Pharmacokinetics and Pharmacodynamics of Diclofenac in the Presence and Absence of Glibenclamide in the Rat

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ABSTRACT – Purpose. There are evidences that glibenclamide, a sulfonylurea antidiabetic agent, reduces the analgesic action of non-steroidal anti-inflammatory drugs (NSAIDs), opioids and neuromodulators in animal models. The purpose of this work was to examine in the rat if such interaction involves pharmacokinetic mechanisms or is solely limited to the pharmacodynamic level. **Methods**. All studies were carried out in female Wistar rats. Analgesia was assessed using the formalin test. Fifty microliters of diluted formalin was injected subcutaneously 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 and a reduction in formalin-induced flinching was interpreted as an analgesic response. Rats were treated with oral diclofenac (3-18 mg/kg) in presence and the absence of oral glibenclamide (1-30 mg/kg). To evaluate the possibility of a pharmacokinetic interaction, the oral bioavailability of diclofenac (18 mg/kg) was studied in presence and the absence of glibenclamide (10 mg/kg). **Results**. Oral administration of diclofenac produced a dose-dependent antinociceptive effect in the formalin test. Coadministration of glibenclamide significantly reduced diclofenac-induced antinociception. Notwithstanding, the interaction does no appear to involve pharmacokinetic mechanisms, as oral glibenclamide failed to produce any significant alteration in oral diclofenac bioavailability. **Conclusion**. Concomitant systemic administration of glibenclamide and diclofenac results in a reduction of the analgesic effect of the NSAID in the formalin test in the rat. This interaction, however, appears due solely to a pharmacodynamic mechanisms as diclofenac pharmacokinetics are not altered.

INTRODUCTION

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID), widely used in therapeutics, that exhibits potent analgesic and anti-inflammatory properties (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 the injured tissues 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 regard, it has been documented that diclofenac activates the nitric oxide-cyclic GMP-potassium channel pathway in primary nociceptive neurons. As a result of potassium channel opening, potassium leaks out of the neuron resulting in hyperpolarization and reduced excitability (4-8). Therefore, perhaps concomitant medication affecting potassium channel activity may interfere with diclofenac-induced analgesia. Oral hypoglycemic agents, such as glibenclamide (glyburide) and tolbutamide, produce their therapeutic effect by the blockade ATP-sensitive potassium channels, and therefore exhibit a certain potential to interact with diclofenac.

Diclofenac is readily absorbed after oral administration and undergoes a considerable first-pass metabolism, its bioavailability ranging from 54 to 90% in humans (9). Diclofenac is highly bound to serum proteins (≥ 99.5%) and it has a relatively small volume of distribution in humans (1, 9). Data about its tissue distribution are scarce.

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Notwithstanding, it has been documented that diclofenac is able to penetrate into the synovial fluid and to cross the placental barrier (1, 10). Diclofenac is also transferred across the blood-brain barrier, although it has been reported that concentrations in cerebrospinal fluid are less than 10% compared to those in plasma (11). It has been shown that diclofenac analgesic response is delayed with respect to drug appearance in the circulation (1, 12). Using pharmacokinetic-pharmacodynamic modeling, our group has shown that diclofenac analgesic response can be related to drug concentration in an effect compartment (12). That is, diclofenac needs to be transferred to its site of action to be effective. It is possible, however, that the delay between the appearance of diclofenac in blood and its effects also involves a cascade of biochemical effects, as it has been proposed for other NSAIDs, such as tolmetin (13).

We have previously shown that there is a pharmacodynamic interaction between glibenclamide and diclofenac involving the blockade of potassium channels. This was shown by the direct injection of both drugs into the inflamed tissue of injured rats, which is directly into the effect compartment (4). Notwithstanding, to our knowledge the diclofenac-glibenclamide interaction in the rat after drug systemic administration has not been characterized. Note that drug interactions are not limited to pharmacodynamics, but frequently involve pharmacokinetic mechanisms. In the case of diclofenac, it has been reported that its bioavailability is decreased by colestipol (14) and increased by aspirin (1). Therefore, the purpose of the present study was to further examine the interaction between the potassium channel blocker glibenclamide (15) and diclofenac by determining if the hypoglycemic agent alters both, the pharmacokinetics and analgesic effect of the NSAID following oral administration to experimental animals.

