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Pharmacological evidence for the participation of NO–$_c$GMP–K_{ATP} pathway in the gastric protective effect of curcumin against indomethacin-induced gastric injury in the rat

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ABSTRACT

Curcumin, main compound obtained from rizhoma of *Curcuma longa*, shows antitumoral, antioxidant, anticarcinogenic and gastric protective properties. Recently, it has been demonstrated that curcumin exerts its gastric protective action due to an increase in gastric nitric oxide (NO) levels. However, it is unknown whether these increased NO levels are associated with activation of intracellular signaling pathways. Thus, the purpose of this study was to investigate the role of NO–$_c$GMP–K_{ATP} pathway in the gastric protective effect of curcumin during indomethacin-induced gastric injury in the rat. Adult female Wistar rats were gavaged with curcumin (3–300 mg/kg, p.o.) or omeprazole (30 mg/kg, p.o.) 30 min before indomethacin insult (30 mg/kg, p.o.). Other groups of rats were administered L-NAME (70 mg/kg, i.p.; inhibitor of nitric oxide synthase), ODQ (10 mg/kg, i.p.; inhibitor of soluble guanylate cyclase) or glibenclamide (1 mg/kg, i.p.; blocker of ATP-sensitive potassium (K_{ATP}) channels) 30 min before curcumin (30 mg/kg, p.o.). 3 h after indomethacin administration, rats were sacrificed and gastric injury was evaluated by determining total damaged area. A sample of gastric tissue was harvested and processed to quantify organic nitrite levels. Curcumin significantly protected against indomethacin-induced gastric injury and this effect was comparable to gastroprotective effect by omeprazole. L-NAME, ODQ and glibenclamide significantly prevented the curcumin-mediated gastric protective effect in the indomethacin-induced gastric injury model. Furthermore, curcumin administration induced a significant increase in gastric nitric oxide levels as compared to vehicle administration. Our results show for the first time that curcumin activates NO/$_c$GMP/K_{ATP} pathway during its gastro protective action.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin are prescribed worldwide for the relief of pain and inflammation. However, their use is limited by the induction of gastric and renal toxicity. NSAIDs-induced gastric damage in large part is due to cyclooxygenases (COXs) inhibition (Lanza, 1989). However, there are other mechanisms that may be activated in response to COXs inhibition to defend gastric mucosa, for example, increased gastric nitric oxide (NO) levels. Nitric oxide shares functions with prostacyclin, a COX product, to increase gastric blood flow, mucus and bicarbonate secretion (Tarnawski et al., 2012; Wallace and Devchand, 2005).

Different intracellular signaling cascades have been suggested to be involved in the gastric protective actions of NO including an increase in cyclic guanosine monophosphate ($_c$GMP) content (Denninger and Marletta, 1999; Medeiros et al., 2008). Then, $_c$GMP may activate different $_c$GMP-dependent protein kinases which in turn activate ATP-sensitive potassium channels (K_{ATP} channels). K_{ATP} channels are functionally expressed in smooth muscle cells of the guinea pig stomach (Sim et al., 2002) and their blockade with glibenclamide reversed the gastro protective effect of sildenafil (Medeiros et al., 2008), L-cysteine (Medeiros et al., 2009), hydrogen sulfide (H_2S), (Medeiros et al., 2009) and carbenoxolone (Chavez-Pina

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et al., 2011) in different animal models. These findings together suggest that activation of NO–$_c$GMP–K_{ATP} pathway has a participation in gastric defense.

