Evaluation of Analgesic and Anti-inflammatory Activity of Novel β -Lactam Monocyclic Compounds

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We have examined the *in vivo* anti-inflammatory and analgesic activity of a new series of monocyclic β -lactams (azetidinones), similar to others which have been demonstrated to be inhibitors of human leukocyte elastase (HLE), an enzyme involved in degradation processes of connective tissue. Our new compounds have been administered orally (15 mg/kg) to albino rats 30 min before injecting carrageenin in the plantar aponeurosis. Tested compounds have demonstrated a certain activity and stability to gastric hydrolysis, in particular two of them markedly reduced paw edema formation, even if slightly less effectively than indomethacin (reference compound, 5 mg/kg). To evaluate the analgesic activity we carried out the acetic acid writhing test, pretreating rats orally with our compounds 30 min before injecting the acid solution *i.p.* The same two molecules which showed the anti-inflammatory activity demonstrated a very light analgesic activity. These results suggest the possibility of carrying out further studies, particularly *in vitro*, on the mechanism of action of our compounds, mechanism which could be the HLE inhibition.

Key words inflammation; monocyclic β -lactam; human leukocyte elastase

The last ten years has seen the fruition of using β -lactam compounds to inhibit some enzymes belonging to the family of serine proteinases, in particular human leukocyte elastase (HLE, EC 3.4.21.37). This is an enzyme stored in the azurophilic granules of polymorphonuclear leukocytes that increases blood vessel permeability hydrolyzing basement membrane elastine and that intervenes in the degradation processes of connective tissue, processes that underlie diseases such as emphysema,¹⁾ chronic bronchitis,²⁾ cystic fibrosis³⁾ and acute respiratory distress syndrome.⁴⁾ Agents able to inhibit this enzyme could thus be of use in treating these inflammatory diseases. The first researchers studying β -lactam HLE inhibitors were Merk ones,⁵⁾ some of the potent resulting compounds⁶⁾ belong to the class of **1a** structure (Fig. 1). The same research team has synthesized azetidinones, as **1b** structure,⁷⁾ which seem able to inhibit HLE by simple formation of a stable acyl-enzyme (due to the attack of Ser-195 in the active site on the lactam carbonyl) which does not undergo hydrolysis.⁸⁾ A further important monocyclic β -lactam inhibitor, which lacks the C-4 side chain, was obtained from Reboud-Ravaux and co-workers and it is compound **1c**.^{9,10)}

Taking these results into account, we decided to design a new type **1d** structural model; it is an azetidinone with some replaced aromatic substituents. We assumed that the characteristics of type **1d** model should have made it able to act as an orally active anti-inflammatory drug and in particular as a serine proteinase inhibitor. We obtained it through cyclization of appropriate *N*-arylpropionamides^{11,12)}; which gave the *N*-aryl-3,3-dimethylazetidin-2-ones **2a–f** reported in Fig. 2.

Preliminary *in vivo* tests were done to evaluate any anti-inflammatory activity (carrageenin-induced edema test)¹³⁾ and analgesic activity was also evaluated (writhing test).^{14,15)}

MATERIALS AND METHODS

a) Anti-edemigenic activity was evaluated through the car-

rageenin test of Winter *et al.*¹³⁾ The effects of pretreating rats with products **2a–f** (30 min previously), administered orally *via* gastric intubation (15 mg/kg), were studied on localized edematous reaction; 0.1 ml of 1% suspension of carrageenin in distilled water was injected subcutaneously (*s.c.*) into the plantar sub-aponeurosis. The paw volume was measured by a mercury plethysmograph (differential gauge, manufactured by Basile) at the moment of carrageenin injection and then 1, 2, 3 and 4 h later (always taking care to immerse the paw at the same level, that is the tibio-astragalic joint, ascertained and marked with a line before the test).

Indomethacin (5 mg/kg *per os*) was used as reference compound. Albino rats (180–200 g) of both sexes were used, excepting pregnant females; each group comprised six animals. The mean increase of paw volume, at the above-said time intervals, of the control group (receiving carrageenin alone) and drug-treated groups was calculated. The most significant results are given in Table 1. MANOVA test was used in the statistical evaluation.

