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SPECIAL REPORT

Anti-inflammatory actions of an N-terminal peptide from human lipocortin 1

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An acetylated polypeptide corresponding to residues 2–26 of human lipocortin 1 was synthesized and the anti-inflammatory activity assessed in three models of acute inflammation in rat and mouse. In the carrageenin rat paw oedema test, the peptide produced a maximal inhibition of approximately 41% at the 3 h time point with a 10 μg dose. When rat paw oedema was induced by the injection of venom phospholipase A_2, the peptide produced a significant inhibition (31%) at the top dose of 20 μg per paw. In the mouse air-pouch model, systemic treatment with the peptide produced a dramatic reduction in cytokine-induced leukocyte migration with an ID_{50} of approximately 40 μg per mouse.

The N-terminal peptide 2–26 shares the actions of lipocortin 1 in these acute models of inflammation.

Keywords: Lipocortin 1/annexin 1; acute inflammation; N-terminus; anti-inflammatory actions

Introduction Human recombinant lipocortin 1 suppresses the release of proinflammatory eicosanoids *in vitro* (Cirino & Flower, 1987; Cirino *et al.*, 1987) and exhibits strong anti-inflammatory actions in rat (Cirino *et al.*, 1989) and mouse (Perretti & Flower, 1993). An intact C-terminus is apparently not necessary for biological activity since the 1–188 recombinant fragment suppresses cytokine induced fever (Carey *et al.*, 1990) and ischaemic brain damage in the rat (Relton *et al.*, 1991). Conversely, removal of the 3kDa N-terminal fragment from lipocortin 1 by proteolytic plasmin cleavage inactivated the molecule (Cirino, Browning & Flower unpublished results).

To elucidate further the role of the N-terminus in lipocortin 1 action we have synthesized a peptide representing the entire N-terminus (residues 2–26). To retard proteolytic degradation, this peptide was synthesized with an acetyl blocked N-terminus.

Methods *Carrageenin and phospholipase A_2 paw oedema* Oedema was induced in male wistar rats weighing 130–150 g (Nossan, Italy) by a single subplantar injection of either 100 μl of a 1% (w/v) solution of carregeenin or 5 μg per paw of phospholipase A_2 from *Naja mocambique mocambique* into the right hind paw (Cirino *et al.*, 1989). The peptide was dissolved in 100 μl sterile saline while the control received saline only (100 μl). Paw volume was measured with a water plethysmometer (Basile, Italy) immediately before and after the injection and at appropriate intervals thereafter.

Interleukin 1β induced neutrophil accumulation in mouse air pouch Male Swiss albino mice (22–25 g; Tuck U.K.) were injected with 2.5 ml of air subcutaneously on day 0 and day 3. On day six, animals received either PBS or the peptide (10–400 μg per mouse) in different doses prepared in 200 μl of PBS by intravenous injection, 20–30 min before interleukin-1β (20 ng) injection into the air-pouch. Pouches were washed with 2 ml of PBS containing heparin and counted after staining in Turk's solution (1/10 dilution). The data are expressed as a percentage of the control migration observed in the PBS-treated groups (Perretti & Flower, 1993).

Materials The peptide 2–26 (N-acetyl-AMVSEFLKQAW-FIENEEQEVVQTVK) was obtained from three different sources Bachem (U.S.A.), Multiple Peptide Systems (U.S.A.) and Biogen (U.S.A.). The latter was a generous gift of Dr J.L. Browning. The purity was always greater than 95%. Phospholipase A_2 from *Naja mocambique mocambique* (EC 3.1.1.4.), carrageenin and other routine buffers and salts were purchased from the Sigma Chemical Company. Interleukin-1β was a generous gift from Dr L. Parente (I.R.I.S., Siena, Italy).

Results Figure 1a shows that the peptide produced a dose-related inhibition of the carrageenin paw oedema at 3 h. Indeed 5 μg and 10 μg per paw produced 20% and 41% inhibition respectively. However 20 μg did not produce any further inhibitory activity (data not shown). At doses of 5 μg and 10 μg per paw the peptide was without effect on the oedema produced by phospholipase A_2 (data not shown). However subplantar injection of 20 μg of the peptide produced a weak (31%) but significant inhibition of the inflammation (Figure 1b).

Figure 2 shows that the peptide produced a dose-related reduction of polymorphonuclear leukocyte (PMN) migration into the pouch with an IC_{50} of approximately 40 μg per mouse.

No overt toxicity of the peptide was observed at the doses used in this experiment in either the rat or mouse.

Discussion We have tested a peptide corresponding to residues 2–26 of lipocortin 1 in three models of inflammation which are known to respond to the inhibitory action of the full length protein. In the carrageenin paw oedema test and phospholipase A_2-induced oedema, human recombinant lipocortin 1 has an ID_{50} of 10–20 μg respectively (Cirino *et al.*, 1989). Thus on a molar basis the peptide is at least one order of magnitude less effective than the native species. In the mouse neutrophil migration test, lipocortin 1 itself has an ID_{50} of approximately 4 μg per mouse when given intravenously (Perretti & Flower, 1993) and the peptide is approximately two orders of magnitude less potent on a molar basis than the parent molecule.