Curcumin is the main compound obtained from rizhoma of *Curcuma longa*, also known as turmeric (Rivera-Espinoza and Muriel, 2009). Curcumin displays a vibrant yellow color and is widely used as an additive in Indian food (Basnet and Skalko-Basnet, 2011). *C. longa* has largely been used in Asian traditional medicine in the treatment of a variety of inflammatory disorders. It has a wide spectrum of biological and pharmacological effects including antitumor, antioxidant and anticarcinogenic properties (Aggarwal and Harikumar, 2009; Aggarwal and Sung, 2009; Chavez-Pina et al., 2011; Goel et al., 2008; Thong-Ngam et al., 2012). There is evidence that curcumin generates gastric protective action against indomethacin-induced gastric injury (Chattopadhyay et al., 2006; Sharma et al., 2012) and this gastric protective effect has been related to an increase in gastric nitric oxide levels (Morsy and El-Moselhy, 2013). Given that the activation of the NO–$_c$GMP–K_{ATP} pathway is involved in the gastric protective actions of different drugs such as sildenafil (Medeiros et al., 2008) and carbenoxolone (Chavez-Pina et al., 2011), the purpose of this study was to investigate the role of NO–$_c$GMP–K_{ATP} pathway in the gastric protective effect of curcumin during indomethacin-induced gastric injury in the rat.

2. Material and methods

2.1. Drugs

Curcumin (Cat no: C7727), N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, Cat no: N5751), 1H-Oxadiazolo [4,3-a] quinoxalin-1-one (ODQ, Cat no: O3636), glibenclamide (Cat no: G0639), indomethacin (Cat no: I7378) and omeprazole (Cat no: O104) were purchased from SigmaAldrich (Toluca, México).

2.2. Animals

All experiments were performed with adult female Wistar rats (180–220 g) obtained from Centro de Investigación y de Estudios Avanzados del IPN (Mexico, City). Procedures involving rats and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with the international rules on care and use of laboratory animals. The sample size per group was five to seven animals. Rats were fed with standard laboratory chow and tap water ad libitum. The rats were placed in cages with wire-net floors and fasted 18 h before experimentation, but were allowed free access to tap water while fasting.

2.3. Acute gastric injury induced by indomethacin

Groups of rats received oral administration of indomethacin (30 mg/kg, 0.1 ml/100 g) with an orogastric feeding tube (Socorex Ibsa S.A., Switzerland; Catalog number 3761251B). The control group received the same volume of vehicle (0.5% sodium bicarbonate). 3 h later, rats were euthanized in a CO_2 chamber. The stomach was removed, and opened along the greater curvature. An observer, blind to the experimental treatment status of the animal, measured the area (mm^2) of each gastric lesion. The damaged area was determined by measuring macroscopically the width and the length of each lesion with digital calipers. The area of all lesions in the corpus of the stomach for each animal was calculated by summing the values and reported as gastric lesions (mm^2) (Pineda-Pena et al., 2012; Wallace et al., 2000).

2.4. Gastric protective effect of curcumin on indomethacin-induced gastric injury

Rats received oral administration of carboxymethyl cellulose (CMC, as vehicle) or curcumin (3–300 mg/kg, 0.4 ml/100 g). After 30 min, all rats were administered with indomethacin (30 mg/kg, p.o.) to produce gastric mucosal lesions. Finally, gastric damage was measured as described above. For subsequent experiments performed curcumin was gavaged in a dose of 30 mg/kg, p.o. This dose was selected due to the effect observed in curcumin dose–response curve after 100 and 300 mg/kg, which was not significantly greater than that produced by 30 mg/kg of curcumin. No adverse effects were observed after the administration of the highest dose of curcumin.

2.5. Gastroprotective effect of omeprazole on indomethacin-induced gastric injury

Rats received oral administration of saline solution (as vehicle for omeprazole, 0.1 ml/100 g) or omeprazole (30 mg/kg, 0.1 ml/100 g) (Navarrete et al., 2005). 30 min later, all rats received oral administration of indomethacin (30 mg/kg, p.o.) to induce gastric mucosal lesions. Finally, gastric damage was determined 3 h later as described above.

2.6. Role of the NO–$_c$GMP–K_{ATP} pathway in the gastric protective effect of curcumin

To investigate the involvement of NO–$_c$GMP–K_{ATP} pathway in the gastric protective effect of curcumin, selective inhibitors or blockers of this pathway were individually administered before curcumin treatment. L-NAME, a nitric oxide synthase (NOS) inhibitor (70 mg/kg, dissolved in saline solution), or ODQ, a soluble guanylyl cyclase enzyme inhibitor (10 mg/kg, dissolved in 1% dimethylsuphoxide), or glibenclamide (K_{ATP} channels blocker, 1 mg/kg, dissolved in 0.05 M NaOH) was intraperitoneally administered 30 min before curcumin. 30 min after curcumin administration the gastric mucosal lesions were induced and measured as described above. Schedules of administration and doses of these inhibitors were selected based on previous studies (Chavez-Pina et al., 2011; Medeiros et al., 2008). Proper controls with the combination of each inhibitor and indomethacin were performed and gastric injury was not significant from indomethacin alone (Table 1).