Fig. 1

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b) Analgesic activity was evaluated through the acetic acid test (writhing test) as described by Davies *et al.*^{14,15} The activity of **2a–f** was evaluated in rats, administering the compounds (15 mg/kg) orally by gastric intubation 30 min before acetic acid (0.25 ml intra peritoneum (i.p.), per rat, of a 0.5% aqueous solution). For each animal, the number of writhing movements was measured, in the 25 min immediately after administration of acetic acid. The mean number of writhes for each group of animals and the percentage variation com-

pared with the control group were calculated. Mice (*Mus musculus*) of both sexes were used, excepting pregnant females, weighing 20–25 g; each group comprised six animals. The most significant results are given in Table 2. Wilcoxon test was used for the statistical evaluation.

RESULTS AND DISCUSSION

All tested compounds showed a certain activity; in particular we give in Table 1 the results of **2a** and **2f**, which showed a marked anti-inflammatory activity on rats at a dose of 15 mg/kg, which was above that of indomethacin, used as reference drug in these tests.

The second test demonstrated that these monocyclic β -lactams have an analgesic activity inferior to the anti-inflammatory one, since response to **2a** was modest and response to **2f** was not really significant.

It is noteworthy that **2a** and **2f** retained their activity after oral administration, showing they did not undergo hydrolysis in the gastro-intestinal tract or in the blood stream, a problem common to β -lactam structures and to many *in vitro* HLE inhibitors.

Even if we cannot state that these new azetidinones are HLE inhibitors we can draw some preliminary conclusions: *in vitro* tests are now necessary in order to verify with certainty if the anti-inflammatory activity of these compounds is due to a mechanism of inhibition of HLE, as could be hypothesised. If true, this result should give some useful suggestions for the design strategy of HLE monocyclic β -lactam inhibitors. If not true, it would be interesting to further investigate the mechanism of action of these new azetidinones.

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Table 2. Evaluation of the Analgesic Activity of Indomethacin and of **2a** and **2f** by the Acetic Acid Test

Treatment	Dose (mg/kg)	Mean writhing movements $\pm$ E.S. in 25 min	Percentage variation over control
Control		44.72 $\pm$ 4.35	
Indomethacin	5	24.13 $\pm$ 5.62 ^{a)}	–46.0
2a	15	32.45 $\pm$ 6.73 ^{b)}	–27.4
2f	15	35.34 $\pm$ 6.21 ^{b)}	–21.0

a) $p < 0.01$ vs. control value (Wilcoxon test). b) $p < 0.05$ vs. control value.

Table 1. Evaluation of the Anti-inflammatory Activity of Indomethacin and of **2a** and **2f** by the Carrageenin Paw Edema Test

Treatment	Dose (mg/kg)	Paw volume (ml)				
		0	1 h	2 h	3 h	4 h
Control		1.55 $\pm$ 0.12	2.36 $\pm$ 0.21 (+52)	2.63 $\pm$ 0.16 (+69)	2.75 $\pm$ 0.19 (+77)	2.88 $\pm$ 0.18 (+86)
Indomethacin	5	1.59 $\pm$ 0.1	1.82 $\pm$ 0.20 (+17) ^{a)}	1.83 $\pm$ 0.17 (+18) ^{a)}	1.86 $\pm$ 0.22 (+20) ^{a)}	1.88 $\pm$ 0.19 (+21) ^{a)}
2a	15	1.62 $\pm$ 0.11	2.10 $\pm$ 0.16 (+30) ^{b)}	2.12 $\pm$ 0.17 (+31) ^{b)}	2.22 $\pm$ 0.15 (+37) ^{b)}	2.27 $\pm$ 0.15 (+40) ^{b)}
2f	15	1.73 $\pm$ 0.14	1.92 $\pm$ 0.18 (+11) ^{b)}	2.21 $\pm$ 0.15 (+28) ^{b)}	2.22 $\pm$ 0.19 (+28) ^{b)}	2.51 $\pm$ 0.17 (+45) ^{b)}

Paw volume was measured after carrageenin administration, at the times (h) written at the top of the table. In brackets are the mean percentage increases in volume over time zero. a) $p < 0.01$ vs. control value (MANOVA test). b) $p < 0.05$ vs. control value.

Fig. 2

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