CLINICAL–ALIMENTARY TRACT

Gastrointestinal Safety of NO-Aspirin (NCX-4016) in Healthy Human Volunteers: A Proof of Concept Endoscopic Study

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See editorial on page 842.

Background & Aims: **NCX-4016 is a nitric oxide–releasing derivative of aspirin with antiplatelet activity. The aim of this study was to investigate the effect of NCX-4016 on gastrointestinal mucosa and platelet functions in healthy human volunteers.** ***Methods:*** **This was a parallel-group, double-blind, placebo-controlled study. Forty healthy subjects were randomly allocated to receive 7 days of treatment with NCX-4016 (400 and 800 mg twice daily), equimolar doses of aspirin (200 and 420 mg twice daily), or placebo. Upper endoscopies were performed before and at the end of the treatment period, and gastroduodenal lesions were graded using a predefined scoring system. Basal and posttreatment platelet aggregation in response to arachidonic acid (AA) and serum thromboxane (TX) B_2 and AA-stimulated platelet TXB_2 production were investigated.** ***Results:*** **Mucosal endoscopic injury score on day 7 was 0.63 ± 0.16 in the placebo group and 11.0 ± 3.0 and 16.1 ± 1.6 in healthy volunteers treated with 200 and 420 mg aspirin twice daily ($P < 0.0001$ vs. placebo). NCX-4016 was virtually devoid of gastric and duodenal toxicity, resulting in a total gastric and duodenal endoscopic score of 1.38 ± 0.3 and 1.25 ± 0.5 ($P < 0.0001$ vs. aspirin, not significant vs. placebo). NCX-4016 inhibited AA-induced platelet aggregation as well as serum TXB_2 and platelet TXB_2 generation induced by AA to the same extent as aspirin (not significant vs. aspirin).** ***Conclusions:*** **In this study, we have proven the concept that addition of an NO-donating moiety to aspirin results in a new chemical entity that maintains cyclooxygenase-1 and platelet inhibitory activity while nearly avoiding gastrointestinal damage.**

Aspirin is widely used for its antiplatelet, anti-inflammatory, and analgesic activities.[1] However, a major disadvantage of aspirin use is the potential for severe adverse gastrointestinal effects, such as bleeding and perforation.[2] Even low doses of aspirin used on a long-term basis inhibit gastric cyclooxygenase (COX) activity[3] and significantly increase the risk of gastrointestinal adverse events, with estimated rates of major bleeding episodes of 2–4 per 1000 middle-aged persons (4–12 per 1000 for older persons) given aspirin for 5 years.[2]

COX exists in several isoforms.[4] The discovery that COX-2 is the prevalent isoform expressed in inflamed tissues has been the basis for developing selective COX-2 inhibitors.[5] However, although coxibs exert anti-inflammatory and analgesic effects and their use is associated with ≈50% reduction of major adverse events in the gastrointestinal tract, they lack efficacy on platelet aggregation and are devoid of cardioprotective activity.[6,7]

Nitro-aspirin (NCX-4016), 2-acetoxy-benzoate 2-(2-nitoxy-methyl)-phenyl ester, is a nitric oxide–releasing derivative of aspirin (for review, see Wallace et al.[8]). Both the aspirin and the NO moieties of this compound contribute to its effectiveness[8]; thus, NCX-4016 not only inhibits formation of COX-1– and COX-2–derived prostanoids in vivo and in vitro[9–13] but also exerts a number of NO-mediated activities. NCX-4016 increases platelet guanosine 3′,5′-cyclic monophosphate concentrations,[9–12] a marker of NO formation, inhibits platelet aggregation induced by adenosine diphosphate and thrombin agonist (i.e., aspirin-resistant aggregation),[9,11,12] reduces proinflammatory cytokine production and apoptosis by a mechanism that involves caspases S-nitrosylation,[8,10,13–15] and mitochondrial protection.[16] Consistent with the fact that NO exerts protective effects on the

Abbreviations used in this paper: AA, arachidonic acid; COX, cyclooxygenase; TX, thromboxane.

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stomach and mediates many components of gastrointestinal mucosal defense,[17] preclinical studies have shown that NCX-4016 is virtually devoid of gastrointestinal toxicity[9,10] while retaining, and in some cases extending, the pharmacologic properties of the parent drug, including the anti-inflammatory,[13–15] analgesic,[18] and antithrombotic[9,11,12,19] activities. Although these studies suggest that nitro-aspirin could replace aspirin in human settings, the gastrointestinal safety and antiplatelet activity of this drug have never been investigated in humans.

