

Pharmacokinetic of Diclofenac in the Presence and Absence of Glibenclamide in the Rat

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ABSTRACT - Purpose. There is evidence that the sulfonylurea antidiabetic agent glibenclamide reduces the analgesic action of non-steroidal anti-inflammatory drugs (NSAIDs), opioids and neuromodulators in animal models. Therefore, in view of the vast clinical uses and interactions of NSAIDs with commonly used therapeutic agents, the interaction of the NSAID diclofenac and glibenclamide was investigated about pharmacokinetic profile and antinociceptive effect in rats. **Methods.** Antinociception was assessed using the formalin test. Fifty microliters of diluted formalin was injected s.c. into the dorsal surface of the right hind paw. Nociceptive behavior was quantified as the number of flinches of the injected paw during 60 min after injection. Rats were treated with oral administration of vehicle or increasing doses of diclofenac (3-18 mg/kg) before formalin injection. To determine the pharmacodynamic interaction between diclofenac and glibenclamide, the effect of oral administration of glibenclamide (1-30 mg/kg) on the antinociceptive effect induced by diclofenac (18 mg/kg, p.o.) was assessed. To evaluate the pharmacokinetic interaction between diclofenac and glibenclamide, the effect of glibenclamide (10 mg/kg, p.o.) on the pharmacokinetic of diclofenac (18 mg/kg, p.o.) was studied in the rat. Blood samples were taken over 8 h and analyzed using a validated high-performance liquid chromatography method to generate the pharmacokinetic profile of diclofenac. Pharmacokinetic parameters were estimated using noncompartmental analysis. **Results.** Systemic administration of diclofenac produced a dose-dependent antinociceptive effect in the formalin test. Systemic treatment with glibenclamide prevented diclofenac-induced antinociception. In pharmacokinetic interaction study, no significant ($P>0.05$) change in diclofenac concentration-time profiles in the presence of glibenclamide was detected. **Conclusion.** The experimental findings suggest that systemic glibenclamide is able to block the diclofenac-induced antinociception in the rat formalin test. Besides, this antagonism was not produced by diminution in the bioavailability of diclofenac. Likewise, the validated assay had sufficient accuracy and precision for pharmacokinetic determination of diclofenac in the rat.

INTRODUCTION

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) that exhibits potent analgesic and anti-inflammatory properties. Diclofenac administered orally, rectally or intramuscularly has showed to have clinical efficacy in patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and other inflammatory conditions (1). It is known that diclofenac, as other nonselective NSAIDs, is able to impair prostaglandin synthesis by the inhibition of the cyclooxygenase isozymes COX-1 and COX-2 in both, the injured tissue and the central nervous system (2, 3). However, there is evidence that additional prostaglandin-independent

mechanisms are involved in the antinociceptive action of diclofenac at both, the peripheral and central levels. In this respect, it has been proven that the local peripheral administration of glibenclamide is able to block the peripheral antinociceptive effect of diclofenac in the rat (4-6),

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suggesting the participation of ATP-sensitive K^+ channels in its antinociceptive effect at peripheral level.

Diclofenac undergoes first-pass metabolism and the oral bioavailability of diclofenac ranges from 54 to 90% (7). Diclofenac is highly bound to serum proteins ($\geq 99.5\%$) and it has a relatively low volume of distribution (0.12 to 0.17 L/kg) (1, 7). There are little data about the distribution of diclofenac into other tissues and fluids. About this, diclofenac easily penetrates the synovial fluid and cross the placenta (1, 8). However, diclofenac does not easily cross the blood-brain barrier. It has been tested that the diclofenac concentrations in cerebrospinal fluid are 8.22% of those in plasma (9). There are reports wherein the anti-inflammatory and antinociceptive effect of diclofenac cannot be directly explained by circulating concentrations in animals (10) or in humans (1). Therefore, we think the diclofenac effects are mainly mediated via a local action in target tissues.

Drug interaction is a modification of the effect of a drug when administered with another drug. The interaction between drugs may be pharmacokinetic and/or pharmacodynamic. The effect may be an increase or a decrease in the action and/or bioavailability of either substance. In this respect, it has been shown that colestipol and cholestyramine decreased the area under the concentration-time curve (AUC) and the maximum concentration (C_{max}) of diclofenac in humans (11). Likewise, administration of aspirin in healthy subjects reduced diclofenac plasma concentrations, peak plasma levels, and AUC values (1). However, there is not any pharmacokinetic study on the interaction between the ATP-sensitive K^+ channels inhibitor glibenclamide (12) and diclofenac. Therefore, the main of the present study was determine whether the systemic administration (oral) of the sulfonylurea antidiabetic agent and ATP-sensitive K^+ channel blocker glibenclamide is able to modify the pharmacokinetic and pharmacodynamic of diclofenac in the rat.

