In Vitro and In Vivo Effects of Nonsteroidal Anti-inflammatory Drugs and Aspirin on Rabbit Esophageal Epithelium

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ABSTRACT

Background: *Gastroesophageal reflux disease has a high incidence of 23%, with 29% of those with gastroesophageal reflux disease consuming nonsteroidal anti-inflammatory drugs. There are insufficient data concerning the effects of nonsteroidal anti-inflammatory drugs on the esophageal tissue. We aimed to examine the effects of well-known nonsteroidal anti-inflammatory drugs using electrophysiologic criteria on the rabbit esophageal epithelium.*

Methods: *Esophageal epithelium mounted on Ussing chambers enabled in vitro investigation of the electrophysiological properties. Doses of 1 mg/mL, 2.5 mg/mL, 5 mg/mL ibuprofen, naproxen, and aspirin were dissolved in dimethyl sulfoxide and added to the luminal side. Esophagi were cannulated from both sides for the administration of high-dose ibuprofen in vivo, and the potential difference was monitored.*

Results: *Ibuprofen and aspirin inhibited tissue transport functions in a dose-dependent manner. pH 4 acid and 0.1 mg/mL ibuprofen alone were not harmful; however, the combination of these agents had an additive and significance effect: 78% decrease in the potential difference and 85% decrease in the short-circuited current (Isc). The change in the potential difference in the in vivo experiments (5 mg/mL ibuprofen) was similar (52 ± 7% decrease) with in vitro experiments in the first 30 minutes.*

Conclusion: *Nonsteroidal anti-inflammatory drugs were harmful to the rabbit esophageal epithelium in both the in vitro and in vivo experiments. Even though aspirin and ibuprofen affected the transport mechanisms of the esophageal epithelium, the dose-dependent decrease of tissue potential difference and Isc with ibuprofen was more pronounced than those with aspirin. The combination of harmless doses of ibuprofen and acid demonstrated that even low acidic conditions can create a disruptive environment.*

Keywords: *Aspirin, electrical potential difference, esophagus, GERD, NSAIDs, short-circuit current, tissue resistance, ussing chamber system*

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used drugs for various purposes among all age groups. They are indicated as analgesics to relieve minor aches and as antipyretics to reduce fever.[1] Nonsteroidal anti-inflammatory drugs are the mainly used over-the-counter nonprescription medications. Epidemiologic data and clinical studies have shown that NSAIDs have gastric adverse effects, are hepatotoxic, and affect the intestines and colon.[2] A proportion of 25% of NSAID users experience drug-related gastropathy, which is a serious health problem in some cases.[3] In a multicenter study conducted in Turkey, 54.3% of cases of gastrointestinal (GI) system bleeding were related to NSAIDs.[4] Gastroesophageal reflux disease (GERD) is a highly prevalent disease at a rate of between 8.5% and 26% in different countries.[5] We showed that 22.2% of patients without GERD symptoms and 29% of patients with GERD used NSAIDs.[6] Nonsteroidal anti-inflammatory drugs are weakly acidic, and their pharmacologic properties affect their distribution in the body. They act through cyclooxygenase (COX) metabolism, blocking prostaglandin production.[7] Aspirin (acetylsalicylic acid (ASA)) is a salicylate; ibuprofen (IBU) and naproxen (NPRX) are propionic acid derivatives. Although they differ in chemical formulation and structure, they have similar pharmacokinetic and physicochemical properties. The COX selectivity, which is the ratio of the COX-2 IC_{50} to the COX-1 IC_{50} (a ratio less than 1 is interpreted as COX-2 selective), of ASA was the highest (3.12), meaning it was the least selective compared to NPRX (1.79) and IBU (1.69).[8] The COX-1 : COX-2 selectivity of these NSAIDs was the primary concern since both their toxicity and therapeutic effects depend on this characteristic.

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It is known that 75 mg ASA causes endoscopically visible injury in the stomach, and development should be expected with low doses. Higher doses of ASA (600 mg) are known to cause gastric injury with erosions 2 hours after ingestion.[9] The effects of other NSAIDs are slower, but recovery is longer. Naproxen and IBU are known to have slightly lower relative risk for the development of GI bleeding.[10] Nonsteroidal anti-inflammatory drugs are capable of forming a complex with cell membrane phospholipids and disrupting the hydrophobicity of tissues, leading to the loss of tissue integrity. Goddard et al[11] showed that gastric mucosa perfused with an acidified ASA solution loses its hydrophobic properties.[11] The toxic effects of NSAIDs are known to be due to H+ ions trapped inside the cell membrane, decreasing the pH and affecting membrane permeability, which leads to mucosal injury. This effect was observed with a rapid decrease in the transmucosal potential difference (PD), which steadily recovered in this instance but did not recover in acidified conditions in the stomach.[12] In a canine experimental model, ASA disrupted the tight junction morphology in the gastric epithelium, affecting permeability.[13]

Studies have mainly addressed the effects of NSAIDs on Barrett's esophagus and adenocarcinoma in the esophagus.[14] However, the noxious effects of NSAIDs on healthy esophageal epithelial tissue and esophageal epithelial tissue damaged by acid and pepsin are underestimated. We aimed to evaluate the effects of widely used NSAIDs using electrophysiological parameters. The agents preferred for this evaluation were chosen due to their extensive clinical usage in all age groups for various reasons.

MATERIALS AND METHODS

Ussing chamber experiments were designed to understand the electrophysiologic effects of different commercially available NSAIDs on rabbit esophageal epithelia. In vivo experiments were also employed in this respect. Nonsteroidal anti-inflammatory drugs were obtained from commercial drug companies. Aspirin was obtained from Bayer AG (Leverkusen, Germany), IBU was obtained from BASF (Ludwigshafen, Germany), and NPRX was obtained from Sigma Aldrich (Burlington, MA, United States). All animal experiments in this study were approved by the Ethics Committee for Animal Studies of the Ege University in İzmir, Turkey (08-036).

Ussing Chamber Studies

Male New Zealand White rabbits weighing 2.5-3 kg were used as animal models and were dissected after the administration of an intravenous overdose of pentobarbital (60 mg/mL). The esophagi were excised and opened lengthwise, and the mucosal surface was pinned down on a paraffin tray containing ice-cold oxygenated normal Ringer's solution. The submucosa was dissected free of the underlying mucosa yielding a tissue sheet consisting of stratified squamous epithelium. The epithelium was cut into 4 equal sections, and each piece was mounted to Lucite half-chambers (with a diameter of 1.2 cm^2) of the Ussing system, which enabled tissues bathing in separate luminal and basolateral solutions for the rest of the experiment protocols. Tissues were bathed with normal Ringer's solution (composed of 140 Na^+, 119.8 Cl^-, 5.2 K^+, 25 HCO_3^-, 1.2 Ca^+, 1.2 Mg^{2+}, 2.4 HPO_4^{2-}, and 0.4 $H_2PO_4^-$ (in mmol/L) with an osmolality of approximately 275-285 mosmol/kg H_2O and pH 7.5) for stabilization and exposed to air with 95% O_2 and 5% CO_2 at 37°C. The junctional potential of acidic Ringer's solutions was measured before the experiments, and calculations were performed accordingly. The electrical resistance was calculated using Ohm's law (PD = Isc × R).

