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• Research Article

Evidence against the participation of a pharmacokinetic interaction in the protective effect of single–dose curcumin against gastrointestinal damage induced by indomethacin in rats

Liliana Zazueta-Beltrán[1], Lorena Medina-Aymerich[1], Nadia Estela Díaz-Triste[1], Aracely Evangelina Chávez-Piña[2], Gilberto Castañeda-Hernández[1], Leticia Cruz-Antonio[3]

1. Department of Pharmacology, Center for Research and Advanced Studies of the National Polytechnic Institute, 07360 Mexico City, Mexico
2. Laboratory of Pharmacology, Institutional Program in Molecular Biomedicine, National School of Medicine and Homeopathy of the National Polytechnic Institute, Academic Area of Medicine of the Institute of Health Sciences, 07320 Mexico City, Mexico
3. Superior Studies Faculty of Zaragoza, Universidad National Autonomous of Mexico, 09230 Mexico City, Mexico

ABSTRACT

OBJECTIVE: To determine the role of a pharmacokinetic interaction in the protective effect of curcumin against the gastric damage induced by indomethacin administration as such or as its prodrug acemetacin.

METHODS: Wistar rats orally received single dose of indomethacin (30 mg/kg) with and without curcumin (30 mg/kg); gastric injury was evaluated by determining the total damaged area. Additional groups of rats received an oral single dose of indomethacin (30 mg/kg) or its prodrug acemetacin (34.86 mg/kg) in the presence or absence of curcumin (30 mg/kg). Indomethacin and acemetacin concentrations in plasma from blood draws were determined by high-performance liquid chromatography. Plasma concentration-against-time curves were constructed, and bioavailability parameters, maximal concentration (C_{max}) and area under the curve to the last sampling time (AUC_{0-t}) were estimated.

RESULTS: Concomitant administration of indomethacin and curcumin resulted in a significantly reduced gastric damage compared to indomethacin alone. However, co-administration of curcumin did not produce any significant alteration in the bioavailability parameters of indomethacin and acemetacin after administration of either the active compound or the prodrug.

CONCLUSION: Curcumin exhibits a protective effect against indomethacin-induced gastric damage, but does not produce a reduction of the bioavailability of this nonsteroidal anti-inflammatory drug, indomethacin. Data thus suggest that a pharmacokinetic mechanism of action is not involved in curcumin gastroprotection.

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Correspondence: Leticia Cruz-Antonio, PhD; E-mail: letycruza@yahoo.com.mx

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1 Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world. However, NSAID use is frequently curtailed by gastrointestinal irritation. Present strategies to diminish NSAID risk include co-administration of gastroprotective agents such as H2-receptor antagonists, misoprostol or proton pump inhibitors.[1,2] Proton pump inhibitors appear to have the most favorable outcome in terms of efficacy and quality of life.[3] However, the use of proton pump inhibitors is not without risk. It has been reported that these agents may exacerbate NSAID-induced small intestine injury by dysbiosis.[4] Therefore, the quest for gastroprotective agents is still ongoing.

It has been reported that several plant-derived products may reduce NSAID-induced gastrointestinal damage.[5–7] There is evidence that curcumin (diferuloylmethane), the most abundant curcuminoid isolated from *Curcuma longa* L (Zingiberaceae), exhibits a protective action against indomethacin-induced gastric and small intestinal damage.[8,9] Morsy et al.[10] observed that curcumin increases nitric oxide levels in the gastric mucosa. Further research by our group has shown that curcumin activates the nitric oxide-cyclic GMP-potassium channel pathway, yielding a gastroprotective effect comparable to that of omeprazole.[11]

