Methods and Findings in Experimental and Clinical Pharmacology 2010, 32(2): ???-???

CCC: 0379-0355/2010
DOI: 10.1358/mf.2010.32.2.1423883

ORIGINAL ARTICLE

ACEMETACIN ANTINOCICEPTIVE MECHANISM IS NOT RELATED TO NO OR K^+ CHANNEL PATHWAYS.

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SUMMARY

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) used for the treatment of acute gout and inflammation. However, its use is limited due to side effects. Acemetacin is a prodrug of indomethacin that exhibits better gastric tolerability in preclinical and clinical trials. The aim of this study was to examine if the systemic administration of acemetacin involved the sequential participation of nitric oxide (NO) or K^+ channel pathways to confer its antinociceptive effect, as compared to indomethacin. The antinociceptive effect of both drugs was studied with the formalin test. Equimolar doses of acemetacin or indomethacin were administered orally. The intraplantar administration of either L-NAME, glibenclamide, apamin or charybdotoxin plus indomethacin or acemetacin was studied using the formalin test and the anti-inflammatory and antihyperalgesic effects were measured. The antinociceptive effect of acemetacin or indomethacin was not significantly different when pretreatment with L-NAME, glibenclamide, apamin or charybdotoxin was done. The antihyperalgesic and anti-inflammatory effects were also similar for both indomethacin and acemetacin. Our results suggest that the antinociceptive effect of indomethacin or acemetacin is not mediated by NO or K^+ channel activation.

INTRODUCTION

Several drugs have been developed for the treatment of pain and inflammation. Despite their adverse effects, nonsteroidal anti-inflammatory drugs (NSAIDs) are the first option for the treatment of inflammatory diseases such as acute gout and arthritis. Even with the market introduction of selective cyclooxygenase COX-2 inhibitors, the gastrointestinal risk observed with the use of NSAIDs has not been completely abolished. Gastric damage induced by NSAIDs is due to the suppression of prostaglandins (PGs) (1, 2), in particular PGE_2, synthesized from COX-2, which induces edema at the inflammation site. In addition, PGE_2 acts synergistically with other mediators to sensitize receptors on afferent nerve endings to cause inflammatory pain (3). The inhibition of PG synthesis by NSAIDs is the main mechanism whereby most of them induce anti-inflammatory and antinociceptive effects. Furthermore, it has been shown that the antinociceptive effect of NSAIDs is due to a blockade of the local synthesis of PG induced by inflammatory stimuli, thus preventing the development of hyperalgesia (4). However, there is evidence that additional PG-independent mechanisms are involved in the antinociceptive action of some NSAIDs. Tonussi and Ferreira reported that the local administration of diclofenac, but not indomethacin, resulted in antinociception and that this effect could be blocked by local pretreatment with inhibitors of the synthesis of either nitric oxide (NO) or cyclic guanosine monophosphate (cGMP) (5). Furthermore, the activation of the NO–cGMP–potassium channel pathway is involved in the antinociceptive effects produced by certain NSAIDs, such as diclofenac, meloxicam and metamizol (6-9). However, not all NSAIDs follow the same mechanism. We have shown that the NO–cGMP–potassium channel pathway is not involved in the antinociceptive effect of indomethacin (9).

Recently, some studies on the anti-inflammatory effects of acemetacin have been performed. Acemetacin is a carboxymethyl ester derivative of indomethacin (10, 11). Substitution on the carboxylic group of indomethacin has resulted in a favorable efficacy–safety profile for acemetacin as compared to indomethacin (12, 13) and similar gastric safety as celecoxib (14). It has been further demonstrated that acemetacin and indomethacin differentially affect LTB_4 synthesis in the zymosan air pouch model, but both drugs display a similar anti-inflammatory effect and PG inhibition (15). In this model, orally administered indomethacin but not acemetacin significantly increased LTB_4 levels, even though acemetacin was completely biotransformed to indomethacin after 1 h of administration (15). Acemetacin has been reported to act by different mechanisms as compared to indomethacin in previous reports. The aim of this study

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was therefore to examine whether the antinociceptive effect of systemically administered acemetacin involved the sequential participation of NO followed by K^+ channel opening, as compared to indomethacin.

