Formalin assay parameters differ in confirming the antinociceptive mechanism of domperidone in mice

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Domperidone, a prokinetic drug with minimal extrapyramidal side-effects was investigated for its antinociceptive response in mice using formalin assay procedure. Two parameters namely the pain score and the time spent by the animal in licking/biting the formalin injected paw were considered. Domperidone (1, 2.5 or 5 mg/kg; ip) injected 15 min prior to formalin effectively reduced the pain score bringing it to zero at the 15th minute and was also effective till 30 min but to a lesser degree. This effect of domperidone (2.5 mg/kg) was significantly attenuated in naloxyne pretreated mice indicating a partial role for opioid pathways. In the other parameter i.e. time spent in licking/biting, domperidone in all the doses employed failed to modify significantly the same by the animal in the early phase. In contrast, a dose related inhibition of the time spent was recorded in the late phase. Besides, a trend towards the enhancement of the inhibitory effect of domperidone (2.5 mg/kg) in the late phase was noticed in naloxyne pretreated mice. Possibly, the peripheral analgesic mechanisms may play a role in this response since the late phase was considered akin to inflammation. The results confirm the antinociceptive effect of domperidone and suggest that caution be exercised while selecting the parameters when formalin assay is employed.

Keywords: Domperidone, Formalin assay, Antinociception, Naloxone

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Metoclopramide, a prokinetic drug, has been documented to exhibit opioid mediated anti-nociceptive effect in experimental models. It is now being used clinically in some of the surgical procedures like ureterolithiasis, termination of pregnancy and prosthetic hip surgery either as an independent or adjunct analgesia. As it crosses the blood brain barrier, metoclopramide is known for its extrapyramidal side-effects based on its interference with central dopaminergic neurotransmission on prolonged use. Therefore, more safer prokinetic drug domperidone which has minimal access into central nervous system was investigated earlier and found to possess antinociceptive response as tested by acetic acid induced abdominal constriction assay. To qualify for a clinical trial for domperidone, it is essential that this effect is confirmed at least in two different experimental procedures. Keeping this in view, in the present study, domperidone has been investigated for its antinociceptive effect in mice using formalin assay procedure. This assay procedure was preferred since it differentiates acute tonic pain and chronic inflammatory one and the pain experienced is akin to human pain.

Animals—Male Swiss Albino mice (25-30 g), which had free access to food and water, were used. They were housed in polypropylene cages in the departmental animalhouse at room temperature with 12:12 hr light:dark cycle. The experiments were carried out during the light hours. The study was approved by the Institute Animal Ethics Committee. Before the experiment, each animal was acclimatized by placing in transparent plexiglass observation chamber (12x12x25 cm) for 30 min and used only once. Each group contained at least 6 animals.

Drug/chemical treatment—Domperidone (Torrent Pharmaceuticals (P) Ltd, Ahmedabad) was administered (1, 2.5 or 5 mg/kg, ip) 15 min prior to formalin injection and the parameters were measured as described below. In another group of animals, naloxone (Endo Labs, USA, 5 mg/kg) was administered (ip) 10 min prior to domperidone (2.5 mg/kg; ip) to assess the role of opioidergic system. The dose and time duration for naloxone was selected based on earlier study. Normal saline treated animals served as control.

Twenty μl of 0.5% w/v formalin in normal saline was injected, sc using 30 gauge needle into the plantar region of the left hind paw of mice to elicit nociception. Animals were placed individually in observation chambers immediately after injection. The observer was blinded to the treatment given.

Parameters—In the present study, two independent parameters namely the pain score and time (sec) spent in licking/biting the left hind paw both in early and late phases were recorded. This is mainly to identify the differences, if any, in the antinociceptive response attributable to the parameters.
**Pain score**—The pain was measured by ascribing different scores based on locomotion during 0-30 min after formalin injection as given below: 0 = no change from normal locomotion, 1 = difficulty in locomotion, 2 = elevation of the injected paw, 3 = injected paw is groomed or bitten.

**Licking/biting time**—Simultaneously, the time (sec) spent by the animal in licking/biting the injected paw during initial 0-10 min (early phase) and during the late 20-30 min (late phase) was recorded.