After being mounted, tissues were perfused in normal Ringer's solution for equilibration for 45 minutes. Experiments were initiated if tissues had *R* values >1000 ohms cm^2 and PD values >10 mV. Measurements were taken every 10 minutes during a 1-hour period. The values before starting the experiments were used as a reference point and defined as 100%. All PD, Isc, and *R* values measured after the addition of agents were calculated from these as a percentage change.

Having 4 sheets of tissue from each rabbit enabled us to experiment with 4 different bathing solutions. Different concentrations were employed for solubility tests in normal Ringer's solution and dimethyl sulfoxide (DMSO). A volume of 500 µL DMSO was used as a solvent, and all experiments included 1 tissue with luminal DMSO perfusion as a control. The same volume (500 µL) was also added to the serosal side.

Luminal perfusion of IBU, NPRX, and ASA was done with 1 mg/mL, 2.5 mg/mL, and 5 mg/mL in 10 mL Ringer's solution. A total of 50 mg of all drugs was dissolved in 500 µL DMSO and added to 10 mL Ringer's solution (at 37°C, oxygenated for 30 minutes with 95% O_2/5% CO_2) to measure the pH of these drug solutions. Perfusion solutions were acidified with luminal HCl titration (3 M) to make non-noxious concentrations of weakly acidic Ringer's solution (pH 4 = weakly acidic (WA)) to experiment in combination with 0.1 mg/mL IBU. Low doses of IBU (0.1 mg/mL,

0.25 mg/mL, and 0.5 mg/mL) were also tested and added to the basolateral side of the tissues.

Tissues were also perfused with Na-free Ringer's solution (2.4 K_2HPO_4, 0.4 KH_2PO_4, 1.2 $MgCl_2$, 1.2 $CaCl_2$, and 115 NMDG [in mmol/L], titrated with HCl to achieve a pH of 7.4) to understand the mechanisms involved in ion transport through epithelial tissues. Tissues were bathed with Na-free Ringer's solution (0-Na-bicarbonate Ringer's solution), and IBU (1 mg and 5 mg) was added to the luminal side.

In Vivo Experiments

In vivo experiments were performed under anesthesia with ketamine (35 mg/kg) and xylazine (5 mg/kg). A tracheotomy was performed to facilitate breathing. A drainage cannula was inserted 2 cm into the distal esophagus after laparotomy, and the Ringer-agar bridge for transepithelial PD measurement was also inserted 3 cm. Both tubes were secured together with a surgical suture. An NSAID or Ringer's solution was administered as a 1-mL bolus using a catheter orally placed into the upper esophagus. A reference Ringer-agar bridge was also placed in the laparotomy site in the peritoneum so that it contacted the peritoneal fluid. Both Ringer-agar bridges were inserted into separate beakers containing 3 M KCl solution and a calomel electrode. Calomel electrodes were connected to the voltage clamp system (World Precision Instruments) for PD recording. Test solutions and Ringer's solution were kept at 37°C. Before starting the experiments, the esophagi were flushed with Ringer's solution, and the baseline PD was recorded. The exit of the flushed solution was also observed for adequate outflow. The esophagi were then pulsed 5 mg/mL with IBU every 5 minutes for 1 hour via syringe (1 mL/bolus).

Control rabbits were intermittently administered normal Ringer's solution. Air was administered after each liquid bolus until no solution emerged from the outlet cannula. At the end of each experiment, 3 boluses of normal Ringer's solution were administered, and a final PD was recorded. Rabbits were sacrificed using the in vitro method. The esophageal catheter, cannula, and Ringer-agar bridge positions were verified.

Statistical Analysis

Statistical significance was determined using Student's *t*-test for parametric data (electrical parameters). All data were reported as the mean ± standard error of mean, and $P < .05$ was used to denote statistically significant differences between groups.

RESULTS

Different concentrations of ASA, NPRX, and IBU were tested in different conditions in the in vitro and in vivo models. Doses of 1 mg, 2.5 mg, and 5 mg of the drugs were chosen to observe the concentration-based effects for in vitro experiments.

The controlled model of the Ussing chamber system allowed us to determine the direct effects of different concentrations of ASA and IBU and their combined effects with HCl to mimic GERD. Tissue property was measured and recorded as mV (PD), and ion transport was measured as μA by short-circuit current in voltage-clamped tissue at 10-minute intervals (Isc). Baseline recordings were performed before drug addition at 0 minutes, and post-measurement values were converted to the percentage change from these first measurements.

Dimethyl sulfoxide alone slightly increased the tissue PD (111 ± 7%) in the first 10 minutes, leveling PD values to pre-experiment values after 60 minutes of perfusion (Figures 1A and 2A).

Low concentrations of ASA (1 mg and 2.5 mg) did not change in the PD (15 ± 7% decrease), but 5 mg caused a statistically significant decrease in the PD after 20 minutes, which decreased to 30 ± 7% at the end of the 60 minutes (Figure 1A). The change in tissue PD with 5 mg significantly differed from that of the control tissues ($P < .05$).

Figure 1B shows the change in net ion transport; Isc significantly decreased after 20 minutes (38 ± 8%, $P < .05$ compared to control tissues) when tissues were perfused with 5 mg ASA compared to tissues perfused with lower concentrations of 1 mg and 2.5 mg (18 ± 14% and 23 ± 8%, respectively). The inhibition of ion transport and decrease in the PD did not have any effect on tissue resistance (Figure 1C).

Ibuprofen was chosen for further experiments to understand the effect of the drug on transepithelial ion transport. The dose-dependent change in PD of tissues perfused with IBU was much more prominent. The

