ELSEVIER

European Journal of Pharmacology 274 (1995) 101–107

ejp

Collagenase-induced oedema in the rat paw and the kinin system

Jose Carlos Souza Pinto [a,1], Gaby Remacle-Volon [a,2], Claudio A.M. Sampaio [b], Jacques Damas [a,*]

[a] *Department of Human Physiology, University of Liège, 17 Place Delcour, B-4020 Liège, Belgium*
[b] *Department of Biochemistry, Escola Paulista de Medicina, Sao Paulo, SP, Brazil*

Received 14 July 1994; revised 24 November 1994; accepted 25 November 1994

Abstract

Collagenase (100 μg) induced a large plasma extravasation, during the first 15 min after its injection in rat paw, associated with the rapid development of oedema which subsided after 6 h. The extent of the oedema was similar in normal and kininogen-deficient rats. The swelling induced in normal rats was reduced by HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin), a bradykinin B_2 receptor antagonist, and by three serine protease inhibitors, soybean trypsin inhibitor (SBTI), *Leucaena leucocephala* trypsin inhibitor 1 (LLTI-1) and *Leucaena leucocephala* trypsin inhibitor 2 (LLTI-2). These agents had no effect on the oedema induced in kininogen-deficient rats. The swelling was also reduced by methysergide, indomethacin, ketoprofen and methylprednisolone. It was increased by heparin, but it was not modified by mepyramine, WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]-triazolo-[4,3-*a*][1,4]-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) and N^G-nitro-L-arginine. In vitro, collagenase did not release kinins from rat plasma or from purified T-kininogen. LLTI-1 and LLTI-2 did not inhibit collagenase activity for one of its specific substrates. Kinins are thus involved in the development of collagenase oedema in normal rats. Their generation would be indirect following changes in matrix proteins in extravascular spaces. Nevertheless, kinins are not the decisive mediators of the swelling. Serotonin, possibly released from platelets, and prostanoids participate in the inflammatory process.

Keywords: Collagenase; Inflammation; Kinin; Nitric oxide (NO); Serine protease inhibitor; Platelet

1. Introduction

Collagenases may play an important role in some acute and chronic inflammatory reactions. During inflammatory processes, several types of cells, such as activated macrophages, fibroblasts, neutrophils and endothelial cells, can provide the inflammatory site with collagenases (Henson et al., 1988). Some of these enzymes are released in a latent form and can be activated by plasmin, tissue kallikrein or cathepsin B (Wahl, 1988). Some bacteria, such as *Clostridium perfringens* and *Clostridium histolyticum*, are another important potential source of this destroying and remodelling activity (Hatheway, 1990). The injection of collagenase in rat paw induces the development of a large swelling which reaches a maximal volume within 30 min. This oedema is reduced by HOE 140, a long-acting bradykinin antagonist. This oedema would thus depend on activation of the kinin system (Legat et al., 1993).

Bradykinin is a pro-inflammatory nonapeptide which is released from high molecular weight kininogen by plasma kallikrein. Plasma kallikrein is one component of the contact system of plasma and is activated by Hageman factor, factor XII of the intrinsic coagulation system, during contact activation (Bhoola et al., 1992). Several pieces of evidence show that bradykinin is the main mediator responsible for paw oedema induced by carrageenan, kaolin or urate crystals in rats (Garcia Leme, 1978; Damas, 1987; Damas and Remacle-Volon, 1992). However, these inflammatory swellings have a typical slow onset and reach a peak in 3–5 h. Indeed, bradykinin has a small oedema-promoting effect in rats, but its effect is greatly increased by several fac-

* Corresponding author. Tel. 41.665924, fax 41.665912.

[1] Present address: Department of Biochemistry, Escola Paulista de Medicina, Sao Paulo, SP, Brazil.

[2] This paper is dedicated to Mrs. G. Remacle-Volon, who unfortunately died from lung cancer during the realization of this study.

SSDI 0014-2999(94)00723-3

tors, like some prostanoids (Williams and Morley, 1973; Oh-Ishi et al., 1986), which are progressively released by leucocytes (Van Arman, 1979).

This difference between the rapid development of collagenase-induced oedema and the relatively slow evolution of carrageenan-induced oedema led us to reinvestigate the importance and the mechanism of the involvement of the kinin system in collagenase-induced oedema by using HOE 140, plasma kallikrein inhibitors and kininogen-deficient rats.

2. Materials and methods

2.1. Animals

We used Wistar rats bred in our laboratory and kininogen-deficient Brown Norway rats (Damas and Adam, 1980) from the farm of the Katholiek University of Leuven (Heverlee, Belgium). The mean weight of the animals was 220 g. They were fed on a standard commercial laboratory diet and allowed free access to water except for the hours of the study. Animals of both sexes were used as there was no difference between the swellings induced by collagenase in male or in female rats.

2.2. Paw oedema

Paw oedema was induced by subplantar injection of 0.1 ml saline containing 100 μg of collagenase or 1% λ-carrageenan. Foot volumes were measured 3–5 times by water plethysmometery (Ugo Basile) immediately before the injection of the irritants and at different intervals thereafter. The swelling was calculated as the mean percentage increase in volume of the injected paw compared to the value at 0 h.