With this background in mind, we designed a parallel-group, double-blind, placebo-controlled pilot study to assess the effect of NCX-4016 and aspirin on gastrointestinal mucosa and platelet aggregation in healthy human volunteers. The results of this study prove the concept that addition of the NO-donating moiety to aspirin results in a new chemical entity that suppresses COX-1 activity while nearly abolishing gastrointestinal toxicity.

Materials and Methods

The primary aim of this study was to compare the gastrointestinal safety of NCX-4016 with equimolar doses of aspirin in a randomized, double-blind, placebo-controlled, parallel-group study. The protocol was approved by a local ethical committee, and the study was performed at CROSS-Alliance facilities (Arzo, Switzerland). All subjects gave written informed consent before entering the study.

Subjects

Forty healthy subjects (26 men and 14 women; mean age, 29.5 years; range, 18–40 years) were enrolled. Before entering the study, all subjects were required to provide a complete medical history and undergo a physical examination and clinical laboratory tests. Subjects were excluded from the study if they met the following criteria: gastrointestinal symptoms, alcohol or drug abuse, pregnancy, known intolerance or hypersensitivity to aspirin, or a history of liver or kidney disease, peptic ulceration, or upper digestive tract surgery. Subjects were also excluded if they received any medications in the 2 weeks preceding the study that would influence the gastric or duodenal mucosa, such as nonsteroidal anti-inflammatory drugs, sucralfate, proton pump inhibitors, histamine type 2 receptor antagonists, or misoprostol. Moreover, subjects were excluded if they had abnormal findings on baseline endoscopy (total endoscopic score >1) or an abnormal baseline hematobiochemical profile (i.e., anemia, signs of coagulation disorders, serum creatinine level >1.5 mg/dL, and so on). A serum sample was obtained from each subject and analyzed for immunoglobulin G antibodies to *Helicobacter pylori* (EIAgen kit; Biochem Immunosystem Italia, SpA, Bologna, Italy). Serum antibody was reported as positive (present) or negative (absent). The sensitivity and specificity of the test, as reported by the manufacturer, were 95.7% and 98.4%.

Study Design

Subjects who fulfilled the admission criteria were randomized into 5 groups with 8 subjects each to receive the following: (1) placebo, (2) 400 mg NCX-4016 twice daily, (3) 800 mg NCX-4016 twice daily, (4) 200 mg aspirin twice daily, and (5) 420 mg aspirin twice daily. These doses contain the same amount of aspirin and release comparable amounts of salicylates and were chosen based on previous studies showing that 400 mg twice daily is the lower dose of NCX-4016 that inhibits COX-1 activity after oral administration.[20] Randomization was performed in blocks of 4 from a computer-generated list. The study medications were taken orally at 8 AM and 8 PM at the clinical site for 7 consecutive days. On the mornings of the first and last endoscopic examination, blood samples were collected for basal and posttreatment hematobiochemical analyses, platelet aggregation studies, serum thromboxane (TX) B_2 levels, and plasma salicylate and nitrite/nitrate levels. The last dose of study medication was taken the evening preceding the second (posttreatment) endoscopy. The use of any drug not included in the study was prohibited.

Endoscopy

After an overnight fast, endoscopic examination was performed using an Olympus Exera GIF-Q160 (Olympus Europe, Hamburg, Germany), with premedication with 3–5 mg intravenous midazolam (Dormicum; Roche, Basel, Switzerland). All subjects received a topical anesthesia with lidocaine 2% (Xylocaine; AstraZeneca, London, England) immediately before endoscopic intubation. Three experienced endoscopists (S.F., L.S., and A.M.) who were blinded to the assigned treatment performed all examinations. The entire stomach and duodenum was systematically examined from the fundus to the duodenum in a proximal to distal manner to minimize errors that might result from a misinterpretation of mucosal damage caused by passage of the instrument. Each endoscopic procedure was completely recorded using a VHS recorder. Hemorrhagic and erosive mucosal lesions were graded using a predetermined scale ranging from 0 to 4: grade 0, normal mucosa; grade 1, 1–3 erosions or submucosal hemorrhages; grade 2, 4–10 erosions or submucosal hemorrhages; grade 3, >10 erosions or submucosal hemorrhages; grade 4, ulcer or diffuse submucosal hemorrhages. Separate endoscopic injury scores, both for hemorrhagic and erosive lesions, were assigned for the esophagus, gastric fundus, gastric body, gastric antrum, and duodenum. Hemorrhagic lesions ranged from the presence of a few single submucosal hemorrhages to profuse bleeding, whereas erosive lesions ranged from one erosion to frank ulceration. Erosions and ulcers were defined as white-based, well-circumscribed mucosal breaks and were measured by close apposition of an endoscopic forceps with defined dimensions. An erosion was defined as a flat lesion with no discernible depth, and an ulcer was defined as a mucosal break ≥ 3 mm in greatest diameter with unequivocal depth.