MATERIAL AND METHODS

Animals

Female Wistar rats (of mixed estrous phases) aged 8-10 weeks (weight range, 180-200 g) from our own breeding facilities were used in this study.

Animals had free access to drinking water, but food was restricted 12 h before the 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 the rats were sacrificed in a CO_2 chamber. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (13). Additionally, the study was approved by the Institutional Animal Care and Use Committee (CINVESTAV-IPN, México, D.F., Mexico).

Pharmacodynamic study

Drugs

Diclofenac and glibenclamide were purchased from Sigma (St. Louis, MO, USA). Diclofenac was dissolved in saline. Glibenclamide was dissolved in 0.05 % carboxymethylcellulose.

Measurement of antinociceptive activity

Pain and antinociception were assessed by the formalin test, as previously described (4, 5, 14). Briefly, fifty microliters of diluted formalin (1%) were injected subcutaneously (s.c.) to the dorsal surface of the right hind paw, and the resulting flinching behavior was considered as an expression of nociception. Numbers of flinches against time curves were constructed, these curves being biphasic. The initial acute phase (0-10 min) was followed by a short quiescent period followed by a prolonged tonic response (15-60 min). The area under the curve for both phases was estimated, and a significant area reduction was interpreted as antinociception.

Study design

To evaluate the effect of glibenclamide on the systemic antinociceptive action of diclofenac in the formalin test, rats received the oral administration (p.o.) of the appropriate vehicle or increasing doses of diclofenac (3-18 mg/kg) 30 min before formalin injection. After formalin injection, flinching behavior was assessed for the next 60 min. To determine whether diclofenac-induced systemic antinociception was mediated by K^+ channel activation, effect of co-treatment with the appropriate vehicle or glibenclamide (1-30 mg/kg, p.o.) on the systemic antinociceptive effect induced by diclofenac (18 mg/kg, p.o.) was assessed. Drugs were administrated in a volume of 4 mL/kg, p.o.

Doses and drug administration schedule of diclofenac and glibenclamide were selected based on previous reports (4-6, 14, 15) and on pilot experiments in our laboratory. Rats in all groups were observed regarding behavioral or motor function changes induced by the treatments. This change was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture. All observations were carried out by a blinded investigator.

Data analysis and statistics

Results are given as the mean \pm SEM for 6 animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule. Reduction of number of flinches or AUC of the second phase is reported only, because diclofenac did not produce any change on phase 1. Analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $p < 0.05$.

Pharmacokinetic study

Surgery

The rats were anesthetized with ethyl ether. Then, PE catheters (a combination of PE-10 and PE-50 was used; I.D. 0.28 mm, O.D. 0.61 mm; I.D. 0.58 mm, O.D. 0.965 mm, respectively; Clay Adams, Parsippany, NJ) were surgically implanted into the caudal artery for the collection of blood samples as reported previously (16).

Chemicals

Diclofenac, naproxen and glibenclamide were purchased from Sigma (St. Louis, MO, USA). Acetonitrile and methanol were chromatographic grade (Merck, Darmstadt, Germany). Deionized water was obtained using a Milli-Q system (Continental Water Systems, El Paso, TX). Other reagents used in the study were of analytical grade. Diclofenac and glibenclamide were dissolved in saline and 0.05 % carboxymethylcellulose, respectively.

Study design

In this study, only circulating concentrations of diclofenac were estimated in the animals. To address this issue, the pharmacokinetic parameters of diclofenac in rats at its antinociceptive dose were generated for baseline information. Thereafter, the pharmacokinetic of diclofenac was investigated in the presence of glibenclamide. For this purpose, following a single oral dose of diclofenac (18 mg/kg) with or without glibenclamide (10 mg/kg, p.o.), blood samples (100 μ L) were drawn before and at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 480 min after dosing. Blood samples were frozen at -70°C until analyzed for diclofenac by HPLC.

