

Possible analgesic and anti-inflammatory interactions of aspartame with opioids and NSAIDs

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The purpose of the present study was to investigate analgesic and anti-inflammatory properties of aspartame, an artificial sweetener and its combination with various opioids and NSAIDs for a possible synergistic response. The oral administration of aspartame (2-16mg/kg, po) significantly increased the pain threshold against acetic acid-induced writhes in mice. Co-administration of aspartame (2mg/kg, po) with nimesulide (2 mg/kg, po) and naproxen (5 mg/kg, po) significantly reduced acetic acid-induced writhes as compared to effects *per se* of individual drugs. Similarly when morphine (1 mg/kg, po) or pentazocine (1 mg/kg, po) was co-administered with aspartame it reduced the number of writhes as compared to their effects *per se*. Aspartame (4.8, 16 mg/kg, po) significantly decreased carrageenan-induced increase in paw volume and also reversed the hyperalgesic effects in rats in combination with nimesulide (2 mg/kg, po). The study indicated that aspartame exerted analgesic and anti-inflammatory effects on its own and have a synergistic analgesic response with conventional analgesics of opioid and non-opioid type, respectively.

Keywords: Aspartame, NSAIDs, Opioids, Synergism

Aspartame (N-L-alpha-aspartyl-L-phenylalanine-1-methyl ester), a non-caloric compound is widely used as an artificial sweetener in various food preparations and beverages. Sweetening agents have been known to have food-drug interactions, particularly with commonly used drugs like analgesics¹. However, exact mechanism of action of sweetening agents-induced antinociception remains uncertain. Previous research suggested a connection between the intake of palatable foods and endogenous opioid peptide system. Several studies with animals also suggest that aspartame may possess antinociceptive properties. The anti-inflammatory and analgesic actions of aspartame may involve similar mechanisms of action as aspirin possibly through the interference of prostaglandin (PG) synthesis^{2,3}. The co-administration of aspartame with opiates or non-steroidal anti-inflammatory drugs (NSAIDs) may have clinical significance both in terms of desired and undesired consequences. In the present study, attempts have been made to study the interactions between aspartame and opioids and NSAIDs in animals for its effect on antinociception caused by these agents.

Materials and Methods

Animals—Laca mice and Wistar rats of either sex (bred in Central Animal House, Panjab University, Chandigarh), weighing 18-25 g and 150-200g, respectively were used. They were housed in plastic cages maintained at 25±0.5°C 12/12 hour natural light/dark cycle and were given food and water *ad libitum*. All experiments were carried out between 1000 and 1700 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Drugs and regimen—Aspartame, naproxen, rofecoxib, nimesulide (Panacea Biotech Ltd., New Delhi, India), pentazocine (Ranbaxy Labs Ltd., AP), morphine (Govt. Labs, Chandigarh), naloxone, carrageenan (type IV), (Sigma, USA), saccharin, and acetic acid (S.D. fine Chemicals, Biosar) were used. All the drugs used were dissolved in distilled water except nimesulide and naproxen which were suspended in 0.25% sodium CMC. Drugs were injected intraperitoneally 60 min before acetic acid injection in the writhing test. Carrageenan (type IV, 1% w/v) was injected (0.1 ml per paw) in the plantar region of the paw for anti-inflammatory test.

Writhing test (acetic acid induced writhing)—Antinociceptive response was assessed by counting the

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number of writhes (constriction of abdomen, turning of trunk (twist) and extension of hind limbs) in mice as described by Koster *et al.*⁴. Acetic acid solution (1%, 1ml/100g) was used to produce writhing response in mice. Number of writhes per animal was counted during a 20 min test period, beginning 3 min after the injection of acetic acid⁴.

