

## Modulatory effect of diclofenac on antispasmodic effect of pitofenone in cholinergic spasm

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Biliary, ureteric and intestinal colic are extremely common clinical conditions associated with smooth muscle spasm. In the present study, antispasmodic activity was carried out against acetylcholine (10-640 ng/ml)-induced contractions on guinea pig ileum. Acetylcholine (10-640 ng/ml) induced concentration-dependent contraction of smooth muscle. Diclofenac, in varying concentration ( $9.4 \times 10^{-5}$  mol/l and  $14.1 \times 10^{-5}$  mol/l) shifted the concentration response curve of acetylcholine to the right without suppressing the maximal response. However, in higher concentration diclofenac ( $18.9 \times 10^{-5}$  mol/l) blocked the response in an unsurmountable fashion. Further, analgin ( $11.09 \times 10^{-5}$ ,  $16.63 \times 10^{-5}$  and  $22.18 \times 10^{-5}$  mol/l) in equimolar concentrations did not alter the concentration response curve of acetylcholine, but in higher concentration analgin ( $44.36 \times 10^{-5}$  mol/l) also blocked the response in an unsurmountable fashion. Pitofenone ( $2.5 \times 10^{-6}$  mol/l) also, shifted the concentration response curve of acetylcholine to right in a parallel fashion with no change in maximal response. The present study confirms the potent antispasmodic activity of diclofenac-pitofenone combination in comparison to analgin-pitofenone in molar equivalent concentration (in comparison to diclofenac) against acetylcholine-induced contractions of guinea pig ileum.

**Keywords:** Antispasmodic effect; Guinea pig ileum; Analgesics

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Acute abdominal pain is generally associated with renal or ureteric colic and has been described as one of the most excruciating pains in humans, needing immediate medical attention presenting a challenge, for diagnosis and therapeutic intervention. The main pathological hallmark for the abdominal pain is colic, which is attributed to spasm or obstruction of the intestinal, biliary, or ureteric smooth muscles. Besides cholinergic involvement, prostaglandins are also known to contract smooth muscles of gastrointestinal tract, sensitize the nerve endings and inhibits fluid absorption thereby causing colic pain<sup>1</sup>.

The inhibition of prostaglandin synthesis by use of nonsteroidal antiinflammatory drugs like diclofenac has been shown to be effective in the treatment of renal colic both in placebo-controlled and comparative trial versus narcotic analgesics alone or combined with spasmolytics<sup>2-5</sup>. A study on the isolated human preparations of the upper urinary tract showed that pitofenone (a non-specific smooth muscle relaxant) has

direct relaxant effect on smooth muscle<sup>5</sup>. There were several formulations containing analgesics in combination with muscle relaxants and anticholinergics available as antispasmodics for the treatment of abdominal spasm and colic. Baralgin<sup>TM</sup> one such preparation, which contains analgin in combination with pitofenone and fempiverinium, has been banned due to growing apprehension about the safety of analgin. The present study was designed to determine the comparative antispasmodic efficacy of diclofenac and analgin *per se*, and their combination with pitofenone against acetylcholine induced specific spasm of guinea pig ileum. This preparation has been regularly used to study the spasmogenic and antispasmodic activities of drugs<sup>6</sup>.

### Materials and Methods

**Animals**—Guinea pigs, of either sex, weighing 300-400 g and bred in the animal facility of Panacea Biotech Ltd., Lalru were used.

**Procedure**—The animals were anesthetized with overdose of ketamine. The abdomen was cut open and the ileum identified. A long piece of terminal ileum was cut after discarding 10 cm length nearest to ileo-

cecal junction. The cut piece of ileum was gently cleaned and about 2-3 cm long segment was mounted in 10 ml organ bath containing Tyrode perfusion fluid (pH 7.4) was maintained at 37°C and bubbled with carbonated air. The preparation was allowed to equilibrate for 30 min under 1 g tension. Concentration dependent contractions due to acetylcholine were recorded using a 2-channel Gemini recorder (Ugo Basile, Italy). A contact time of 60 sec was allowed. The concentration-response curves due to acetylcholine were noted in absence and presence of diclofenac, analgin, pitofenone, and their combinations. The tissue was irrigated with drugs for 20 min during which period, preparation was washed every 5 min with bath solution.

