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Synergistic effect of the interaction between curcumin and diclofenac on the formalin test in rats

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ABSTRACT

The association of non-steroidal anti-inflammatory drugs with certain plant extracts can increase antinociceptive activity, permitting the use of lower doses and thus limiting side effects. Therefore, the aim objective of the current study was to examine the effects of curcumin on the nociception and pharmacokinetics of diclofenac in rats. Antinociception was assessed using the formalin test. 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 antinociceptive response. Rats were treated with oral diclofenac (1–31 mg/kg), curcumin (3.1–100 mg/kg) or the diclofenac–curcumin combination (2.4–38.4 mg/kg). To determine the possibility of a pharmacokinetic interaction, the oral bioavailability of diclofenac (10 mg/kg) was studied in presence and the absence of curcumin (31 mg/kg). Diclofenac, curcumin, or diclofenac–curcumin combination produced an antinociceptive effect on the formalin test. ED_{30} values were estimated for the individual drugs, and an isobologram was constructed. The derived theoretical ED_{30} for the antinociceptive effect (19.2 mg/kg) was significantly different from the observed experimental ED_{30} value (9.8 mg/kg); hence, the interaction between diclofenac and curcumin that mediates the antinociceptive effect was synergistic. Notwithstanding, the interaction does not appear to involve pharmacokinetic mechanisms, as oral curcumin failed to produce any significant alteration in oral diclofenac bioavailability. Data suggest that the diclofenac–curcumin combination can interact at the systemic level and may have therapeutic advantages for the clinical treatment of inflammatory pain.

Introduction

Pain is a warning mechanism allowing the preservation of the integrity of the organism. However, under certain circumstances, pain does not longer have a beneficial effect and becomes a pathological process that requires treatment. Drugs used for pain relief, such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs), induce several adverse effects (O'Neil et al., 2012). A strategy that may allow an increase in analgesic drug efficacy and a reduction of adverse effects is combining drugs with different mechanism of action. Diclofenac is a NSAID prescribed for its anti-inflammatory and antipyretic effects, besides it has been shown to be effective in treating a variety of acute and chronic pains (Gan, 2010). Extensive research has shown that the pharmacological activity of diclofenac goes beyond COX inhibition, including multiple mechanisms of action (Björkman, 1995; Ortiz et al., 2003; Ortiz, 2012; Vellani et al., 2013). Even though diclofenac is an effective analgesic, the inhibition of COX enzymes can lead to a range of undesirable and sometimes fatal short-and long-term organ toxicities, including gastrointestinal ulceration, bleeding, nefro- and hepatotoxic effects (O'Neil et al., 2012). Therefore, several approaches have been adopted to reduce the risk of NSAID-induced complications; these include reducing the NSAID dose, switching

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to NSAIDs that are perceived to be less toxic, or the concomitant use of other drugs or plant extracts with minor toxicity and with analgesics effects.

In recent years curcumin (diferuloylmethane), the most abundant curcuminoid isolated from *Curcuma longa* L (Zingiberaceae) has demonstrated anti-inflammatory, immunomodulatory, hypolipidemic, antiviral, antiprotozoal, antifungal and antibacterial properties (Srinivas et al., 1992; Mesa et al., 2000; Chainani-Wu, 2003; Kohli et al., 2005). Furthermore, previous reports have demonstrated analgesic effects of curcumin in formalin-induced pain (De Paz-Campos et al., 2012; Mittal et al., 2009) and chronic constriction injury-induced neuropathic pain (Zhao et al., 2011). The mechanisms underlying the analgesic action of systemic curcumin have been suggested to be associated with suppression of brain nitrite and serum tumor necrosis factor α levels, activation of K_{ATP} channels and the descending monoamine system coupled with opioid receptors (De Paz-Campos et al., 2012; Zhao et al., 2011). A recent study showed that the intrathecal administration of curcumin decreased inflammatory pain in rats (Han et al., 2012). Moreover, it appears that curcumin is able to improve the effect of subanalgesic doses of diclofenac in the formalin-induced orofacial pain in rats (Mittal et al., 2009). However, no information is available on the kind of interaction between diclofenac and curcumin. Therefore the main objective of the present work was to extend the observations on the interaction between curcumin and diclofenac and to determine if this interaction between these two drugs is pharmacodynamic or pharmacokinetic.

Materials and methods

Animals

Female Wistar rats aged 7–10 weeks (weight range: 180–200 g) from our own breeding facilities were used in this study. Efforts were made to minimize animal suffering and to reduce the number of animals used. Each rat was used in only one experiment and sacrificed in CO_2 chamber at the end of the experiment. All experiments followed the Guidelines on Ethical Standards for Investigation in Animals (Zimmermann, 1983), and the protocol was approved by the Institutional Animal Care and Use Committee (CINVESTAV, IPN).