MATERIALS AND METHODS

Animals

Male Wistar rats aged 8-9 weeks (weight range, 200-220 g) from our own breeding facilities were used in this study. Animals had free access to food and drinking water before experiments. Efforts were made to minimize animal suffering and to reduce the number of animals used. Rats were used once only. At the end of the experiments, rats were sacrificed in a CO_2 chamber. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (16) and the protocol was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F., México).

Drugs

Acemetacin, indomethacin, glibenclamide, charybdotoxin and apamin were obtained from Sigma Aldrich (St. Louis, MO, USA). N^G—nitroarginine methyl ester (L-NAME) was purchased from RBI (Natick, MA, USA). Acemetacin and indomethacin were dissolved in Tween 20 and buffer solution (sodium hydroxide and monobasic potassium phosphate). Charybdotoxin, apamin and L-NAME were dissolved in saline. Glibenclamide was dissolved in 20% dimethylsulfoxide (DMSO).

Measurement of antinociceptive activity

Antinociception was assessed using the formalin test. Rats were placed in open Plexiglas observation chambers for 30 min to allow them to adjust to their surroundings; they were then removed for formalin administration. Fifty µL of diluted formalin (1%) was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers and nociceptive behavior observed immediately after formalin injection. Mirrors were placed behind each chamber to enable unhindered observation of the formalin-injected paw. Nociceptive behavior was quantified as the number of flinches of the injected paw at 1-min periods for 5 to 60 min after injection (9, 17, 18). Flinching was readily identified and characterized as a rapid and brief withdrawal of or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. The initial acute phase (0-10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15-60 min).

Effect of K^+ channel blockers and L-NAME on the antinociceptive effect of acemetacin and indomethacin

Rats received vehicle (1 mL) or increasing oral doses of acemetacin or indomethacin (8.4-279.5 µmol/kg), 60 min before formalin injection. To determine whether the acemetacin- and indomethacin-induced antinociception was mediated by either NO or K^+ channels, the effect of local peripheral pretreatment (10 min before formalin injection) with the appropriate vehicle or L-NAME (100 µg/paw), glibenclamide (100 µg/paw), charybdotoxin (1 µg/paw) and apamin (1 µg/paw) on the systemic antinociceptive effect induced by acemetacin and indomethacin (83.8 µmol/kg) was assessed. Glibenclamide blocks ATP-sensitive potassium channels (19), whereas charybdotoxin and apamin block large- and small-conductance Ca^{2+}-activated potassium channels, respectively (20). Drugs were injected in a volume of 1 mL for systemic administration and 50 µL for local peripheral administration. Doses and drug administration schedules were selected based on previous reports (21). The observer was unaware of the treatment in each animal. Rats in all groups were tested for possible side effects such as reduction of righting, stepping and corneal reflex before and 30 min after formalin injection.

Carrageenan paw-induced inflammation

Carrageenan (100 µL of a 1% w/v solution, prepared in sterile saline) was injected into the hind footpad of rats under halothane anesthesia. Paw volume was measured prior to any treatment, immediately before carrageenan administration, and at intervals of 1 h for 6 h thereafter using an Ugo Basile Model 7140 plethysmometer (Comerio, Italy). The person performing these measurements was unaware of the treatments that the rats had received. Groups of at least 6 rats each were treated orally 60 min before carrageenan administration with vehicle or increasing doses of indomethacin or acemetacin (8.4-279.5 µmol/kg).

Thermal hyperalgesia

Thermal hyperalgesia was determined according to Hargreaves et al. (22) using a Plantar Test Apparatus (Ugo Basile, Verese, Italy). Rats were placed in plastic cages (22x17x14 cm) with a glass floor. After a 30-min habituation period, the rats were removed for vehicle or carrageenan administration. One hundred mL of 0.9% saline or carrageenan solution (1%) was injected s.c. into the plantar surface of the right hind paw. The animals were then returned to the chambers and the treated paw was exposed to a beam of radiant heat through the glass floor. A photoelectric cell detected light reflected from the paw and turned off the lamp when paw movement interrupted the reflected light. The paw withdrawal latency (PWL) was automatically displayed to the nearest 0.1 s. The cut-off time was 25 s. PWLs were determined at various time points following vehicle or carrageenan administration up to 6 h. Rats received vehicle (carboxymethylcellulose 0.5% p.o.) or increasing doses of indomethacin (8.4-279.5 µmol/kg p.o.) or acemetacin (8.4-279.5 µmol/kg p.o.) 20 min before carrageenan administration into the plantar surface of the right hind paw.