Symptoms

Each subject received a diary card on which to record onset and intensity of daytime and nighttime heartburn, dysphagia, regurgitation, bloating, and epigastric pain. An arbitrary score from 0 (no symptoms) to 3 (severe symptoms that interfered with daily activity or nocturnal rest) was allocated.

Platelet Aggregation Studies and Platelet TXB_2 Generation

Blood was collected in the morning, just before the first (basal) and the second (posttreatment) endoscopy, in sodium citrate 3.8% (1:10 vol/vol) and centrifuged at 150*g* for 15 minutes to obtain platelet-rich plasma and at 900*g* for a further 10 minutes to obtain platelet-poor plasma. The platelet count in platelet-rich plasma was adjusted to 250×10^6/mL with platelet-poor plasma. Platelet aggregation induced by 600 μmol/L arachidonic acid (AA) was evaluated by the photometric method with an automated platelet aggregometer analyzer (HELENA PACKS-4, Beaumond, TX) as previously described.[21] The maximal amplitude of platelet aggregation at 5 minutes, as automatically calculated by the aggregometer, was recorded. After 5 minutes, aggregation was stopped and the sample immediately transferred to Eppendorf tubes and centrifuged at 12,000*g* for 5 minutes. The resulting supernatants were recovered and stored at −80°C for subsequent measurement of AA-stimulated platelet TXB_2 generation (see below).

Serum TXB_2

For the measurement of serum TXB_2 levels, the stable metabolite of TXA_2, blood samples were taken in the morning just before the first (basal) and the second (posttreatment) endoscopy. Every blood sample was drawn by a 21-gauge cannula inserted into the antecubital vein of each subject in Vacutainer tubes (Becton Dickinson BV, Leiden, The Netherlands). Non-anticoagulated blood samples were collected in a dry syringe and immediately transferred in a glass tube. The samples were allowed to clot for 1 hour at 37°C and then centrifuged at 2000*g* for 15 minutes. The supernatant serum was recovered and snap frozen at −80°C until assayed. Serum and AA-stimulated platelet TXB_2 concentrations were measured using a specific radioimmunoassay as previously described.[22]

Serum Salicylate and Nitrite/Nitrate Levels

For measurement of serum salicylate and nitrite/nitrate levels, blood samples were taken before and 12 hours after the last dose of each drug. Blood samples were centrifuged at 4200*g* for 10 minutes at 4°C. Specimens were subsequently stored at −20°C and assayed by high-performance liquid chromatography (Prostar 330; Varian, Rome, Italy) according to a previously published method.[22] Serum nitrite and nitrate concentrations were measured using a chemiluminometer according to a previous published method.[23]

Statistical Analysis

The data were analyzed using the SAS/STAT version 6.12 and SASonline version 8, html format (SAS Institute Inc., Cary, NC). The primary end points of the study were to compare the mucosal damage scores, platelet aggregation, and TXB_2 levels at day 7. The results for each treatment group are expressed as mean ± SE. Differences between endoscopic scores, TXB_2 levels in serum and platelet-rich plasma, and plasma salicylate and nitrite/nitrate concentrations among the treatment groups were tested using analysis of variance with contrast protected by Bonferroni-adjusted level for $\alpha = 0.05/2$ for each contrast or Kruskal–Wallis when more appropriate. Differences between treatment groups were considered significant with P values ≤ 0.05. The calculation of the sample size and power for one-way analysis of variance with contrast[24] was made with macro UnifyPow program (SAS Institute, Inc.) developed for Unifyed power analysis in SAS.[24] The planned sample size (total, n = 40) allocated 8 in the placebo group, 16 in the aspirin-treated group, and 16 in the NCX-4016–treated group, providing at least 79% power to detect a statistically significant difference in mucosal damage scores between aspirin and NCX-4016.