2.7. Measurement of organic nitrite levels produced by nitric oxide

Five groups were formed and received the following treatment: 1) saline solution + CMC + sodium bicarbonate, 2) L-NAME + vehicle + sodium bicarbonate, 3) CMC + indomethacin + sodium bicarbonate, 4) curcumin + indomethacin + sodium bicarbonate and 5)

Table 1
Gastric area lesions (mm^2) in control groups for the combination of inhibitors and indomethacin in the indomethacin-gastric injury model in the rats.

Treatment	Gastric injury (mm^2)
Indomethacin	30.76 ± 2.7
Indomethacin + L-NAME	28.00 ± 5.4
Indomethacin + ODQ	38.40 ± 6.8
Indomethacin + GBC	38.00 ± 8.7

N^G-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg i.p.), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, soluble guanylate cyclase inhibitor, 10 mg/kg i.p.) and glibenclamide (GBC, K_{ATP} inhibitor, 1 mg/kg i.p.) on indomethacin induced gastric injury in rats. Data are presented as mean ± S.E.M. (n = 5–7). There is no statistical difference between groups.

L-NAME + curcumin + indomethacin. After 3 h rats were sacrificed in a CO_2 chamber. The stomach was removed, and a sample of the corpus region of the stomach was then excised, weighed, and added to a tube containing 1 ml of sodium phosphate buffer (10 mmol/l; pH 7.4). The tissue sample was minced with scissors for 30 s, and then placed in a shaking water bath (37 °C) for 20 min. The samples were centrifuged (9000 g) for 1 min, and the concentration of organic nitrites in the supernatant was determined by nitrate/nitrite colorimetric assay using the Griess reaction at 540 nm (Giustarini et al., 2008).

2.8. Statistical analysis

All data are expressed as mean ± S.E.M. Comparisons among groups were performed using a one-way analysis of variance followed by Neuman's Keuls's test. $P \leq 0.05$ was considered as a statistically significant difference between means.

3. Results

Oral administration of either vehicle or curcumin did not produce gastric ulcers by themselves (Fig. 1A). In contrast, oral administration of indomethacin resulted in a significant gastric injury as evidenced by multiple gastric lesions (Fig. 1A). Pretreatment with curcumin at doses of 30, 100 and 300 mg/kg, but not at 3 and 10, resulted in a gastric protective effect against indomethacin-induced gastric injury. Moreover, a plateau effect was reached at the doses of 30 mg/kg. Thus, this dose was used for further studies (Fig. 1A). The gastric protective effect of curcumin was similar in magnitude to that produced by omeprazole, a proton pump inhibitor (Fig. 1B).

In order to investigate the role of NO–$_c$GMP–K_{ATP} pathway during the gastric protective effect of curcumin, L-NAME, ODQ or glibenclamide was administered. L-NAME, a NOS inhibitor, significantly reversed the gastric protective effect of curcumin during indomethacin-induced gastric damage (Fig. 2A). Rats treated only with L-NAME and vehicles did not show any gastric hemorrhagic lesions (Fig. 2A).

To investigate whether $_c$GMP participates in curcumin's gastric protective effect, we administered ODQ, a soluble guanylate cyclase inhibitor. ODQ reversed curcumin gastric protective effect, while ODQ and vehicles administered alone did not generate any gastric lesions (Fig. 2B). Finally, to assess the involvement of K_{ATP} channels in curcumin's gastric protective effect, a selective blocker of K_{ATP} channels (glibenclamide) was administered. Glibenclamide decreased curcumin's gastric protective effect (Fig. 2C).