Each experiment was performed using at least six control solvent-treated rats and six drug-treated animals. The involvement of kinins in the inflammatory reaction elicited by collagenase was examined by using either kininogen-deficient rats or several agents. The rats were treated with doses of HOE 140 which inhibit bradykinin-induced oedema (Damas and Remacle-Volon, 1992), or with doses of soybean trypsin inhibitor (SBTI) which reduce kinin release in sponge exudates (Damas et al., 1990b) or with doses of *Leucaena leucocephala* trypsin inhibitor 1 (LLTI-1) or LLTI-2 which reduce carrageenan oedema (Souza Pinto et al., 1992). The roles of mast cell amines, prostanoids, platelet-activating factor (PAF) and nitric oxide (NO) in collagenase oedema were also explored. The rats were treated with doses of mepyramine and/or methysergide which suppress the oedemas induced by histamine and by 5-hydroxytryptamine (Damas and Remacle-Volon, 1986), or with doses of ketoprofen or of indomethacin which abolish the hypotensive activity of arachidonic acid and which reduce carrageenan oedema (Damas and Mousty, 1978), with doses of WEB 2086 which inhibit the hypotensive and oedema-producing effects of PAF (Damas, 1991) or with doses of N^G-nitro-L-arginine which induce an increase in blood pressure in rats (Damas and Remacle-Volon, 1990).

For studies of plasma exudation induced by collagenase in the paw, the animals were anaesthetized with sodium pentobarbital (45 mg/kg) at various times after collagenase injection and 1.0 μCi of ^{125}I-labelled bovine serum albumin dissolved in 0.5 ml of saline was injected into a tail vein. Fifteen minutes later, a blood sample was withdrawn by cardiac puncture and mixed with 0.1 vol. 3.8% trisodium citrate and the paws were cut at the ankle. Blood samples were centrifuged at $1500 \times g$ for 10 min and the plasma separated. The radioactivity of plasma samples and paws was counted in a gamma spectrometer (Packard Instrument Co.). The results were calculated as μl of plasma extravasated in the collagenase-treated paw after subtracting the radioactivity present in the control paw.

2.3. Kinin formation and collagenolytic activity in vitro

Collagenase (10–100 μg) or trypsin (0.1 or 2 mg) was incubated with Wistar rat plasma (0.2 ml) and EDTA (5%) or with T-kininogen (10 μg) in Tris-HCl (0.15 M, pH 7.8; final volume 1 ml) for 30 min. The kinin level in the incubates was determined by bioassays using rat stomach strip, rat uterus and rat duodenum superfused with Tyrode solution (2.5 ml/min) at 37°C and aerated with oxygen (95%) and carbon dioxide (5%). The threshold level of reaction of the uterus and duodenum to bradykinin varied from one assay to the other from 0.1 to 1 ng/ml. T-kininogen has been purified by a method previously described (Adam et al., 1989).

The influence of two inhibitory proteins, LLTI-1 and LLTI-2, on collagenase activity in vitro was examined by the method of Van Wart and Steinbrink (1981). Briefly, collagenase was incubated with FALGPA (*N*-(3[2-furyl]acryloyl)-Leu-Gly-Pro-Ala; 0.03 or 2 mM) in Tricine (50 mM; pH 7.5) containing NaCl (0.4 M) and $CaCl_2$ (10 mM). The assay was carried out spectrophotometrically by continuously monitoring the decrease in absorbance of the substrate at 345 nm after addition of the enzyme (8 μg), preincubated alone or with one of the two protease inhibitors (50 or 250 μg).

2.4. Materials

Collagenase (EC 3.4.24.3) was isolated from *Clostridium histolyticum* and purified by Sigma (product number C 9891). We also used bradykinin, trypsin,

N^G-nitro-L-arginine, λ-carrageenan, soybean trypsin inhibitor (SBTI) and FALGPA (*N*-(3[2-furyl]acryloyl)-Leu-Gly-Pro-Ala) from Sigma (St. Louis, MO, USA), methysergide from Sandoz (Basel, Switzerland), mepyramine from Specia (Paris, France), heparin from Leo Pharmaceutical Products (Vilvoorde, Belgium) and methylprednisolone from Upjohn (Puurs, Belgium). WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*] [1,2,4]-triazolo-[4,3-*a*] [1,4]-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) and HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) were kind gifts of Boehringer Ingelheim (Ingelheim am Rhein, Germany) and of Hoechst (Frankfurt, Germany), respectively. The proteins *Leucaena leucocephala* trypsin inhibitor 1 (LLTI-1) and *Leucaena leucocephala* trypsin inhibitor 2 (LLTI-2) have been previously purified from *Leucaena leucocephala*. They are inhibitors of serine proteases, trypsin, Hageman factor, plasma kallikrein and plasmin (Souza Pinto et al., 1992). All these products were dissolved in physiological saline, with the aid of HCl for WEB 2086. Indomethacin and ketoprofen were kind gifts from Merck Sharp and Dohme (Brussels, Belgium) and Specia (Paris, France) respectively. They were dissolved in Tris-HCl (0.15 M; pH 7.4).