Indomethacin is one of the most effective NSAIDs. It is also highly damaging to the gastrointestinal tract. Hence, administration of its prodrug, acemetacin, allowing maintaining indomethacin efficacy while attenuating, but not eliminating gastrointestinal damage, is an alternative.[12–14] The available evidence suggests that co-administration of indomethacin or acemetacin with a single oral dose of curcumin may lead to favorable results in terms of gastrointestinal safety. It has been reported that the gastroprotective effect of curcumin against indomethacin-induced gastric damage is due to a pharmacodynamic mechanism of action.[10,11] However, it is also plausible that diminished gastric damage could be due to a reduction in indomethacin bioavailability after its administration as indomethacin, or as the prodrug acemetacin. It has been reported that curcumin co-administration increases the absorption and retention of certain drugs, such as glibenclamide,[15] impairs gastric emptying,[16] and reduces presystemic drug metabolism.[17] Hence, in order to determine if pharmacokinetic changes are involved in the gastroprotective effect of single-dose curcumin, we examined gastric injury after oral administration of indomethacin in the presence and absence of curcumin, as well as indomethacin bioavailability in rats after oral administration of either indomethacin or acemetacin in the presence and absence of curcumin.

2 Materials and methods

2.1 Animals

Fifty-four male Wistar rats, aged 10–12 weeks (weight range: 200–250 g), from our own breeding facilities, were used in this study. Rats were housed in cages at the animal holding unit under controlled temperature and 12-hour dark/light cycle. Each rat was used in only one experiment and euthanized in a carbon dioxide (CO_2) chamber at the end of the experiment. The protocol was approved by the Institutional Animal Care and Use Committee (Approval number 0169-15, CINVESTAV-IPN).

2.2 Drugs

Curcumin (≥ 80% curcumin, ≥ 94% curcuminoids content), acemetacin and indomethacin were purchased from Sigma Aldrich (Saint Louis, MO, USA). For oral administration to the experimental animals, drugs were suspended in 5% carboxymethyl cellulose (CMC) and given by gavage. Indomethacin and acemetacin solutions for analytical determinations were prepared by dissolution in a 5% bicarbonate solution. All solvents used were of chromatographic grade. Other reagents were of analytical grade. High-quality water, employed to prepare solutions, was obtained using a Milli-Q Reagent Water System (Merck Millipore Corporation, Mexico City, Mexico).

2.3 Gastric protective effect of curcumin on indomethacin-induced gastric injury

Gastric damage and gastroprotection were determined as described previously.[11] Independent groups of 7 rats received an oral administration of the vehicle, indomethacin (30 mg/kg) or curcumin (30 mg/kg) 30 min before receiving indomethacin (30 mg/kg). Three hours after the administration of the drugs, each rat was euthanized in a

CO_2 chamber. The stomach was removed and the extent of hemorrhagic damage was scored by an observer who was unaware of the treatments that each rat had received. The length (mm) of all hemorrhagic lesions was measured, to yield a gastric damage score for each rat stomach, that was obtained by summing these values.[11,18]

2.4 Pharmacokinetic study design

Animals were divided into four groups of eight rats each. Groups 1 and 2 received a single oral dose 30 mg/kg of indomethacin, while groups 3 and 4 received a single oral dose 34.86 mg/kg of acemetacin. Indomethacin and acemetacin doses were equimolar in terms of indomethacin equivalents.[12,19] Groups 2 and 4 were pre-treated with a single oral dose of (30 mg/kg) curcumin 30 min before receiving the NSAIDs. The curcumin dose was selected due to the gastrointestinal effect observed by Díaz-Triste et al.[11] Groups 1 and 3 were pre-treated with 5% CMC (CMC as vehicle).

A cannula was inserted in the caudal artery to allow blood sampling.[19] Blood samples (200 μL) were drawn 5, 10, 15, 20, 30, 45, 60, 120, 240, 480, 720, 1 440, 1 620 and 1 800 min after drug administration. The extracted volume was immediately replaced with isotonic saline to avoid changes in the circulating volume. Whole blood was immediately centrifuged at 12 000×*g* for 15 min for plasma separation. Plasma samples were frozen at –20 °C until assayed.

