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Probable role of spinal purinoceptors in the analgesic effect of *Trigonella foenum* (TFG) leaves extract

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Abstract

In our previous work, we demonstrated that *Trigonella foenum* (TFG) leaves extract can exert analgesic effects in both formalin (F.T.) and tail flick (T.F.) tests. Spinal serotonergic system, but not endogenous opioid system, was involved in TFG induced analgesia (in the second phase of formalin test). Some reports concern the similarity between NSAIDs and TFG extract in many pharmacological effects or the interaction between NSAIDs and purinergic system; so the present study was designed to investigate the relationship between TFG extract and purinergic system or the inhibition of cyclo-oxygenase (COX). We examined the effect of TFG extract on: (1) the response of rabbit platelets to ADP induced aggregation, (2) the contraction of mouse vas deferens induced by α,β-Me-ATP (a P_2 receptor agonist; this receptor mediates the rapid phase of ADP- and ATP-evoked influx of Ca^{2+} through a non-specific cation channel in platelets), (3) α,β-Me-ATP induced hyperalgesia in tail flick test in male rats and (4) the specific inhibition of COX-1 and COX-2. Our results showed that TFG extract (0.5, 1, 1.5, 3 mg/ml) inhibited ADP (10^{-5} mol) induced platelet aggregation (IC_{50} = 1.28 mg/ml). α,β-Me-ATP (30 μM) induced isometric contraction in vas deferens while suramin (a P_2 receptor antagonist, 50, 150, 300 μM) or TFG extract (0.5, 1, 2, 3 mg/ml) inhibited this effect significantly (IC_{50} were 91.07 μM and 1.57 mg/ml, respectively). Moreover, α,β-Me-ATP (3 μg/rat, i.t.) induced hyperalgesia in tail flick test, but it was prevented by co-injection of α,β-Me-ATP with suramin (120 μg/rat, i.t.) or TFG extract (1 mg/rat, i.t.). Effective concentrations of TFG extract in the above mentioned experiments did not inhibit COX enzymes in EIA tests. In conclusion, these results indicate that the blocking of spinal purinoceptors may contribute in the analgesic effect of TFG leaves extract.

Keywords: Antinociception; P_2X purinoceptor antagonist; TFG; Tail flick; Rabbit platelet aggregation; Vas deferens contraction

1. Introduction

Opioids and NSAIDs are the most widely used antinociceptive drugs; however, they both produce different toxic or side effects. So, it is of great interest to find less harmful antinociceptive drugs. The plant *Trigonella foenum-graecum* (Fenugreek), from the family of Papillonaceae, has been demonstrated to produce antinociceptive (Javan et al., 1997; Parvizpur et al., in press), anti-inflammatory and antipyretic (Ahmadiani et al., 2001) effects. We have reported that intraperitoneal (i.p.) and intrathecal (i.t.) administration of TFG leaves extract produced significant antinociceptive effects in both the first and second phases of formalin test (Parvizpur et al., in press). We showed that the spinal serotonergic system was involved in the analgesia induced by i.t. administration of TFG extract in the second but not in the first phase of formalin test. The analgesic effect of the extract in the second phase of formalin test was partially reduced after lesioning of spinal 5-HT system by 5,7-dihydroxytryptamine (5,7-DHT), but there was still significant analgesic effect in the first and second phases of this test, that indicates the existence of other antinociceptive mechanism(s) of action. It seems that there are some similarities between the effects of NSAIDs and TFG extract. Moreover, it has been

Abbreviations: ADP, adenosin-5′-diphosphate; COX, cyclo-oxygenase; 5,7-DHT, 5,7-dihydroxytryptamine; EIA, enzyme immunoassay; F.T., formalin test; i.p., intraperitoneal; i.t., intrathecal; LPS, lipopolysaccharide; NSAIDs, non-steroid anti-inflammatory drugs; PGE_2, prostaglandin E2; PRP, platelet-rich plasma; PPR, platelet poor plasma; PBS, phosphate-buffered saline; T.F., tail flick test; TFG, *Trigonella foenum*; TXA_2, Tromboxan A2