Analysis of diclofenac in blood

Blood concentrations of diclofenac were determined by a HPLC method developed in our laboratory. Briefly, whole-blood samples (100 μ L) were placed into 2 mL Eppendorf tubes, and 0.1 ng/mL of naproxen (internal standard) was added. Blood was then acidified by the addition of 20 μ L of 0.5 M NaH_2PO_4 (pH 3). Next 1 mL of ethyl acetate was added, and samples were extracted by agitation in vortex at maximal speed for 1 min. After centrifugation at 10,000 rpm for 10 min, the organic layer was transferred into a clean conical glass tube and evaporated to dryness in a water bath at 50°C under a gentle nitrogen stream. The dry residue was redissolved in 200 μ L of a mixture of 0.075 M Na_2HPO_4 buffer (pH 7) and methanol (1:1), and 100- μ L aliquots were injected into the chromatographic system.

The chromatographic system consisted of a model 510 solvent delivery system (Waters Assoc., Milford, MA), a 7125 Rheodyne injector with a 100-mL loop (Cotati, CA) and a LC-4B electrochemical detector (BAS, West Lafayette, IN) with a glassy carbon working electrode and an Ag/AgCl reference electrode. Compounds were separated at room temperature on a MicroPak C18 column of 300 mm X 4 mm I.D. and particle size of 10 μm (Varian, Palo Alto, CA) eluted with a mixture of 0.075 M sodium acetate (adjusted to pH 3.3 with glacial acetic acid) and acetonitrile (55:45, v/v) at a flow rate of 2 mL/min. The detector was operated at +1.1 V, and the chromatograms were registered in a Servogor 120 (Norma Goerz Instruments, Elik Grove Village, IL). Diclofenac pharmacokinetic parameters were performed by

non-compartmental analysis using WinNonlin software, version 3.0 (Pharsight Corp, Mountain View, CA).

Statistical analysis

Data are expressed as mean value \pm SEM. The data obtained were subjected to one-way ANOVA followed by Tukey's test and Student's "*t*"-test to determine the level of significance and a *P* value of <0.05 was considered as statistically significant.

RESULTS

Pharmacodynamic interaction between diclofenac and glibenclamide

Formalin administration produced a typical pattern of flinching behavior. Systemic (oral) administration of diclofenac significantly reduced the number of flinches during phase two ($p < 0.05$) (Figure 1), but not during phase one (data not shown). Systemic pretreatment with the ATP-sensitive K^+ channel inhibitor glibenclamide was able to reverse ($p < 0.05$) diclofenac-induced antinociception (systemic) (Figure 1).

Assay Validation

Diclofenac pharmacokinetic was performed with a high-performance liquid chromatograph in blood. The assayed method was validated before use. The method showed specificity and good precision; the repeatability and reproducibility were within acceptable limits in quality control samples at low, medium and high concentration levels.

The retention times were 1.6 and 3.5 min for naproxen and diclofenac, respectively. The peaks were resolved with no interfering peaks (data not shown). Calibration curves were constructed for diclofenac concentrations in blood ranging from 0.1 to 12 $\mu\text{g}/\text{mL}$. A linear relationship ($r^2 = 0.9996$) was obtained when peak-height ratios of diclofenac to the internal standard were plotted against diclofenac blood concentration. Coefficients of variation were always lower than 10%, whereas accuracy ranged from 90% to 115%. The detection limit of the method was 50 ng/mL.

Pharmacokinetic interaction between diclofenac and glibenclamide

Following a single oral dose of 18 mg/kg diclofenac alone, the drug was monitored up to 8 h. Figure 2 shows the mean (\pm SEM) diclofenac plasma

concentration vs. time profiles after oral dosing of diclofenac at 18 mg/kg alone, or when co-administered with glibenclamide (10 mg/kg, p.o.) in rats. The pharmacokinetic parameters of diclofenac with and without co-administered glibenclamide are summarized in table 1. No significant ($P > 0.05$) change in diclofenac concentration-time profiles in the presence of glibenclamide was detected.

DISCUSSION

Pharmacodynamic interaction study

The results reported here suggest that modulation of some K^+ channels at systemic level represent an important step in the mechanism of antinociception induced by diclofenac. Our data demonstrate that the systemic administration of the ATP-sensitive K^+ channels inhibitor glibenclamide prevented the diclofenac-induced antinociception. In according to these results, it has been proven that local peripheral administration of glibenclamide was able to prevent the antinociceptive and antihyperalgesic effects of diclofenac (4-6). To our knowledge, this is the first report indicating that systemic glibenclamide is able to block the analgesic effect induced by systemic diclofenac in the rat formalin test. In the same line, recently we had demonstrated that systemic glibenclamide blocked the antihyperalgesic effect of diclofenac in the mouse (17). At the concentrations used in this work, the K^+ channel blocker glibenclamide did not modify the flinching behavior of rats in comparison with that of control rats. The lack of effect of the K^+ channel blocker is consistent with the results of studies in which glibenclamide not modify the nociceptive activity of chemical, thermal noxious stimuli and mechanical hyperalgesia (4-6, 15, 18), thus excluding the possibility that the prevention of diclofenac antinociception could be due to a hyperalgesic or nociceptive effect of glibenclamide.

