A Comparison of the Inhibitory Activity of Selective PDE4 Inhibitors on Eosinophil Recruitment in Guinea Pig Skin

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The elevation of intracellular cyclic AMP by phosphodiesterase (PDE)4 inhibitors in eosinophils is associated with inhibition of the activation and recruitment of these cells. We have previously shown that systemic treatment with the PDE4 inhibitor rolipram effectively inhibt eosinophil migration in guinea pig skin. In the present study we compare the oral potency and efficacy of the PDE4 inhibitors rolipram, RP 73401 and CDP 840 on allergic and PAF-induced eosinophil recruitment. Rolipram and RP 73401 were equally effective and potent when given by the oral route and much more active than the PDE4 inhibitor CDP 840. We suggest that this guinea pig model of allergic and mediator-induced eosinophil recruitment is both a sensitive and simple tool to test the efficacy and potency of PDE4 inhibitors in vivo.

Key words: eosinophils - phosphodiesterase inhibitors - rolipram - allergic inflammation

EOSINOPHILS AND ALLERGIC DISEASES

There is much evidence to support a role for the eosinophil in the pathophysiology of allergic diseases, such as asthma and atopic dermatitis (Butterfield & Leiferman 1993). In these conditions, eosinophil numbers and eosinophil-derived secretory products (eg. eosinophil major basic protein) are elevated in inflamed tissue and appear to correlate positively with the severity of the diseases (Djukanovic et al. 1990, Gleich et al. 1993). In addition, the activation status of eosinophils as assessed by monoclonal antibodies such as EG2 (against the secreted form of eosinophil cation protein) also correlates with functional indices of disease severity (Djukanovic et al. 1990, Corrigan & Kay, 1992). Inasmuch as the secretory products of eosinophils may cause tissue damage (eg. to epithelial cells) in concentrations which are found *in vivo* (Djukanovic et al. 1990), the development of drugs which inhibit eosinophil recruitment and activation in the tissues may be of therapeutic value in the treatment of allergic diseases.

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Received 3 September 1997
Accepted 30 September 1997

CYCLIC AMP, PHOSPHODIESTERASE ISOENZYMES AND INHIBITORS

One interesting therapeutic possiblity relates to the capacity of adenosine 3',5'-monophosphate (cAMP) to modulate the activity of leukocytes involved in the inflammatory process (reviewed by Nicholson et al. 1991, Nicholson & Shahid 1994). The mechanisms by which cAMP modulates leukocyte function are not completely understood but appear to depend on the activation of protein kinase A and subsequent phosphorylation of various other proteins in the target cell (Kammer 1988). The intracellular levels of cAMP are regulated by the rate of cAMP production by receptor-coupled adenylate cyclase and the rate of cAMP degradation by 3',5'-cyclic nucleotide phosphodiesterases (PDEs). It is now appreciated that PDEs are a diverse group of enzymes of which at least seven different families have been described (Giembycz & Kelly 1994, Beavo et al. 1994). Of these, PDE3, PDE4 and PDE7 appear to be most important for the regulation of cAMP in different cell types. However, most cells implicated in the pathogenesis of inflammation express one or more representatives of the PDE4 isoenzyme family which are primarily or even exclusively responsible for the degradation of cyclic AMP in these cells (see Torphy & Undem 1991, Giembycz 1992). Accordingly, PDE4 inhibitors are capable of increasing cyclic AMP levels and inhibiting various functional responses in most leukocytes that are considered pro-inflammatory (reviewed in Torphy & Undem 1991, Giembycz 1992). For example, the elevation of cyclic AMP in eosinophils has been associ-

ated with inhibition of functions including the respiratory burst, degranulation, aggregation and lipid mediator production (Teixeira et al. 1996 and references therein).

ANTI-INFLAMMATORY EFFECTS OF PDE4 INHIBITORS *IN VIVO*

Not only have PDE4 inhibitors shown to be effective inhibitors of leukocyte activation *in vitro* but a number of studies have evaluated the effects of these drugs on models of inflammation *in vivo* (Teixeira et al. 1997). While PDE4 inhibitors are capable of inhibiting a number of inflammatory conditions, special interest has been placed on investigating the effects of these drugs on eosinophil recruitment in animal "models" of allergic diseases, particularly asthma (Table I). The great interest in the use of this drugs in the context of asthma is not surprising inasmuch PDE4 inhibitors may provide the additional benefit of bronchodilatation and synergism with ß$_2$-adrenoceptor agonists (Nicholson & Shahid 1994, Giembycz & Kelly 1994).