**Data analysis**—Values are expressed as mean ± SE of pain score and licking/biting time. Data were subjected to statistical analysis initially by ANOVA followed by unpaired Student’s ‘t’ test to analyse the difference between mean of control and treated groups. A value of $P<0.05$ was considered statistically significant.

Administration of domperidone significantly reduced the pain score. This reduction was appreciable ($P<0.01$) in all the doses employed and was found to be dose related. A sharp reduction in the pain score was recorded during the initial 15 min period (Fig. 1). At 15th min, domperidone irrespective of the dose used, completely antagonized ($P<0.01$) the nociception induced by formalin. In the next 15 min schedule, though the antagonism was not 100%, a statistically significant ($P<0.01$) attenuation of the pain score was observed (Fig. 1).

Domperidone in all the doses tested did not alter significantly the time (sec) spent in licking/biting during the early phase (Fig. 2). In contrast, domperidone elicited a significant reduction ($P<0.01$) in this parameter during the late phase (Fig. 3). This effect was found to be dose-dependent. Naloxone (5 mg/kg; ip) per se failed to modify significantly the time spent in licking/biting in both the phases (unpublished data). In naloxone pretreated animals, domperidone (2.5 mg/kg; ip) still significantly reduced the time spent by the animals in licking/biting the hind paw in the late phase (Fig. 3). Conversely, a trend towards enhanced reduction in time was evident.

Analgesics are most commonly used but are not without side-effects. The commonly used non-steroidal anti-inflammatory analgesics are potential ulcerogenic whereas the opioids induce tolerance to and dependence on them. Prokinetics have proved to be beneficial over these drugs as they do not produce the aforesaid alarming side-effects. In contrast they ameliorate the associated nausea and vomiting with pain. This effect has projected prokinetics either as a
better substitute or add on drug to analgesics in some of the surgical procedures cited in the introduction.

The first reported prokinetic agent exhibiting analgesic effect is metoclopramide. This effect has been proposed to be mediated by opioidergic system involving dopaminergic mechanisms, independent of $\alpha_2$ adrenergic receptors$^{4,12}$. Metoclopramide in higher doses produces extrapyramidal side-effects. Therefore, domperidone which has minimal side-effects by virtue of its inability to access mesolimbic system in the brain has been chosen in the present study.

The formalin assay procedure was identified to explore into the mechanism of domperidone antinociception. The results of the present study confirm the antinociceptive action of domperidone especially in the late phase of this model. As mentioned earlier this phase was related to inflammatory pathway and is akin to that experienced by humans$^{10}$. Therefore, it can be extrapolated that the therapeutic utility of domperidone is more likely in painful conditions associated with inflammation. Similarly, the absence of antinociceptive response in early phase suggests that domperidone may not be effective in acute painful situations.

The effect of domperidone, when analysed using pain score, suggests that the effect is dose related and was maximum in the initial 15 min exhibiting 100% efficacy which was reduced in the second 15 min of observation period. This clearly suggest the existence of biphasic pattern in the pain score parameters too as noticed in licking/biting parameter.

The antagonism of the effect of 2.5 mg/kg domperidone in n aloxone pretreated animals in pain score parameter suggest a role for opioid pathways in the effect of domperidone.

However, the data generated from the naloxone pretreated animals in licking/biting response is interesting. In contrast to pain score an enhanced antinociceptive response for domperidone was recorded in the late phase of licking/biting. The possibility that domperidone potentiates the peripheral opioidergic pain relieving mechanisms which is independent of central pathways$^{11}$ cannot be excluded.

Currently, the data pertaining to interaction between opioidergic analgesics and prokinetic agents in analgesic response, to the best of our knowledge, is not available and is open for further investigation. However, the prokinetic agents may not be efficacious but sufficient enough to produce analgesia in clinical situation. It can significantly reduce the dose requirement of the conventional analgesics and thereby their side-effects$^{35}$.

It can be hypothesized that whenever formalin assay procedure is employed, measurement of both pain score as well as time spent in licking/biting parameters be included as mechanism appears to be different in these phases.

To summarize, domperidone considering its lack of extrapyramidal side-effects can comprehensively be considered as a substitute to metoclopramide whenever the later is employed as analgesic either alone or as an adjuvant$^{5}$.

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References