Pharmacokinetic interaction study

In the present study, rats were orally administered with diclofenac and glibenclamide and the former was monitored up to 8 h. Although the administration of glibenclamide increased the C_{max} of diclofenac from $10.1 \pm 1.1 \mu\text{g}/\text{mL}$ to $11.9 \pm 0.9 \mu\text{g}/\text{mL}$, this was not a significant effect ($P > 0.05$). On the contrary, glibenclamide was able to decrease the T_{max} of diclofenac of $12.1 \pm 3.6 \text{ min}$ to $9.6 \pm 0.4 \text{ min}$, however, this change was not significant

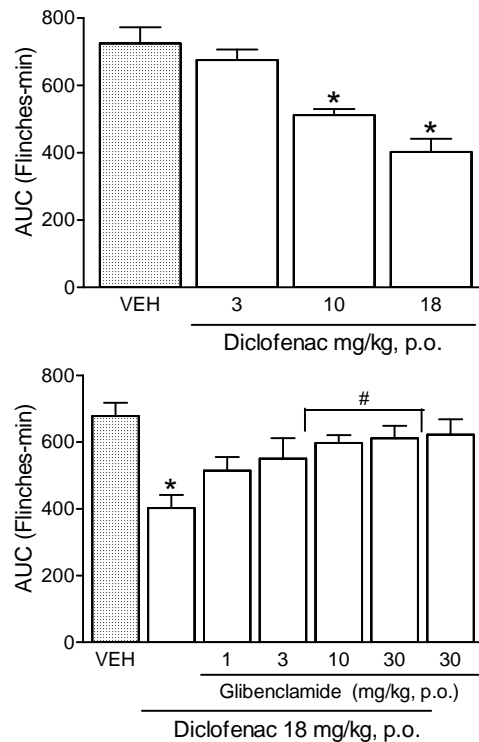


Figure 1. Antinociceptive effect induced by systemic diclofenac during the second phase of the formalin test (top panel) and effect of the ATP-sensitive K^+ channel inhibitor glibenclamide on the systemic antinociception produced by diclofenac during the second phase of the formalin test (bottom panel). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean \pm SEM for 6 animals. * Significantly different from vehicle group ($P < 0.05$) and #significantly different from the diclofenac group ($P < 0.05$), as determined by analysis of variance followed by Tukey's test.

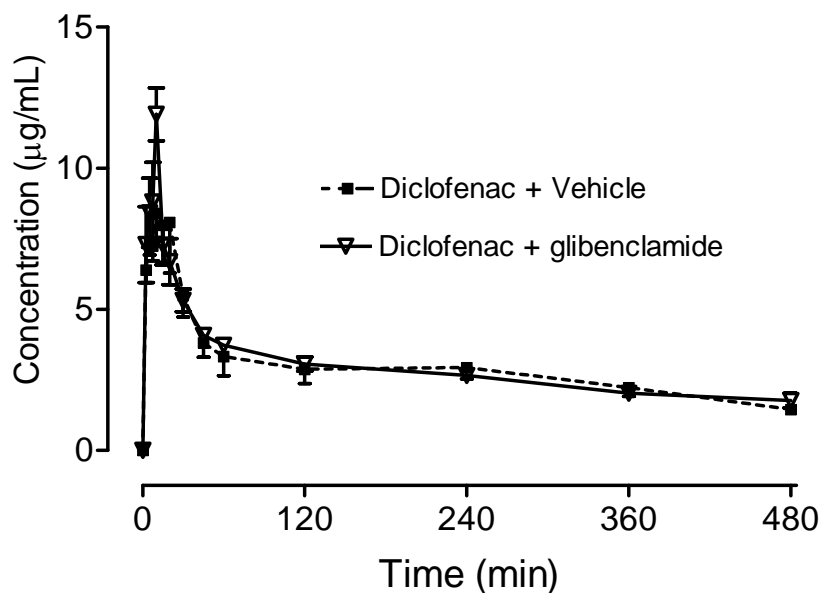


Figure 2. Mean plasma concentration–time curves in rat after single oral administration of 18mg/kg diclofenac or with 10mg/kg oral dose of glibenclamide. Data are the mean \pm SEM for 6 rats.