2.5 Analysis of acemetacin and indomethacin in plasma

Plasma concentrations of acemetacin and indomethacin were determined by a high-performance liquid chromatography method previously described.[12,19] Briefly, whole plasma samples (100 μL) were placed into 1.5 mL Eppendorf tubes and spiked with 75 μL of a 16 μg/mL carbamazepine solution, as an internal standard. Then, 825 μL of methanol was added to precipitate the proteins; samples were vortexed at maximum speed for 1 min, and centrifuged at 12 000×*g* for 15 min. Finally, the supernatant was transferred to a clean tube and 60 μL aliquots were injected into the chromatographic system.

The chromatographic system consisted of an Xterra MS C_{18} column (4.6 mm × 100 mm ID, particle size 3.5 μm; Waters Assoc., Milford, MA, USA) eluted with a mobile phase consisting of a mixture of 0.025 mol/L phosphate buffer (pH 6.0) and methanol, 45:55 (v/v) at a constant flow of 1.2 mL/min and at room temperature. Retention time was 2.04, 3.80 and 4.90 min for carbamazepine (internal standard), indomethacin and acemetacin, respectively.

2.6 Data analysis and statistics

Gastric injury data are expressed as scores (*vide supra*). Comparisons of gastric damage scores between different experimental groups of rats were performed by analysis of variance followed by the Newman-Keuls test.

Plasma concentration-against-time plots were constructed for each rat; the maximum concentration (C_{max}) and the time to achieve this peak (T_{max}) were directly determined from these plots. The area under the curve to the last sampling time (AUC_{0-t}) was estimated by the trapezoidal rule.[7] Data are expressed as mean ± standard error of mean. Comparisons of acemetacin and indomethacin bioavailability parameters, observed in absence and presence of curcumin, were carried out by the Student's *t*-test. Differences were considered to be statistically significant when $P < 0.05$.

3 Results

3.1 Gastric protective effect of curcumin

Gastric damage scores are shown in Figure 1. The vehicle did not induce any gastric damage, yielding a score of zero. Indomethacin resulted in the formation of hemorrhagic erosions in gastric mucosa, producing a significant increase of the gastric damage score. Curcumin co-administration significantly reduced indomethacin-induced gastric injury (Figure 2).

3.2 Effect of curcumin on indomethacin oral bioavailability

Indomethacin plasma concentrations in the absence and presence of curcumin are shown in Figure 3. Plasma concentration-against-time curves were comparable in the presence and absence of curcumin. Bioavailability parameters are given in Table 1. Curcumin co-administration did not induce any significant differences in C_{max} or AUC_{0-t}. Notwithstanding, it induced a significant longer time (T_{max}) to reach the maximum concentration of indomethacin.

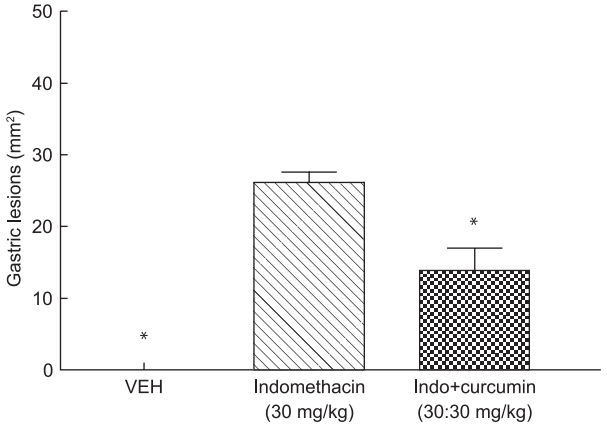

Figure 1 Protective effect of curcumin against indomethacin-induced gastric damage

Animals received vehicle (VEH), indomethacin and indomethacin plus curcumin (indo+curcumin). Data are expressed as the mean ± standard error of mean of 7 rats per group. Statistically significant difference was determined by analysis of variance followed by the Newman-Keuls test. * $P < 0.05$, *vs* indomethacin-treated group.

