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Anti-inflammatory and analgesic activity of the bradykinin antagonist, icatibant (Hoe 140), against an extract from *Porphyromonas gingivalis*

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1 *Porphyromonas gingivalis* is one of the bacteria likely to be related to pain in periodontitis. Several enzymes isolated from *P. gingivalis* have been reported to have kininogenase activity. Since kinin release could be held responsible for inflammatory symptoms and pain in periodontitis, we investigated whether the inflammatory and algesic effects of a sonic extract from *P. gingivalis* (PGSE) could be inhibited by the potent bradykinin B_2 receptor antagonist, icatibant (Hoe 140).

2 In anaesthetized rats, the subplantar injection of PGSE (0.1 and 1.0 mg) caused a dose-dependent oedema of the hind paws. The net increase of the paw volume 60 min after the injection was 23 ± 5% and 77 ± 12%, respectively. The oedema was rich in plasma proteins as determined by the Evans blue method. Pretreatment with icatibant (300 nmol kg^{-1}, s.c.) significantly reduced the effect of 1.0 mg of PGSE whereas the effects of 0.1 mg of PGSE remained unaffected.

3 The subplantar injection of 1.0 mg of PGSE in unanaesthetized rats caused nociceptive behavioural responses which started about 5 min after the injection and lasted for about 10–15 min. These responses were completely prevented by pretreatment with icatibant (300 nmol kg^{-1}, s.c.).

4 The present results show that the plasma extravasation induced by non-algesic doses of a sonic extract from *P. gingivalis* are caused by mechanisms other than B_2 kinin receptor activation whereas inflammatory effects of algesic doses are due to the action of kinins. The pain elicited by the extract is solely mediated by kinins and can be prevented by icatibant. The bradykinin antagonist could thus have a potential for a clinical use against pain associated with periodontal inflammation.

Keywords: Bradykinin antagonists; icatibant (Hoe 140); inflammation; pain; *Porphyromonas gingivalis*

Introduction

Porphyromonas gingivalis is a bacterium found in the bacterial flora of infected dental root canals and is significantly related to percussion pain in periodontitis (Hashioka *et al.*, 1992). Several proteinases isolated from *P. gingivalis* have been shown to possess potent kininogenase activity (Hinode *et al.*, 1992; Scott *et al.*, 1993; Sojar *et al.*, 1993). One of these enzymes, lys-gingivain, is apparently the most potent kininogenase reported to date (Scott *et al.*, 1993). In order to elucidate the contribution of kinin release to the inflammatory and algesic effects of the bacterium, we tested the potent and specific bradykinin B_2 receptor antagonist, icatibant (D-Arg-[Hyp^3-Thi^5-D-Tic^7-Oic^8]-bradykinin; formerly named compound I [Lembeck *et al.*, 1991] or Hoe 140 [Hock *et al.*, 1991; Wirth *et al.*, 1991]), against the effects of a sonic extract from *P. gingivalis* (PGSE) following its subplantar injection in rats.

Methods

Experimental procedure

Female Sprague-Dawley rats (200–250 g, Forschungsanstalt für Versuchstierzucht, Himberg, Austria) were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). Icatibant (300 nmol kg^{-1}) was injected s.c.; control animals received a corresponding volume (3 ml kg^{-1}) of a 154 mM solution of NaCl (saline). Twenty min later, a subplantar injection of a sonic extract from *Porphyromonas gingivalis* (PGSE; Scott *et al.*, 1993; 0.1 or 1.0 mg in 50 µl) was given into one hindpaw; the contralateral paw received an equal volume of saline. The paw volumes were measured before, and 5, 10, 20, 25, 30, 45 and 60 min after the injection with a plethysmometer (Ugo Basile, Italy).

For the determination of the extravasation of plasma proteins, Evans blue (20 mg kg^{-1}), an azo dye binding to plasma proteins, was administered i.v. 5 min prior to the subplantar injection of the PGSE. The rats were killed 1 h later by perfusion with 40 ml heparinized saline via the aorta. The hindpaws were cut off, placed in 8 ml formamide and incubated for 36 h at 55°C for the extraction and photometric determination of Evans blue (Gamse *et al.*, 1980).

Nociceptive reactions of unanaesthetized rats were monitored over a period of 30 min following the subplantar injection of PGSE (0.1 and 1.0 mg) under brief nitrous oxide analgesia. The behavioural responses were rated with scores (0 = no reaction; 1 = favouring the uninjected paw; 2 = elevating the injected paw; 3 = licking the injected paw; modifed from Cohen *et al.*, 1984). For each 1 min period a mean score was calculated from the times spent within each score during that period. Pretreatment with icatibant or saline was performed as above.