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reported that there are some relationships between NSAIDs and purinergic system and that the latter plays a role in pain and platelet aggregation (Puri and Colman, 1997). Intrathecal P2X purinoceptor antagonist can suppress both the first and second phases of the formalin induced nociceptive behaviour (Tasuda et al., 1999). Platelet aggregation is induced by ADP acting through purinoceptors (Puri and Colman, 1997) and in the spinal cord, ATP is involved in the initiation of pain through purinoceptors (Kennedy and Leff, 1995; Burnstock, 1996). It has been shown that a brief challenge of rat astrocytes with an ATP analogue, α,β-ME-ATP, results in increased COX-2 expression which may play a role in a chronic neurological disease characterized by inflammation and astrogliosis and a selective COX-2 inhibitor abolishes α,β-Me-ATP induced astrocyte activation (Brambilla et al., 1999). ATP evoked a transient increase in intracellular Ca^{2+} in rat endothelial cell line and indomethacin, an inhibitor of prostaglandin synthesis, inhibited the response to ATP (Nobles and Abbott, 1998) and decreased the response to α,β-Me-ATP in rat urinary bladder smooth muscle cells (Naramatsu et al., 1997). Taken together, in the present study, we examined the probable roles that COX inhibition and purinergic system play in the effects of TFG extract using different in vitro and in vivo experiments.

2. Materials and methods

2.1. Plant material

Trigonella foenum-graecum L (Fenugreek) belongs to the family of Papilionaceae. The leaves of the plant were obtained from the local market. The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran) and voucher specimen coded 417 has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Preparation of the plant extract

Fresh green leaves were separated, cleaned and dried in shade at room temperature. Dried leaves (100 g) were decocted in water for 30 min. Thereafter, the extract was filtered and concentrated using a rotary evaporator apparatus (Heidolph, Germany). The final weight of the crude extract was 21 g. The extract was maintained at 4 °C throughout the experiments.

2.3. Experimental animals

Male NMRI rats (200–250 g), male mice (20–25 g) and albino rabbits (2–2.5 kg) from both sexes were used. Rats and mice were housed in plexiglass cages in groups of four under a temperature range of 21–25 °C and 12-h light/12-h dark cycle. Rabbits were kept separately. Food and water were available ad libitum. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Pub. No. 85-23, revised 1985).

2.4. Drugs

TFG extract was prepared as described previously (Javan et al., 1997). Adenosin-5′-diphosphate (ADP), α,β-Me-ATP and suramin were purchased from Sigma. EIA kits (for the evaluating of PGE_2 and TXA_2) were purchased from Amersham.

2.5. Platelet aggregation

Blood was collected from either sex of albino rabbits. Venous blood was collected from marginal vein in siliconized glass tubes containing 3.8% trisodium citrate (1:10) as anticoagulant and centrifuged at approximately $150 \times g$ for 15 min at room temperature to obtain platelet-rich plasma (PRP), which was collected in plastic tubes. Platelet poor plasma (PPP) was prepared by centrifugation of the remaining blood at approximately $1500 \times g$ for 20 min and collected as mentioned above. PRP and PPP were kept at 37 °C. Platelets were counted by a light microscope and adjusted to a total count of approximately $3 \times 10^9\ l^{-1}$. Platelet aggregation was quantified by impedance method, i.e. the increase in electrical impedance onto two electrodes immersed in the sample. ADP induced aggregation was measured by a chrono-log aggregometer (model 560) and chrono-log recorder (model 707). One milliliter of the aliquots of adjusted PRP were incubated at 37 ° C and centrifuged at 70–80 × *g*. When a stable base line was obtained, 10 μl of adenosin-5′-diphosphate (ADP) with various concentrations (3×10^{-7} to 10^{-5} mol, freshly prepared in 0.9%, w/v, NaCl) was added immediately to 1 ml of PRP in a plastic cuvate containing a stirring rod coated with plastic to initiate the aggregation (Dehpour et al., 1995).

In the next experiment, after recording the response of adjusted PRP to ADP (10^{-5} mol) and obtaining a stable base line for adjusted PRP, TFG extract (0.5, 1, 1.5 and 3 mg/ml) was added to come into contact with PRP for 5 min before adding ADP (10^{-5} mol). The response produced by ADP as an agonist in the presence of TFG extract was expressed as the percentage of response to ADP alone.

2.6. Vas deferens contraction

The whole vas deferens was dissected from mature male mice (20–25 g) killed by cervical dislocation. Each vas deferens was stripped of adhering fat and mesenteric investment in a 20 ml tissue bath containing gassed (5% CO_2 in O_2) Kerebs solution at 37 °C. Following tissue preparation (resting tension of 0.5 g for 40 min), the response to α,β-Me-ATP (30 μM) was measured (Mallard et al., 1992). Then vas deferens was incubated with different doses of suramin or TFG extract for 20 min and after these treatments, the responses to α,β-Me-ATP were measured again. The response produced by α,β-Me-ATP (30 μM) as an agonist in the presence of TFG extract or suramin was expressed as the percentage of response to α,β-Me-ATP alone.

2.7. Tail flick test

Antinociception was assessed by a tail flick apparatus (HSE, Germany). The tail flick latencies were evaluated twice just