Data and statistical analysis

All results are presented as mean ± S.E.M. for at least six animals per group. Curves were made plotting the pharmacological effects against time. In the formalin test the area under the number of flinches against time curves was calculated by the trapezoidal rule. Analysis of variance followed by Tukeys test was used to compare the differences between treatments. Differences were considered to achieve statistical significance when $P < 0.05$.

RESULTS

Effect of L-NAME, glibenclamide, apamin and charybdotoxin on the systemic antinociceptive effect of acemetacin and indomethacin

Local injection in rats of 1% formalin produced a typical flinching behavior. The first phase started immediately after the administration of formalin and then diminished gradually in about 10 min. The second phase started at about 15 min and lasted until 1 h. Oral acemetacin or indomethacin produced a dose-dependent reduction in the flinching behavior otherwise observed after formalin injection (Figs. 1 and 2). Treatments with the NSAIDs significantly reduced the number of flinches during phase 2 ($P < 0.05$), but not during phase 1 ($P > 0.05$). Therefore, only data from phase 2 were submitted for further analysis. Pretreatment with L-NAME, glibenclamide, charybdotoxin or apamin did not abolish antinociception due to acemetacin or indomethacin treatment (Fig. 3). No side effects were observed in any of the studied groups of animals.

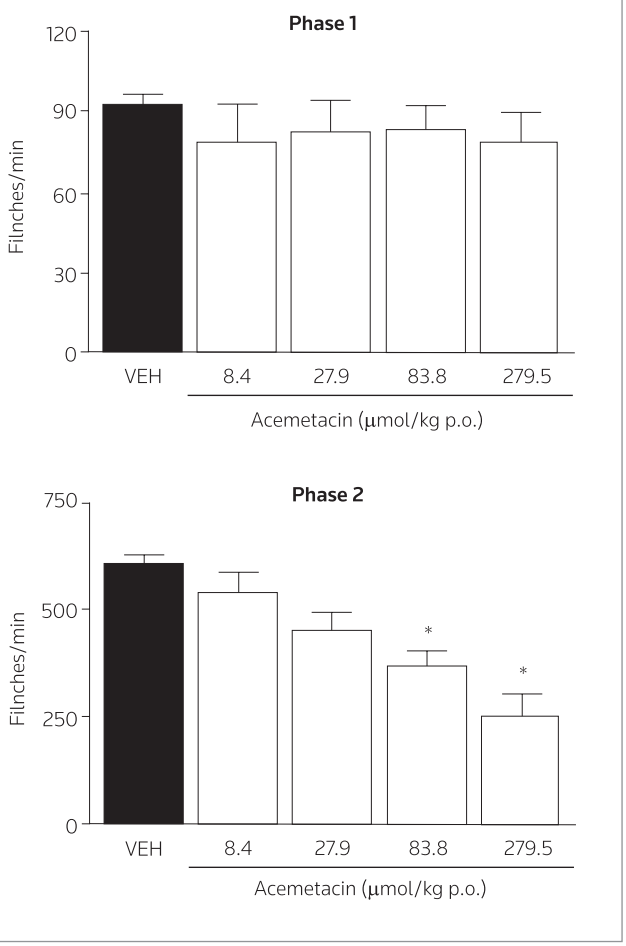

Figure 1. Antinociceptive effect observed after oral administration of acemetacin in the formalin test. Rats were pretreated with vehicle or acemetacin p.o. before formalin injection. Data are expressed as the area under the number of flinches against time curve. Bars are the mean ± SEM for 6 animals. *Significantly different from vehicle group ($P < 0.05$), as determined by analysis of variance followed by Tukeys test. VEH, vehicle.