Results

All volunteers completed the study protocol. There were no statistically significant differences between the basal and end-of-treatment hematobiochemical values in each of the 5 groups (data not shown). Serum immunoglobulin G antibodies to *H. pylori* were found in 10 subjects: 3 in the placebo group, 4 in the aspirin-treated group, and 3 in the NCX-4016–treated group (not significant among groups).

Effect of Aspirin and NCX-4016 on Gastrointestinal Mucosa

No injury was visible in the stomach or duodenum in any of the 8 participants who received placebo (mean endoscopic score, 0.63 ± 0.16). In contrast, as shown in Figure 1, gastric and/or duodenal injury was found in all participants treated with aspirin 200 and 420 mg twice daily for 7 consecutive days; mean total endoscopic score was 11.0 ± 3.0 and 16.1 ± 1.6, respectively ($P < 0.0001$ vs. placebo). Administration of NCX-4016 caused only minimal gastrointestinal injury, similar to that observed in the placebo group, with a mean total endoscopic score of 1.38 ± 0.5 and 1.25 ± 0.3, respectively ($P < 0.0001$ vs. equimolar doses of aspirin) (Figure 1). Although lesions caused by aspirin were mainly located in the gastric antrum (Figure 2*A* and *B*), duodenal erosions and/or submucosal hemorrhages were observed in 60% of participants treated with aspirin (Figure 2*C* and *D*). Mucosal lesions induced by

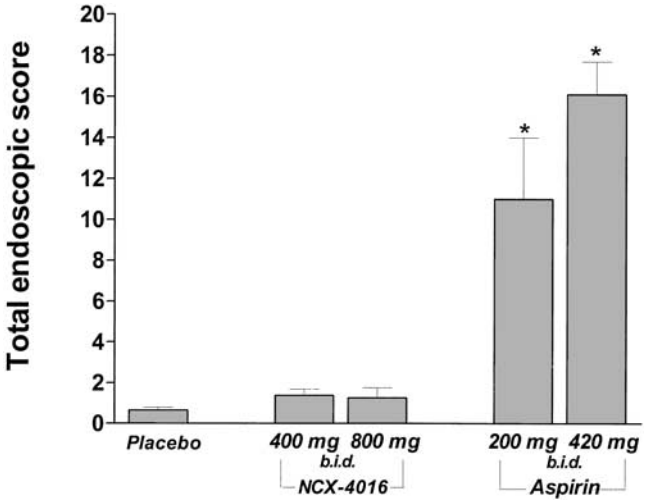

Figure 1. NCX-4016 spares gastrointestinal mucosal in healthy human volunteers. Mean total, gastric, and duodenal endoscopic damage score (erosive and hemorrhagic lesions) after 7 days of administration of placebo, NCX-4016 (400 and 800 mg twice daily), and aspirin (200 and 420 mg twice daily). n = 8 per group. $*P < 0.0001$, aspirin vs. placebo and NCX-4016. No significant difference was found between placebo and NCX-4016.

treatment with NCX-4016 followed a similar topographic distribution, mainly found in the antrum (Figure 2*A* and *B*), whereas the duodenum was almost completely spared (Figure 2*C* and *D*). Gastric and/or duodenal ulcers were seen in 50% of subjects treated with aspirin but not in participants treated with NCX-4016. In some subjects, aspirin-induced erosive/ulcerative lesions showed modest bleeding; this was never observed in subjects treated with NCX-4016.

Effect of Aspirin and NCX-4016 on Platelet Aggregation

After 7 days of treatment, AA-induced platelet aggregation was almost completely inhibited by aspirin and NCX-4016 (Figure 3*A*). The lower dose of NCX-4016 caused an 80% reduction of platelet aggregation, which was not statistically different ($P > 0.1$ between groups) from that observed with the equimolar dose of aspirin.

