Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 349-354

# POTENT AND SPECIFIC INHIBITION OF IL-8-, IL-1α- AND IL-1β-INDUCED *IN VITRO* HUMAN LYMPHOCYTE MIGRATION BY CALCIUM CHANNEL ANTAGONISTS

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Received September 11, 1989

SUMMARY The role of calcium in interleukin- (IL) 8-, IL-1α- and IL-1β-induced lymphocyte migration has been investigated by using the calcium channel antagonists, verapamil, nifedipine, diltiazem (IL-8) and the optical isomers of the dihydropyridine analogue SDZ 202-791 (IL-8, IL-1α and IL-1β). Potent inhibition of IL-8-induced migration was observed in response to nifedipine ($IC_{50}$=10nM), verapamil ($IC_{50}$=60nM) and diltiazem ($IC_{50}$=10nM). The (+)-isomer of SDZ 202-791 was without effect on any of the agonists tested, however, the (-)-isomer induced dose-related inhibition of stimulated migration, $IC_{50}$ values being 0.1nM, 10pM and 1.0nM, for IL-8-, IL-1α- and IL-1β-induced migration, respectively. Reversal of the inhibitory effects of the (-)-isomer was obtained in the presence of increasing concentrations of (+)-isomer. The induction of lymphocyte migration by IL-8, IL-1α and IL-1β therefore appears to be a process dependent on calcium channel activation. 

The directed migration of lymphocytes infiltrating inflammatory sites may involve receptor-mediated, calcium-dependent mechanisms similar to those operating following antigenic stimulation of the T-cell receptor, where early events include the generation of inositol-1,4,5-trisphosphate, transiently increased intracellular calcium concentration and protein kinase C activation (1). A more sustained rise in intracellular calcium ensues, probably mediated by influx of extracellular calcium through specific calcium channels. Whether this flux occurs through voltage-dependent (2-4) or voltage-independent (5) channels is unclear. It is likely that the functional integrity of the lymphocyte motor apparatus, involved in adherence and migration, is dependent on a sustained rise in the concentration of intracellular calcium, therefore calcium channel antagonists may be a useful pharmacological tool to investigate this calcium-dependency. The aim of the present study, therefore, was to determine the effect and specificity of calcium

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ABBREVIATIONS: PBL, peripheral blood lymphocytes; MEM, Eagle's Minimal Essential Medium buffered with 30 mM Hepes; IL, interleukin; $IC_{50}$, concentration of antagonist which inhibited maximal migration by 50%; fMLP, N-formyl-methionyl-leucyl-phenylalanine; 12(R)-HETE, 12(R)-hydroxy, 5,8,10,14-eicosatetraenoic acid.

0006-291X/89 $1.50

channel blockers on periperal blood lymphocyte (PBL) migration. This report describes the potent lymphocyte chemoattractant effects of IL-8, IL-1α and IL-1β and their inhibition by specific calcium channel blockers.

## MATERIAL AND METHODS

Human Recombinant cytokines. IL-1α and β were obtained from Immunex Corporation, Seattle, Washington, U.S.A. and human recombinant IL-8 (NAP) was a gift from Dr. I. Lindley, Sandoz Forschungsinstitut, Vienna, Austria (6).
Calcium Channel antagonists. Nifedipine, verapamil and diltiazem HCl were obtained from Sigma Chemical Company Ltd., Poole, Dorset, England. The (+) and (-) isomers of SDZ 202-791 (7) were obtained from Dr. U. Rüegg, Sandoz Ltd., Basle, Switzerland. All antagonists, except diltiazem HCl, were dissolved in ethanol at a concentration of 10mM, the final concentration of ethanol in medium being 1% when top concentrations ($10^{-4}$M) of antagonist were used.
Mixed PBL Purification. Mixed human PBL were isolated from heparinized whole venous blood by density gradient centrifugation on ficoll. Monocytes were removed from the mixed mononuclear cells by plastic adherence over 18 h in MEM containing 10% heat-inactivated foetal calf serum, 100 $Uml^{-1}$ penicillin and 100 $\mu g\ ml^{-1}$ streptomycin. The non-adherent cells (>99% lymphocytes) were removed and resuspended in serum-free MEM prior to assay (8).
Lymphocyte Migration assay. In vitro PBL migration was assayed in a 48-well microchemotaxis chamber as previously described (8). Activity was expressed as a migration index (area of lower surface of filter occupied by cells in response to agonist [$mm^2$] / area of lower surface of filter occupied by unstimulated cells [$mm^2$]). Effects of antagonists on lymphocyte migration were assessed in the presence of a sub-optimal dose of agonist, which gave approximately 80% maximal migration, and in the absence of agonist as a control. In three experiments using all three agonists, reversal of the antagonistic effects of the (-)-isomer of SDZ 202-791 by the (+)-isomer was attempted. The dose of inhibitory isomer used was one which gave approximately 80% inhibition (10nM). The approximate $IC_{50}$ values for inhibition of agonist-induced lymphocyte migration, were calculated as the concentration of antagonist which inhibited stimulated lymphocyte migration in the presence of a suboptimal concentration of agonist, by 50%. A value of 1 was used as the migration index for unstimulated migration.