Carrageenan-induced paw edema—Acute edema was induced in the hind right paw of rats by injecting 0.1 ml of freshly prepared 1% solution of carrageenan (type IV). The left paw (non-inflamed paw) served as control for comparison (0.9%, 0.1 ml saline injected). The carrageenan was injected under the planter region of right hind paw and paw volumes were measured using plethysmometer (Ugo Basil, Italy) at 30, 60, 120, 180, 240 and 300 min after carrageenan challenge. Percent change in paw volume was calculated and expressed as amount of inflammation⁵.

Antihyperalgesic activity—Acute edema was induced in the right hind paw of rats by injecting 0.1 ml of freshly prepared 1% solution of carrageenan (type IV). The left paw served as control (non-inflamed paw) for comparison (0.9% 0.1 ml saline injected). Each paw was dipped alternatively in the water bath maintained at $47^{\circ} \pm 0.3^{\circ}\text{C}$ and paw withdrawal latencies were noted at 30, 60, 120, 180, 240 and 300 min after carrageenan challenge. Three trials were recorded for each animal for calculating the mean basal paw withdrawal latencies. A reduction (time) in the paw withdrawal latency (sec) from the basal reaction time is referred to as the hyperalgesic effect⁶.

Statistical analysis—Results are expressed as mean $\pm$ S.E. Data were analysed statistically using analysis of variance (ANOVA) followed by Dunnett's t-test to assess the significance. $P < 0.05$ was considered as statistically significant.

Results

Effect of aspartame against acetic acid-induced writhing in mice—Oral administration of aspartame dose dependently decreased the number of writhes in acetic acid-induced writhing assay in mice (Fig. 1a). Saccharin (4, 8, 16 mg/kg, po) did not alter the number of writhes as compared to the control (Fig. 1b). Aspartame (4 mg/kg, po) however, significantly reduced the number of writhes at 15, 30 and 60 minutes and the maximum analgesic effect was observed at 60 min (Fig. 2).

Effect of aspartame on NSAIDs-induced analgesia in acetic acid-induced writhing in mice—Nimesulide

(1 and 2 mg/kg, po) and naproxen (5 mg/kg, po) significantly ($P < 0.05$) reduced the number of writhes in mice as compared to the control group (Fig. 3). Naproxen also significantly ($P < 0.05$) reduced the number of writhes in mice as compared to control animals (Fig. 3). The co-administration of aspartame (2 mg/kg, po) with nimesulide (2 mg/kg) or naproxen (5 mg/kg, po) significantly ($P < 0.05$) decreased the number of writhes in mice when compared to the effect *per se* of individual drugs (Fig. 3).

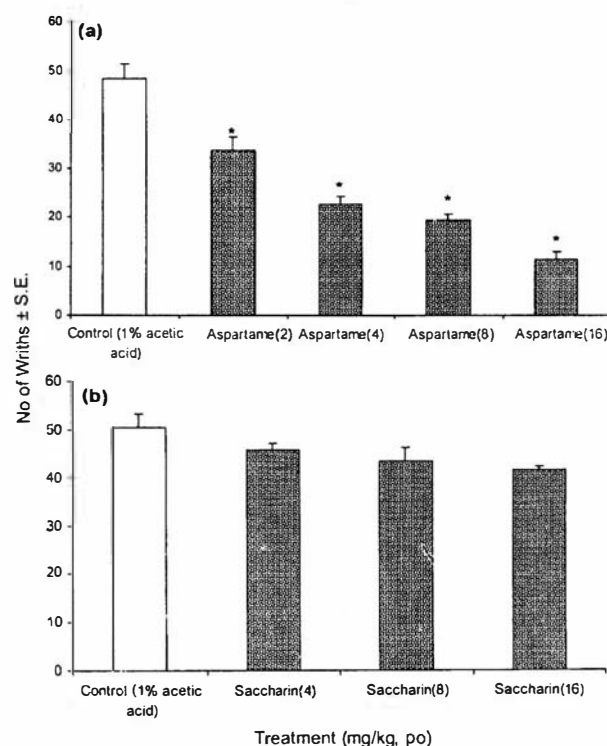


Fig.1—Effect of (a) aspartame (2-16 mg/kg) and (b) saccharin against acetic acid-induced writhing in mice. [Vertical lines shows S.E. * $P < 0.05$ compared with control.]

