EFFECT OF INDOMETHACIN ON THE BINDING OF THE CHEMOTACTIC PEPTIDE FORMYL-MET–LEU–PHE ON HUMAN POLYMORPHONUCLEAR LEUKOCYTES

Hélène COST, Christian GESPACH and Jean-Pierre ABITA*
Unité 204 de l'INSERM Hôpital Saint-Louis, 2 Place du Docteur Fournier, 75475 Paris Cedex 10, France

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1. Introduction

Indomethacin, a non-steroidal anti-inflammatory-agent, is known to interfere with prostaglandin synthesis by inhibiting the enzyme cyclooxygenase [1,2]. This drug can also inhibit the phospholipase A2 of rabbit polymorphonuclear leukocytes (PMN) in vitro [3]. However, in vitro, indomethacin can block many of the responses of rabbit and human PMN elicited by the synthetic chemotactic peptide formyl-Met–Leu–Phe (FMLP), such as aggregation, lysosomal enzyme release or superoxide anion generation [4–7]. These in vitro effects of indomethacin which do not appear to be correlated with its inhibition of cyclooxygenase and/or phospholipase A_2 activities [5] have not been adequately explained [4,5].

One possibility not explored, is that indomethacin, owing to its hydrophobic nature, interferes with the binding of FMLP to its specific receptor present on the PMN plasma membrane [8–10].

These studies were undertaken to test this hypothesis directly by using the radiolabelled peptide [^{3}H]FMLP. They show that indomethacin is a competitive and reversible inhibitor of the binding of the chemotactic peptide. The concentration of indomethacin which inhibits 50% of the binding of [^{3}H]FMLP, 7×10^{-5} M, was found to be of the same order of magnitude than that which was shown to inhibit 50% of the in vitro effects of FMLP [4,5]. We have also demonstrated that this effect of indomethacin is highly dependent on the presence of albumin in the incubation medium.

* To whom correspondence and reprint requests should be addressed

2. Materials and methods

PMN were isolated from peripheral blood of normal human volunteers by means of Ficoll-Hypaque density separation (≥98% PMN). After hypotonic lysis of erythrocytes, PMN were washed twice and, if not stated otherwise, resuspended in Hanks' balanced salt solution (HBSS). FMLP was obtained from UCB (Brussels) and [^{3}H]FMLP (46.4 Ci/mmol) from New England Nuclear. Indomethacin and bovine or human serum albumin (fraction V) were supplied by Sigma (St Louis MO). FMLP was dissolved in DMSO at 2×10^{-3} M and diluted in HBSS before use. Indomethacin was dissolved in absolute ethanol.

The radioligand binding assay for [^{3}H]FMLP was done as in [10], except that cells were preincubated for 10 min with or without indomethacin before adding the labelled peptide. The final concentration of ethanol was 5% (v/v).

3. Results

The direct binding isotherms of [^{3}H]FMLP to human PMN at 22°C in the absence and in the presence of 10^{-4} M indomethacin are presented in fig.1. They demonstrate that the drug acts as a competitive inhibitior of the binding of [^{3}H]FMLP. In the presence of indomethacin the dissociation constant (K_d) increased from $2.2–7.6 \times 10^{-8}$ M. From these data, the inhibition constant (K_i) of indomethacin for the binding of [^{3}H]FMLP was calculated to be 4×10^{-5} M using the equation:

$$K_i = I/(K'_a/K_a \quad 1) \text{ [11]}$$

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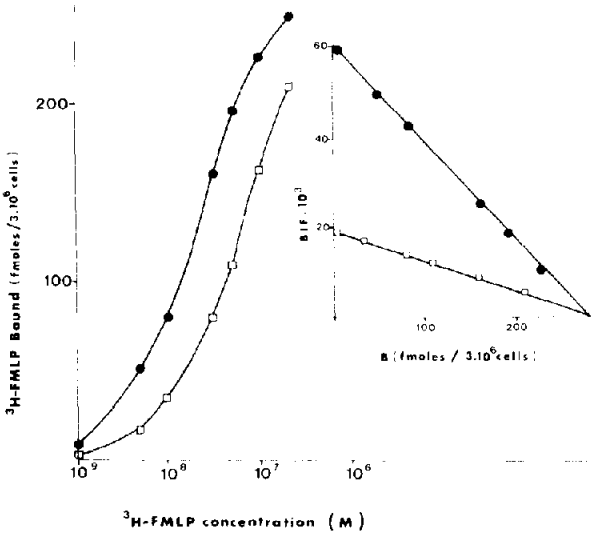

Fig.1. Binding of [^{3}H]FMLP to PMN as a function of [^{3}H]-FMLP concentration. Binding was measured after 30 min incubation of PMN (3×10^6 cells) with increasing concentration of labelled FMLP at 22°C in HBSS. The non-specific binding (measured in the presence of a 1000-fold excess of unlabelled FMLP) has been subtracted from each point; (•) binding measured in the absence of indomethacin; (□) binding measured in the presence of 10^{-4} M indomethacin; (insert) data plotted according to Scatchard analysis. Each point is the mean of triplicate determinations in a single experiment. Similar results were obtained in 2 separate experiments.