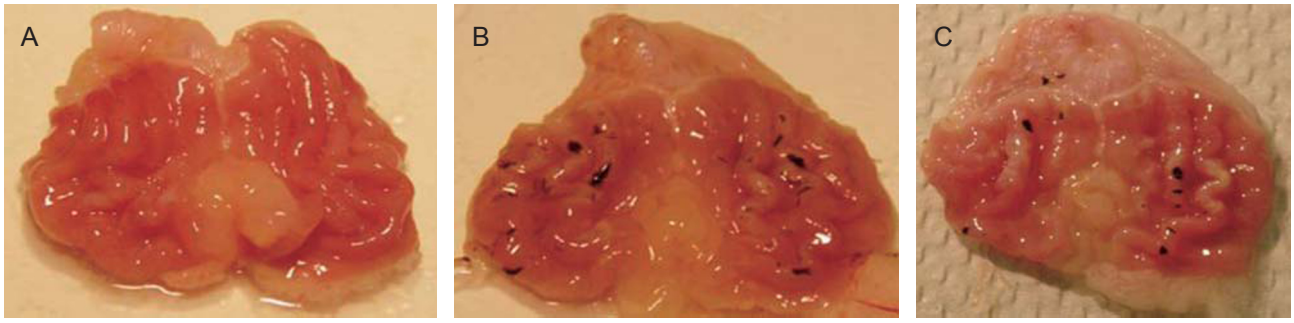

Figure 2 The representative gross appearance with gastric mucosal lesions in rats treated with vehicle, indomethacin and indomethacin plus curcumin
A: vehicle group; B: indomethacin (30 mg/kg) group; C: curcumin (30 mg/kg) plus indomethacin (30 mg/kg) group.

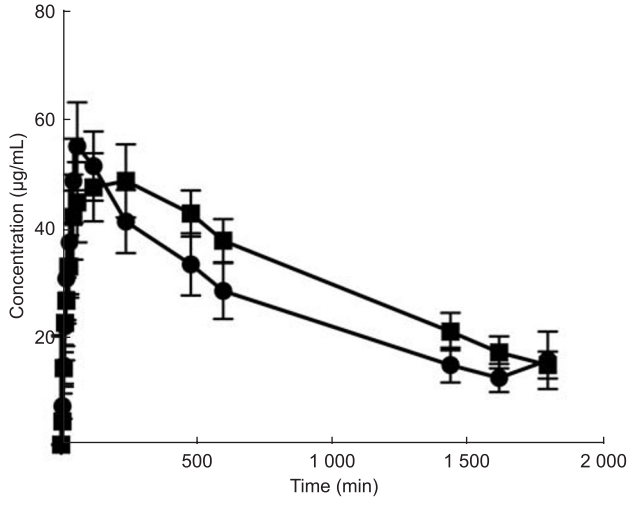

Figure 3 Mean plasma concentration-time curves in rats
Rats were orally administered with 30 mg/kg of indomethacin alone or with 30 mg/kg oral dose of curcumin. Data are mean ± standard error of mean for 8 rats per group.

3.3 Effect of curcumin on indomethacin and acemetacin bioavailability

Plasma concentrations of acemetacin and its active metabolite indomethacin are plotted against time after acemetacin administration in Figure 4A and 4B, respectively. For each of the two measured compounds, concentrations were comparable in presence and absence of curcumin. Hence, curcumin pre-treatment did not significantly affect bioavailability of either acemetacin or indomethacin (Table 2).

4 Discussion

It has been observed that curcumin exhibits a significant protective effect in experimental models of indomethacin-induced gastric damage.[8–10] Our group has described the potent gastroprotective effect of single-dose curcumin against indomethacin and provided evidence for the involvement of the nitric oxide-cyclic GMP-potassium channel pathway,[11] who's role in gastroprotection is well established.[20–24] The ability of curcumin to activate the nitric oxide-cyclic GMP-potassium channel pathway after a single administration has been documented in other experimental models, such as antinociception in the formalin pain model.[25] Therefore, it is likely that curcumin reduces indomethacin-induced gastric damage by activation of the nitric-oxide-cyclic GMP-potassium channel pathway at the gastrointestinal level. However, it should be noted that curcumin exhibits a wide variety of actions *in vivo*.[26] It is thus probable that other mechanisms of action also play a role in curcumin gastroprotection against indomethacin.