Doses of diclofenac ( $9.4 \times 10^{-5}$ ,  $14.1 \times 10^{-5}$  and  $18.9 \times 10^{-5}$  mol/l), and pitofenone ( $2.5 \times 10^{-6}$  mol/l) were selected on the basis of the published reports<sup>7</sup>. Doses of analgin ( $11.09 \times 10^{-5}$ ,  $16.63 \times 10^{-5}$ ,  $22.18 \times 10^{-5}$  and

$44.36 \times 10^{-5}$  mol/l) were calculated on the molar equivalent basis with diclofenac. All the experimental protocols were approved by the Institutional Animal Ethics Committee of Panjab University, Chandigarh.

## Results

Acetylcholine (10-640 ng/ml organ bath fluid) produced concentration dependent contractions of guinea pig ileum. Diclofenac ( $9.4 \times 10^{-5}$  and  $14.1 \times 10^{-5}$  mol/l) shifted the concentration response curve of acetylcholine to right with no change in the maximal response. However a higher dose of diclofenac ( $18.9 \times 10^{-5}$  mol/l) produced blockade, which was unsurmountable (Fig. 1 A.). Equimolar concentration of analgin ( $11.09 \times 10^{-5}$ ,  $16.63 \times 10^{-5}$  and  $22.18 \times 10^{-5}$  mol/l) did not alter the concentration response curve of acetylcholine. However, in higher concentration analgin ( $44.36 \times 10^{-5}$  mol/l) also produced blockade, which was unsurmountable (Fig. 1B). Further