To confirm NO participation in the gastric protective effect of curcumin, gastric organic nitrite levels were measured. Administration of either L-NAME or indomethacin by themselves significantly decreased organic nitrite levels content as compared to basal levels (Fig. 3). Also, the results showed that administration of curcumin prevented the decrement of organic nitrite levels induced by indomethacin (Fig. 3). When L-NAME was administered to rats

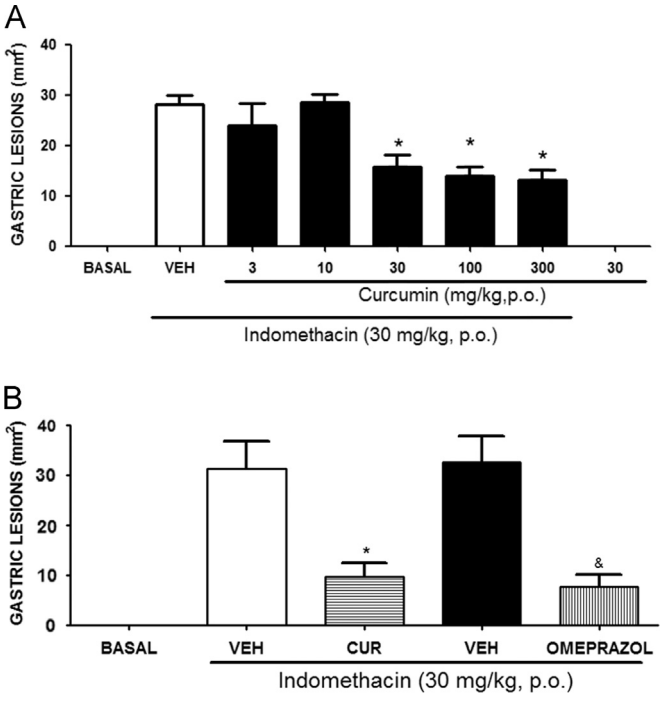

Fig. 1. Gastroprotective effect of CUR (curcumin) on the indomethacin-induced gastric injury model in rats. (A) Dose–response curve of curcumin (CUR). CUR was gavaged 30 min before indomethacin administration. (B) Comparative gastroprotective CUR (curcumin, 30 mg/kg, p.o.) and omeprazol (30 mg/kg, p.o.). Data are presented as mean ± S.E.M. ($n=5$–7) $^{*}P \leq 0.05$ vs vehicle (carboxymethylcelluose (CMC)), $^{\&}P \leq 0.05$ vs vehicle (saline solution (SS)).