TABLE I

Experimental situations in which PDE4 inhibitors have been shown to inhibit eosinophil recruitment

1. Skin
. Eosinophilia after allergen challenge of passively sensitized animals
. PAF-, LTB_4-, C5a-, histamine-induced cutaneous eosinophilia

2. Lung
. Eosinophilia after allergen challenge of actively sensitized animals
. Cytokine (IL-5 and IL-8)-induced lung eosinophilia
. PAF- and LTB_4-induced lung eosinophilia

3. Eye
. Leukotriene- and histamine-induced ocular eosinophilia

We have previously shown that the intraperitoneal administration of the PDE4 inhibitor rolipram, but not a PDE3 or a PDE5 inhibitor, effectively inhibited eosinophil, but not neutrophil, recruitment into skin sites of allergic and mediator-induced inflammation in the guinea pig (Teixeira et al. 1994). In the present study, we have evaluated the comparative efficacy and potency of oral administration of the PDE4 inhibitors rolipram, RP 73401 and CDP 840 on allergic and PAF-induced eosinophil recruitment in guinea pig skin.

EFFECT OF ORAL TREATMENT WITH PDE4 INHIBITORS ON EOSINOPHIL RECRUITMENT IN GUINEA PIG SKIN

The accumulation of ^{111}In-eosinophils in response to i.d. injection of PAF and in a passive cutaneous anaphylactic (PCA) reaction were measured in guinea pig skin as previously described (Teixeira et al. 1994). Briefly, eosinophils were obtained from the peritoneal cavity of horse serum-treated ex-breeder guinea pigs and purified on a discontinuous Percoll gradient. The purified eosinophils (>95 pure and >98% viable) were then labelled with $^{111}InCl_3$ and 2.5 x 10^6 ^{111}In-eosinophils injected intravenously. This was followed 10 min later by the i.d. injection of PAF (10^{-9} mol/site) and antigen (0.1 to 1.0 µg of bovine gamma globulin/site) in sites previously sensitized with antigen. After 1 hr the animals were sacrificed and the number of ^{111}In-eosinophils recruited in the skin sites quantified. The PDE4 inhibitors rolipram, RP 73401 and CDP 840 were given by oral gavage 30 minutes prior to the i.v. injection of ^{111}In-eosinophils. The drugs were dissolved initially in dimethyl sulphoxide (DMSO) and diluted further in saline. Control animals received DMSO only (10% in saline).

Fig. 1a shows the inhibitory effects of oral administration of the PDE4 inhibitor rolipram (2 mg/kg) on ^{111}In-eosinophil recruitment in a PCA reaction in guinea pig skin. When used at a similar dose, RP 73401 also abolished ^{111}In-eosinophil recruitment (Fig. 1b). In contrast, CDP 840 failed to alter significantly ^{111}In-eosinophil recruitment in a PCA reaction when used at 2 mg/kg (Fig. 1c). Full dose-response studies conducted with these PDE4 inhibitors (0.125 to 32 mg/kg) showed the order of potency for the inhibition of ^{111}In-eosinophil recruitment in a PCA reaction to be RP 73401 >= rolipram >> CDP 840. The effects of 2 mg/kg (oral treatment) of rolipram, RP 73401 and CDP 840 on ^{111}In-eosinophil induced by 10^{-9} M PAF is given in Table II. Similar to the inhibitory effects on the PCA reaction, the order of potency for the inhibition of ^{111}In-eosinophil recruitment induced by PAF was RP 73401 >= rolipram >>

TABLE II

Effect of the PDE4 inhibitors rolipram, RP 73401 and CDP 840 on ^{111}In-eosinophil recruitment induced by PAF

PDE 4 inhibitor	^{111}In-eosinophils per skin site	
	Control	Treated
Rolipram	5347 ± 58	657 ± 360[a]
RP 73401	1287 ± 128	480 ± 104[a]
CDP 840	1958 ± 245	1625 ± 335

PDE4 inhibitors (2 mg/kg) or vehicle were given orally 30 min prior to i.v. injection of ^{111}In-eosinophils. PAF was given i.d. at the dose of 10^{-9} mol/site and the number of ^{111}In-eosinophils per skin site assessed after 1 hr. *a:* for $P < 0.01$.