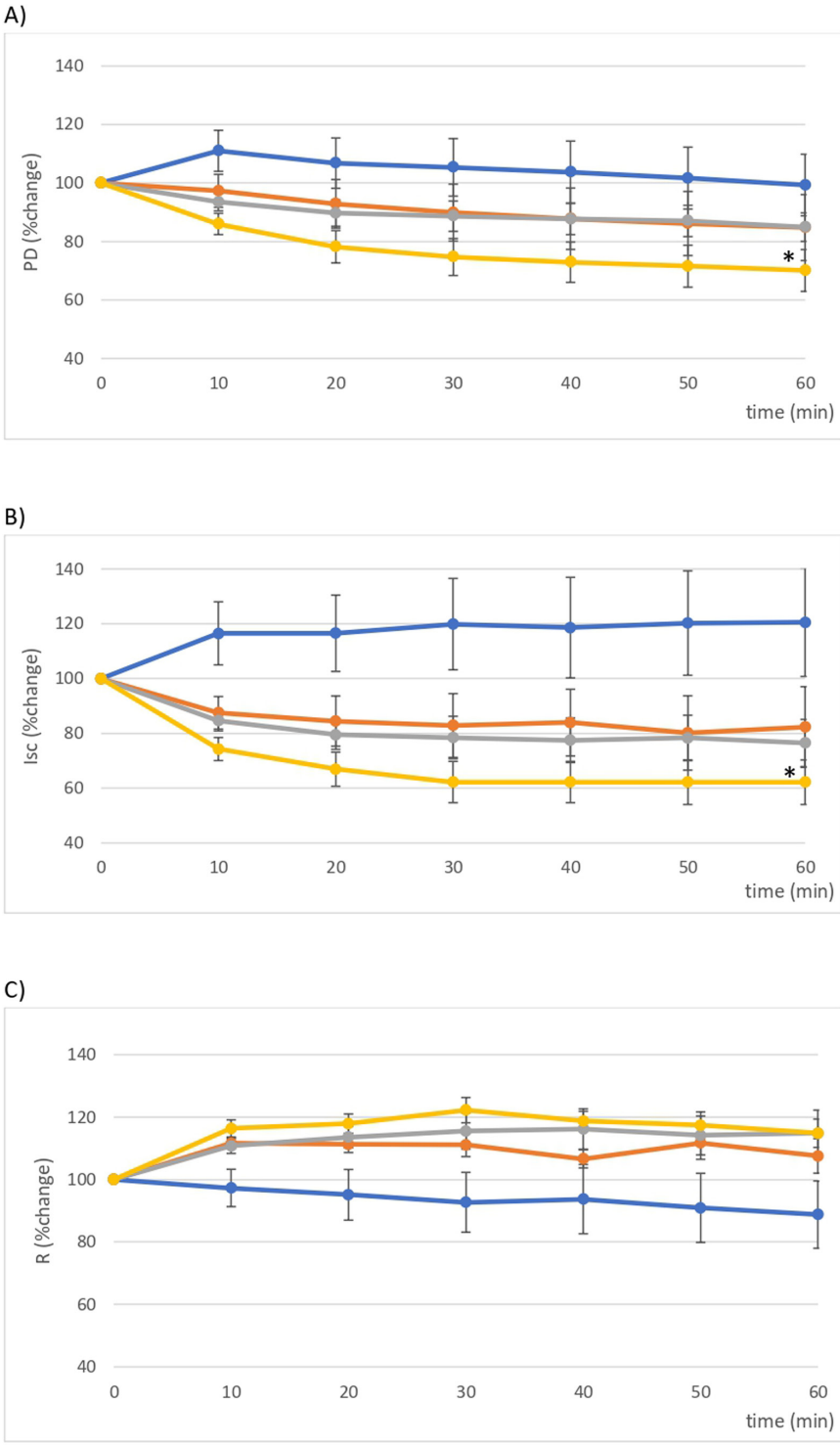

Figure 1. (A) Effects of DMSO and 1 mg, 2.5 mg, and 5 mg ASA solution on the PD. (B) Effects of DMSO, 1 mg, 2.5 mg, and 5 mg ASA solution on the short-circuit current (Isc) and (C) tissue resistance (R). Data are expressed as the percentage of the initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; n = 5/group. Luminal perfusion with (—•—) 500 µL DMSO; (—•—) 1 mg ASA; (—•—) 2.5 mg ASA; and (—•—) 5 mg ASA in Ringer's solution. ASA, aspirin; DMSO, dimethyl sulfoxide; PD, potential difference; SEM, standard error of mean.

effect of 1 mg IBU on tissue PD (24 ± 3% decrease after 60 minutes) was similar to that of 5 mg ASA (30 ± 7%). The 5 mg dose of IBU caused the most drastic change in the PD during the 60-minute duration (88 ± 4% decrease, $P < .05$ compared to control tissues). Even 2.5 mg caused a pronounced 60 ± 10% decrease ($P < .05$ compared to control tissues) (Figure 2A).

The dose-dependent change in Isc was evident for all dosages compared to control tissues with the initiation of experiments. Isc was inhibited starting from the addition of 1 mg IBU to the luminal side of tissues, and the Isc decreased 33 ± 7% (Figure 2B). The decreases with 2.5 mg and 5 mg (41 ± 4% and 50 ± 11%, respectively) were similar in the first 20 minutes, but the Isc for tissues perfused with 5 mg continued to decline (85 ± 4% after 60 minutes of perfusion), while the Isc in tissues perfused with 2.5 mg did not change after 20 minutes. There was no significant change in tissue resistance (Figure 2C).

The effects of the same concentration of another NSAID, NPRXNPRX, were also examined with the same experimental settings. Low dosages of NPRX (1 and 2.5 mg) slightly decreased the PD (Figure 3A). Luminal addition of 1 mg NPRX caused a 7 ± 10% decrease, whereas 2.5 mg caused a 13 ± 10% initial decrease, which recovered to 7 ± 12% toward the end of the 60 minutes. The effects of 5 mg NPRX were similar to those of ASA, which demonstrated a steady decrease to 74 ± 7% (loss of 26% of tissue PD throughout the experiment). However, no statistical significance was found between the groups ($P > .05$).

The initial decrease in Isc with 1 mg NPRX (12 ± 8%) in the first 10 minutes was recovered in the following 20 minutes, returning to normal values (Figure 3B). Similarly, 2.5 mg NPRX caused a 21 ± 9% inhibition of Isc, which steadily recovered as well. However, 5 mg luminal perfusion caused a gradual inhibition of 24 ± 11% in the Isc properties of the tissue and there was no significant change in tissue resistance (Figure 3C).

Basolateral Perfusion Experiments

Nonsteroidal anti-inflammatory drugs might affect the esophageal epithelium (EE) from the basolateral side following absorption via the bloodstream. For that reason, the luminal and basolateral sides of tissues were perfused with low concentrations (0.1 mg, 0.25 mg, 0.5 mg, and 1 mg) of IBU (Figures 4 and 5). Basolateral perfusion of the EE with 0.5 mg and 1 mg IBU caused a marked decrease in tissue PD (the PD decreased 43 ± 3% and 66 ± 5%, respectively). These changes in PD were more than twice that of luminally perfused tissues (Figures 4A, 5A). Lower doses (0.1 and 0.25 mg) had minimal effects on PD. The basolateral perfusion of rabbit EE with low doses (0.1 mg and 0.25 mg) of IBU caused no change in Isc, inhibiting only 14 ± 10% (0.1 mg) and 18 ± 12% (0.25 mg). The decrease in Isc was highly similar to that of luminally perfused tissues: 14 ± 6% (0.1 mg) and 26 ± 8% (0.25 mg). However, higher concentrations caused more dramatic inhibition of Isc when tissues were perfused basolaterally with 0.5 mg or 1 mg IBU (48 ± 4% and 75 ± 8%, respectively) (Figure 5A). The respective concentrations caused 36 ± 5% and 50 ± 7% decreases in the Isc when perfused luminally (Figure 5B).

Acidic Condition Experiments

The effects of non-noxious concentrations of IBU were investigated in combination with a weak acid (Figure 6). Figure 6B shows the change in the Isc when tissues were perfused with harmless concentrations of IBU (0.1 mg) in a WA Ringer's (pH 4) preparation. The slight decrease in the Isc with WA Ringer's solution (25 ± 7%) and with 0.1 mg IBU (18 ± 7%) was amplified 4-fold (85 ± 3%) with the combination of these agents ($P < .05$, pH 4 Ringer's solution + 0.1 mg IBU combination vs 0.1 mg IBU and pH 4 Ringer's solution). The effect of a low dose of IBU +WA on PD was examined with harmless dose combinations, which significantly decreased (78 ± 6% decrease in the PD) (Figure 6B), whereas both low-dose IBU and WA only diminish PD 19% ($P < .05$, pH 4 Ringer's solution + 0.1 mg IBU combination vs. 0.1 mg IBU and pH 4 Ringer's solution). There was no significant change in tissue resistance (Figure 6C).