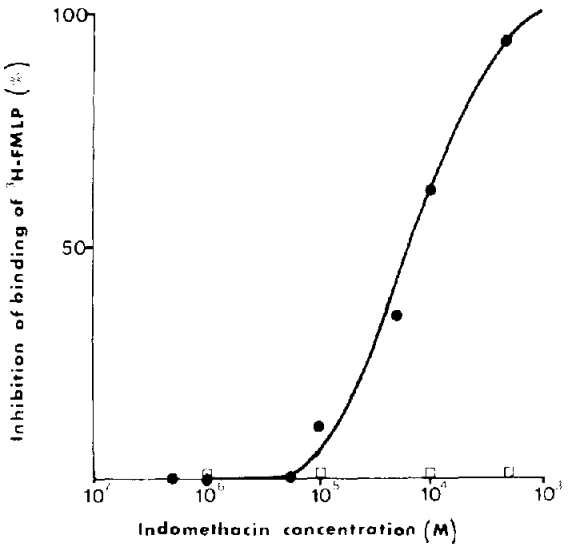

Fig.2. Effect of various concentrations of indomethacin on the binding of [^{3}H]FMLP. PMN (3×10^6 cells) were first preincubated with or without indomethacin for 10 min at 22°C in HBSS. [^{3}H]FMLP (2×10^{-8} M) was then added and the incubation was continued for another 30 min. The amount of [^{3}H]FMLP bound was then determined as in section 2. The non-specific binding has been subtracted from each point and inhibition of binding is expressed in terms of % inhibition of specific binding of labelled ligand. Each point is the mean of 3 different determinations done in duplicate: (□) PMN preincubated with indomethacin then washed before starting binding experiments.

where K_a and K'_a are the dissociation constants of [^{3}H]FMLP binding in the absence and presence of indomethacin, respectively, and I is the concentration of the drug. The Scatchard plots presented in the insert of fig.1 show that the 3×10^6 PMN can bind 275 fmol [^{3}H]FMLP which represent ~50 000 binding sites/cell. In [10] we had found a value of 40 000 binding sites/cell. This difference can be explained by the fact that this time the binding experiments were performed in the presence of 5% (v/v) ethanol, which increases the number of available binding sites for [^{3}H]FMLP on human PMN (not shown). Similar qualitative results have been found with rabbit neutrophils [12].

Fig.2 shows the effects of various concentrations of indomethacin on the binding of 2×10^{-8} M [^{3}H]-FMLP. The concentration required for half-maximal inhibition was determined to be 7.1×10^{-5} M, and K_i was calculated, assuming competitive inhibition by the drug at the [^{3}H]FMLP receptor, from the equation:

$$K_i = IC_{50}/(1 + S/K_a) \quad [11]$$

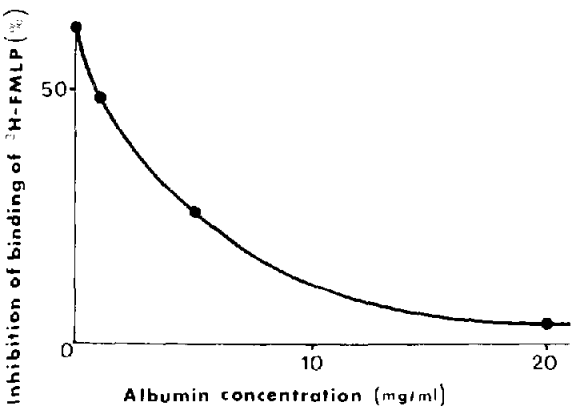

Fig.3. Effect of the presence of bovine serum albumin on the inhibition of the binding of [^{3}H]FMLP due to indomethacin. Binding of [^{3}H]FMLP was measured as in fig.2 in the presence of 10^{-4} M indomethacin in HBSS containing different concentrations of bovine serum albumin. Each point is the mean of 3 different determinations done in duplicate.

where $IC_{50} = 7.1 \times 10^{-5}$ M, S is the concentration of [^{3}H]FMLP (2×10^{-8} M) and K_a is the dissociation constant for the binding of [^{3}H]FMLP (2.2×10^{-8} M). In these conditions $K_i = 3.7 \times 10^{-5}$ M. This value is, as expected, in close agreement with that determined from the experimental design of fig.1. PMN which were preincubated for 10 min with indomethacin and then washed before addition of [^{3}H]FMLP bind the same amount of tritiated peptide as PMN incubated in HBSS alone (fig.2).