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. We have previously shown (4, 5, 16) that female rats provide reliable results in the formalin assay of analgesia (*vide infra*), while García-López and Salas (17) have shown that diclofenac pharmacokinetics can be accurately determined in female rats. 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 (18). Additionally, the study was approved by the Institutional Animal Care and Use Committee (CICUAL/VESTAV-IPN, México, D.F., Mexico).

Drugs

Diclofenac and glibenclamide were purchased from Sigma (St. Louis, MO, USA). Diclofenac was dissolved in saline. Glibenclamide was suspended in 0.05 % carboxymethylcellulose. Drugs were administered orally in a volume of 4 ml/kg.

Pharmacodynamic study

Measurement of antinociceptive activity

Pain and analgesia were determined using the formalin test in the rat. This is a widely used assay for analgesic agents, which has shown to yield reliable NSAIDs (4, 5, 14, 16, 19). Rats were placed in open Plexiglas observation chambers for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. Fifty microliters 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 was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paws during 1-min periods every 5 min up to 60 min after injection (4, 5, 14, 16, 19). Flinching was readily discriminated and was characterized as rapid and brief withdrawal 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). A reduction of formalin-induced flinching behavior observed after administration of a given drug is interpreted as an analgesic response.

Study design

Rats were treated orally with 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 evaluate the effect of glibenclamide on the analgesic action of orally administered diclofenac, the NSAID was given orally at a dose of 18 mg/kg concomitantly with vehicle or glibenclamide (1-30 mg/kg, p.o.). Doses and drug administration schedules were selected based on previous reports (4-6, 14, 15) and pilot experiments conducted 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 ± SD for 6 animals per group. Curves were constructed plotting the number of flinches as a function of time, these curves being biphasic (Fig. 1). The area under the effect (number of flinches vs. time) curves (AUEC), an expression of the duration and intensity of the effect, was calculated for both the first and second phases of the assay, by the trapezoidal rule. 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

Blood sampling

Animals were lightly 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 (20).

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.

Study design

Diclofenac (18 mg/kg) was given orally to two groups of rats. Animals in one group were concomitantly treated with oral glibenclamide (10 mg/kg), the other group receiving vehicle. Blood samples (100 µL) were drawn before and at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 480 min after diclofenac administration. Blood samples were frozen at -70°C until analyzed for diclofenac by high-performance liquid chromatography (HPLC).

Analysis of diclofenac in blood

Diclofenac blood concentrations were estimated by HPLC by a procedure developed and validated in our laboratory. This method has been previously described in detail (21).

Pharmacokinetic and Statistical analyses

Diclofenac pharmacokinetic parameters were estimated by standard non-compartmental analysis using WinNonlin software, version 3.0 (Pharsight Corp, Mountain View, CA). Data are expressed as mean value ± SD. Comparisons between diclofenac bioavailability parameters was carried out by the Student's "*t*"- test and a *P* value of <0.05 was considered statistically significant.

RESULTS

Analgesic effect of oral diclofenac in presence and the absence of glibenclamide

Formalin administration produced a typical pattern of flinching behavior (Fig. 1).