Substances

The sonic extract from *P. gingivalis* was generously supplied by Cheryl F. Scott (Sol Cherry Thrombosis Research Center, Temple University, Philadelphia, PA, U.S.A.) and Dr Benjamin F. Hammond (Medical College of Pennsylvania, Philadelphia, PA, U.S.A.). Icatibant (D-Arg-[Hyp^3-Thi^5-D-Tic^7-Oic^8]-bradykinin) was a gift from Hoechst AG (Frankfurt am Main, Germany). Pentobarbitone sodium (Nembutal) was from Sanofi (Libourne, France). Evans blue was purchased from Sigma (St. Louis, MO, U.S.A.). Formamide was from E. Merck (Darmstadt, Germany).

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Statistical analysis

All values presented are means ± s.e.mean. Comparisons of the results obtained in icatibant-treated rats with those from control animals were made by the Mann-Whitney U test.

Results

Rat paw oedema

The subplantar injection of PGSE (0.1 and 1.0 mg) into the hindpaws of anaesthetized rats elicited a dose-dependent paw oedema which reached a plateau after 30 min and thereafter remained constant (Figure 1a). The oedema induced by the lower dose of PGSE (23 ± 5% net volume increase at 60 min) was not affected by pretreatment with icatibant (300 nmol kg^{-1}, s.c.), whereas the oedema caused by the higher dose (77 ± 12%) was significantly ($P<0.05$) attenuated (to 24 ± 4%) in rats which had been injected with the bradykinin antagonist.

The extravasation of plasma proteins into the tissue was quantified with the Evans blue method. Similar to the results obtained when the volume of the paw oedema was measured, icatibant did not modify the extravasation of Evans blue caused by 0.1 mg PGSE, whereas the amount of Evans blue determined in the paws following injection of 1.0 mg PGSE was significantly ($P<0.05$) smaller in icatibant-treated rats than in controls.

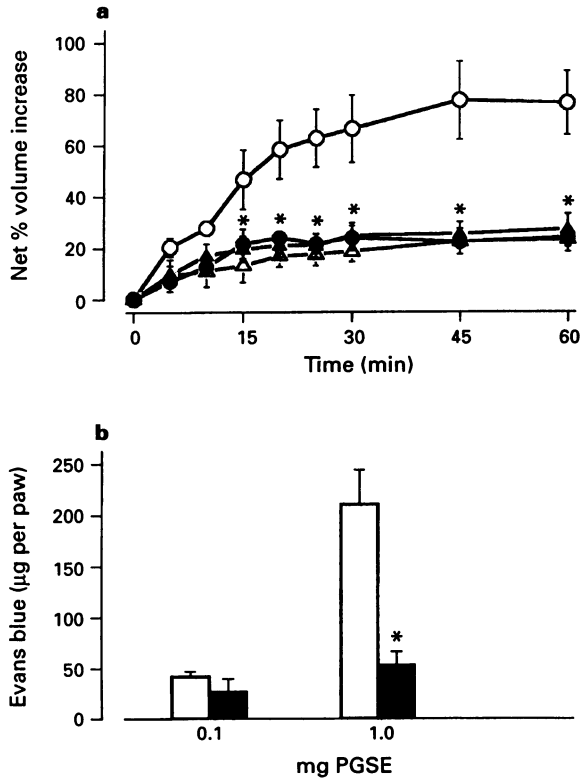

Figure 1 Paw oedema following subplantar injection of a sonic extract from *P. gingivalis* in anaesthetized rats: the increase in paw volume (a) following injection of 0.1 mg (triangles) or 1.0 mg (circles) of the extract was measured repeatedly during a period of 1 h. Extravasation of plasma proteins, quantified by the Evans blue method (b) was determined 60 min after the subplantar injection of the extract. The rats were pretreated s.c. with icatibant (300 nmol kg^{-1}; closed symbols and columns) or with saline (3 ml kg^{-1}; open symbols and columns) 20 min prior to the experiment. Significance of difference of effects of 1.0 mg PGSE in icatibant-treated rats vs control rats: $*P<0.05$. Means ± s.e.mean; $n = 4-6$.

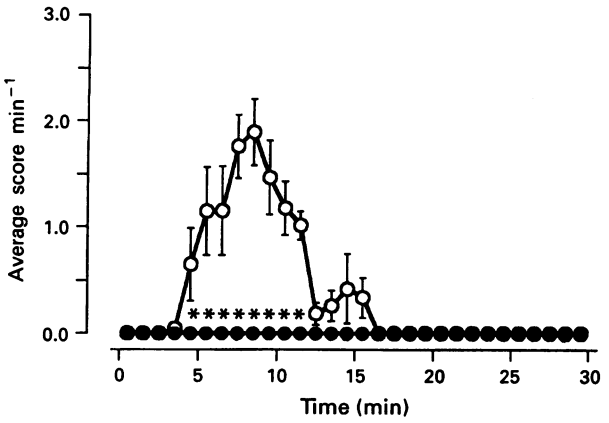

Figure 2 Nociceptive responses to subplantar injections of a sonic extract (1.0 mg) from *P. gingivalis* in unanaesthetized rats: the rats were pretreated s.c. either with icatibant (300 nmol kg^{-1}; ●) or with saline (3 ml kg^{-1}; ○) 20 min prior to the experiment. Behavioural responses were monitored for a period of 30 min after the subplantar injection and rated with scores as described in the Methods section. For each 1 min period an average score value was calculated. Significance of difference to control rats: $*P<0.05$. Means ± s.e.mean; $n = 4-5$.