2.5. Statistics

Results are means ± S.E.M. of 6–34 determinations. Means were compared by Student's unpaired *t*-test or by one-way analysis of variance (ANOVA) followed by Fisher's PLSD (protected least significant difference) test, as appropriate. A difference was accepted as significant when $P < 0.05$.

3. Results

3.1. Collagenase oedema

Collagenase oedema was elicited by injecting 100 μg of the enzyme in the rat paws. This dose has been used in previous works (Vinegar et al., 1969; Legat et al., 1993). In normal rats, paw oedema rapidly developed, following collagenase injection, and was already marked after 15 min. It reached its maximum (about 50% increase in paw volume) after 2 h and remained relatively stable up to 4 h after the injection. Thereafter, the swelling slowly decreased (Fig. 1) and disappeared after 24 h. The injection of the same volume of physiological saline, in the rat paw, only induced a 12% increase in paw volume after 1 h. A similar small swelling (11 ± 5%, $n = 6$) was elicited by collagenase (100 μg) when the enzyme was previously inactivated by heating for 10 min at 75°C.

Collagenase-induced oedema was associated with local haemorrhages and with an increase in vascular permeability. Plasma protein extravasation, measured by the accumulation of ^{125}I-labelled albumin, was very large during the first 15 min after collagenase injection. Protein extravasation progressively declined during the first 1 h and thereafter vascular permeability remained slightly elevated (Fig. 1).

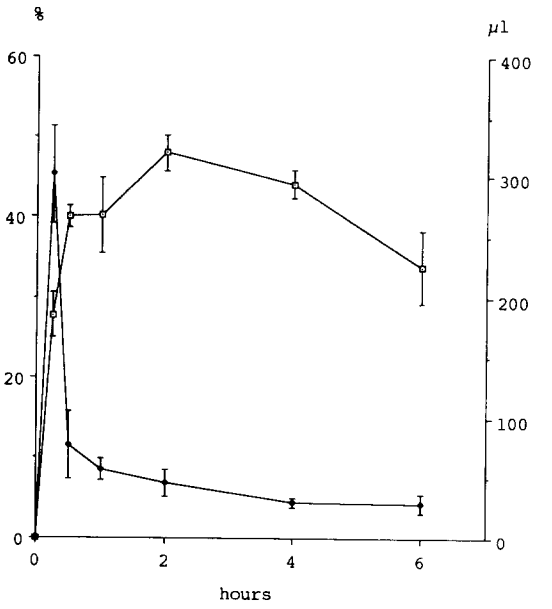

Fig. 1. Swelling and exudation of ^{125}I-labelled albumin after injection of collagenase in the paws of normal Wistar rats. The swelling (left-hand scale) was calculated as the percentage increase in the weight of the paw (□), the exudation of albumin (right-hand scale) was calculated as μl of plasma (◆) extravasated in collagenase-treated paws after subtracting the radioactivity present in control paws. Each value is the mean ± S.E.M. of 6 determinations.

In kininogen-deficient rats, collagenase (100 μg) induced a rather similar inflammatory reaction: the swelling of the paws developed as quickly as in normal rats. It reached a similar size during the first 2 h but it increased up to 4 h after collagenase injection (Fig. 2). It was also associated with macroscopically visible haemorrhages.

3.2. Effect of inhibitors of kinin activity

In rats, bradykinin is involved in the inflammatory process by stimulation of bradykinin B_2 receptors (Marceau et al., 1983; Whalley, 1987). To investigate the involvement of bradykinin in collagenase oedema occurring in normal rats, the animals were treated with HOE 140, a bradykinin B_2 receptor antagonist (Wirth et al., 1991). HOE 140 (2–8 mg/kg) was injected i.p. 30 min before the injection of collagenase. In normal rats, HOE 140 dose dependently reduced the oedema (Table 1). The maximal reduction reached 25–30%. However, the simultaneous injection of HOE 140 (0.24 mg) with collagenase in the paw significantly increased the oedema during the first 1 h from a 40.4 ± 1.8% in-

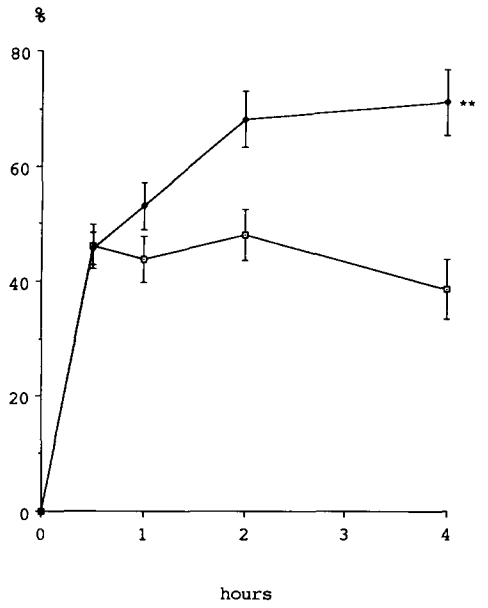

Fig. 2. Swelling induced by collagenase in the paws of normal Wistar rats (□) and of kininogen-deficient Brown Norway rats (♦). The swelling was calculated as the percentage increase in the volume of the paw. Each value is the mean ± S.E.M. of 12 determinations. ** $P < 0.001$ versus Wistar rats.

