

Statistics: The data are expressed as the mean $\pm$ S.D. in the text and illustrations, and the significance of the differences between mean values was obtained by Student's *t*-test for parametric data and Welch's *t*-test for nonparametric data.

Results

Time-course change of gastric mucosa damaged by NSAID (observation with a gastroscope): Time-course changes of gastric mucosa damaged by NSAID, observed with a gastroscope and expressed by CHI, are shown in Fig. 1.

Aspirin (300 mg/kg): A few points of congestion and hemorrhage were seen in the gastroduodenal region, at 30 min after a forced oral administration of aspirin; and several points were observed at 60 min after the treatment, of which the CHIs were 2.2 ± 0.8 and 8.4 ± 2.5 , respectively. The damage on gastric mucosa proceeded thereafter, and CHI at 3 hr was 13.4 ± 3.6 . No particular

change was seen during 3 to 6 hr, and then the CHI again progressed to reach the maximum (33.4 ± 4.8) at 18 hr after the treatment, with clear point- and linear hemorrhage. Healing started after the peak (18 hr), and CHIs at 24 and 36 hr were 29.2 ± 5.8 and 17.8 ± 4.0 , respectively. No hemorrhage could be seen at 72 hr after the treatment; the CHI value was 2.8 ± 0.8 .

Indomethacin (60 mg/kg): No specific change was seen 30 min after a forced oral administration of indomethacin, and slight changes were observed during the next 3 hr. A gradual progress of damage was then seen around the greater curvature, it reached the maximum CHI of 22.4 ± 4.1 at 24 hr. Healing started after the peak (24 hr), and CHIs at 48 hr and 72 hr were 11.4 ± 2.3 and 8.4 ± 2.6 , respectively. Disappearance of hemorrhage was noticed at 96 hr after the treatment.

Mefenamic acid (300 mg/kg): No specific damage on gastric mucosa was seen during 9 hr after a forced oral administration of mefenamic acid. Point hemorrhage started to appear at 12 hr after the treatment and hemorrhage at 18 hr, reaching the maximum CHI of 11.0 ± 2.7 at 24 hr after the treatment. Hemorrhage disappeared by 72 hr after the treatment. Melena was seen at 24 hr to 72 hr after the treatment.

Fenoprofen calcium (300 mg/kg): No specific damage on gastric mucosa was observed for the first 1 hr after forced oral administration of fenoprofen calcium. However, erosion started to be seen 3 hr after the treatment; and with further lapse of time, severe linear hemorrhage was observed around the greater curvature. The peak CHI of 30.2 ± 5.7 was found at 24 hr after the treatment. CHI values then decreased and were 10.2 ± 3.4 and 6.8 ± 3.0 at 72 hr and 96 hr after the treatment, respectively. It took 120 hr for the complete disappearance of hemorrhage. Melena was seen 24 hr to 72 hr after the treatment.

Determination of ulcer indexes obtained at 6 hr and 24 hr after NSAID administration: UIs of the stomach that were obtained at 6 hr and 24 hr after forced oral administrations of aspirin, indomethacin, mefenamic acid and fenoprofen calcium are summarized in Fig. 2. Mean UIs at 6 hr and 24 hr after the

Fig. 1. Time-course change of rat gastric mucosa, observed with an endoscope and expressed as the Congestion-Hemorrhage Index (CHI), damaged by NSAIDs, aspirin, indomethacin, mefenamic acid and fenoprofen calcium. Observation was started immediately after administration of NSAID. —○—: aspirin (300 mg/kg, *n*=5). —△—: indomethacin (60 mg/kg, *n*=5). —□—: mefenamic acid (300 mg/kg, *n*=5). —◇—: fenoprofen calcium (300 mg/kg, *n*=5). —●—: control (intact animal, *n*=5).

treatment of aspirin (300 mg/kg) were 8.6 ± 3.9 and 20.8 ± 5.3 , respectively. In the case of indomethacin (60 mg/kg), UIs were 5.2 ± 2.2 and 14.6 ± 5.7 , respectively. In both cases, spot and linear hemorrhages were seen in the gastropyloric region at 24 hr after the treatments. Mean UIs at 6 hr and 24 hr after the treatment of mefenamic acid (300 mg/kg) were 2.0 ± 1.5 and 8.6 ± 3.7 ,