Nociceptive behavioural responses

Following the subplantar injection of 0.1 mg PGSE into the paws of unanaesthetized rats, no behavioural responses, indicative of nociception, could be observed. However, 1.0 mg PGSE induced such responses (favouring the uninjected paw, elevating or licking the injected paw) starting about 5 min after the injection. For the subsequent 10–15 min, nociceptive behaviour, frequently interrupted by short periods of normal behaviour, was seen. Thereafter, the rats did not show any signs of pain. This pattern of behaviour was completely prevented by pretreatment with icatibant (Figure 2).

Discussion

Porphyromonas gingivalis is an anaerobe bacterium frequently found in severe periodontal inflammatory disease. It is significantly related to subacute clinical symptoms such as percussion pain in apical periodontitis (Hashioka *et al.*, 1992). Several proteinases isolated from *P. gingivalis* were shown *in vitro* to have kininogenase activity (Hinode *et al.*, 1992; Scott *et al.*, 1993; Sojar *et al.*, 1993). One of these enzymes, lys-gingivain, is apparently the most potent kininogenase reported to date (Scott *et al.*, 1993). Unlike gingipain, another proteinase isolated from *P. gingivalis* (Chen *et al.*, 1992), which can be purified from the culture medium and is also capable of initiating kinin production (Travis *et al.*, 1993), lys-gingivain is a membrane-bound enzyme. Lys-gingivain represents about 1% of the total protein of a sonic extract from *P. gingivalis* (PGSE) and seems to be a major component of its proteinase activity (Scott *et al.*, 1993). In order to elucidate the contribution of the kinin release to the inflammatory and algesic effects of *P. gingivalis*, the bradykinin B_2 receptor antagonist, icatibant was tested against effects of PGSE *in vivo*.

The subplantar injection of PGSE caused a dose-dependent increase in vascular permeability as quantified by the increase in paw volume and accumulation of plasma proteins in the tissue. The effects of the lower dose of PGSE (0.1 mg) remained unaffected by icatibant, suggesting that this part of the inflammatory response was due to mechanisms other than kininogenase activity. On the other hand, icatibant

significantly reduced the effects of the higher dose of PGSE (1.0 mg) to approximately the level of responses induced by the lower dose. Since icatibant is a specific bradykinin B_2 receptor antagonist without any inhibitory effects on the actions of other inflammatory mediators (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991) it can be concluded that this part of the inflammatory response is due to activation of B_2 receptors in the tissue. Although we did not use a B_1 receptor antagonist, an involvement of B_1 receptors is not likely since B_1 receptor antagonists have been shown to be ineffective against the oedema-producing actions of bradykinin (Whalley, 1987) or kinin-releasing enzymes (Cirino *et al.*, 1991; Legat *et al.*, 1994) in the rat paw.

The remaining part of the oedema may be due to other components of the extract. Proteinases purified from *P. gingivalis* also have other actions which may subserve inflammatory responses such as disruption of basement membranes of epithelial cells (Shah *et al.*, 1992), cleavage of collagen (Sojar *et al.*, 1993) and fibrinogen (Scott *et al.*, 1993) or activation of complement components (Wingrove *et al.*, 1992).

In contrast to its inflammatory effects, the lower dose of PGSE did not have algesic properties. However, nociceptive behavioural responses could be observed readily when 1.0 mg of PGSE was given as a subplantar injection in unanaesthetized rats. This algesic effect was completely abolished by icatibant which shows that the pain induced by PGSE was entirely due to the action of kinins in the tissue. The nociceptive responses to PGSE (1.0 mg) may appear to be somewhat more short-lived than the effects on plasma extravasation (compare Figures 1 and 2). However, the increase in paw oedema observed at a time when the nociceptive responses have disappeared, i.e. later than 20 min after the subplantar injection of PGSE, was small. This may indicate that tissue kinin concentrations required to induce nociception were higher than those leading to increased permeability.

In summary, the two doses of PGSE (0.1 and 1.0 mg) used in the present investigation represent a non-algesic and an algesic dose of PGSE, respectively. While the inflammatory effects of the non-algesic dose of PGSE are not due to kinin action in the tissue, the increase in vascular permeability caused by the algesic dose of PGSE are caused predominantly by the action of kinins acting on bradykinin B_2 receptors. The pain induced by PGSE is solely mediated by kinins and can be completely prevented by the bradykinin B_2 antagonist, icatibant. Thus, icatibant could be potentially useful for the treatment of pain in severe periodontal disease.

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