Table 1
Inhibitory effects of HOE 140, SBTI, LLTI-1 and LLTI-2 on collagenase-induced oedema in normal Wistar rats

Treatment	30 min	1 h	2 h	4 h
HOE140				
Saline (34)	40.4 ± 1.8	50.8 ± 2.5	62.3 ± 3.1	56.6 ± 3.4
2 mg/kg (10)	35.1 ± 2.2	40.8 ± 2.6 [a]	53.6 ± 3.3	50.3 ± 4.5
4 mg/kg (25)	30.0 ± 1.5 [b]	34.9 ± 2.0 [b]	48.2 ± 2.8 [b]	46.0 ± 3.2 [a]
8 mg/kg (6)	28.2 ± 2.2 [b]	36.7 ± 2.9 [a]	45.5 ± 4.7 [b]	44.5 ± 4.0 [a]
SBTI				
Saline (30)	46.2 ± 1.8	54.0 ± 1.7	62.1 ± 2.5	58.0 ± 3.0
2.5 mg/kg (12)	43.2 ± 3.0	42.6 ± 3.0 [a]	42.5 ± 3.4 [b]	38.4 ± 3.0 [b]
5 mg/kg (12)	39.8 ± 2.6	41.0 ± 2.4 [a]	42.5 ± 2.9 [b]	35.5 ± 3.1 [b]
10 mg/kg (12)	35.7 ± 2.7 [a]	39.3 ± 2.4 [b]	49.1 ± 2.6 [a]	41.9 ± 3.0 [a]
LLTIs				
Saline (24)	45.4 ± 2.2	45.1 ± 1.9	48.9 ± 2.4	43.5 ± 2.6
LLTI-1 (16)	27.5 ± 3.0 [b]	40.3 ± 3.4	59.4 ± 4.7	63.5 ± 4.0 [a]
LLTI-2 (15)	24.4 ± 2.1 [b]	31.6 ± 3.0 [a]	48.6 ± 3.5	49.9 ± 3.3

The swelling is expressed as a percentage of the control paw volume. Each result is the mean ± S.E.M. Numbers in parentheses are numbers of observations. HOE 140 was administered i.p. 30 min before collagenase, SBTI was administered i.v. 10 min before collagenase, LLTI-1 and LLTI-2 were locally administered in the paw with collagenase at the dose of 1 mg.
[a] $P < 0.05$; [b] $P < 0.01$: significant difference from saline-treated rats.

crease in paw volume ($n = 30$) to 52.1 ± 4.0% ($n = 9$; $P < 0.05$). This latter potentiating effect suggests that HOE 140 at very high doses (15 μmol) can act as an agonist, as described for other bradykinin analogues (Grant and Zeitlin, 1987; Kindgen-Milles and Klement, 1992, for examples).

The mechanism of kinin release was also investigated by using the plasma kallikrein inhibitors, SBTI, LLTI-1 and LLTI-2 (Vogel and Werle, 1970; Souza Pinto et al., 1992). SBTI was intravenously injected 10 min before collagenase. It dose dependently reduced the oedema by about 25–35% (Table 1). The two other serine protease inhibitors, LLTI-1 and LLTI-2, were administered in the paws with collagenase at the dose of 1 mg. This dose has previously given maximal inhibition of carrageenan oedema (Souza Pinto et al., 1992). Both inhibitors reduced the swelling by about 40–45% during the first 30 min after collagenase injection but this inhibitory effect had disappeared after 2 h and the oedema was increased, in some cases, at 4 h (Table 1).

These agents, HOE 140, LLTI-1 and LLTI-2, had no influence on collagenase oedema elicited in kininogen-deficient rats (Table 2).

3.3. Effects of antagonists of mast cell amines

Intraperitoneal pretreatment of Wistar rats with mepyramine (5 mg/kg, 30 min before collagenase) failed to inhibit the oedema. However, the swelling was greatly reduced during the first 1 h by methysergide (2 or 5 mg/kg i.p., 30 min before collagenase) but this inhibitory effect had disappeared at 4 h (Table 3). Pretreatment of the animals with the mixture of methysergide and mepyramine did not increase the inhibitory effect of the serotonin antagonist (Table 3). In Wistar rats, the swelling was nearly suppressed during the first 30 min by pretreatment of the animals with methysergide associated with the local administration of LLTI-1 or LLTI-2 in the paw (Table 3).

Methysergide also reduced the swelling induced in kininogen-deficient rats. At 1 h, the swelling reached a 43 ± 4.4% increase in paw volume ($n = 6$) after saline but 22.5 ± 2.5% ($n = 6$; $P < 0.005$) after methysergide (5 mg/kg) in these kininogen-deficient animals.