In the present study we confirmed the gastroprotective effect of curcumin and explored the possibility of a pharmacokinetic interaction between single doses of curcumin and indomethacin, administered as indomethacin or as its prodrug acemetacin. The hypothesis was that curcumin would diminish indomethacin bioavailability, thus reducing its effects. It has been reported that curcumin may alter the pharmacokinetics of certain drugs. Further, it is known that curcumin increases the exposure of several pharmacological agents by inhibition of drug transporters[17,27] as well as of metabolic clearance by cytochromes P450, UDP-glucuronosyltransferase and sulfotransferase.[17,28,29] However, there is also evidence that curcumin exerts actions that can result in a reduced bioavailability, including impairment of gastric emptying[16] and increase in volume of distribution.[15] Curcumin is also able to inhibit presystemic drug metabolism[17] and thus to reduce conversion of a prodrug to its active metabolite. Therefore, we examined the possibility of pharmacokinetic interactions between curcumin and indomethacin and its prodrug acemetacin in rats.

Single oral doses of indomethacin and acemetacin (30 and 34.86 mg/kg, respectively) were selected as they are equimolar, in terms of indomethacin equivalents, and have

Table 1 Pharmacokinetic parameters of indomethacin after single oral dose of 30 mg/kg alone or co-administered with curcumin (30 mg/kg) in rats

Treatment	C_{max} (μg/mL)	T_{max} (min)	AUC_{0-t} (μg min/mL)
Indomethacin	62.03 ± 6.90	72.85 ± 12.43	44 986.00 ± 7 034.93
Curcumin + indomethacin	57.23 ± 5.61	203.57 ± 56.30*	56 722.07 ± 5 429.88

Results are given as mean ± standard error of mean for 8 rats per group. * $P < 0.05$, *vs* indomethacin-treated group (determined by the Student's *t*-test). C_{max}: maximal concentration; T_{max}: time to achieve maximal concentration; AUC_{0-t}: area under the curve to the last sampling time.

Table 2 Pharmacokinetic parameters of acemetacin and indomethacin in rats and the effect of curcumin

Treatment	C_{max} (μg/mL)	T_{max} (min)	AUC_{0-t} (μg min/mL)
Acemetacin	10.02 ± 3.74	48.12 ± 12.92	434.28 ± 102.27
Curcumin + acemetacin	9.06 ± 2.66	26.87 ± 16.70	373.16 ± 87.88
Indomethacin	63.84 ± 6.24	217.50 ± 74.30	51 623.42 ± 2 964.12
Curcumin + indomethacin	59.05 ± 3.93	110.62 ± 20.38	46 078.99 ± 2 895.55

Results are given as mean ± standard error of mean for 8 rats per group. C_{max}: maximal concentration; T_{max}: time to achieve maximal concentration; AUC_{0-t}: area under the curve to the last sampling time.