## RESULTS

Cytokine-induced PBL migration. Figure 1 (a and b) shows the migration obtained in response to IL-8 and IL-1α and IL-1β, respectively. IL-8 stimulated migration over the dose-range 1 fM to 1μM, the increase in activity being dose-related up to a maximum of 0.1nM (maximal migration index [MMI] ± S E being 2.76 ± 0.2, n = 5). The responses to IL-1α and β (Figure 1b) also demonstrate dose-related increases in migratory activity, between 14pM and 0.57nM (MMI ± S E being 2.6 ± 0.2 and 2.4 ± 0.26, respectively, n = 8).
Calcium channel antagonist modulation of migration. Figure 2 (a, b and c) shows the dose-dependent inhibition of IL-8-induced PBL migration by nifedipine, verapamil and diltiazem, the approximate $IC_{50}$ values being 10nM, 60nM and 10nM, respectively. Unstimulated PBL migration was not significantly affected by the antagonists, as demonstrated in three duplicate assays using a $10^{-4}$M concentration of each antagonist in the absence of agonist (MMI ± SE were 1.07 ± 0.03, 1.02 ± 0.10 and 1.02 ± 0.07 for nifedipine, verapamil and diltiazem, respectively, under these conditions). Figure 3 shows the effect of the (+) and (-) isomers of SDZ 202-791 on a) IL-8-, b) IL-1α- and c) IL-1β-induced migration, respectively. The (-)-isomer, in all cases, caused a dose-dependent inhibition of cytokine-induced migration. The approximate $IC_{50}$ values were 0.1nM, 10pM and 1nM, for IL-8, IL-1α and IL-1β, respectively (n=4). The (+)-isomer

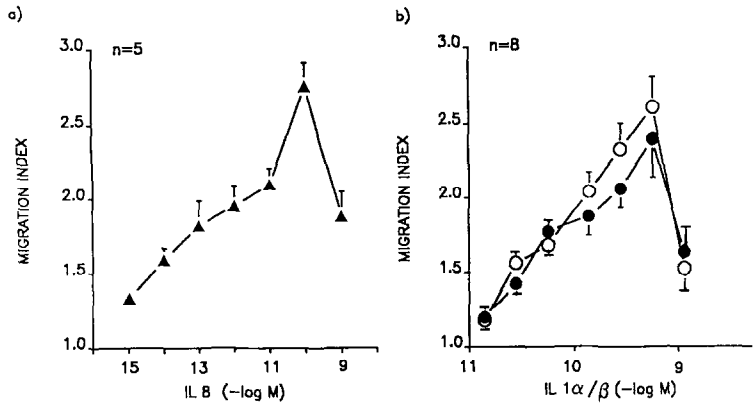


FIGURE 1 Dose-related PBL migration obtained in the presence of increasing concentrations of a) IL-8 and b) IL-1α ○ and IL-1β ●. Activity is represented as mean ± S E migration index for 5 duplicate (IL 8) and 8 duplicate (IL 1) experiments.

neither stimulated nor inhibited agonist induced PBL migration (Figure 3a, b and c) and did not enhance unstimulated migration when a top concentration ($10^{-6}$M) was used in the absence of agonist (MMI ± SE being 1.10 ± 0.08, n=4). The (-)-isomer reduced agonist-induced migration to