It is interesting to note that the N-terminal peptide shares some of the actions of lipocortin 1 in these models of

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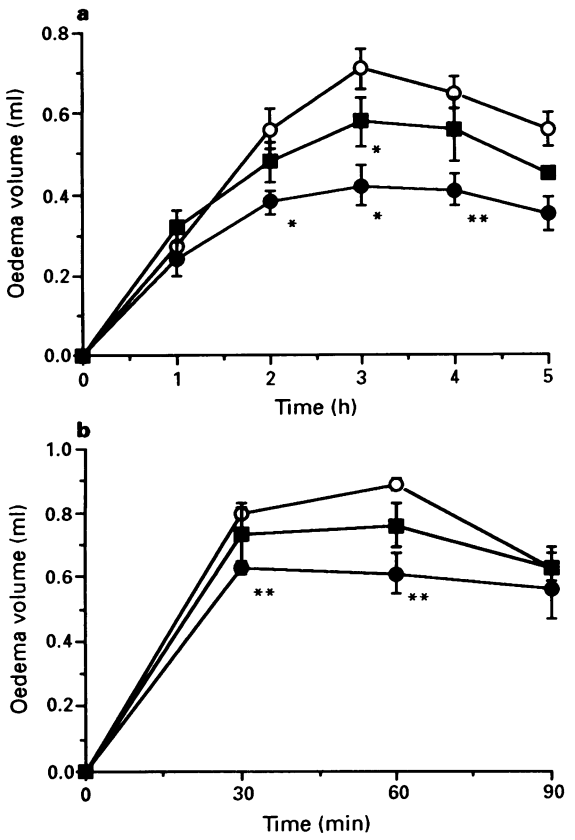

Figure 1 (a) The inhibitory effect of N-acetyl 2–26 lipocortin 1 on the oedema (ml) produced in rat paw by subplantar injection of carrageenin: (○) 0.1 ml 0.1% carrageenin; (■) carrageenin + 5 μg N-acetyl 2–26; (●) carrageenin + 10 μg N-acetyl 2–26. Each point is a mean value ± s.e.mean (vertical bars); $n = 15$ rats per group. (b) The inhibitory effect of N-acetyl 2–26 lipocortin 1 on the oedema (ml) produced in rat paw by subplantar injection of phospholipase A_2: (○) 5 μg phospholipase A_2 only; (■) phospholipase A_2 + 10 μg N-acetyl 2–26; (●) phospholipase A_2 + 20 μg N-acetyl 2–26. Each point is the mean ± s.e.mean.
Significance (Student's t test) is indicated by $*P<0.05$; $**P<0.01$.

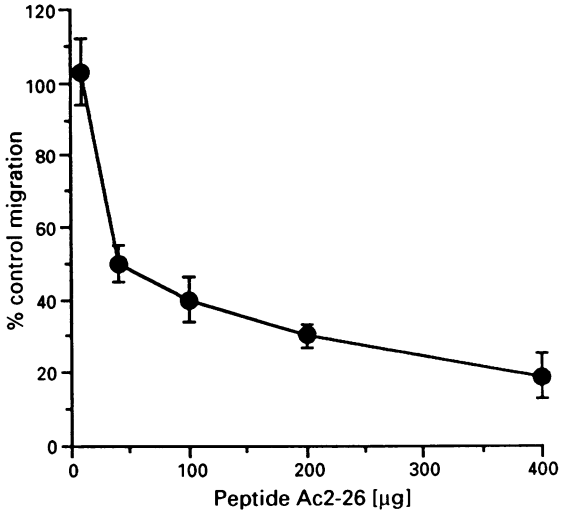

Figure 2 The inhibitory effect of N-acetyl 2–26 lipocortin 1 on neutrophil migration into the mouse air pouch elicited by interleukin 1. The control migration ($9.63 \pm 0.84 \times 10^6$ per mouse; $n = 15$) was measured in mice treated with intravenous saline only and the effect of the peptide tested at different concentrations by i.v. injection. All points are significantly different from control with a minimum significance of $P<0.05$.

inflammation. That it does so is consistent with our previous unpublished observations that N-terminal clipped molecule is without biological activity and with work demonstrating that lipocortin 1-188 seems fully effective in several models (Carey *et al.*, 1990; Relton *et al.*, 1991). All these data now point to the N-terminus as a functionally important domain and suggest that the remaining core sequence, which is able to bind calcium and phospholipid, is somehow important for the attachment of the molecule to its target cell.

Phosphorylation of the N-terminus at Tyr-21 leads to a change in the physico-chemical properties of the protein with enhanced proteolysis and biological inactivation (Chuah & Pallen, 1989). Interestingly, several peptides with a homologous structure to the lipocortin 1 N-terminus, such as those of middle T-antigen and synthetic peptides from pp60, have previously been shown to alter neutrophil chemotactic properties and to posses lipocortin-like effects on phospholipase A_2 (Notsu *et al.*, 1985).

The most likely explanation for the activity of the peptide is that it interacts with the cell surface lipocortin 1 binding protein (Goulding *et al.*, 1990) thus bringing about a biological effect similar to the native protein. However, the peptide could also be interfering with the phosphorylation or turnover of naturally occurring lipocortin 1 thus prolonging and producing a *de facto* anti-inflammatory effect.

Further studies will examine this possibility as well as testing other peptides from different regions of the N-terminus to define more closely the active site.

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