Table 2
Lack of effect of HOE 140, LLTI-1 and LLTI-2 on collagenase-induced oedema in kininogen-deficient rats

Treatment	30 min	1 h	2 h	4 h
HOE 140				
Saline	45.5 ± 4.8	55.1 ± 4.2	58.5 ± 1.8	55.6 ± 5.8
4 mg/kg	42.0 ± 5.1	48.2 ± 5.8	67.0 ± 4.3	64.4 ± 5.5
LLTIs				
Saline	77.6 ± 9.6	84.1 ± 9.4	82.6 ± 10.7	74.8 ± 7.5
LLTI-1	64.5 ± 4.2	82.1 ± 5.8	89.3 ± 7.1	93.0 ± 7.1
LLTI-2	65.1 ± 7.0	77.8 ± 9.1	78.5 ± 10.2	87.7 ± 4.9

The swelling is expressed as a percentage of the control paw volume. Each result is the mean ± S.E.M. of 6 observations. HOE 140 was injected i.p. 30 min before collagenase. LLTI-1 or LLTI-2 (1 mg) was administered with collagenase in the paw.

Table 3
Effects of mepyramine and methysergide on collagenase-induced oedema in normal Wistar rats

Treatment	30 min	1 h	2 h	4 h
Saline (20)	48.9 ± 2.4	49.5 ± 2.5	61.0 ± 2.6	49.8 ± 3.4
Mepyramine 5 mg/kg (8)	52.0 ± 7.2	54.1 ± 7.2	59.7 ± 6.8	55.0 ± 6.9
Methysergide 2 mg/kg (8)	38.1 ± 0.6 [a]	45.2 ± 1.7	59.3 ± 2.3	48.1 ± 3.0
Methysergide 5 mg/kg (22)	32.4 ± 1.8 [b]	38.3 ± 2.0 [b]	50.5 ± 2.3 [b]	47.0 ± 2.5
Methysergide + mepyramine 5 mg/kg (6)	33.6 ± 2.8 [b]	37.1 ± 4.1 [b]	42.8 ± 4.3 [b]	44.1 ± 5.5
Methysergide 5 mg/kg + LLTI-1 (14)	15.5 ± 2.4 [b]	32.7 ± 4.7 [b]	54.7 ± 6.4	58.7 ± 5.1
Methysergide 5 mg/kg + LLTI-2 (14)	21.3 ± 4.6 [b]	32.6 ± 3.9 [b]	53.7 ± 4.8	57.2 ± 5.2

The swelling is expressed as a percentage of the control paw volume. Each result is the mean ± S.E.M. Numbers in parentheses are numbers of observations. Mepyramine and methysergide were administered i.p. 30 min before collagenase. LLTI-1 or LLTI-2 (1 mg) was injected with collagenase in the paw.
[a] $P < 0.05$; [b] $P < 0.01$: significant difference from saline-treated rats.

3.4. Effects of miscellaneous agents

The oedema induced by collagenase in Wistar rats was partly inhibited by indomethacin and ketoprofen, two cyclo-oxygenase inhibitors (5 mg/kg i.p., 1 h before collagenase). This inhibitory effect was more apparent with indomethacin and was maximal 2 h after collagenase injection (Table 4). Methylprednisolone (5 or 20 mg/kg i.p., 3 h before collagenase) partly reduced the development of the swelling (Table 4). N^G-Nitro-L-arginine, an inhibitor of nitric oxide synthase (40 or 100 mg/kg i.p., 30 min before collagenase or 100 μg in the paw with collagenase), did not affect collagenase-induced oedema in Wistar rats, though it largely inhibited the development of carrageenan oedema (Table 5). Similarly, the oedema was not modified in rats pretreated by WEB 2086, a PAF receptor antagonist (20 or 40 mg/kg i.p., 30 min before collagenase) (Table 6). However, heparin (5 IU) administered in the paw with collagenase increased the oedema. At 1 h, the swelling reached 41.3 ± 4.0% increase in paw volume after collagenase injection in control rats ($n = 12$) but 53.7 ± 2.1% after collagenase associated with heparin ($n = 12$; $P > 0.05$).

Table 4
Inhibitory effect of indomethacin, ketoprofen and methylprednisolone on paw oedema induced by collagenase in Wistar rats

Treatment	30 min	1 h	2 h	4 h
Indomethacin-ketoprofen				
Saline (12)	40.6 ± 3.2	46.1 ± 3.4	48.5 ± 4.1	50.9 ± 6.9
Indomethacin (12)	34.5 ± 4.3	30.3 ± 4.1 [a]	27.5 ± 4.0 [b]	31.8 ± 7.8
Ketoprofen (12)	39.8 ± 3.3	37.0 ± 3.0	37.3 ± 3.2 [a]	41.4 ± 5.3
Methylprednisolone				
Saline (18)	51.7 ± 1.8	58.6 ± 1.9	68.0 ± 2.6	66.4 ± 3.9
5 mg/kg (6)	44.3 ± 1.6 [b]	47.3 ± 4.0 [a]	54.5 ± 6.6 [a]	50.1 ± 5.2 [a]
20 mg/kg (12)	43.3 ± 2.1 [b]	46.4 ± 2.8 [b]	49.1 ± 3.0 [b]	42.7 ± 2.9 [b]

The swelling is expressed as a percentage of the control paw volume. Indomethacin or ketoprofen (5 mg/kg) was administered i.p. 1 h before collagenase, methylprednisolone was administered i.p. 3 h before collagenase.
[a] $P < 0.05$; [b] $P < 0.01$: significant difference from saline-treated rats.