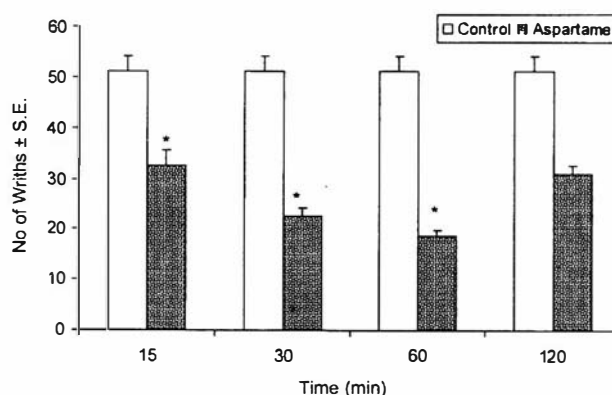


Fig.2—Time dependent effect of aspartame (4mg/kg) against acetic acid - induced writhes in mice. [Vertical lines show S.E. * $P < 0.05$ compared with control]

Effect of aspartame on opioid-induced analgesia in acetic acid-induced writhing in mice—Morphine (1 mg/kg, po) significantly reduced the number of writhes as compared to control and pentazocine (1 mg/kg, po) had no significant analgesic effect in this pain assay. However, co-administration of morphine (1 mg/kg, po) or pentazocine (1 mg/kg, po) with aspartame (2 mg/kg) significantly ($P < 0.05$) reduced the number of writhes in mice as compared to their effects *per se* (Fig. 4). Naloxone (1 mg/kg) when co-administered with aspartame (2 mg/kg) and morphine (1 mg/kg), significantly ($P < 0.05$) reversed the facilitatory effect of morphine but not that of aspartame (2 mg/kg) (Fig. 4). The analgesic response of aspartame (4 mg/kg, po) in the writhes-test was not altered by naloxone (1 mg/kg; Fig. 5).

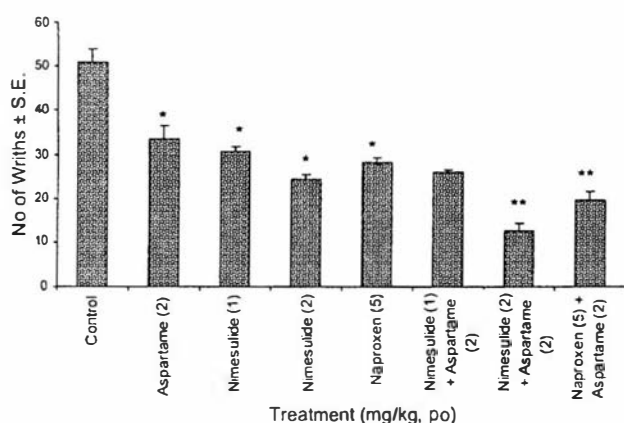


Fig.3—Antinociceptive effects of aspartame with NSAIDs in mice. [Vertical lines shows SE. * $P < 0.05$ as compared with control; ** < 0.05 compared with *per se* effects]

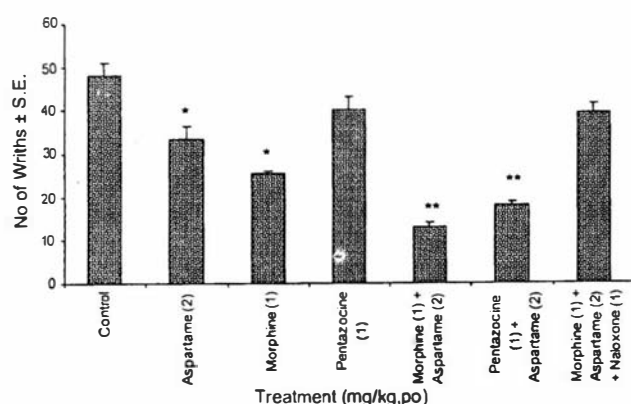


Fig.4—Interaction of aspartame (2 mg/kg) with morphine (1 mg/kg) and pentazocine (1 mg/kg) and the reversal of antinociceptive effect by naloxone (1 mg/kg) in mice. [Vertical lines show SE. * $P < 0.05$ compared with control; ** < 0.05 compared with *per se* effects]