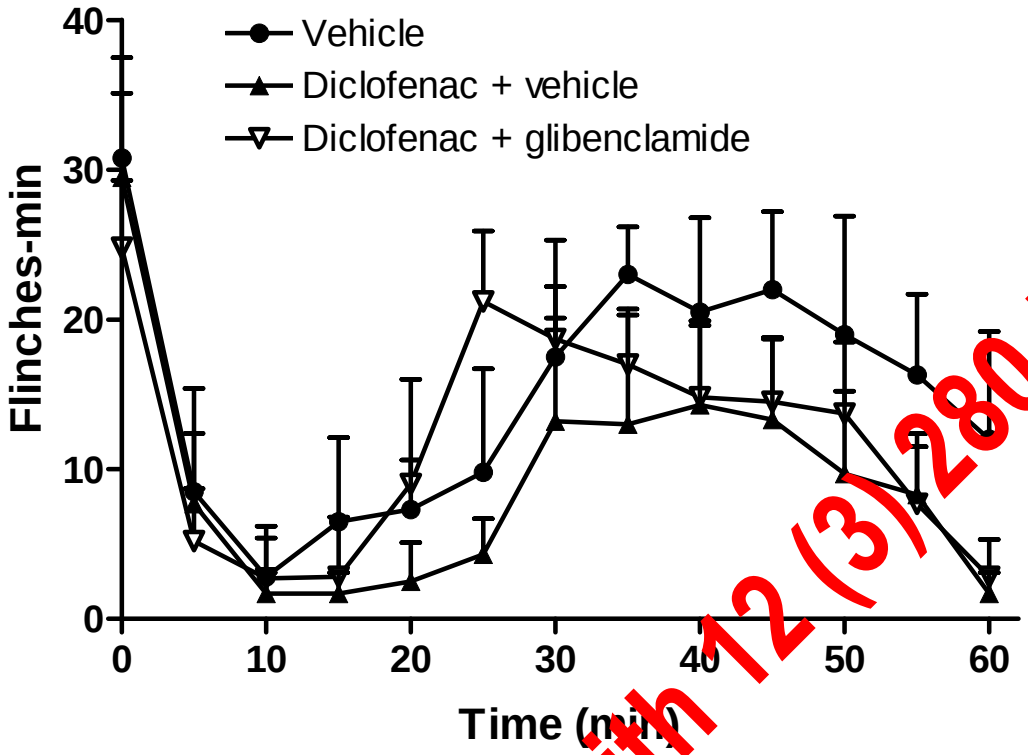

Figure 1. Time course of the systemic antinociceptive effect of diclofenac in the presence and absence of glibenclamide in the formalin test. Rats were pretreated with the oral administration of diclofenac (18 mg/kg) and glibenclamide (10 mg/kg) before formalin injection. Data represent the mean ± SD of six animals.

Table 1. Effect of glibenclamide on the antinociception produced by diclofenac during the first and second phases of the formalin test. Data are expressed as the mean ± SD of the area under the effect curve (AUEC) of the number of flinches vs. time.

	Vehicle	Diclofenac (18 mg/kg, p.o.) plus Glibenclamide vehicle	Diclofenac (18 mg/kg, p.o.) plus Glibenclamide (10 mg/kg, p.o.)
AUEC (Phase 1)	126.7 ± 28.8	116.3 ± 25.8	102.9 ± 32.7
AUEC (Phase 2)	667.9 ± 96.9	401.7 ± 97.5*	596.7 ± 59.4$^{\#}$

* 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. n= 6 animals.

An initial phase was observed immediately after the formalin insult, flinching decreasing gradually after 5 min. Then, a second flinching phase occurred, being observed from 15 to 60 min. Oral diclofenac was effective on the second, but not on the first phase of the formalin tests (Fig. 1). When data are presented as the AUEC (Fig. 2, Table 1), it can be clearly seen that oral diclofenac exhibited a significant dose dependent analgesic effect in phase 2, but not in phase 1. These results are in agreement with previously reported data (4, 5). When diclofenac was administered concomitantly with glibenclamide, the hypoglycemic agent prevented the analgesic response of the NSAID in a dose-dependent manner in the second phase of the formalin test. Glibenclamide, however, did not exhibit any significant effect on the first phase of the assay (Figs. 1 and 3, Table 1).