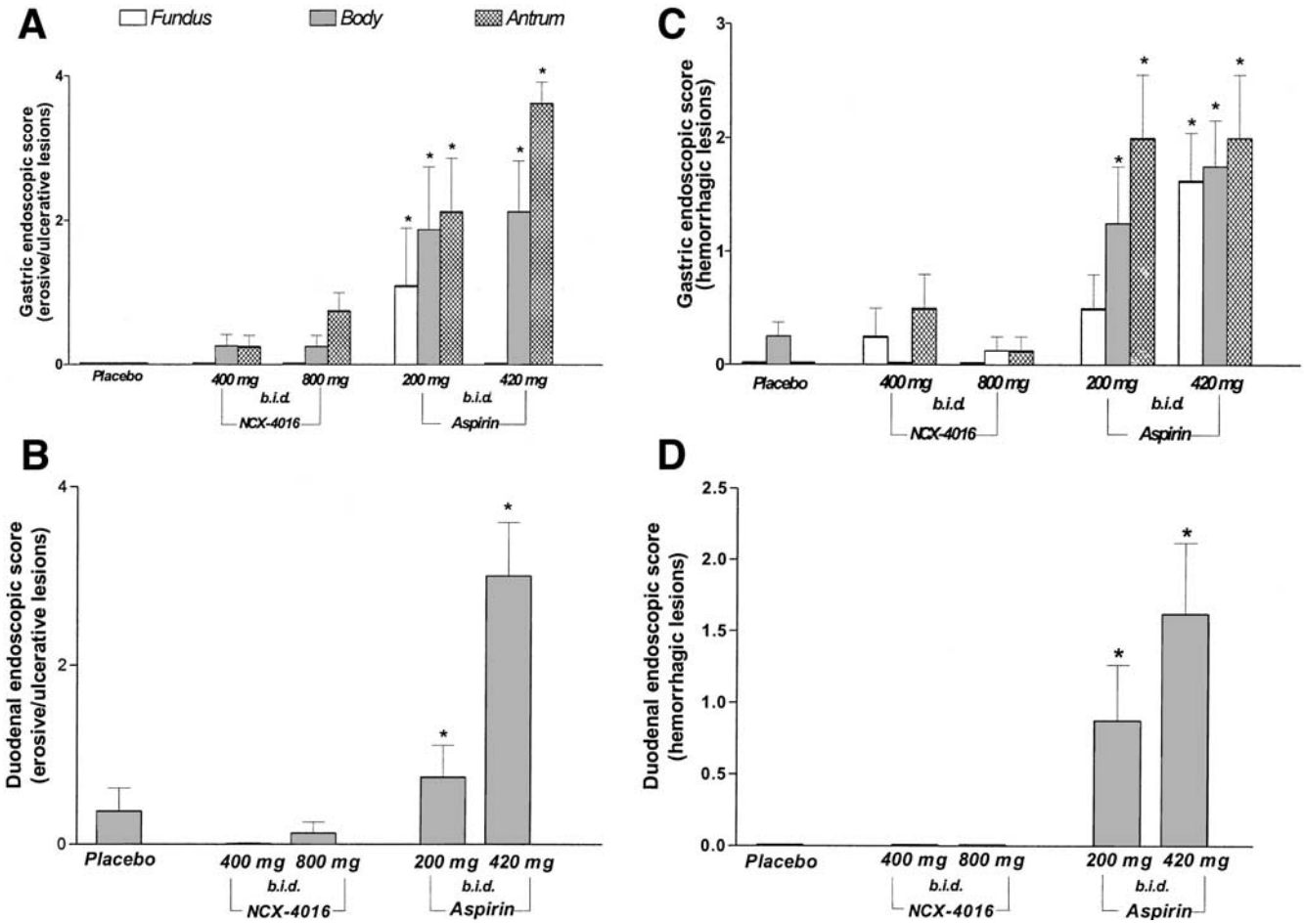

Figure 2. Gastric and duodenal endoscopic damage scores after 7 days of administration of placebo, NCX-4016 (400 and 800 mg twice daily), and aspirin (200 and 420 mg twice daily). (*A* and *B*) Gastric and duodenal erosive/ulcerative lesions. (*C* and *D*) Gastric and duodenal hemorrhagic lesions. n = 8 per group. $*P < 0.0001$, aspirin vs. placebo and NCX-4016. □, fundus; ■, body; ▨, antrum.