Drugs

Curcumin (≥80% curcumin, ≥94% curcuminoid content), diclofenac and formaldehyde were purchased from Sigma–Aldrich, Saint Louis, MO, USA. Curcumin was suspended in a 0.5% carboxymethylcellulose (CMC). Diclofenac was dissolved in saline solution.

Isobolographic analysis study

Measurement of antinociceptive activity

Pain and antinociception were assessed using the paw formalin test, as previously described (Ortiz et al., 2003, 2012; Ortiz and Castañeda-Hernández, 2008). Briefly, 50 μl of diluted formalin (1%) were injected subcutaneously (s.c) into the dorsal surface of the right hind paw, and the resulting flinching behavior was considered to be an expression of nociception. Certain groups of animals received an oral administration (p.o.) of vehicle or increasing doses of curcumin (3.1, 10, 31 and 100 mg/kg) sixty min before the formalin insult. Other groups of rats were treated with diclofenac (1, 3.1, 10 and 31 mg/kg, p.o.) 15 min before formalin injection. Finally, the curcumin–diclofenac combination was also administered at increasing doses (2.4, 4.8, 9.6, 19.2 and 38.4 mg/kg p.o.). The oral volumes administered were 4 ml/kg. Rats in all groups were observed for changes in behavioral or motor function that could have been induced by the treatments. The ability of the animals to stand and walk with a normal posture was assessed, but this was not quantified.

Data analysis

The time courses of the antinociceptive responses resulting from the administration of the individual drugs and the drug combinations were constructed by plotting the mean number of flinches as a function of time. The areas under the resulting curves (AUC) were calculated using the trapezoidal rule (Tallarida and Murray, 1981). AUC was calculated for the two phases of the assay and the percent of antinociception for each phase was calculated according to the following equation (Ortiz and Castañeda-Hernández, 2008): *Percent of antinociception* = $[(AUC_{vehicle} - AUC_{post\ compound})/AUC_{vehicle}] \times 100$.

Dose–response curves were constructed using least-squares linear regression, and the antinociceptive $ED_{30} \pm$ standard error (SE) values were calculated according to Tallarida (2000). The interaction between curcumin and diclofenac was characterized by isobolographic analysis in which it was assumed that the combinations are comprised of equieffective dose of the individual component drugs (Berenbaum, 1989). Thus, from the dose–response curves of each individual agent, the dose resulting in 50% of the effect (ED_{50}) could be determined. However, considering a maximal effect to be 100% of the total suppression of formalin-induced nociception, it appeared that diclofenac was incapable of exerting a 50% suppression of the nociceptive response; thus, the calculation of ED_{50} in the antinociceptive effect was not feasible. Therefore, we estimated the antinociceptive ED_{30} instead of the antinociceptive ED_{50}. It has been demonstrated that ED_{30} values are suitable for isobologram construction with several agents (Tallarida, 2000; Ortiz and Castañeda-Hernández, 2008), including diclofenac (Jiménez-Andrade et al., 2003). Subsequently, a dose–response curve was obtained by concurrent delivery of two drugs (curcumin plus diclofenac) in a fixed-ratio mixture (1:1) that was based on the ED_{30} values of each individual agent. To construct the experimental antinociceptive effect–dose curve each group of rats received one of the following dose of the combination: curcumin ED_{30} (18.6 mg/kg) + diclofenac ED_{30} (19.8 mg/kg); curcumin $ED_{30}/2$ (9.3 mg/kg) + diclofenac $ED_{30}/2$ (9.9 mg/kg); curcumin $ED_{30}/4$ (4.65 mg/kg) + diclofenac $ED_{30}/4$ (4.95 mg/kg); curcumin $ED_{30}/8$ (2.33 mg/kg) + diclofenac $ED_{30}/8$ (2.48 mg/kg); or curcumin $ED_{30}/16$ (1.16 mg/kg) + diclofenac $ED_{30}/16$ (1.24 mg/kg). The experimental ED_{30} value for the curcumin–diclofenac combination was calculated from this curve.

The theoretically additive effect of the antinociceptive ED_{30} was estimated from the dose–response curves obtained by sole administration of each drug (*i.e.*, considering that the effect observed with the combination is the result of the sum of the effects of each individual drug). This theoretical ED_{30} value was then compared with the experimentally derived ED_{30} value to determine whether there was a statistically significant difference (Tallarida, 2000). The theoretical and experimental ED_{30} values of the combinations were also contrasted by calculating the interaction index. This was calculated as experimental ED_{30}/theoretical ED_{30}. If the value is close to 1, the interaction is additive. Values lower than 1 are an indication of the magnitude of supra-additive or synergistic interactions, and values higher than 1 correspond to sub-additive or antagonistic interactions (Berenbaum, 1989; Tallarida, 2000, 2001, 2002; Ortiz and Castañeda-Hernández, 2008).

Statistical analysis

Dose–response data were analyzed by one-way analysis of variance (ANOVA) with Tukey's test *post hoc* comparison (Jiménez-Andrade et al., 2003; Ortiz and Castañeda-Hernández, 2008). The