3.5. Enzyme activity in vitro

The influence of LLTI-1 and LLTI-2 (50 and 250 μg) on collagenase activity in vitro was evaluated using a synthetic peptide substrate, FALGPA. Though the two protease inhibitors reduced collagenase oedema in vivo, they did not modify the activity of the enzyme on one of its specific substrates in vitro.

The potential kinin-forming activity of collagenase in vitro was evaluated by incubating either rat plasma or purified rat T-kininogen with collagenase and measuring the kinin release by bioassays. Trypsin was used as control kinin-forming agent. Collagenase (10–100 μg) failed to release a significant amount of kinins from rat plasma or from purified T-kininogen. Bioassays only showed a small direct myostimulating activity of collagenase on rat duodenum. In the same conditions, trypsin released large amounts of kinins from Wistar rat plasma and 125 ng of T-kinin from 10 μg of T-kininogen.

Table 5
Effect of N^G-nitro-L-arginine (NOARG) on oedema induced by collagenase or carrageenan in normal Wistar rats

Treatment	30 min	1 h	2 h	4 h
1. Collagenase				
A. Saline	54.0 ± 1.1	68.0 ± 2.8	75.1 ± 3.8	68.1 ± 5.6
NOARG 40 mg/kg	55.3 ± 7.9	64.3 ± 5.7	69.0 ± 5.0	57.5 ± 4.2
NOARG 100 mg/kg	56.5 ± 2.9	62.8 ± 3.2	71.1 ± 3.7	57.5 ± 2.1
B. Saline	70.6 ± 4.6	78.3 ± 2.5	79.8 ± 5.8	80.3 ± 6.0
NOARG 100 μg/paw	70.8 ± 4.0	77.8 ± 5.6	90.5 ± 2.8	87.5 ± 4.0
2. Carrageenan				
Saline		24.0 ± 2.9	55.6 ± 5.2	84.1 ± 9.1
NOARG 25 μg/paw		11.0 ± 2.3 [c]	28.1 ± 5.3 [c]	84.3 ± 8.4

The swelling is expressed as a percentage of the control paw volume. Each result is the mean ± S.E.M. of 6 determinations. N^G-Nitro-L-arginine was administered either i.p. (40 or 100 mg/kg) 30 min before collagenase or in the paw (25 or 100 μg/paw) with collagenase or carrageenan.
[c] $P < 0.005$: significant difference from saline-treated rats.

Table 6
Lack of effect of WEB 2086 on collagenase-induced oedema in normal Wistar rats

Treatment	30 min	1 h	2 h	4 h
Saline	65.8±6.3	73.6±8.5	80.5±7.7	69.1±5.9
WEB 2086				
20 mg/kg	59.8±2.8	61.5±1.9	73.0±2.8	63.0±3.4
40 mg/kg	57.6±4.5	67.3±5.5	71.3±5.3	60.0±6.0

The swelling is expressed as a percentage of the control paw volume. Each result is the mean±S.E.M. of 6 observations. WEB 2086, a PAF receptor antagonist, was administered i.p. 30 min before collagenase.

4. Discussion

The rapid development of collagenase oedema was inhibited in normal rats by HOE 140, a bradykinin antagonist, and by three serine protease inhibitors. These results are in accordance with previous findings of Vargaftig et al. (1976) and Legat et al. (1993). These agents had no influence on the oedema elicited in kininogen-deficient rats. They are thus specific agents inhibiting the effects or the formation of bradykinin. Therefore, a large part – about 30% – of the swelling induced by collagenase in normal rats depends on kinin formation.

Collagenase does not release kinins in vitro from rat plasma and from T-kininogen, the third kallikrein-resistant form of rat kininogens. The formation of kinins during the inflammatory reaction elicited by this enzyme is thus indirect. Extravasation of plasma into extravascular spaces is thought to activate in vivo the kinin system. However, as demonstrated by Yamamoto et al. (1989), Hageman factor, plasma prekallikrein and kininogens are present in the normal extravascular dermal tissue. Moreover, Hageman factor is not activated by a variety of purified components of vascular basement membranes (Griffin et al., 1975). Further, kinin formation is not triggered during plasma exudation in zymosan-induced inflammation in rat (Damas et al., 1990a). Thus, other mechanisms must activate the kinin system. Collagenase oedema is associated with local haemorrhages and blood coagulation. It was increased by heparin. Therefore, the generation of kinins during collagenase oedema would mostly result from a short-lasting blood extravasation into interstitial spaces in which collagenase has induced modifications of the matrix proteins. These modifications would activate the plasma contact system. Indeed, collagenase oedema was reduced by proteins, LLTI-1 and LLTI-2, that inhibit Hageman factor and plasma kallikrein and not tissue kallikreins (Souza Pinto et al., 1992).