Table 1. Pharmacokinetic parameters of diclofenac after single oral dose of 18 mg/kg alone or in the presence of glibenclamide at 10 mg/kg orally in rat.

Treatment	Cmax ($\mu\text{g/mL}$)	Tmax (min)	AUC _{0-t} ($\mu\text{g min/mL}$)	AUC _{0-∞} ($\mu\text{g min/mL}$)
Diclofenac + vehicle	10.1 ± 1.1	12.1 ± 3.6	1400.8 ± 170.0	1985.5 ± 184.2
Diclofenac + Glibenclamide	11.9 ± 0.9	9.6 ± 0.4	1403.0 ± 62.5	2399.1 ± 305.8

The results for Cmax, Tmax and AUC are given as mean ±SEM of six repetitions for each treatment.

($p > 0.05$). Likewise, there was no significant change in the AUC of diclofenac due to glibenclamide co-administration.

Most drug-drug interactions are caused by the inhibition of metabolism of one drug by a concomitantly administered drug. Clinically, relevant drug-drug interactions are caused mainly by an inhibition of P450-dependent reactions (19). In this respect, there is experimental evidence that cytochrome P450 (CYP) 1A1, CYP2C9, CYP2C19 and CYP3A4 are the main enzymes catalyzing the biotransformation of glibenclamide (20); while diclofenac is extensively metabolized by CYP2C9 and CYP3A4 enzymes (7, 21-23). In the present study, diclofenac concentrations were not affected significantly by the concomitant administration of 10 mg/kg of glibenclamide. The possible metabolic interaction was not sufficient to modify the diclofenac bioavailability. A partial explanation for this result is that both drugs are metabolized by several enzymes and the doses occupied in the present study (diclofenac 18 mg/kg, p.o. plus glibenclamide 10 mg/kg, p.o.) were not able to saturate the enzymes. This result indicates that glibenclamide does not interfere with pathways responsible for the metabolism of diclofenac.

The results showed a lack of pharmacokinetic drug interaction about diclofenac, but because the data were derived from single-dose administrations care should be followed in extrapolation to steady-state situations. Likewise, perhaps higher doses of glibenclamide could modify the diclofenac pharmacokinetic. Nevertheless, previous studies have demonstrated that systemic glibenclamide at the doses ≤ 10 mg/kg were sufficient to block the effects of analgesics (15, 17).

Practical implications of these results

The most important drugs in use for Type 2 Diabetes are the biguanide metformin and the sulfonylurea group. Glibenclamide, a potent second-generation sulfonylurea, which has been widely used in the management of non-insulin dependent diabetes mellitus in Europe since 1969 and in the United States since 1984, improves glucose tolerance mainly by augmenting insulin secretion (24). The action mechanism of glibenclamide consists in the inhibition of the ATP-sensitive K^+ channels (12). In the present work, the sulfonylurea antidiabetic agent and ATP-sensitive K^+ channel blocker glibenclamide decreased the antinociceptive effect produced by diclofenac in the rat. According to our pharmacokinetic results, this effect was not due to an alteration in the diclofenac bioavailability. With this evidence, it is possible now to propose that after the systemic administration of diclofenac and glibenclamide, both drugs are absorbed and distributed to the action sites. When diclofenac is only administered, it produces an antinociceptive effect and when it is administered along with glibenclamide, the antinociceptive action is blockade. Up to now, there is not any clinical study about the pharmacokinetic interaction between diclofenac and glibenclamide. Likewise, it is necessary to realize a clinical study to evaluate the possible alteration of the analgesic effect when diclofenac is administered with glibenclamide.

A limitation of our study was that we did not use diabetic rats. In this respect, it has been demonstrated that chronic hyperglycemic states are able to alter the pain threshold and the renal function (25, 26). Nevertheless, the objective of the

present study was not to evaluate the diclofenac-glibenclamide interaction with these alterations. However, it would be of interest to examine that pharmacologic interaction in diabetic rat.

CONCLUSION

The results showed here displayed that systemic glibenclamide is able to block the diclofenac-induced antinociception in the rat formalin test. Besides, this antagonism was not produced by diminution in the bioavailability of diclofenac.

Finally, although in the present study we did not find alteration in the pharmacokinetics of diclofenac due to the glibenclamide treatment, this pharmacological agent should be further investigated to ensure its safe administration in diabetic patients with concurrent diclofenac administration.

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