Effect of aspartame in carrageenan-induced paw oedema and hyperalgesia in rats—Carrageenan (1%) produced significant increase in paw edema in control group. Aspartame (4, 8 and 16 mg/kg, po) significantly ($P < 0.05$) decreased the carrageenan-induced increase in paw volume as compared to the control rats ($P < 0.05$) (Fig. 6). Aspartame (4 mg/kg, po) also significantly reversed the carrageenan-induced hyperalgesia in rats (Fig. 7).

Modification of aspartame-induced anti-inflammatory and anti-hyperalgesic effect by opioids or NSAIDs—Both morphine (0.5 mg/kg) and nimesulide (2 mg/kg, po) failed to alter the aspartame-induced (4 mg/kg) anti-hyperalgesic effect in rats (Fig. 7). Co-administration of nimesulide (2 mg/kg, po) but not morphine significantly enhanced the anti-inflammatory effect of aspartame (4 mg/kg, po) (Fig. 8).

Discussion

Aspartame is a widely known artificial sweetener and is about 160 times sweeter than sucrose in aqueous solutions. There have been reports in recent

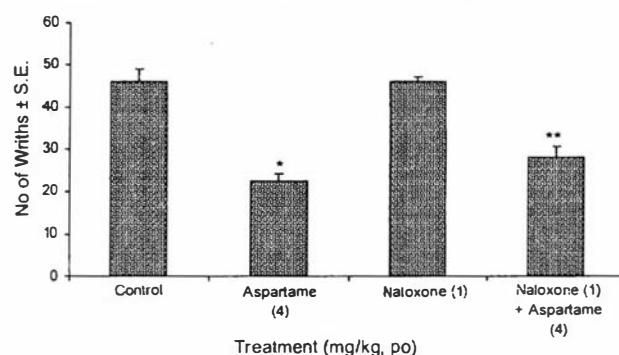


Fig.5—Effects of naloxone (1 mg/kg) on aspartame (4 mg/kg) induced analgesia in mice. [Vertical lines show SE. * $P < 0.05$ compared with control; ** < 0.05 compared with *per se* effects]

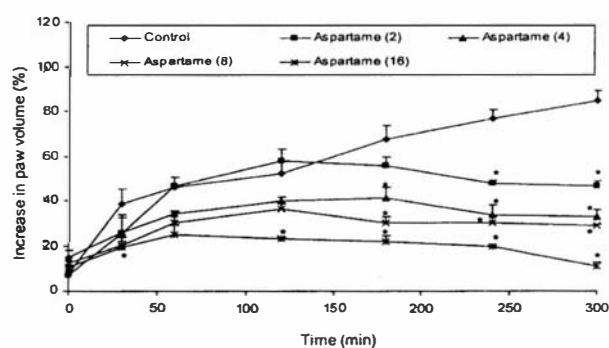


Fig.6—Anti-inflammatory effect of aspartame (2-16 mg/kg, po) against carrageenan-induced paw edema. [Vertical lines show SE. * $P < 0.05$ compared with control]