Fig.3 shows that the inhibition of binding of [^{3}H]-FMLP produced by 10^{-4} M indomethacin is highly dependent on the presence of bovine serum albumin, being almost totally blocked at 2% (w/v) BSA. Very similar results were found with human albumin (not shown).

4. Discussion

In [4–7], the anti-inflammatory drug, indomethacin, inhibited, in vitro, the responses of rabbit and human PMN elicited by the synthetic chemotactic peptide FMLP. The level of indomethacin giving half-maximal inhibition was between 10^{-5}–10^{-4} M depending on the response tested. These findings led to the inference that the inhibitory effect of indomethacin was due to its already known action on the enzymes cyclo-oxygenase and phospholipase A_2 [1–3] and to the conclusion that the intermediates in the synthesis of prostaglandins were important in the action of the peptide FMLP on PMN. However, as the concentrations of indomethacin necessary to inhibit the action of FMLP were higher than that which inhibited cyclo-oxygenase and since, in the same conditions, another anti-inflammatory drug, aspirin, was not inhibitory this interpretation was not shared [5].

Thus, to tentatively explain the in vitro inhibitory effect of indomethacin on FMLP actions, we have explored another hypothesis which was that indomethacin could act by impeding the binding of FMLP to receptors which had been demonstrated to exist on the plasma membranes of human PMN [9,10].

These data show without any doubt that indomethacin is indeed an inhibitor of [^{3}H]FMLP binding on human PMN. They indicate that our hypothesis was relevant since the concentration of indomethacin which gives 50% inhibition of binding is the same as that found to inhibit 50% of the effects of FMLP [4,5]. Moreover, indomethacin does not inhibit the binding of [^{3}H]FMLP when albumin is present in the incubation medium [4,5]. The most probable explanation for this last result is that indomethacin has a greater affinity for albumin [13] than for PMN and that it no longer acts when bound to the protein.

Our hypothesis is strengthened by the data in [14]. They found that two other anti-inflammatory drugs, phenylbutazone and sulfinpyrazone possess in vivo, as well as in vitro, potent antagonistic properties against FMLP-induced PMN alteration in rabbits, and in vitro in human. They found also that both drugs were inhibitors of the binding of [^{3}H]FMLP on human PMN with IC_{50} of 1.97×10^{-4} M and 7.2×10^{-5} M, respectively.

Indomethacin did not inhibit the binding of [^{3}H]-FMLP in vitro [14]. However, this result is not surprising since 100% autologous heat-inactivated plasma was used in the radioligand binding assay [14].

Our hypothesis is further strengthened by the fact that indomethacin is not an inhibitor when the responses of PMN (lysosomal enzyme release, aggregation), are elicited by another chemotactic factor, C5a, [4,5,13] which binds to PMN at receptor sites distinct to that of FMLP [15].

From this study we cannot explain the mechanism by which indomethacin interferes with the binding of FMLP to the human neutrophil. This could involve competition for a common receptor site or binding of the drug at separate sites such that the binding of FMLP to its proper sites would be impeded. In favor of receptor identity for FMLP and indomethacin are the observations that the other two anti-inflammatory drugs [12] suppress the responses of PMN ellicited by pepstatin. This pentapeptide is a potent chemotaxin for human PMN and shares a common receptor with FMLP on these cells [16]. It does so despite its primary structure being unrelated to that of FMLP [16]. However FMLP, pepstatin and indomethacin have one feature in common, they are all very hydrophobic. This suggests that one part of the receptor site for FMLP is constituted by an hydrophobic pocket which would be able to bind hydrophobic molecules of unrelated primary structures. It is interesting to note that if the K_d for the binding of FMLP to its receptor is around 10^{-8} M [10] the K_i for both pepstatin [16] and indomethacin are around 10^{-5} M.

While this manuscript was in preparation, indomethacin was reported to inhibit the binding of the peptide hormone angiotensin to its receptors in bovine umbilical artery with an ID_{50} of 1.6×10^{-4} M [17].

These data demonstrate that indomethacin, in the absence of albumin, is a competitive inhibitor of the chemotactic peptide FMLP for its binding on human PMN. This finding, which adds a new role for indomethacin, is the most probable explanation for in vitro inhibition, by the drug, of the responses of PMN stimulated by FMLP.

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