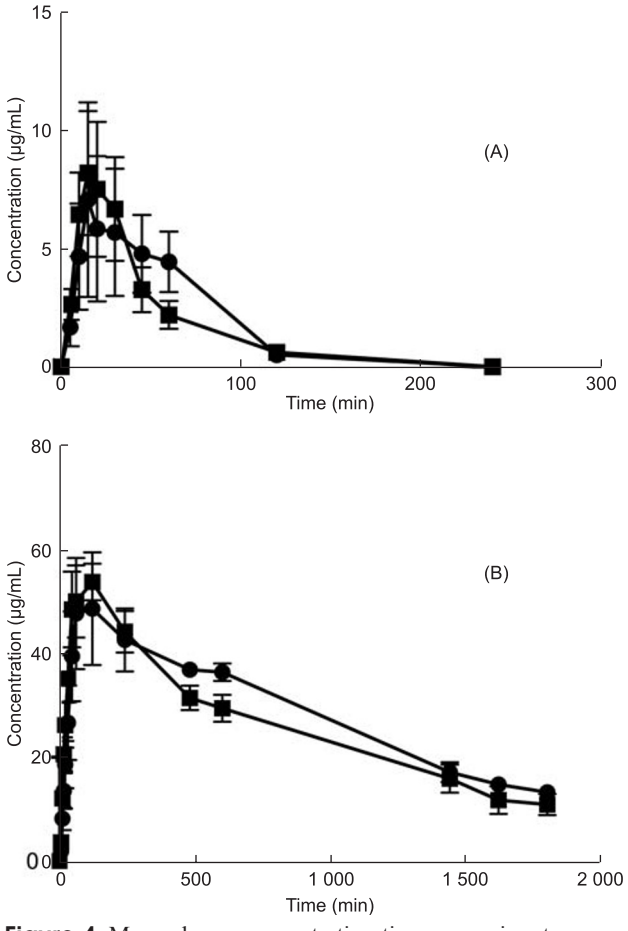

Figure 4 Mean plasma concentration-time curves in rats

Mean plasma concentration-time curves in rats of acemetacin (A) and its active metabolite indomethacin (B) after single oral administration of 34.86 mg/kg of acemetacin alone or with 30 mg/kg oral dose of curcumin. Data are mean ± standard error of mean for 8 rats per group.

been shown to yield comparable indomethacin exposure and produce significant anti-inflammatory responses of comparable magnitude in experimental models.[12,19] Curcumin was administered as a single 30 mg/kg dose, as we have provided evidence that this dose provides significant gastroprotection against indomethacin.[11]

Our results showed that single-dose curcumin did not produce significant alterations in indomethacin bioavailability parameters of C_{max} or AUC_{0-t}, although there was an extension of T_{max}, observing that it requires more time to reach C_{max}. It has been reported that curcumin impairs gastrointestinal motility,[30] and thus may delay drug absorption. However, this was not observed with acemetacin. Moreover, as both, acemetacin and indomethacin are acidic drugs,[31] gastric emptying does not appear to be a critical determinant of the rate and extent of drug absorption.[32] Hence, the reason why indomethacin T_{max} was longer in rats receiving curcumin is not clear at present.

Sakunthala et al.[15] recently reported that pre-treatment for two months with curcumin (50 mg/kg once daily) resulted in a slight, but statistically significant, increase in the volume of distribution of glibenclamide. Notwithstanding, they did not observe any statistically significant differences in glibenclamide exposure. Hence, it appears unlikely that a single-dose of curcumin, as the one used in the present study, could result in an increase in indomethacin volume of distribution, leading to reduced bioavailability. It is well documented that curcumin inhibits drug metabolism by multiple enzymatic systems[17,28,29] and therefore can impair both systemic and presystemic drug metabolism.[17] In the latter case, the bioavailability of an active metabolite after administration of a prodrug will be diminished. It should be considered,

however, that curcumin actions on drug metabolism are due to a down-regulation of enzyme expression produced by repetitive administration.[28] In other research, a single curcumin dose was not sufficient to make significant alterations in the metabolic clearance of several co-administrated agents;[28,29] this corroborates our data for indomethacin and acemetacin in the present study. The fact that indomethacin bioavailability after acemetacin administration was similar in the presence and absence of curcumin suggested that curcumin, at this dose, had no significant effects on the first-pass effect. Moreover, these results also suggested that curcumin did not alter hepatic blood flow, as acemetacin is a high hepatic extraction drug and its clearance depends on flow of blood through the liver.

It should be noted that the purpose of the present study was to examine whether a pharmacokinetic interaction, leading to reduced indomethacin exposure, occurs with co-administration of a single curcumin oral dose and therefore plays a role in the gastroprotective effect of single-dose curcumin against indomethacin. Our results strongly suggest that this is not the case. Therefore, gastroprotection of single-dose curcumin against indomethacin is likely exclusively due to a pharmacodynamic mechanism. Our results, however, do not preclude a pharmacokinetic interaction of either indomethacin or acemetacin with repetitive curcumin dosing. This is an issue that requires further investigation.

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6 Conflict of interests

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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