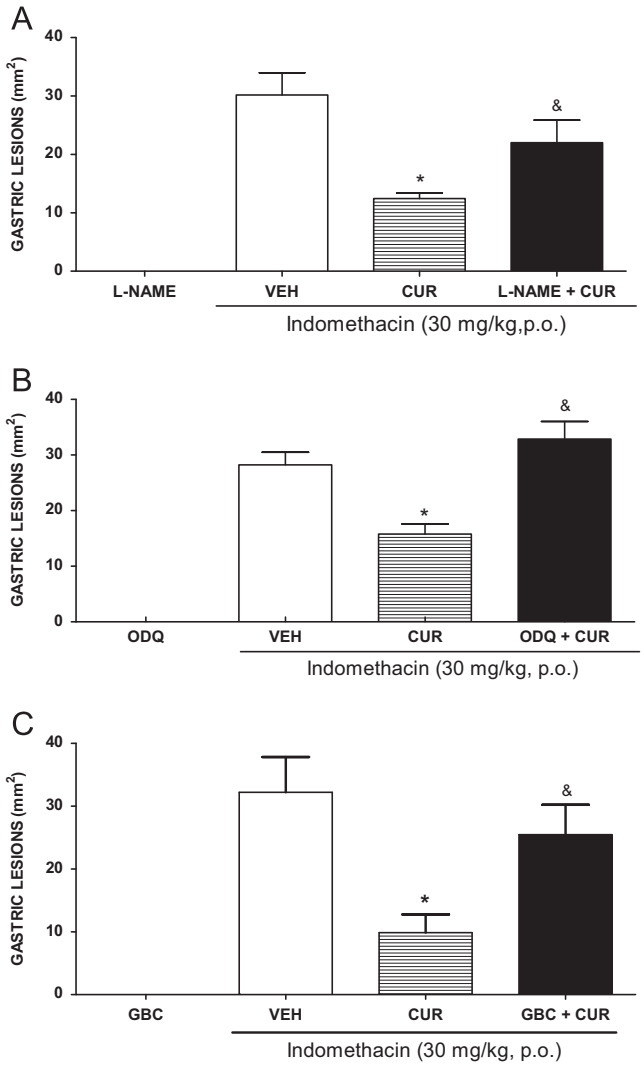

Fig. 2. Participation of the NO/$_c$GMP/K_{ATP} channels pathway on the gastric protective effect of curcumin. (A) N^G-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg i.p.), (B) 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, soluble guanylate cyclase inhibitor, 10 mg/kg i.p.) and (C) glibenclamide (K_{ATP} inhibitor, 1 mg/kg i.p.) on curcumin (30 mg/kg p.o.) gastroprotection on gastric lesions induced by indomethacin in rats. Data are presented as mean ± S.E.M. ($n=5$–7) $^{*}P \leq 0.05$ vs vehicle (VEH). $^{\&}P \leq 0.05$ vs curcumin (CUR).

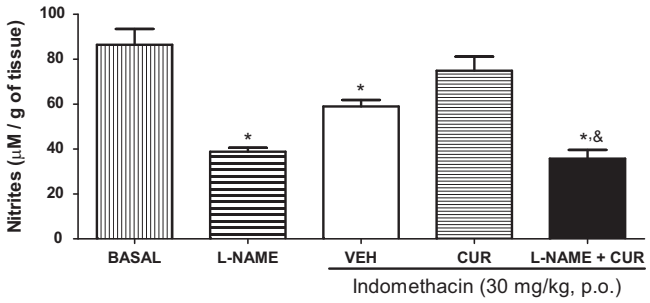

Fig. 3. Levels of gastric organic nitrites during curcumin treatment in the indomethacin-induced gastric injury model in the rat. Data are presented as mean ± S.E.M. (n=5–7) $^{*}P \leq 0.05$ vs basal, $^{\&}P \leq 0.05$ vs curcumin+indomethacin.

with gastric injury and treated with curcumin, organic nitrite levels were significantly reduced (Fig. 3).

4. Discussion

The present study shows for the first time that curcumin administration reduces indomethacin-induced gastric injury due to the activation of NO–$_c$GMP–K_{ATP} channels pathway.

It is well known that curcumin is used for the treatment of several disorders such as inflammation, cancer (Aggarwal and Harikumar, 2009; Aggarwal and Sung, 2009), pain (De Paz-Campos et al., 2012; Sharma et al., 2006; Tajik et al., 2008; Yeon et al., 2010) and gastric injury (Chattopadhyay et al., 2006; Morsy and El-Moselhy, 2013; Sharma et al., 2012; Thong-Ngam et al., 2012). Regarding the mechanism(s) behind the gastric protective effect, it is suggested that curcumin activates multifactorial mechanisms. For example, curcumin prevents gastric peroxidase and scavenging reactive oxygen species (Chattopadhyay et al., 2006), diminishes leukocyte infiltration, attenuates intercellular adhesion molecule (ICAM)-1 and tumor necrosis factor (TNF)-α levels (Thong-Ngam et al., 2012). In addition, it was recently reported that the gastric protective effect of curcumin against indomethacin-induced gastric injury is associated with increased gastric mucosal NO levels and with an enhanced activity of gastric catalase and superoxide dismutase (Morsy and El-Moselhy, 2013).