0Na Condition Experiments

Rabbit EE is a mostly Na-transporting (80%) tissue, similar to human EE, and the Isc is mostly dependent on the passage of this ion (33). The relationship between Na transfer mechanisms and drug-induced decreases in the Isc with IBU was tested with Na-free Ringer's solution. Tissues were perfused in Na-free bicarbonate Ringer's solution for 30 minutes, and 1 mg or 5 mg of IBU was added to the luminal side of tissues (Figure 7). No IBU was added to control tissues (Na-free bicarbonate Ringer's solution perfusion). Na-free bicarbonate Ringer's solution caused a decrease of tissue PD and inhibition of Isc in the first 10 minutes (the PD decreased 74 ± 4%, and the Isc decreased 49 ± 4%), which remained steady. However, within minutes of IBU addition, both the PD and Isc of tissues significantly decreased (Figure 7A, 7B). The PD of tissues perfused with 1 mg decreased from 71 ± 4% in the first 10 minutes, and Isc was inhibited 18%. The

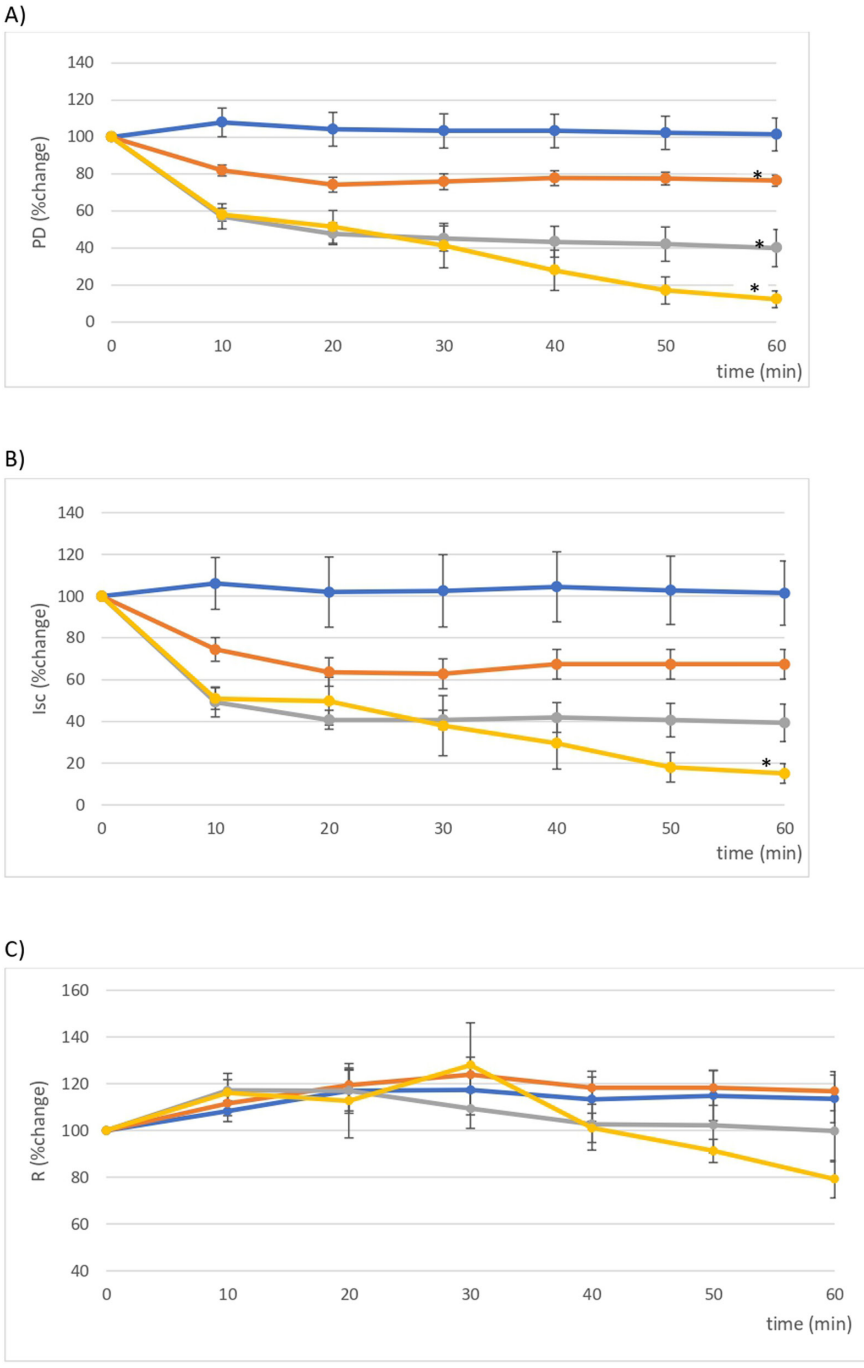

Figure 2. (A) Effects of DMSO and 1 mg, 2.5 mg, and 5 mg IBU solution on the PD. (B) Effects of DMSO and 1 mg, 2.5 mg, and 5 mg IBU solution on the short-circuit current (Isc) and (C) tissue resistance (R). Data are expressed as the percentage of initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; n = 4/group. Luminal perfusion with (—•—) 500 µL DMSO; (—•—) 1 mg IBU; (—•—) 2.5 mg IBU; and (—•—) 5 mg IBU in Ringer's solution. DMSO, dimethyl sulfoxide; IBU, ibuprofen; PD, potential difference; SEM, standard error of mean.