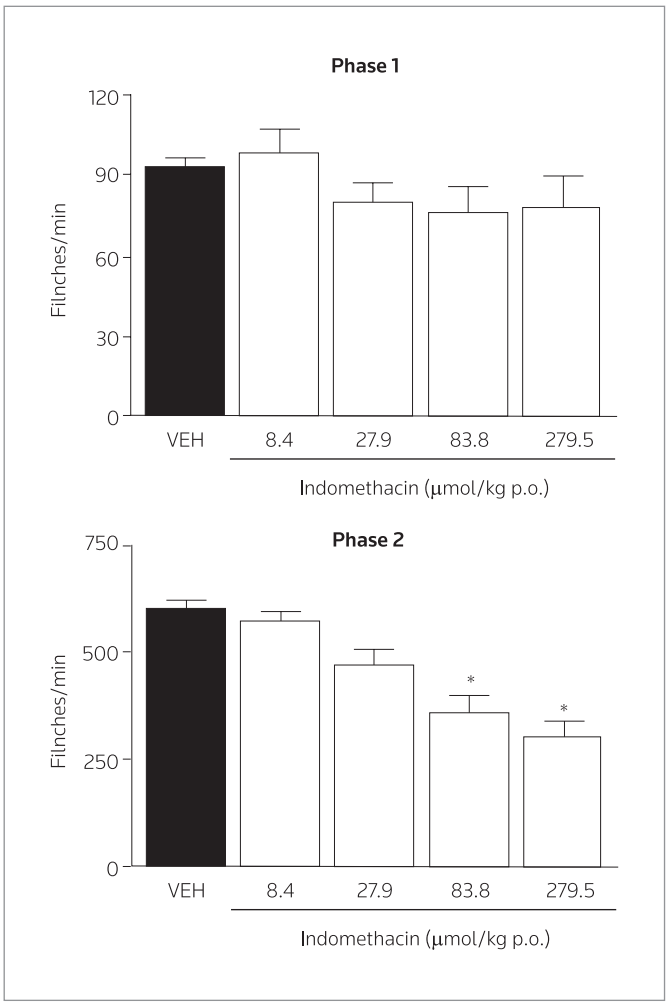

Figure 2. Antinociceptive effect observed after oral administration of indomethacin in the formalin test. Rats were pretreated with vehicle or indomethacin p.o. before formalin injection. Data are expressed as the area under the number of flinches against time curve. Bars are the mean ± SEM for 6 animals. *Significantly different from vehicle group ($P < 0.05$), as determined by analysis of variance followed by Tukeys test. VEH, vehicle.

Anti-inflammatory effect of acemetacin and indomethacin

Injection of carrageenan into the hind footpads of rats resulted in a rapid and marked increase in paw volume as a consequence of edema formation. The increase in paw volume could be significantly reduced by pretreatment with either acemetacin or indomethacin in a dose-dependent manner ($P < 0.05$). Edema formation was reduced by 70% by indomethacin (83.8 μmol/kg) and by 80% using acemetacin (83.8 μmol/kg) (Fig. 4).

Indomethacin and acemetacin effect on carrageenan-induced thermal hyperalgesia

Intraplantar injection of carrageenan (100 μL, 1%) into the right hind paw, but not saline in the contralateral paw, produced a time-dependent thermal hyperalgesic effect. Oral acemetacin or indomethacin administration, but not vehicle, produced a dose-