Fig. 2. Comparative damaging potencies of NSAIDs. Ulcer Index (UI) at gastric mucosa by naked eye observation at 6 hr and 24 hr after the treatment. ASP: Aspirin (300 mg/kg, $n=5$). IND: Indomethacin (60 mg/kg, $n=5$). MEF: Mefenamic acid (300 mg/kg, $n=5$). FENP: Fenoprofen calcium (300 mg/kg, $n=5$). $\square$: 6 hr. ▨ : 24 hr. Mean $\pm$ S.D. *: $P_0 < 0.05$. **: $P_0 < 0.01$.

respectively. The damage was mostly point hemorrhage and was found around the pyloric region, and the damage was the lowest among the four NSAIDs. In the case of fenoprofen calcium (300 mg/kg), UIs at 6 hr and 24 hr after the treatment were 7.0 ± 2.9 and 23.8 ± 3.8 , respectively. Significant differences were found between aspirin and mefenamic acid ($P_0=0.0077$), indomethacin and mefenamic acid ($P_0=0.0276$) and mefenamic acid and fenoprofen calcium ($P_0=0.0109$), at 6 hr, and between aspirin and mefenamic acid ($P_0=0.0129$), indomethacin and fenoprofen calcium ($P_0=0.0170$) and mefenamic acid and fenoprofen calcium ($P_0=0.0002$), at 24 hr.

Comparison between CHI and UI of stomach: The comparison between respective mean CHI and UI on damages caused by each NSAID on gastric mucosa at 6 hr and 24 hr after the treatments are shown in Fig. 3. The correlation coefficient (r) at 6 hr and 24 hr after the treatments were 0.954 and 0.979, respectively, both of which were significant ($P_0 < 0.005$).

Discussion

The present novel approach, the CHI method, was found to be comparable to the conventional method, UI method, in which six to 7 hr after the administration of drugs, the rats were sacrificed and their damaged

Fig. 3. Correlation between endoscopic observation (CHI) and naked eye observation (UI) on lesions of rat gastric mucosa caused by NSAID. $\circ$: 6 hr, $(UI)=0.584 (CHI)+0.886$ ($r=0.953$, $P_0 < 0.05$). $\bullet$: 24 hr, $(UI)=0.747 (CHI)+1.486$ ($r=0.979$, $P_0 < 0.005$). Horizontal and vertical bars: Mean $\pm$ S.D. ($n=5$).

gastric mucosa were examined quantitatively. As shown in Fig. 3, the correlation between the present approach (CHI) and the conventional method (UI) was highly significant.

The present approach has several merits compared to the conventional method. First of all, there was no need to sacrifice animals, this enables one to reduce the number of animals for the experiment. This fact must be also considered with respect to economics and the animal's welfare. Since time-course observation of damaged gastric mucosa was possible, the maximum damage was found approx. 24 hr after administration of NSAIDs (Fig. 1). Thus, in addition to an observation at 6 to 7 hr, it might also be necessary to examine the animals at 24 hr after the treatment in the conventional method.

On the other hand, the present approach might require greater skills for animal experimentation and reliable reading of the endoscope. Since the animal was not sacrificed during the experimental period, it was not possible to identify the precise damaging property, which might be obtainable through histo-pathological examination.

Although a conclusive comparison could not be made, our data suggests the following order of relative damaging potency on the gastric mucosa among the NSAIDs tested under the present experimental conditions: fenoprofen calcium > aspirin > indomethacin > mefenamic acid. Ridolfo et al. (24) reported that the loss of blood in human feces following an oral dose of fenoprofen calcium (400 mg and 600 mg acid equivalent) every 6 hr for 4 days was significantly less than that following administration of an aspirin (650 mg and 1000 mg) preparation. The difference of the results obtained in the present study and the data of Ridolfo et al. (24) might be due to a difference in the doses employed. In the present study, fenoprofen calcium, which is poorly soluble in water, was administered in the form of a 1% CMC suspension and undissolved crystals might directly affect the gastric mucosa, in the same manner as aspirin. On the other hand, in the human study (24), fenoprofen calcium might dissolve completely, so that it would not have great effects on the gastric mucosa. The dose

of indomethacin used in the present experiment is three times the clinical dosage compared to the other NSAIDs. Therefore, the damaging potency on gastric mucosa in a clinical study might be lower than the present result. Mefenamic acid has also been reported to possess a weak damaging property on gastric mucosa (25, 26).

In conclusion, the approach described in the present study might be acceptable for the evaluation of gastric damage; it has the merit of reducing the number of experimental animals required, which is advantageous with regards to economics and animal welfare, and it is a good method because it allows a time course study to be performed in single animals.

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