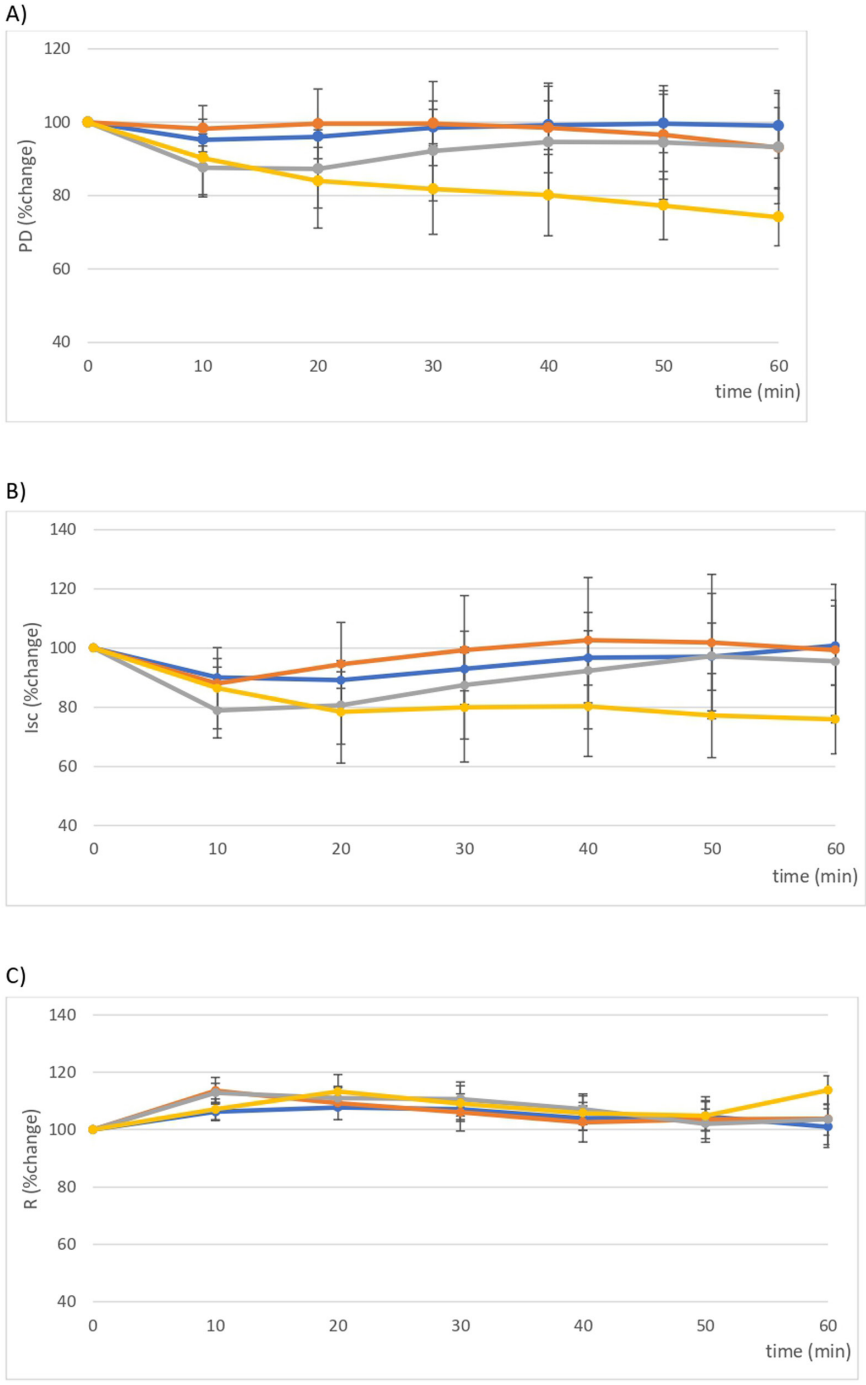

Figure 3. Effects of DMSO and 1 mg, 2.5 mg, and 5 mg NPRX solution on the PD. B) Effects of DMSO and 1 mg, 2.5 mg, and 5 mg NPRX solution on the short-circuit current (Isc) and C) tissue resistance (R). Data are expressed as the percentage of initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; n = 5/group. Luminal perfusion with (—•—) 500 µL DMSO; (—•—) 1 mg NPRX; (—•—) 2.5 mg NPRX; and (—•—) 5 mg NPRX in Ringer's solution. DMSO, dimethyl sulfoxide; NPRX, naproxen; PD, potential difference.

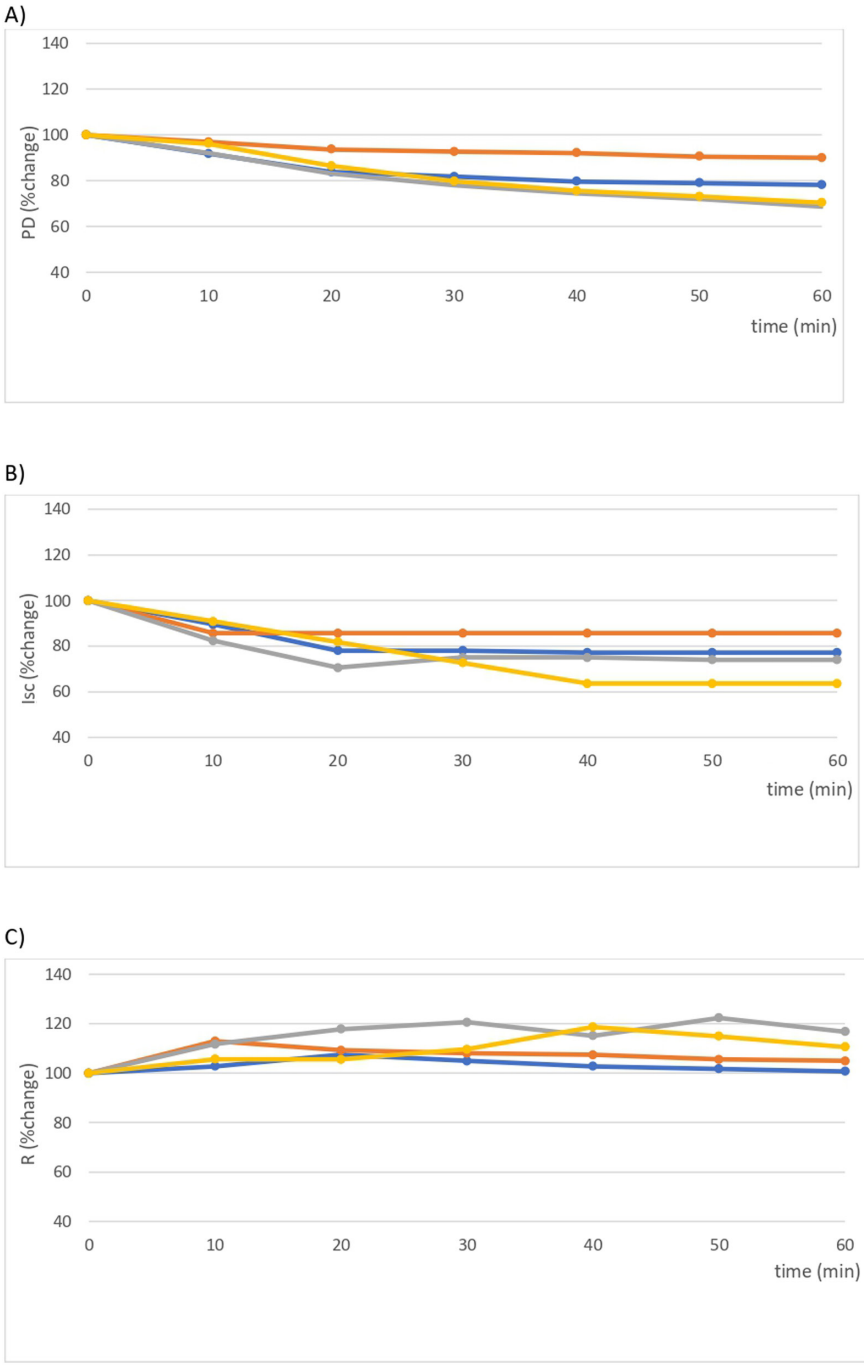

Figure 4. Effects of luminal addition of DMSO and 0.1 mg, 0.25 mg, and 0.5 mg IBU solution on (A) the PD, (B) short-circuit current (Isc), and (C) tissue resistance (R). Data are expressed as the percentage of initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; n = 3/group. Luminal perfusion with (—•—) 500 µL DMSO; (—•—) 0.1 mg IBU; (—•—) 0.25 mg IBU; and (—•—) 0.5 mg IBU in Ringer's solution. DMSO, dimethyl sulfoxide; IBU, ibuprofen; PD, potential difference; SEM, standard error of mean.