According to Legat et al. (1993), bradykinin is the main mediator of collagenase-induced oedema. However, if the kinin system participates in the development of the swelling elicited by collagenase in normal rats, its role is not decisive as collagenase induced similar oedema in normal and kininogen-deficient rats. Inflammatory reactions in which the kinin system plays the leading role are greatly reduced in kininogen-deficient rats, such as the oedemas induced by carrageenan or urate crystals (Damas et al., 1984). Thus, the involvement of the kinin system in collagenase oedema only results from blood extravasation and coagulation. Simultaneously, other inflammatory factors are released or activated. Indeed, a large part of the inflammatory reaction induced by collagenase still occurred in normal rats treated with HOE 140 or with the protease inhibitors. Thus, in this swelling, bradykinin adds its pro-inflammatory effects to those of other factors.

Legat et al. (1993) observed that collagenase oedema is not modified during the first 1 h by mepyramine and indomethacin. They ruled out histamine and prostanoids as being mediators involved in this inflammatory reaction. However, in our hands, though mepyramine had no effect on collagenase oedema, methysergide, a serotonin antagonist, greatly inhibited the reaction. Rat mast cells contain larger amounts of histamine than serotonin, but rat platelets also store serotonin and could be involved in the oedema during blood coagulation. The oedematogen effect of serotonin in rat paw is 20–30 times higher than that of histamine (Damas and Remacle-Volon, 1986). Similarly, Vargaftig et al. (1976) reported that collagenase oedema is reduced by cyproheptadine, another serotonin antagonist. These results show that serotonin, possibly released from platelets, plays a role in collagenase oedema.

This swelling is also inhibited by several non-steroidal anti-inflammatory drugs (Vinegar et al., 1969; Vargaftig et al., 1976; the present results). Though these drugs have many properties beside their inhibitory effect on cyclo-oxygenases (Abramson, 1985), this reduction obtained with several different inhibitors of prostaglandin synthases suggests that prostanoids take part in oedema. The late phase of the swelling is also reduced by steroidal anti-inflammatory agents (Vinegar et al., 1969; Vargaftig et al., 1976; the present results). On the other hand, WEB 2086, a PAF receptor antagonist, and N^G-nitro-L-arginine, a nitric oxide synthase inhibitor, failed to depress this inflammatory reaction. The latter agent was used at sufficient concentration because it reduced carrageenan oedema, as other NO synthase inhibitors (Ialenti et al., 1992). These results would indicate that nitric oxide and platelet-activating factor do not play a significant role in this inflammatory response.

Collagenase oedema was reduced by the two protease inhibitors, LLTI-1 and LLTI-2, in normal but not in kininogen-deficient rats. This kind of inhibition suggests that Hageman factor and plasma kallikrein partic-

ipate in the inflammatory reaction elicited by collagenase only through the formation and the activity of kinins.

Acknowledgements

This work was supported by grant No. 1.5.126.91F of the Fonds National de la Recherche Scientifique de Belgique. J.C.S.P. was supported by a grant from CNPq-Brasilia, Brazil.

References

Abramson, S., 1985, Mechanism of action of nonsteroidal antiinflammatory drugs, in: Advances in Inflammation Research, Vol. 10, eds. F. Russo-Marie, J.M. Mencia-Huerta and M. Chignard (Raven Press, New York) p. 111.

Adam, A., J. Damas, C. Renard, G. Calay and V. Bourdon, 1989, Purification and characterization of plasma T-kininogen from Wistar and Brown Norway rats, Biochem. Cell Biol. 67, 86.

Bhoola, K.D., C.D. Figueroa and K. Worthy, 1992, Bioregulation of kinins: kallikreins, kininogens and kininases, Pharmacol. Rev. 44, 1.

Damas, J., 1987, The novel rat kininogen, T-kininogen. A pro-inflammatory factor or a thiostatin?, Arch. Int. Physiol. Biochim. 95, 67.

Damas, J., 1991, Involvement of platelet-activating factor in the hypotensive response to zymosan in rats, J. Lipid Med. 3, 333.

Damas, J. and A. Adam, 1980, Congenital deficiency in plasma kallikrein and kininogens in the Brown Norway rat, Experientia 36, 586.

Damas, J. and J.C. Mousty, 1978, Inhibition de l'action hypotensive de l'acide arachidonique chez le rat, J. Pharmacol. (Paris) 9, 13.

Damas, J. and G. Remacle-Volon, 1986, Mast cell amines and the oedema induced by zymosan and carrageenans in rats, Eur. J. Pharmacol. 121, 367.

Damas, J. and G. Remacle-Volon, 1990, Platelets and the endotoxin shock in the rat, Life Sci. Adv. 9, 125.

Damas, J. and G. Remacle-Volon, 1992, Influence of a long-acting bradykinin antagonist, HOE 140, on some acute inflammatory reactions in the rat, Eur. J. Pharmacol. 211, 81.

Damas, J., G. Remacle-Volon and A. Adam, 1984, Inflammation in the rat paw due to urate crystals. Involvement of the kinin system, Naunyn-Schmied. Arch. Pharmacol. 325, 76.

Damas, J., V. Bourdon, G. Remacle-Volon and A. Adam, 1990a, Kinins and peritoneal exudates induced by carrageenin and zymosan in rats, Br. J. Pharmacol. 101, 418.