In this study we found that curcumin's gastric protective effect is associated with the activation of an intracellular signaling pathway dependent on NO. The NO–$_c$GMP–K_{ATP} pathway has been found to participate in the cardiovascular system (in the regulation of vascular tone and platelet function), in the nervous system (in neurotransmission and, possibly, long-term potentiation and depression) (Denninger and Marletta, 1999) and in mucosa gastric defense (Chattopadhyay et al., 2006; Morsy and El-Moselhy, 2013; Thong-Ngam et al., 2012). In this regard, sildenafil, an inhibitor of phosphodiesterase-type 5, and carbenoxolone also exert their gastric protective effect on ethanol-induced gastric injury due to the activation of NO–$_c$GMP–K_{ATP} channels pathway (Chavez-Pina et al., 2011; Medeiros et al., 2008).

In this study, we found that curcumin significantly increases gastric NO levels. NO, like other endogenous substances (e.g. prostaglandins), regulates the mucosal microcirculation which is essential for delivery of oxygen and nutrients and removal of toxic substances (Wallace and Granger, 1996). NO is an important vasodilator in the gastric vasculature. It has been reported that a diet rich in nitrates increases gastric blood flow. Nitrate is absorbed in the proximal small intestine and then concentrated in the salivary glands (Tannenbaum et al., 1976). Salivary nitrate is then reduced to nitrite by oral bacteria and is further reduced to NO in the acidic stomach (Duncan et al., 1997; Lundberg et al., 1994). In addition, studies have demonstrated that application of a solution of NO or a NO donor to the mucosa protects it from injury (MacNaughton et al., 1989; Wallace et al., 2004). NO may also modulate the gastric mucosal integrity by interacting with other protective mediators, such as sensory neuropeptides, prostaglandins, and secretion of mucus and bicarbonate (Kim and Hwan Kim, 2001). Finally, endogenous NO produced by constitutive nitric oxide synthase ($_c$NOS) such as neuronal nitric oxide synthase ($_n$NOS) and endothelial nitric oxide synthase ($_e$NOS) regulates mucosal perfusion to protect gastrointestinal mucosa against harmful stimuli. However, overproduction of NO following activation of inducible NOS may be involved in gastrointestinal injury (Hayashi et al., 2004).

Our results suggest that curcumin induces an increase of $_c$GMP due to $_s$GC activation. There are a few reports that describe that curcumin increases $_c$GMP levels. Recently curcumin, like sildenafil, has been shown to be involved in erectile signaling via elevation of cyclic guanosine monophosphate ($_c$GMP) (Abdel Aziz et al., 2012). Curcumin increases the synthesis of NO from vascular endothelial cells and stimulates the activity of $_s$GC which produces $_c$GMP from GTP. Increase of cellular concentrations of $_c$GMP activates different $_c$GMP-mediated PKGs which result in vasodilation (Barnett and Machado, 2006).

Recently, there is evidence showing that curcumin activates K_{ATP} channels in the formalin-induced pain model in the rat (De Paz-Campos et al., 2012). However, De Paz-Campos did not confirm the complete activation of the NO–$_c$GMP–K_{ATP} pathway by curcumin in the pain model. Contribution of K_{ATP} channels to the gastric protective effect of prostaglandins and NO during indomethacin- and ethanol-induced gastric injury has been previously reported (Chavez-Pina et al., 2011; Medeiros et al., 2008; Peskar and Ehrlich, 2002). Furthermore, the gastric protective action of several compounds such as *Zanthoxylum rhoifolium* (Freitas et al., 2011), carbenoxolone (Chavez-Pina et al., 2011), sildenafil (Medeiros et al., 2008), nicorandil (Ismail et al., 2007), calcitonin gene-related peptide (CGRP) (Doi et al., 1998), L-cysteine and H_2S (Medeiros et al., 2009), has been associated with the opening of K_{ATP} channels. It is suggested that K_{ATP} channels participate in the mucosal defense by modulation of primary afferent nerve fibers which in turn regulate the gastric blood flow and gastric mucus secretion (Iwata et al., 1997).

In conclusion, this is the first report that shows curcumin activation of the NO–$_c$GMP–K_{ATP} pathway to induce gastric protection in indomethacin-induced gastric injury. The mechanism of how curcumin increases nitric oxide production is still unknown. These results confirm the idea that curcumin induces gastric protective effect in a multifactorial way and support the gastric protective action of curcumin.

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