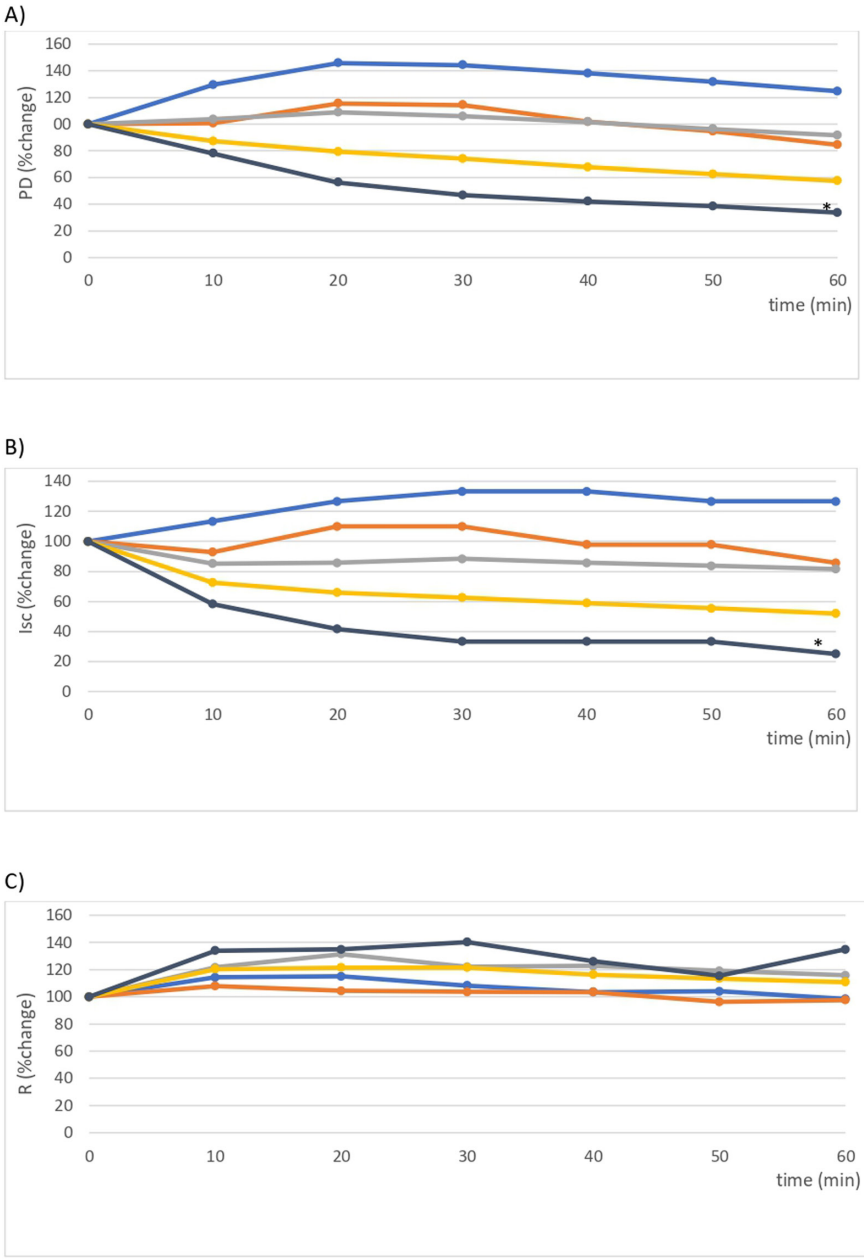

Figure 5. Effects of serosal addition of DMSO and 0.1 mg, 0.25 mg, and 0.5 mg IBU solution on (A) the PD, (B) short-circuit current (Isc), and (C) tissue resistance (R). Data are expressed as the percentage of initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; N = 3/group Serosal perfusion with (—•—) 500 µL DMSO; (—•—) 0.1 mg IBU; (—•—) 0.25 mg IBU; (—•—) 0.5 mg IBU; and (—•—) 1 mg IBU in Ringer's solution. DMSO, dimethyl sulfoxide; IBU, ibuprofen; PD, potential difference; SEM, standard error of mean.

5-mg dose of IBU caused a drastic decrease in PD (57% decrease in the first 10 minutes and 92 ± 2% loss after 30 minutes of perfusion), and 94 ± 2% of Isc activity was inhibited. There was no significant change in tissue resistance (Figure 7C).

In Vivo Experiments

The highest concentration of IBU (5 mg) used during the in vitro experiments was chosen for the in vivo experiments and intermittently administered in 5-minute intervals, as explained in the Methods