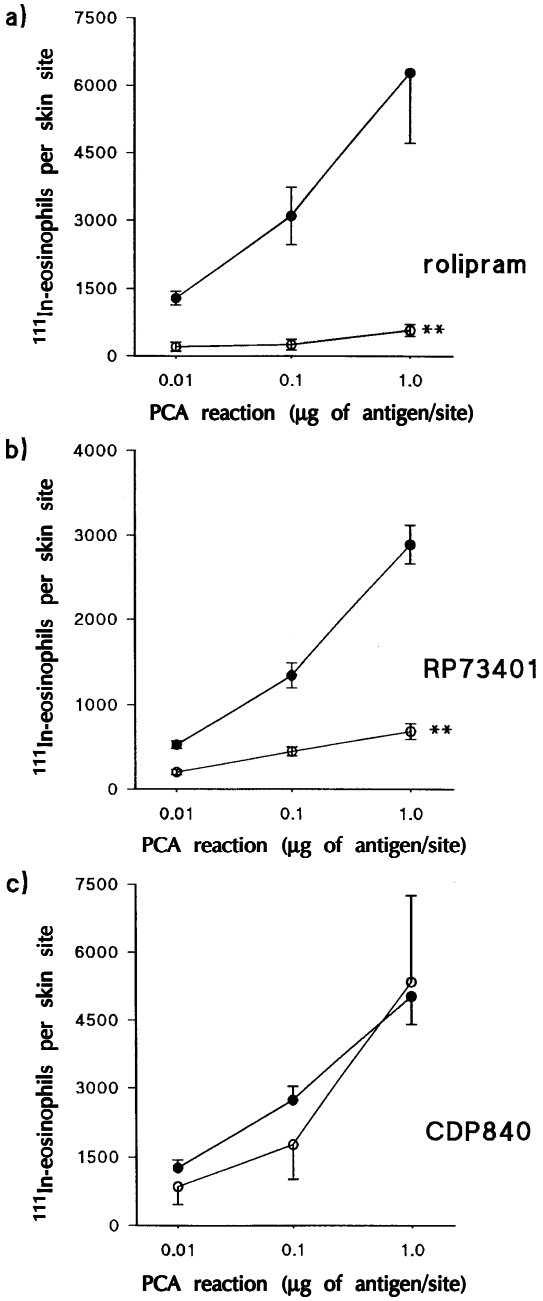

Fig. 1: effect of the PDE4 inhibitors (a) rolipram, (b) RP 73401 and (c) CDP 840 on ^{111}In-eosinophil recruitment in a passive cutaneous anaphylactic (PCA) reaction. PDE4 inhibitors (2 mg/kg, closed symbols) or vehicle (open symbols) were given orally 30 min prior to i.v. injection of radiolabelled eosinophils. Antigen (bovine gamma globulin, 0.01 to 1.0 µg of BGG/site) was given i.d. into sites previously sensitized with an anti-BGG rich IgG_1 antisera and the number of ^{111}In-eosinophils per skin site assessed after 1 hr. * and ** for $P < 0.05$ and $P < 0.01$, respectively.

CDP 840. None of the drugs used significantly affected the levels of circulating ^{111}In-eosinophils at 1 hr (data not shown).

CONCLUSION

There has been an enormous excitement in both industry and academia with the development of selective PDE4 inhibitors. These are efficacious anti-inflammatory agents in animal models with potential widespread use in diverse inflammatory diseases in man. Special interest has been given to the use of these drugs as anti-asthma agents. Not only are PDE4 inhibitors capable of inducing bronchodilatation, but there is increasing evidence that these drugs are effective inhibitors of eosinophil recruitment into the lung and eosinophil activation *in vitro* (Teixeira et al. 1995). Moreover, PDE4 inhibitors have been shown to inhibit eosinophil recruitment in the skin and the eye following allergic and mediator-induced inflammation (Newsholme & Schwartz 1993, Teixeira et al. 1994). This opens the possibility that these agents may also be helpful in the treatment of allergic conditions in the eye and skin where eosinophil activation and recruitment is thought to play a major pathophysiological role. The present study shows that PDE4 inhibitors are effective inhibitors of eosinophil recruitment at skin sites when given by the oral route. In our hands, rolipram and RP 73401 were equally effective and potent when given by the oral route and much more active than the PDE4 inhibitor CDP 840. Much needs to be done at the preclinical stage to develop a safe, potent and effective drug to be used in clinical trials (Teixeira et al. 1997). We suggest that this guinea pig model of allergic and mediator-induced eosinophil recruitment is both a sensitive and simple tool to test the efficacy and potency of PDE4 inhibitors *in vivo*.

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