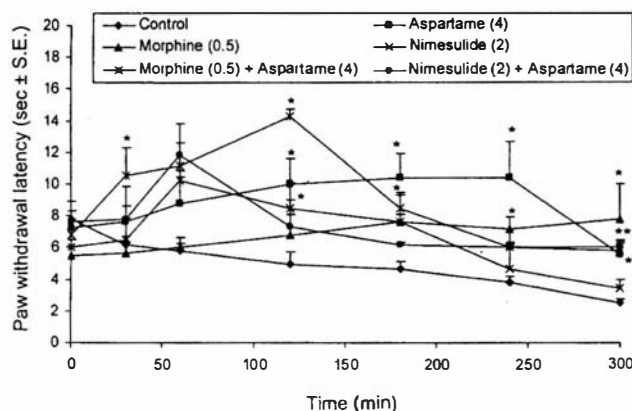


Fig.7—Anti-hyperalgesic effect of morphine and NSAIDs on aspartame. [Vertical lines show SE. * $P < 0.05$ compared with control. ** < 0.05 compared with aspartame]

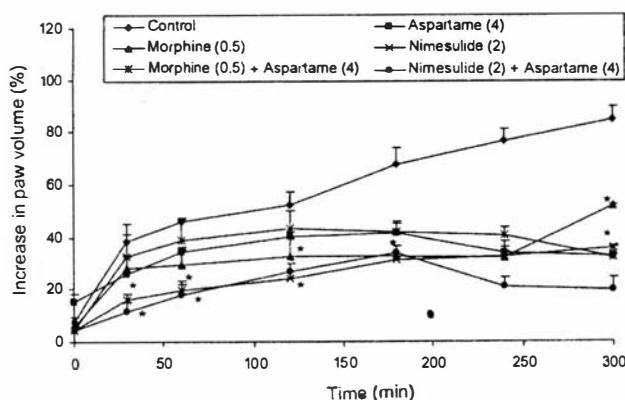


Fig.8—Anti-inflammatory effect of morphine and NSAIDs on aspartame. [Vertical lines show SE. * $P < 0.05$ compared with control. ** < 0.05 compared with aspartame.]

times that aspartame may possess biological response and help in maintaining homeostasis and bone mass regeneration. Aspartame is known to promote calcium deposition in bones, lowers fever in animal models and has also been found to prolong bleeding time in humans⁷. In the present study, the analgesic and anti-inflammatory effects of aspartame and saccharin were investigated. Aspartame significantly and dose dependently (2-16 mg/kg) increased the pain threshold in acetic acid-induced chemonociception, whereas saccharin did not have any such property which correlates with the previous data⁴⁻⁷.

Aspartame is speculated to interact with NSAIDs and endogenous opioid systems and increases levels of β -endorphin in hypothalamus². The anti-inflammatory and analgesic actions of aspartame may involve similar mechanisms of action as aspirin, possibly through the interference of prostaglandin (PG) synthesis. Aspartame's effect on PG synthesis

has not been empirically substantiated, but computer-assisted modeling suggests an action similar to aspirin's inhibition of cyclooxygenase activity⁷. In the present study, saccharin and sucrose decreased the antinociceptive effect of morphine while aspartame increased the effect of morphine-induced analgesia. Nikfar *et al.*⁸ have also shown that aspartame increases the analgesic effect of morphine and naloxone only reversed the action of opioids but had no effect on aspartame.

Aspartame (2 mg/kg) was used in combination with nimesulide (a preferential COX-2 inhibitor) and a conventional NSAID, naproxen, the combination exhibited a synergistic activity. The study showed that besides the interaction of aspartame with opioids and NMDA receptor antagonists it could also interact with some NSAIDs such as nimesulide and naproxen in a sub maximal dose to show a synergistic effect^{9,10}.

Although there is no direct evidence available, to support the present data it can be speculated that aspartame and NSAIDs may act through a central nociceptive mechanism. Another speculation could be that this interaction may influence the pharmacokinetic parameters of the drugs in combination or may reduce the renal clearance of drugs thereby acting at the site of action for a longer duration of time and thus showing synergistic effect.

Aspartame also possessed a significant anti-inflammatory action against carrageenan-induced paw oedema but it was found to be not effective in combination with opioids/NSAIDs in inflammatory and hyperalgesic pain models. The interaction between these drugs is highly speculative at the present and further studies are needed to find out the possible therapeutic implications of such interactions in humans.

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