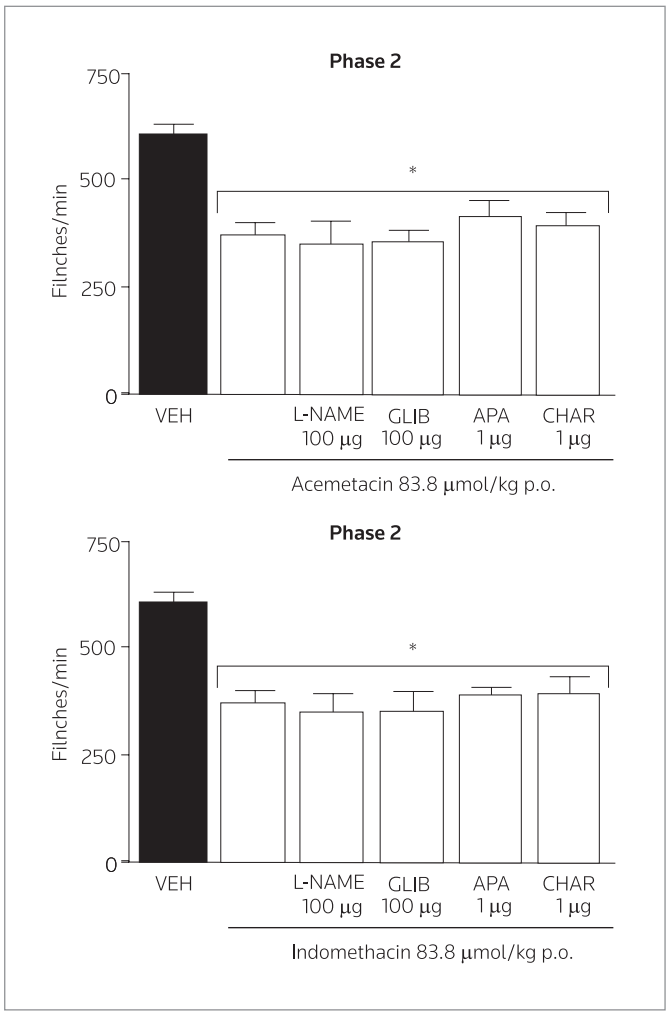

Figure 3. Antinociceptive effects of indomethacin or acemetacin in the absence or presence of the inhibitor of NO synthase L-NAME and the potassium channel blockers glibenclamide (GLIB), apamin (APA) and charybdotoxin (CHAR). Data are expressed as the area under the number of flinches against time curve. Bars are the mean ± SEM for 6 animals. *Significantly different from vehicle group ($P < 0.05$), as determined by analysis of variance followed by Tukeys test.

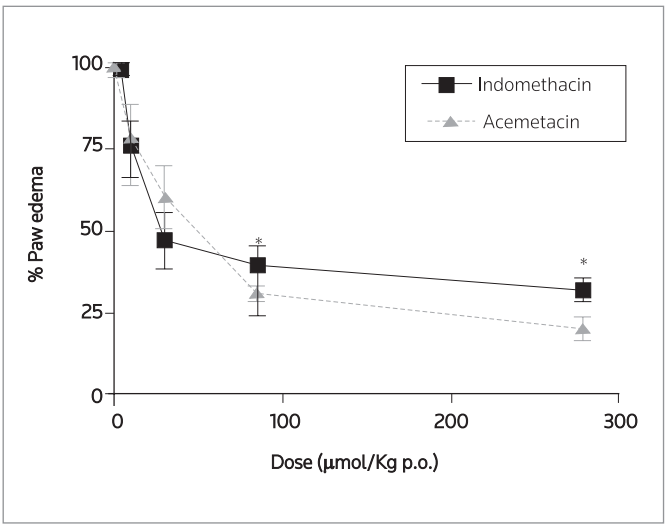

Figure 4. Effect of indomethacin or acemetacin on carrageenan-induced paw edema in the rat. Each point represents the mean ± SEM of 6 rats. The percentage of edema in the paw was calculated from the area under dose?response curves, in comparison to the group of rats treated with the vehicle. *$P < 0.05$ vs. control (no administration of the drug).