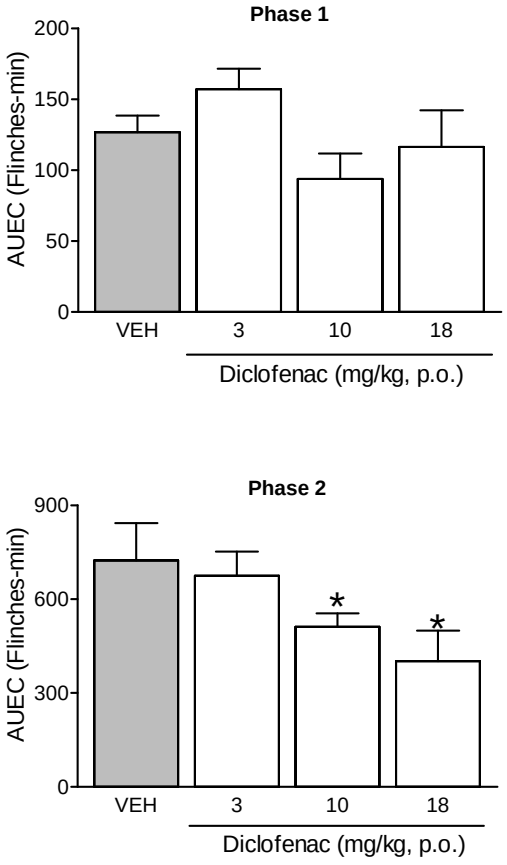

Figure 2. Antinociceptive effect induced by systemic diclofenac during the first and second phases of the formalin test. Rats were pretreated with oral administration of diclofenac before formalin injection. Data are expressed as the area (AUEC) under the number of flinches against time curve. Bars are the mean ± SD for 6 animals. * Significantly different from vehicle group ($P < 0.05$) as determined by analysis of variance followed by Tukey´s test.

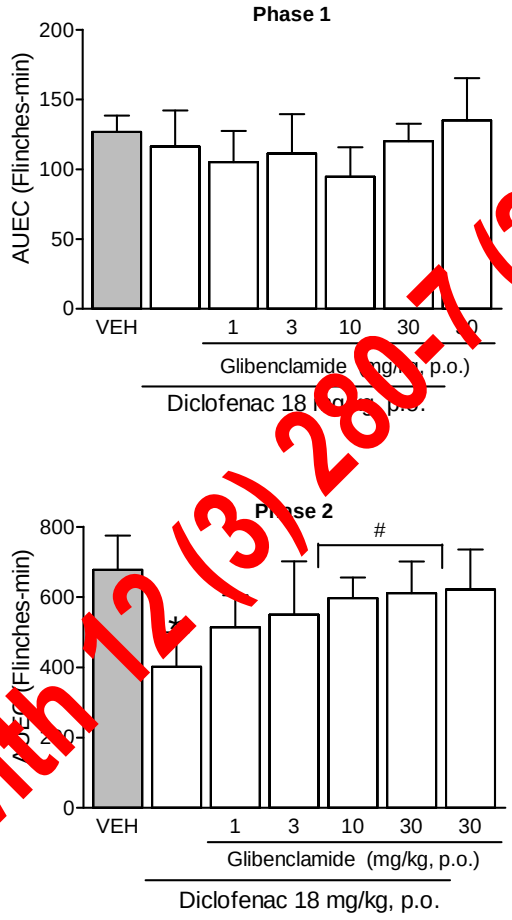

Figure 3. Effect of the ATP-sensitive K+ channel blocker glibenclamide on the systemic antinociception induced by diclofenac during the first and second phases of the formalin test. Data are expressed as the area (AUEC) under the number of flinches vs time curve. Bars are the mean ± SD for 6 animals. * 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.

Effect of glibenclamide on diclofenac pharmacokinetics

Diclofenac blood concentrations determined in rats receiving a single oral dose of 18 mg/kg in either presence or absence of 10 mg/kg of oral glibenclamide are shown in Fig. 4. It can be appreciated that glibenclamide did not altered diclofenac circulating levels. As a result, glibenclamide failed to produce any significant alteration of oral diclofenac bioavailability parameters, as it can be seen in Table 2.

DISCUSSION

Interaction between glibenclamide and diclofenac

It is widely accepted that NSAIDs produce their analgesic effects by the inhibition of prostaglandin synthesis inhibition (2). However, in the last two decades a whole body of experimental evidence has shown that certain NSAIDs, such as diclofenac,

exhibit additional mechanisms of action, which contribute to the analgesic response. At present, it is well documented that the analgesic effect of diclofenac involves not only a prostaglandin-dependent mechanism, but also activation of the nitric oxide-cyclic GMP-potassium channel pathway (4-6). The participation of this pathway has been characterized using mainly models of acute inflammatory pain, such as the formalin test (4, 5). The formalin test is a widely used assay, as it yields results, which allow explaining the effects of analgesic agents used in clinical practice (22). It should be noted however, that observations derived from the formalin test in the rat cannot be directly extrapolated to human patients. At present, it is accepted that the formalin test is a suitable assay for the characterization of mechanisms of analgesic action (19, 22). Furthermore, our group has demonstrated that the formalin test has shown to be adequate for the characterization of analgesic drug interactions, including NSAIDs (4, 5, 16, 23).