Effect of Aspirin and NCX-4016 on TXB_2 Serum Levels and TXB_2 Generation

TXB_2 serum levels at baseline did not differ significantly among groups. The lower dose of NCX-4016 caused a 75% reduction in serum TXB_2 concentration, whereas the higher dose and the 2 doses of aspirin reduced the serum concentrations of TXB_2 by ≈95% ($P < 0.001$ vs. placebo). NCX-4016 and aspirin were also equally effective in reducing the urinary excretion of 11-dehydro-TXB_2, a TXB_2 metabolite (data not shown).

AA-induced platelet TXB_2 generation was completely inhibited by both doses of NCX-4016 and aspirin (Figure 3*C*). The inhibition of TXB_2 generation was 98% with the lower dose and 100% with the higher dose of NCX-4016 and the 2 doses of aspirin ($P > 0.1$ between groups).

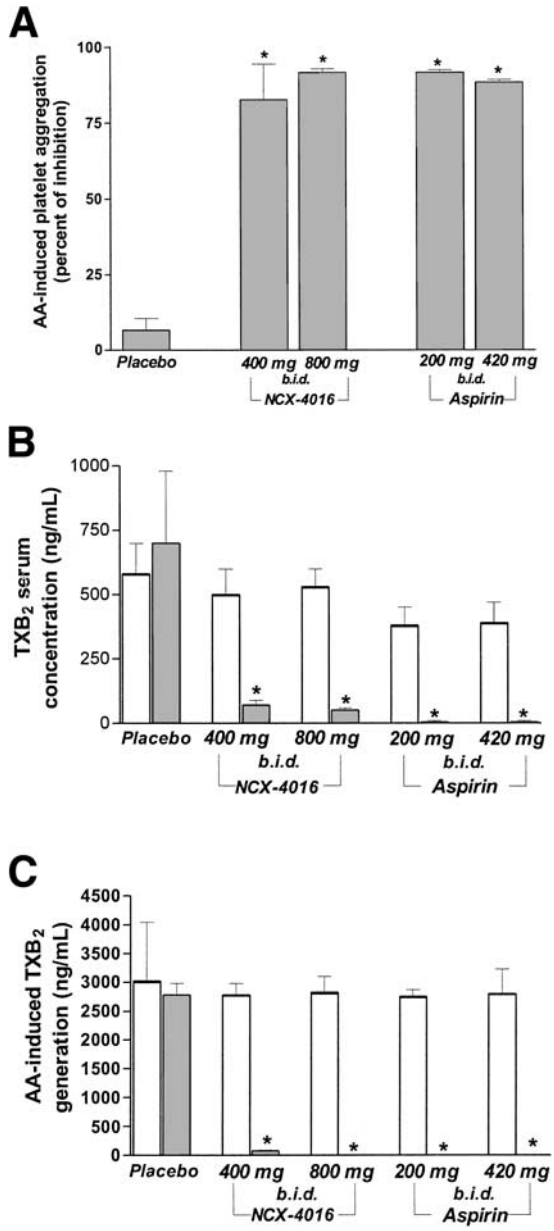

Figure 3. NCX-4016 exerts antiplatelet activity. (*A*) AA-induced platelet aggregation after 7 days of administration of placebo, NCX-4016 (400 and 800 mg twice daily), and aspirin (200 and 420 mg twice daily). n = 8. *$P < 0.0001$, aspirin and NCX-4016 vs. placebo. (*B*) Serum TXB_2 concentration before and 7 days after treatment with placebo, NCX-4016 (400 and 800 mg twice daily), and aspirin (200 and 420 mg twice daily). *$P < 0.0001$ vs. pretreatment. (*C*) AA-induced platelet TXB_2 generation before and 7 days after treatment with placebo, NCX-4016 (400 and 800 mg twice daily), and aspirin (200 and 420 mg twice daily). n = 8 per group. *$P < 0.0001$ vs. pretreatment.