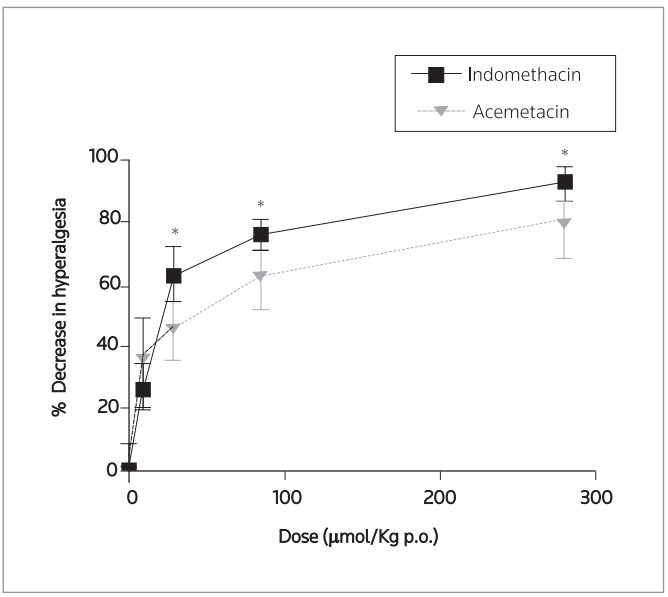

Figure 5. Antihyperalgesic effect of acemetacin and indomethacin using the Hargraves test. Each point represents the mean ± SEM of 6 rats. *$P < 0.05$ vs. control (no administration of the drug).

dependent reduction in the hyperalgesic effect induced by carrageenan ($P < 0.05$). Both treatments exhibited similar efficacy in the carrageenan-induced thermal hyperalgesia model in the rat (Fig. 5).

DISCUSSION

Acemetacin is a carboxymethyl ester derivative of indomethacin (10, 11). In clinical studies, acemetacin exhibits comparable anti-inflammatory efficacy as indomethacin, but displays a better gastric tolerability (12, 15). Acemetacin similarly inhibits COX-1 and COX-2 at the inflammation site (15); however, it exhibits a different mechanism of PG inhibition as compared to indomethacin. Moreover acemetacin has no effect on LTB_4 production, while indomethacin increases the production of LTB_4 (15). Similarly, acemetacin has been reported to cause no rise in TNF-α level as is seen with indomethacin (23). These effects are not related to the delay in the availability of indomethacin after oral acemetacin administration. We have recently reported that acemetacin is biotransformed to indomethacin within a few minutes of oral administration and complete metabolism to indomethacin

occurs within an hour (24). Some years ago, the anti-inflammatory and antinociceptive effects of NSAIDs were attributed exclusively to the inhibition of prostaglandins. Recent reports, however, state that several NSAIDs, such as lumiracoxib, present antinociceptive effects that are independent of PGs (25).

It has been published recently that local or intrathecal pretreatment with L-NAME, ODQ (a guanylyl cyclase inhibitor), glibenclamide, charybdotoxin and apamin significantly prevented lumiracoxib-induced antinociception (26). Our group has shown that the L-arginine–NO–cGMP–potassium channel pathway participates in the peripheral antinociceptive effect of diclofenac but not indomethacin (9). Indomethacin and acemetacin differ in their effects on gastric tolerability (15). In our study, acemetacin exhibited comparable antihyperalgesic, anti-inflammatory and antinociceptive effect at equimolar doses as indomethacin. However, at the doses tested for acemetacin or indomethacin, NO or K^+ channel activation was not involved in the antinociceptive effect following oral administration, in contrast to other NSAIDs such as diclofenac and lumiracoxib (6, 9, 23).

Our group has recently reported equipotent anti-inflammatory effects using acemetacin and indomethacin (15). In our study, acemetacin also showed a similar antihyperalgesic effect as indomethacin at equimolar doses using the Hargreaves model in rats. We have also tested the anti-inflammatory effect of acemetacin in the carrageenan paw edema test, reporting that acemetacin generates comparable anti-inflammatory effects as indomethacin.

To conclude, although acemetacin does not increase LTB_4 synthesis in the zymosan air pouch model (15), its antinociceptive effect is unrelated to NO- or K^+ channel activation. Furthermore, the antihyperalgesic and anti-inflammatory effects of indomethacin and acemetacin are comparable after oral administration. Acemetacin has proven to be an excellent therapeutic option for the treatment of inflammation and pain. It exhibits better gastric tolerability than indomethacin; these properties make acemetacin a safe therapy for pain and inflammation (12, 15).

DISCLOSURES

The authors state no conflict of interest.

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