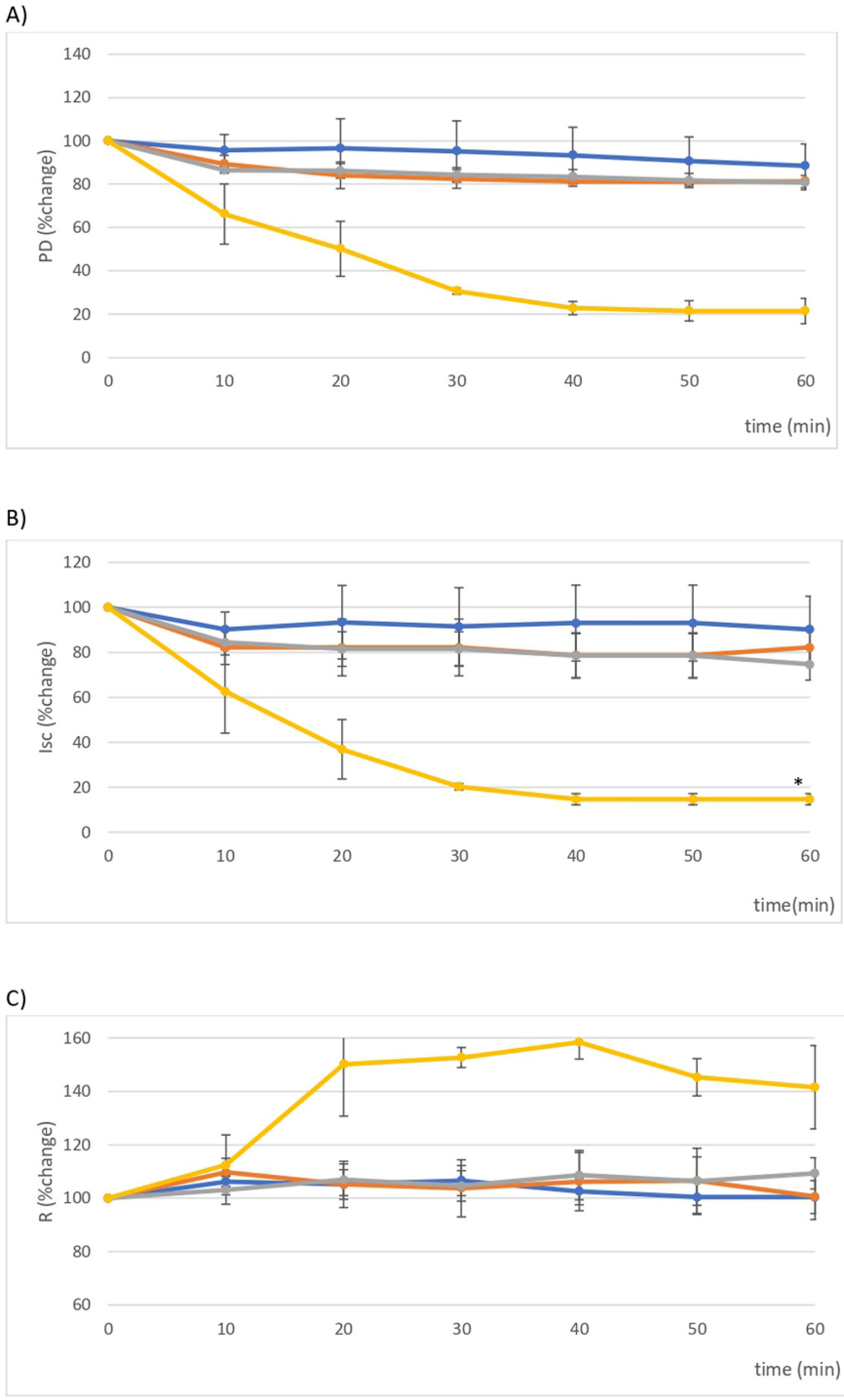

Figure 6. Effects of luminal addition of DMSO, 0.1 mg IBU, weakly acidic Ringer's solution (pH 4), and 0.1 mg IBU in weakly acidic Ringer's solution (pH 4) on (A) the PD and (B) short-circuit current (Isc). Data are expressed as the percentage of the initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; n = 3/group. Luminal perfusion with (—•—) 500 µl DMSO; (—•—) 0.1 mg IBU; (—•—) weakly acidic Ringer's solution (pH 4); and (—•—) 0.1 mg IBU + weakly acidic Ringer's solution (pH 4). DMSO, dimethyl sulfoxide; IBU, ibuprofen; PD, potential difference; SEM, standard error of mean.

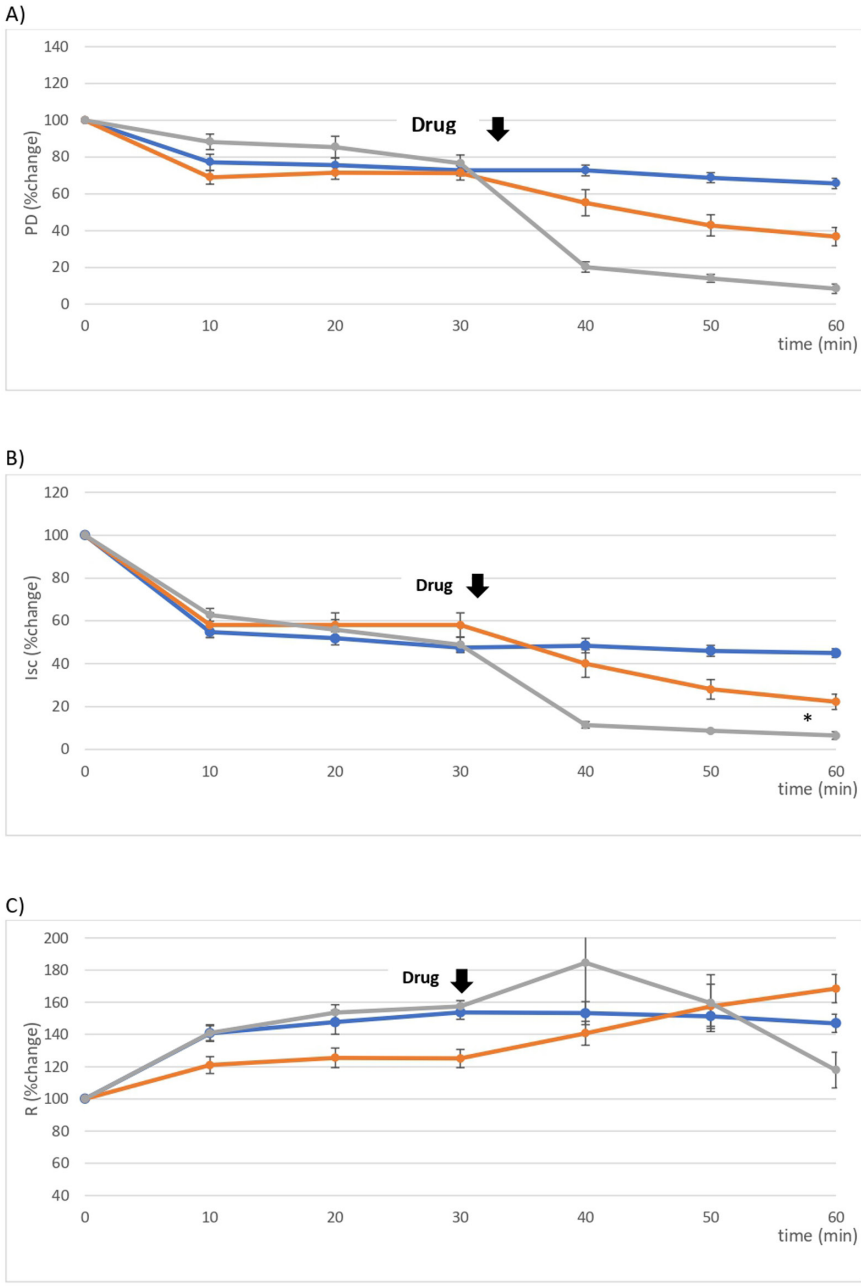

Figure 7. Effects of luminal perfusion with Na-free bicarbonate Ringer's solution and 1 mg and 5 mg IBU in Na-free bicarbonate Ringer's solution on the short-circuit current (Isc). Data are expressed as the percentage of the initial values prior to agent exposure (0-60 minutes). Values are expressed as the mean ± SEM; N = 3/group. Luminal perfusion with (—•—) 0Na-bicarbonate; (—•—) 0Na-bicarbonate + 1 mg IBU; and (—•—) 0Na-bicarbonate + 5 mg IBU in Ringer's solution. IBU, ibuprofen; SEM, standard error of mean.

section. There was no change recorded with the control group (normal Ringer's solution) (Figure 8). The change in the PD (52 ± 7% decrease) was similar to that in the in vitro experiment group (59 ± 12%) in the first 30 minutes. However, even though perfusion continued, tissues started to recover, with PD values improving at the end of the 60 minutes. The final decrease in tissue PD was 39 ± 8%, which significantly differed from the in vitro values (88 ± 4% decrease of tissue PD) ($P < .05$).

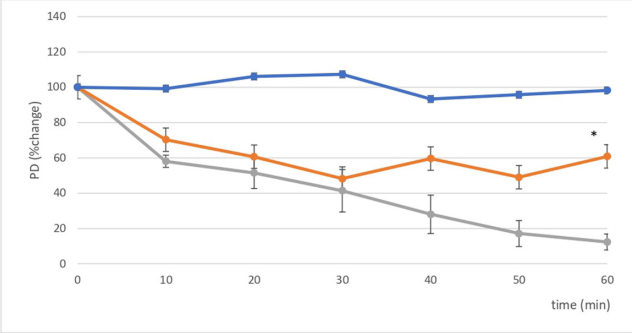

Figure 8. Effects of 1-mL boluses of normal Ringer's solution (control group) and 5 mg IBU on the in vivo PD compared with the in vitro experiment. Data are expressed as the percentage of initial values prior to agent exposure (0-60 minutes). Values are the mean ± SEM; n = 3/group. In vivo perfusion with (— • —) normal Ringer's solution and (— • —) 5 mg IBU in Ringer's solution. n = 4/group. Luminal in vitro perfusion with (— • —) 5 mg IBU in Ringer's solution. IBU, ibuprofen; PD, potential difference; SEM, standard error of mean.