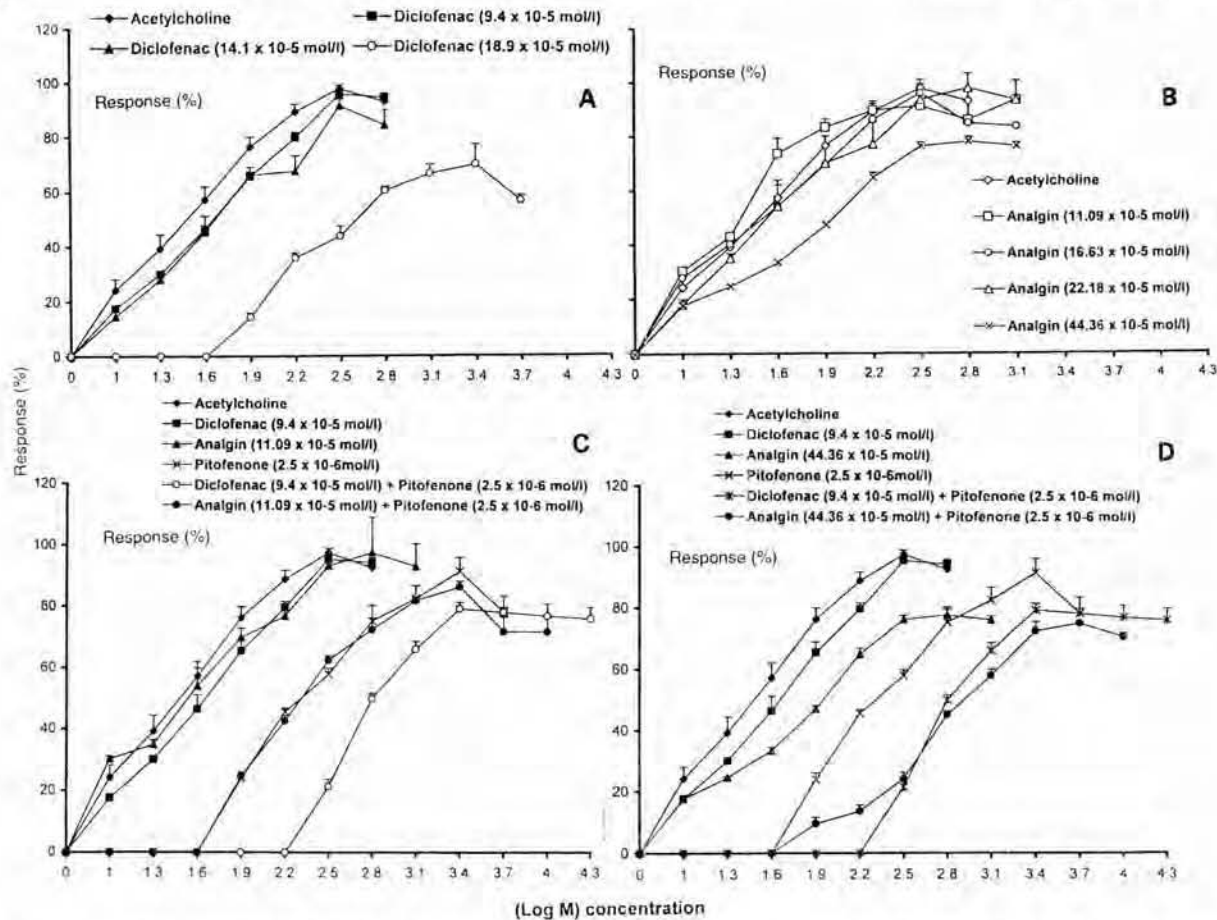


Fig. 1—Antispasmodic activity of (A) diclofenac ( $9.4 \times 10^{-5}$ ,  $14.1 \times 10^{-5}$  and  $18.9 \times 10^{-5}$  mol/l), (B) analgin ( $11.09 \times 10^{-5}$ ,  $16.63 \times 10^{-5}$ ,  $22.18 \times 10^{-5}$  and  $44.36 \times 10^{-5}$  mol/l), (C) diclofenac ( $9.4 \times 10^{-5}$  mol/l) or analgin ( $11.09 \times 10^{-5}$  mol/l) and its combination with pitofenone ( $2.5 \times 10^{-6}$  mol/l) and (D) diclofenac ( $9.4 \times 10^{-5}$  mol/l) or analgin ( $44.36 \times 10^{-5}$  mol/l) and its combination with pitofenone ( $2.5 \times 10^{-6}$  mol/l) against acetylcholine-induced contractions on guinea pig ileum. Each value represents mean  $\pm$  S.E.M. (n=5 guinea pig per group)

pitofenone ( $2.5 \times 10^{-6}$  mol/l) shifted the concentration response curve of acetylcholine to right in a parallel fashion with no change in maximal response (Fig 1 C). Diclofenac ( $9.4 \times 10^{-5}$  mol/l) potentiated the anticholinergic effect of pitofenone ( $2.5 \times 10^{-6}$  mol/l). However, analgin ( $11.09 \times 10^{-5}$  mol/l) in molar equivalent concentrations with that of diclofenac did not alter the antispasmodic effect of pitofenone (Fig. 1 C). In higher concentration analgin ( $44.36 \times 10^{-5}$  mol/l) potentiated the anticholinergic effect of pitofenone and the effect was comparable to diclofenac ( $9.4 \times 10^{-5}$  mol/l) pitofenone combination (Fig. 1 D).

### Discussion

Due to the differences in the pathophysiology of different types of abdominal colic, different remedies have been employed for their treatment. Analgesics such as analgin or dipyron<sup>8</sup> ketorolac<sup>9</sup>, diclofenac<sup>10</sup>, piroxicam<sup>11</sup> have been shown to be effective in the treatment of colic in the past. Amongst all, diclofenac is the most effective and well-tolerated agent used in the treatment of smooth muscle spasm of urinary tract and thus was effective in the treatment of renal colic both alone and in combination with spasmolytics. Analgin (one of the components of Baralgin<sup>TM</sup>) is associated with bone marrow depression and as observed in the present study, it has no direct effect on smooth muscle. The effectiveness of diclofenac could be made dependable in all types of colic by combining it with an anticholinergic agent(s) and smooth muscle relaxant(s) like fempiverinium. As revealed in the present study pitofenone another smooth muscle relaxant along with diclofenac may provide a better control over muscle spasms, as unlike analgin it possesses its own antispasmodic activity against acetylcholine contractions.

In continuation to earlier studies<sup>7,12</sup>, the relative effect of diclofenac and analgin was further investigated on pitofenone induced antispasmodic effect against cholinergic spasm in guinea pig ileum. Diclofenac in lower concentration blocked acetylcholine-induced spasm in guinea pig ileum in a dose dependent manner, however, in higher concentration ( $18.9 \times 10^{-5}$  mol/l) its effect was non-specific. Whereas, analgin in equimolar concentration did not alter acetylcholine-induced spasm indicating that it had no direct effect on smooth muscle activity. Diclofenac at a lower concentration showed potentiation of antispasmodic activity of pitofenone, whereas analgin failed to do so. The observation of

potentiating effect of low concentration of diclofenac plus pitofenone and failure of analgin to do so indicate two important aspects of this synergism: (1) a state of prostaglandin synthesis inhibition in smooth muscle may attenuate acetylcholine-induced spasm, and (2) this effect may play a synergistic role along with a drug, pitofenone which act antispasmodic by a direct action or by some other mechanism which does not utilize prostaglandin synthesis inhibition. In conclusion, these results indicated that diclofenac-pitofenone combination resulted in better and predictable antispasmodic effect than analgin-pitofenone combination.

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