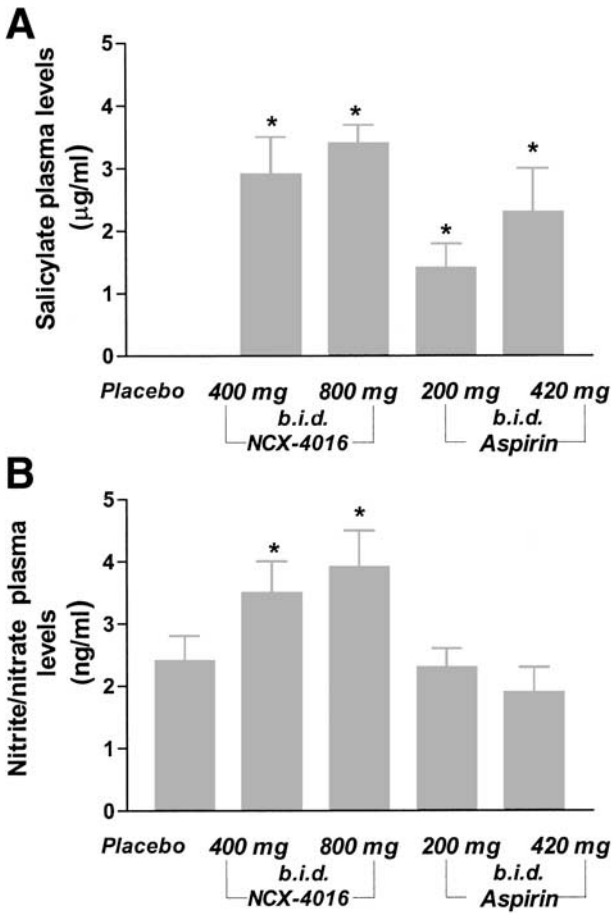

Figure 4. Effect of NCX-4016 and aspirin on salicylate and nitrite/nitrate plasma levels. Blood samples were taken 12 hours after the last dose of each drug. n = 8 per group. *$P < 0.01$ vs. placebo.

Salicylate and Nitrite/Nitrate Plasma Levels

At baseline, plasma salicylate levels were undetectable in all subjects (not shown). At the end of the study (Figure 4), although salicylates were not detected in the plasma of subjects treated with placebo, they were significantly increased in those treated with aspirin or NCX-4016 ($P < 0.001$, NCX-4016 and aspirin vs. placebo). Basal nitrite/nitrate plasma levels were similar in all groups (not shown). Nitrite/nitrate plasma levels

measured 12 hours after the last dose of each drug were significantly different from placebo and baseline only in subjects treated with NCX-4016 ($P < 0.01$, NCX-4016 vs. placebo and aspirin). Thus, NCX-4016 is absorbed and releases a detectable amount of NO in the systemic circulation.

Symptoms and Adverse Events

No subjects reported severe symptoms during the study. The most frequently reported adverse effect was mild heartburn and epigastric pain. No differences were observed among the active treatments with respect to the gastric discomfort ratings compiled after 7 days of treatment, the number of days on which subjects reported symptoms, and the number of subjects reporting gastric discomfort (data not shown). No major adverse events were reported by any subjects, and no relevant changes in vital signs (specifically, blood pressure and heart rate) between baseline and the final visit were observed in any group.

Discussion

The main finding of the present study was that administration of NCX-4016, an NO-releasing derivative of aspirin, to healthy human volunteers inhibits platelet function to the same extent as aspirin while nearly avoiding gastrointestinal mucosal damage. Although assessment of the gastrointestinal safety of NCX-4016 in patients with cardiovascular risk factors will require appropriately designed outcome trials,[2] the results of this pilot study represent the first human evidence that addition of an NO-releasing moiety to aspirin results in a new chemical entity that inhibits COX-1 activity to the same extent as aspirin while nearly avoiding gastrointestinal toxicity. Endoscopic evaluation after 1 week of treatment showed gastric and duodenal damage in 90% of healthy volunteers treated with aspirin, whereas no endoscopic lesions were found in participants administered equimolar doses of NCX-4016. Damage caused by aspirin was mainly localized into the body and antrum of the stomach, but duodenal lesions were recorded in ≈60% of subjects. By comparison, gastric damage caused by NCX-4016 was similar to that documented in subjects treated with placebo, resulting in an ≈90% reduction in the mean endoscopic score in comparison with aspirin, and none of the volunteers developed ulcers or duodenal damage. Consistent with the present results, 2 NO-releasing nonsteroidal anti-inflammatory drugs, NO-flurbiprofen (Fiorucci et al., manuscript in preparation) and NO-naproxen,[25] have recently been found to cause significantly fewer gastric and duodenal lesions than parent drugs, suggesting that addition of the NO-releasing moiety to anti-inflammatory drugs results in a general improvement in their gastrointestinal safety. Further supporting a mechanistic role of the NO moiety in protecting the gastric mucosa, the use of nitrovasodilator drugs in combination with low doses of aspirin or other nonsteroidal anti-inflammatory drugs significantly decreases the risk of upper gastrointestinal bleeding in high-risk patients.[26]