Using the formalin test in the rat, we observed that the analgesic response of diclofenac involves participation of the nitric oxide-cyclic GMP-potassium channel pathway (4, 5). As a result of potassium channel opening, potassium leaks out of the neuron resulting in hyperpolarization and reduced excitability, leading to a decreased pain threshold, i.e, to analgesia (4-8) We have previously reported that local administration of glibenclamide, a blocker of ATP-sensitive potassium channels, was able to inhibit the analgesic response of local diclofenac. That is, both drugs were directly injected in the inflamed tissue. This experimental strategy was designed to produce a pharmacodynamic drug interaction occurring at the effect compartment level allowing to characterize the role of potassium channels in diclofenac-induced analgesia. However, since glibenclamide is widely used in therapeutics, as it is an effective oral hypoglycemic agent, an interaction with diclofenac in clinical practice appears as possible. Nonetheless, it should be noted that glibenclamide is administered systemically and not locally. Moreover, although there are topical formulations of diclofenac, systemic administration of this NSAID is the most frequently used. Therefore, we decided to study if there is an interaction between diclofenac and glibenclamide in the rat after systemic administration, in order to have some insight on the potential effects on a patient taking both medications concomitantly. We decided to study the possibility of both a pharmacodynamic and a pharmacokinetic interaction.

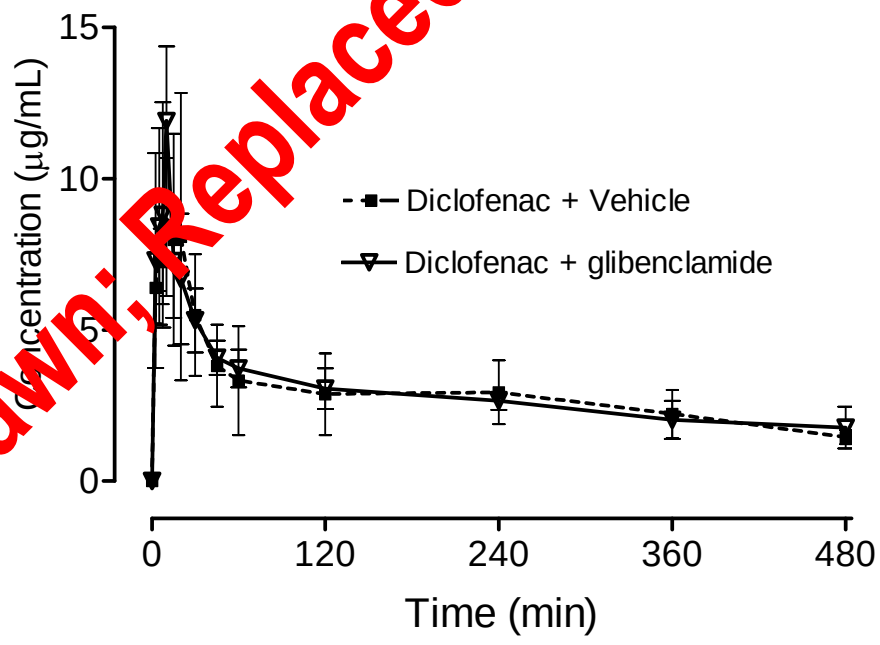

Figure 4. Mean plasma concentration–time curves in rat after single oral administration of 18 mg/kg diclofenac or with 10 mg/kg oral dose of glibenclamide. Data are the mean ± SD for 6 rats.