Damas, J., V. Bourdon, G. Remacle-Volon and A. Adam, 1990b, Proteinase inhibitors, kinins and the inflammatory reaction induced by sponge implantation in rats, Eur. J. Pharmacol. 175, 341.

Garcia Leme, J., 1978, Bradykinin system, in: Inflammation, Handbook of Experimental Pharmacology, Vol. 50/I, eds. J.R. Vane and S.H. Ferreira (Springer-Verlag, New York, Berlin, Heidelberg) p. 464.

Grant, A. and I.J. Zeitlin, 1987, The effects of some analogues of bradykinin on bradykinin-induced rat paw swelling, Br. J. Pharmacol. 92, 626P.

Griffin, J.H., E. Harper and C.G. Cochrane, 1975, Studies on the activation of human blood coagulation factor XII (Hageman factor) by soluble collagen. Fed. Proc. 34, 860.

Hatheway, C.L., 1990, Toxigenic clostridia, Clin. Microbiol. Rev. 3, 66.

Henson, P.M., J.E. Henson, C. Fittschen, G. Kimani, D.L. Bratton and D.W.H. Riches, 1988, Phagocytic cells: degranulation and secretion, in: Inflammation: Basic Principles and Clinical Correlates, eds. J.I. Gallin, I.M. Godstein and R. Snyderman (Raven Press, New York) p. 363.

Ialenti, A., A. Ianaro, S. Moncada and M. Di Rosa, 1992, Modulation of acute inflammation by endogenous nitric oxide, Eur. J. Pharmacol. 211, 177.

Kindgen-Milles, D. and W. Klement, 1992, Pain and inflammation evoked in human skin by bradykinin receptor antagonist, Eur. J. Pharmacol. 218, 183.

Legat, F.J., T. Griesbacherg and F. Lembeck, 1993, Bradykinin is the main mediator of collagenase-induced oedema in the rat paw, Br. J. Pharmacol. 108, 57P.

Marceau, F., A. Lussier, D. Regoli and J.P. Giroud, 1983, Pharmacology of the kinins: their relevance to tissue injury and inflammation, Gen. Pharmacol. 14, 209.

Oh-Ishi, S., M. Hayashi and K. Yamaki, 1986, Inflammatory effects of acetylglycerylether phosphorylcholine: vascular permeability increase and induction of pleurisy in rats, Prostagl. Leukotr. Med. 22, 21.

Souza Pinto, J.C., M.S. Araujo and M.U. Sampaio, 1992, Purification and partial characterization of two trypsin inhibitors from *Leucaena leucocephala* (LLTI-1 and LLTI-2), in: XXI Annual Reunion of the Brazilian Society of Biochemistry and Molecular Biology, Abstract P11-27.

Van Arman, C.G., 1979, Oedema and increased vascular permeability, in: Inflammation, Handbook of Experimental Pharmacology, Vol. 50/II, eds. J.R. Vane and S.H. Ferreira (Springer-Verlag, New York, Berlin, Heidelberg) p. 75.

Van Wart, H.E. and D.R. Steinbrink, 1981, A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase, Anal. Biochem. 113, 356.

Vargaftig, B.B., J. Lefort and E.L. Giroux, 1976, Haemorrhagic and inflammatory properties of collagenase from *C. histolyticum*, Agents Actions 6, 627.

Vinegar, R., W. Schreiber and R. Hugo, 1969, Some characteristics of enzyme-induced inflammation in the rat, Fed. Proc. 28, 357.

Vogel, R. and E. Werle, 1970, Kallikrein inhibitors, in: Bradykinin, Kallidin and Kallikrein, Handbook of Experimental Pharmacology, Vol. 25, ed. E.G. Erdös (Springer-Verlag, New York, Berlin, Heidelberg) p. 213.

Wahl, S.M., 1988, Fibrosis: bacterial-cell-wall-induced hepatic granulomas, in: Inflammation: Basic Principles and Clinical Correlates, eds. J.I. Gallin, I.M. Godstein and R. Snyderman (Raven Press, New York) p. 841.

Whalley, E.T., 1987, Receptors mediating the increase in vascular permeability to kinins: comparative studies in rat, guinea-pig and rabbit, Naunyn-Schmied. Arch. Pharmacol. 336, 99.

Williams, T.J. and J. Morley, 1973, Prostaglandins as potentiators of increased vascular permeability in inflammation, Nature 246, 215.

Wirth, K., F.J. Hock, U. Albus, W. Linz, H.G. Alpermann, H. Anagnostopoulos, St. Henke, G. Breipohl, W. König, J. Knolle and B.A. Schölkens, 1991, HOE 140 a new potent long acting bradykinin-antagonist: in vivo studies, Br. J. Pharmacol. 102, 774.

Yamamoto, T., T. Ishimatsu and T. Kambara, 1989, Hageman factor dependent kinin generation in guinea pig skin: extravascular localization of the components, and prolonged vascular reaction in inhibitor-depleted animal of this system, in: Kinins V, Advances in Experimental Medicine and Biology, Vol. 247A, eds. K. Abe, S. Fujii and H. Moriya (Plenum Press, New York) p. 447.