DISCUSSION

Nonsteroidal anti-inflammatory drugs have been known for more than 80 years to cause mucosal damage to the upper GI system.[15] It remains unclear whether or how NSAIDs damage the EE, especially in the presence of refluxed acid. Nonsteroidal anti-inflammatory drugs and ASA are very widely used drugs and GERD is one of the most common chronic disorders in adults; it is very common that GERD patients who take those medications are under the attack of both refluxed materials and NSAIDs. For that reason, it is essential to evaluate the effects of NSAIDs on the EE.

Short-term and long-term usage of NSAIDs has different effects on the GI system. For example, long-term usage of NSAIDs has a significantly higher risk of upper GI bleeding than those taking short-term NSAIDs or regular-dose ASA.[16] Our study design is an example of the acute model, so it may not reflect problems with long-term effects.

In our experimental setup, IBU, ASA, and NPRX were shown to be capable of inhibiting epithelial tissue transport functions starting at low concentrations. This effect was obvious with IBU in a dose-dependent manner, and the same doses of ASA or NPRX were not as harmful as IBU. These results showed that both agents affected Isc as measured by the short-circuit current and transepithelial PD in esophageal epithelial tissue in vitro, but IBU had a larger effect. This effect on the transport characteristics of the tissue could not be explained by the inhibition of Na transport abilities.

Following GI absorption and circulation through the bloodstream, NSAIDs reach the basolateral side of the tissue and may have a harmful effect. To evaluate this concept, we applied different but low concentrations of IBU on the basolateral side and compared these with the same concentrations on the luminal side. With doses of IBU as low as 0.25 mg and 0.5 mg, which were applied to the basolateral side, significant damage was observed, while these concentrations had no effect on the luminal side.

Another interesting finding was the effect of the combination of 2 non-noxious agents, pH 4 Ringer's solution and 0.1 mg IBU, on the EE. The combination had a profound effect (the PD decreased 78%, and the Isc decreased 85%), although these agents did not separately have any considerable effect on the transport mechanisms of rabbit EE. The combination of harmless doses of IBU with WA conditions demonstrated that even low acidic conditions, which is a very good model for gastroesophageal reflux, can create a disruptive environment. This finding supports the concept that there is no safe dose of NSAIDs.

There are studies showing the effects of NSAIDs on GERD symptoms, although less than in the stomach. In a large community-based retrospective survey study, the prevalence of GERD was evaluated in 63 902 patients taking NSAIDs and 99 183 control patients. Nonsteroidal anti-inflammatory drugs increased the relative risk for absolute GERD development to 2.11.[17] Similar studies have shown an increase in GERD symptoms with an odds ratio (OR) of 1.5-1.7.[18,19] When upper GI endoscopy is used, the new cases of GERD are higher with an OR of 4.23 (CI: 1.66-10.74) with NSAID consumption.[20] However, there are some conflicting studies. Two studies showed no relationship between ulcerative reflux esophagitis and NSAIDs.[21,22]

Aspirin may also cause both systemic and topical damage to the gastroduodenal mucosa. However, in contrast to the results with NSAIDs, the results are less impressive and more conflicting in epidemiologic studies. While no association was observed between the use of ASA and reflux symptoms in 3 case-control studies,[18,21,22] a slightly higher risk was observed by others. A randomized controlled trial with low doses showed an increase in erosive esophagitis compared to the control group.[23] The risk is also related to the dose. While 1-5 tablets per week slightly increase symptoms, exceeding 5 tablets tremendously increases the risk.[24] Development of heartburn in patients using ASA was significantly less than with other NSAIDs in a retrospective multicenter study (OR: 1.44, 95% CI 1.01-2.04).[25]

All these clinical studies are comparable to our basic science results. Both NSAIDs and ASA have noxious effects on the EE in vitro and in vivo, and this effect is dose-dependent. IBU, as an NSAID, is more harmful than ASA. The effects in clinical studies are not very profound or disruptive, and this also explains why tissue resistance does not change with any of the agents we used.

Nonsteroidal anti-inflammatory drugs inhibit the activity of both COX-1 and COX-2, leading to the inhibition of prostaglandin and thromboxane synthesis. Suppression of COX-1 reduces mucosal prostaglandin synthesis and decreases mucosal blood flow. The suppression of COX-2 increases the number of neutrophils adhering to the vascular endothelium in the gastrointestinal microcirculation.[26] Mucosal damage occurs as a result of these changes in mucosal tissue due to inhibition of COX enzymes. Nonsteroidal anti-inflammatory drugs act through a systemic effect. In addition to the inhibition of COX enzymes, topical irritant effects also play an important role in mucosal damage. Their topical irritant effects are reduced by new formulations and enteric coating. However, they still have noxious effects that are most likely due to their acidic properties. To evaluate this theory, we applied IBU to the basolateral side of the tissue at much lower concentrations than the effective concentrations on the luminal side. For example, 1 mg IBU had a harmful effect that was approximately 3 times higher with the application on the basolateral side than that on the luminal side. These data reflect the importance of the systemic effects of these drugs in addition to the topical effects.

The use of high doses of H_2 antagonists or proton pump inhibitors is known to play an important role in preventing NSAID-induced mucosal damage.[27] This approach implicates the importance of acid (and pepsin) in combination with NSAIDs. Acid can contribute to the formation of NSAID-induced mucosal damage with different mechanisms. It may limit the platelet aggregation ability and cause mucosal injuries to progress to deep mucosal necrosis during restitution.[28] In addition, various growth factors that are important in maintaining mucosal integrity and wound healing are unstable in acid, so the presence of acid in the environment may limit their ability to defend and repair such factors.[29] One of the intriguing findings in this study was the combination of 2 relatively non-noxious concentrations of acid, and IBU produced a harmful effect. This finding is particularly important, as many reflux episodes occur between pH 4 and 7 and predispose the EE to the harmful effects of very low doses of NSAIDs.

The Na^+/H^+ antiporter, which is found in the EE, plays an important role in the maintenance of cell homeostasis and pH regulation. Luminal Na channels in various epithelial tissues are inhibited at low pH. Na channels can often be inhibited in the esophagus due to GERD and various agents.[30] We investigated the effects of IBU at various doses using Na-free Ringer's solution. We determined that the absence of Na did not show any additional decrease in tissue PD or Isc.

No significant difference was found in tissue resistance results in all our experimental designs. Since we cannot measure apical and basolateral resistance separately, this is not known whether shunt resistance was responsible for these results.[31]

The smaller injury with 5 mg IBU in vivo (40% decreation of tissue PD) than with 5 mg IBU in vitro (88% decreation of tissue PD) demonstrated that these defense mechanisms are efficient enough to protect the tissue to an extent. The decrease in the PD with in vivo experiments stopped after 30 minutes of intermittent dosing and slightly recovered without reaching significance.

This finding might have been related to some adaptive cytoprotective mechanisms and deserves further investigation.

CONCLUSION

These results showed that ASA and COX-1-selective NSAIDs, especially IBU, cause a substantial noxious effect on rabbit esophageal epithelial transport and tissue property with a simple acid reflux model. This effect was independent of the Na transport abilities of the tissue. The combination of 2 agents had an additive effect and transformed an unharmful concentration to a noxious effect. Even though pepsin was not included in the experimental setup, it is obvious that WA conditions are effective enough to cause a significant loss in these properties. Our results were confirmed at least with IBU, although to a lesser extent. Future studies should address the role of more COX-2 selective NSAIDs, such as celecoxib.

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