Table 2. 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 (µg/mL)	Tmax (min)	AUC_{0-t} (µg min/mL)	$AUC_{0-\infty}$ (µg min/mL)
Diclofenac + vehicle	10.1 ± 2.7	12.1 ± 8.7	1400.8 ± 416.6	1985.5 ± 451.3
Diclofenac + Glibenclamide	11.9 ± 2.5	9.6 ± 0.9	1403.1 ± 165.4	2399.1 ± 809.0
Significance (p)	0.474	0.239	0.990	0.291

The results for Cmax, Tmax and AUC are given as mean ±SD of six repetitions for each treatment. Comparisons of bioavailability parameters observed in presence and the absence of glibenclamide were performed using the Student's *t* test.

In the present study, we observed that systemic glibenclamide was able to reduce the analgesic response of systemic diclofenac. Notwithstanding, glibenclamide, by itself, did not produce any significant effect on formalin-induced pain. The lack of effect of the K^+ channel blocker is consistent with previous studies in which glibenclamide did not modify the nociceptive activity of chemical, thermal noxious stimuli and mechanical hyperalgesia (4-6, 24, 25), and allows excluding the possibility that inhibition of diclofenac antinociception could be due to a hyperalgesic or nociceptive effect of the hypoglycemic agent.

The fact that systemic glibenclamide reduces the analgesic effect of systemic diclofenac could be due to a reduction in the bioavailability of the NSAID. Hence, we considered relevant to examine diclofenac oral bioavailability in presence and the absence of the oral hypoglycemic agent. It is well known that interactions can be produced by inhibition of the metabolism of one drug by another. Clinically, relevant drug-drug interactions are frequently caused by an inhibition of P450-dependent reactions (26). In this respect, there is experimental evidence that cytochromes CYP1A1, CYP2C9, CYP2C19 and CYP3A4 are involved in the biotransformation of glibenclamide (27). On the other hand, diclofenac is metabolized by CYP2C9 and CYP3A4 enzymes (9, 28-30). Notwithstanding, oral glibenclamide did not produce any significant alteration of diclofenac oral bioavailability, suggesting that there is no interaction affecting the absorption, distribution or elimination of the NSAID. Our results show that, although the first pass-effect and elimination by metabolic clearance of both drugs involve common enzymatic pathways, there is no alteration in diclofenac bioavailability. This could be due to the fact that the drug concentrations resulting from the studied doses were far below saturation levels, and thus could be handled without any significant inhibition by the enzymatic systems.

Practical implications of these results

Molecules from the sulfonylurea group, as well as the biguanide metformin, are widely used in the therapeutic management of Type 2 Diabetes. Glibenclamide, a potent second-generation sulfonylurea, has been used in the management of non-insulin dependent diabetes mellitus in Europe since 1969, and in the United States since 1984. Glibenclamide improves glucose tolerance mainly by augmenting insulin secretion (31). The mechanism of action glibenclamide at the cellular level consists of an inhibition of the ATP-sensitive K^+ channels (15). In the present work, systemic administration of glibenclamide decreased the antinociceptive effect produced by systemically administrated diclofenac in the rat. This effect, however, did not involve an alteration of diclofenac bioavailability, and thus a pharmacokinetic interaction appears as unlikely. Our results thus suggest that glibenclamide volume of distribution includes the effect compartment of diclofenac. Once both drugs are distributed into this compartment, a purely pharmacodynamic interaction occurs, likely involving potassium channels.

Our results show that an interaction between glibenclamide and diclofenac, resulting in a reduced analgesic efficacy, is possible in clinical practice. Notwithstanding, it is necessary to further

characterize this issue. Studies on diabetic rats are required, as it is known that hyperglycemic states are able to alter the pain threshold and the renal function (32, 33). Hence, both the pharmacokinetics and pharmacodynamics of glibenclamide and diclofenac could show differences diabetic animals compared to non-diabetic rats, as those studied in the present work. Finally, clinical studies are warranted to establish the relevance of the glibenclamide-diclofenac interaction.

CONCLUSION

Systemic administration of diclofenac reduces the analgesic effect of diclofenac. The interaction does no appear due to an alteration of diclofenac bioavailability, but to a pharmacodynamic interaction involving blockade of potassium channels at site of action of the NSAID.

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