The mechanisms underlying the protective effect of NCX-4016 on the gastrointestinal mucosa were not investigated in the present study. However, animal studies have extensively shown that NCX-4016 spares the stomach of rats at doses that completely inhibit gastric mucosal COX-1 activity,[10] suggesting that mechanisms other than gastric mucosal prostaglandin preservation are involved in the gastrointestinal protection afforded by this drug.[8,10,13,15–17] Topical application of nonsteroidal anti-inflammatory drugs decreases gastric mucosal blood flow, an event that is believed to play a mechanistic role in the pathogenesis of the so-called "nonsteroidal anti-inflammatory drug–induced gastropathy."[17] NO-aspirin not only prevents this detrimental effect but even increases gastric mucosal blood flow in rodents, an effect that is related to the local release of NO.[27–29] Although preservation of gastric mucosal blood flow is an important mechanism, NO is also known for its inhibitory activity on neutrophil function and down-regulates the expression of adhesion molecules required for leukocyte adherence to the endothelium, an important step involved in the process of targeting neutrophils to the gastric microcirculation.[30,31]

Because NCX-4016 consists of 2 active moieties and NO exerts antiplatelet activities, it can exert its antithrombotic effect through several mechanisms involving COX-dependent and COX-independent, NO-mediated pathways.[8] In the present study, we have shown that 800 mg NCX-4016 twice daily inhibits COX-dependent platelet function to a similar extent as an equimolar dose of aspirin. At this dose, NCX-4016 caused a 90% inhibition of AA-induced platelet aggregation and an almost complete suppression of TXB_2 generation. The slight discrepancy between the degree of TXB_2 suppression when assessed in serum or by AA-stimulated platelets suggests that part of the platelet inhibitory activity of NCX-4016 is due to an NO-mediated mechanism.[8,32,33] In contrast to aspirin, NCX-4016 increases platelet guanosine 3′,5′-cyclic monophosphate concentrations[8] and inhibits platelet aggregation in aspirin-resistant assays, such as aggregation induced by adenosine diphosphate and/or U46619.[8,9,11,12,32,33] Furthermore, similar

to conventional NO donors but in striking contrast to aspirin, NCX-4016 inhibits the expression of P-selectin[11,20] and glycoprotein IIb-IIIa on platelet surface[8,11,33] and tissue factor activity and expression on monocytes and endothelial cells.[34,35] Consistent with this extended spectrum of activity, it has been shown that NCX-4016 not only reduces P-selectin plasma concentrations in healthy human volunteers[20,36] but that NCX-4016 is significantly more effective than aspirin in suppressing thromboembolism in a rodent model of pulmonary thrombosis[32] and in preventing carotid restenosis after balloon angioplasty in hypercholesterolemic mice.[37,38] Taken together, these data indicate that NCX-4016 inhibits platelet function through COX-dependent and COX-independent (NO-mediated) mechanisms.[8]

In summary, the present study shows that NCX-4016, an NO-releasing derivative of aspirin, is virtually devoid of gastrointestinal toxicity in healthy human volunteers, while it inhibits AA-induced platelet aggregation to the same extent as aspirin. Whether long-term administration of NCX-4016 is truly safer than aspirin, while maintaining cardioprotection and clinical efficacy, will require long-term appropriately designed outcome trials.

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Riolan of the Arc of Riolan

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Jean Riolan the Younger (1580–1657) was born in Paris, the son of a dean of the medical faculty. Two years after earning his doctorate at the University of Paris, young Riolan was named to the Regius Chair in Anatomy and Botany by Henry IV. In addition, he was appointed principal physician to the Queen Mother Marie de Médici, whom he accompanied on travels throughout Europe. In London, he became acquainted with William Harvey who proclaimed Riolan as "the prince of anatomists." Later, because of his extreme conservatism and stubborn adherance to outmoded Galenic concepts, Riolan allied himself with those who denounced Harvey's elucidation of the circulatory system. Riolan was the only one of his critics to whom Harvey deigned to reply in writing. Ironically, Riolan's name would now be forgotten were it not for his description of the anastomosis between branches of the superior and inferior mesenteric arteries in the mesocolon, now known as the "arc of Riolan."

—Contributed by WILLIAM S. HAUBRICH, M.D.
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