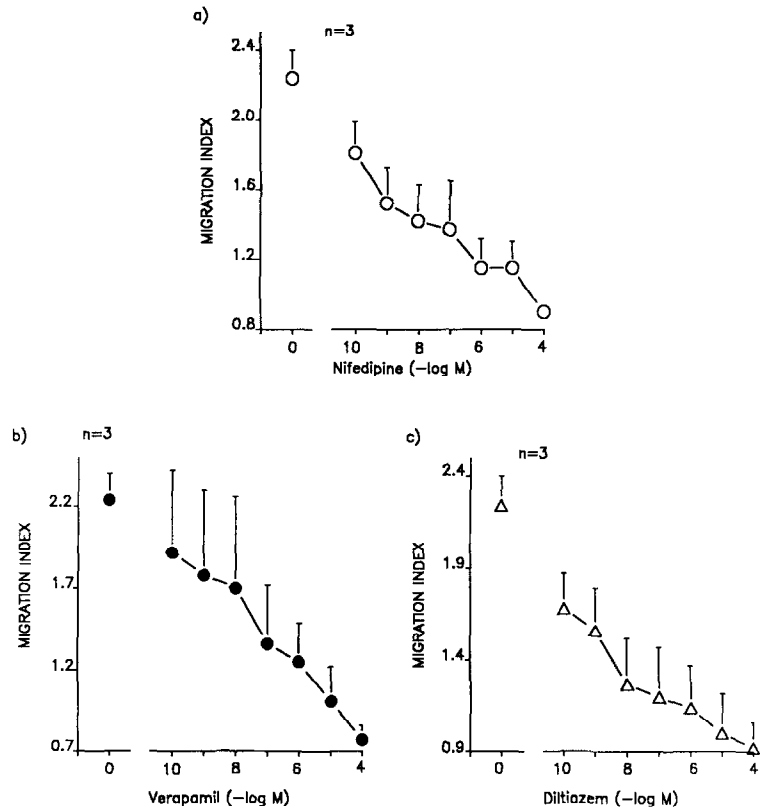


FIGURE 2 Dose-related inhibition of IL-8-induced migration by a) nifedipine, b) verapamil and c) diltiazem, over the dose-range (-log M) 10 - 4 (n=3). Activity is expressed as mean ± S E migration index in response to 0.01nM IL-8, in the presence of antagonist. Maximal migration ± S E in the absence of antagonist is indicated separately on the graphs.

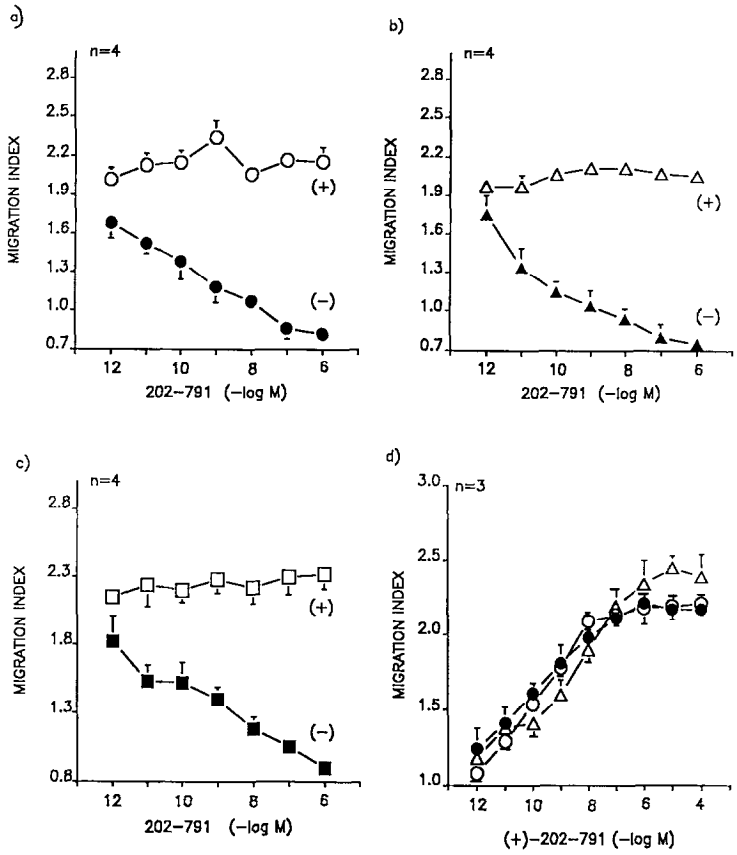


FIGURE 3 The effects of (+)- and (-)-isomers of SDZ 202-791 on a) IL-8-, b) IL-1α- and c) IL-1β- induced PBL migration. A submaximal dose of each agonist was used (0.01nM IL 8; 0.28nM IL 1α and β) and migration was assessed in the presence of the (+), or (-)- isomer of SDZ 202-791 over the dose range (-logM) 12 - 6. Each point represents the mean ± S E for 4 duplicate experiments. d) The dose-dependent reversal of the inhibitory effects of the (-)-isomer of SDZ 202-791 (10nM) on IL- 8- (○), IL-1α- (●) and IL-1β- (△) induced PBL migration, by the (+)-isomer. Each point represents the mean ± S E for 3 duplicate experiments.

below background only at concentrations of $10^{-7}$M or above (Figure 3a, b and c). However, in the absence of agonist, the (-)-isomer was without effect when tested at $10^{-6}$M (MMI ± SE being 1.05 ± 0.11, n=4).

Reversal of (-)SDZ 202-791-induced inhibition by (+)SDZ 202-791. In three separate experiments (figure 3 d), investigating the effects of the presence of increasing concentrations of the (+)-isomer of SDZ 202-791 on inhibition induced by the (-)-isomer, a complete reversal of the inhibitory effects was obtained. In the presence of 10nM (-)-isomer, the approximate $ED_{50}$ values for reversal of the inhibition were 0.1nM, 0.7nM and 7nM for IL-8, IL-1α and IL-1β, respectively.

## DISCUSSION

This study demonstrates the stimulatory effects of IL-8, IL-1α and IL-1β on in vitro PBL migration, IL-8 being the most potent agonist of those we have tested which, apart from IL-1α and

β, include fMLP, $LTB_4$, casein and 12(R)-HETE (8). Characteristic bell-shaped dose-response curves, commonly seen in in vitro leukocyte migration assays, were obtained, possibly indicating increased adherence and reduced migration of cells with the higher doses of agonist. The in vitro lymphocyte chemoattractant activity of IL-1 preparations has been described previously (9-11), although, to our knowledge, this is the first report demonstrating the lymphocyte chemoattractant effects of both recombinant forms of IL-1. IL-8 is a newly described neutrophil activating peptide which is produced by a variety of cell types (12). Monocyte-derived IL-8 has recently been shown to induce T-cell chemotaxis at a concentration of approximately 0.15nM, in a 48-well microchemotaxis chamber similar to that used in the present experiments (13). The results of the present study demonstrate for the first time the potent lymphocyte chemoattractant activity of recombinant IL-8, which was active at femtomolar concentrations and induced maximal activity at 0.1nM.

Assessment of the effects of calcium channel antagonism on IL-8-induced migration revealed potent inhibition by the standard antagonists nifedipine, verapamil and diltiazem. The optical isomers of the dihydropyridine analogue SDZ 202-791 were used in additional experiments to determine the specificity of this inhibitory effect. The (-)-isomer has been shown to inhibit calcium channel-dependent contraction of rabbit aortic ring preparations and the (+)-isomer to enhance this activity (7). In the lymphocyte migration assay, the (-)-isomer inhibited the response to IL-8 , IL-1α and IL-1β in a concentration-related manner (figure 3a,b and c), but the (+)-isomer was without effect, a finding which suggests that the inhibition of migration demonstrated was due to a specific effect on calcium channels. The finding that nifedipine, verapamil, diltiazem and the (-)-isomer of SDZ 202-791 all inhibited stimulated migration in the presence of agonist (Figure 2a, b, c and Figure 3a, b and c) to below the level of unstimulated migration, indicates that the efficacy of these antagonists may be dependent on the functional changes associated with lymphocyte membrane activation (14). The concentration-related reversal of the inhibitory effects of the (-)-isomer by the (+)-isomer and the lack of inhibitory or stimulatory activity on unstimulated PBL migration by either isomer, suggests competition between the isomers for binding at the same, or a closely related site.

These results show for the first time that the activation of calcium channels may be important in the stimulated migration of human PBL. The potent inhibitory effect of calcium channel antagonists on cytokine-induced PBL migration suggests that the specific properties of these drugs in inflammatory, autoimmune and allergic diseases characterized by lymphocyte infiltrates, should be further investigated.

## ACKNOWLEDGMENT

We are grateful to the Wellcome Trust for financial support (KBB).

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