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Newly Synthesized Phosphodiesterase 4 (PDE4) Inhibitor, DWP205505, Inhibits TNF-α Secretion and mRNA Expression

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Abstract The therapeutic potential of phosphodiesterase 4 (PDE4) inhibitors in inflammatory diseases including some autoimmune diseases has been explored recently with some hopeful results. These PDE4 inhibitors are thought to show their anti-inflammatory effect by down-regulating tumor necrosis factor-α (TNF-α) production in lymphocytes and macrophages. A high concentration of TNF-α has been found in rheumatoid arthritis (RA) synovium and reducing TNF-α using biological agents was proven to be an effective RA treatment. To test the possibility of using PDE4 inhibitors for RA treatment, the effects of a newly synthesized PDE4 inhibitor, DWP205505, on TNF-α and IL-10 production was tested in cells isolated from normal peripheral blood and rheumatoid arthritis synovial fluid. Cytokine production was assayed at the protein level by sandwich enzyme-linked immunosorbent assay (ELISA) and at the mRNA expression level by semi-quantitative RT-PCR. Another PDE4 inhibitor, RP73401, was used for comparison. DWP205505 and RP73401 had no harmful effect on cell viability up to 10 μM concentration during the 24 h culture period. DWP205505 as well as RP73401 significantly reduced TNF-α secretion from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC). The effect of DWP205505 or RP73401 treatment on the mRNA expression of TNF-α was also studied in LPS-stimulated PBMC and SFMC. TNF-α mRNA expression was increased by LPS stimulation and both of the PDE4 inhibitors suppressed TNF-α mRNA expression. For interleukin-10 (IL-10), a little different results were obtained from PBMC and SFMC; IL-10 secretion was unaffected by LPS stimulation and only minimally affected by both of the PDE4 inhibitors in PBMC. In unstimulated SFMC, DWP205505 and RP73401 slightly enhanced IL-10 secretion, while they reduced IL-10 secretion from LPS-stimulated SFMC where IL-10 secretion was a lot higher than unstimulated SFMC. These results suggest that the newly synthesized PDE4 inhibitor DWP205505 may have anti-rheumatoid arthritis activity.

Key words: Phosphodiesterase 4 inhibitor, TNF-α, IL-10, Rheumatoid arthritis, Peripheral blood mononuclear cells, Synovial fluid mononuclear cells

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting about 1% of the population. Although close association between certain types of human leukocyte antigen (HLA) and the susceptibility to RA of the individual has been proved, the cause of RA is not clearly known yet. Tumor necrosis factor-α (TNF-α) is one of the principal mediators of the inflammatory response in mammals [1] and increasing evidence implies TNF-α as one of the major players in the inflammation and tissue destruction in RA [2, 3]. TNF-α is present and abundant at the sites of inflammation in

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Abbreviations: PDE4, Phosphodiesterase 4; TNF-α, Tumor necrosis factor-α; RA, rheumatoid arthritis; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; SFMC, synovial fluid mononuclear cells; IL-10, interleukin-10; HLA, human leukocyte antigen; cAMP, cyclic AMP; PBS, phosphate buffered saline; cDNA, complementary DNA; SEM, standard error of the mean.

RA patients and ways to reduce the concentration of TNF-α at the disease site have been researched [4, 5]. Biological agents aimed to inhibit the activity of TNF-α include anti-TNF monoclonal antibodies and soluble TNF receptors combined with human Fc constructs [6, 7]. These biological agents showed promising results in studies using animal models and in short term clinical trials [6, 7]. However, repeated use of chimeric antibody containing murine epitopes could trigger a neutralizing antibody response in patients resulting in less effective treatment. In addition, these biological agents have a limited administration route (injection) and are rather expensive to use. Thus, the development of small synthetic molecules capable of inhibiting TNF-α production would be beneficial.

TNF-α production is inhibited when the intracellular cyclic AMP (cAMP) level is elevated [8] and the intracellular cAMP level is regulated partly by the rate of cAMP degradation by 3',5'-cyclic nucleotide phosphodiesterases (PDEs). Seven totally different PDE isozymes have been reported so far based on distinct substrate specificity and susceptibility to selective inhibitors as well as cell distribution pattern [9]. PDE4 is the major isozyme in many inflammatory cells including lymphocytes, monocytes, macrophages, neutrophils, eosinophils, mast cells, and basophils [9]. Recently, PDE4 inhibitors have been studied actively to examine the possibility of developing them as anti-inflammatory and anti-autoimmune disease drugs. As expected, PDE4 inhibitors suppressed TNF-α production by increasing intracellular cAMP and inhibit the function of several inflammatory cells [10, 11]. The prototype PDE4 inhibitor, rolipram, was effective in inhibiting the synthesis of TNF-α [12]. Rolipram significantly suppressed ankle swelling and cartilage injury in a rat arthritis model [13] and reduced the severity of the disease and inflammatory lesions in the brain of experimental autoimmune encephalomyelitis animal [14]. Another potent PDE4 inhibitor, RP73401, inhibited antigen-induced bronchospasm in guinea-pig and rat models of bronchoconstriction [15]. RP73401 also reduced total inflammatory cells and eosinophil numbers in guinea-pig bronchoalveolar lavage fluid [15] as well as significantly inhibited prostaglandin E2-induced cAMP accumulation and lipopolysaccharide (LPS)-induced TNF-α production from isolated human monocytes [16]. These results demonstrate that PDE4 inhibitors may have a therapeutic potential in many pathological conditions associated with the over-production of TNF-α. Generally, the clinical use of PDE IV inhibitors has been hampered by adverse effects on the central nervous system, such as nausea and vomitting.

To develop new PDE4 inhibitors with less side effects, substituted dialkoxyphenyl derivatives, sharing structure homology with RP73401, were designed and synthesized. Among the derivatives, DWP205505 selectively inhibited purified PDE4 and inhibited TNF-α production in LPS stimulated murine macrophage cell line RAW264.7. *In vivo* experiments using C57BL/6 mice showed that orally administered DWP205505 lowered the LPS-stimulated serum TNF-α level with a comparable potency to RP73401 but with 3~10 times less side effects than RP73401. Based on these results, DWP205505 was selected as a good PDE4 inhibitor having comparable potency and lower side effects than RP73401.

In this study, the inhibitory effect of DWP205505 on the LPS-induced TNF-α production was studied in primary cells isolated from human blood to test whether the results obtained from murine macrophage cell line are repeated in human cells. In addition, to determine whether it can be used as an anti-inflammatory and anti-rheumatoid arthritis drug, the effects of DWP205505 were compared with that of RP73401 in mononuclear cells isolated from synovial fluid withdrawn from RA patients. As some studies suggested that cAMP-elevating agents such as PDE4 inhibitors block TNF-α secretion in part by augmenting IL-10 production [17], the effect of DWP205505 on the production of IL-10 by human mononuclear cells was also studied.

MATERIALS AND METHODS

PDE4 Inhibitors

RP73401 (3-cyclopentyloxy-N-(3,5-dichloro-4-pyridyl)-4-methoxy benzamide), a potent PDE4 inhibitor developed at Rhone-Poulene Rorer Ltd. [15], was provided by Daewoong Pharmaceutical Co. Ltd. DWP205505 (1-oxo-2-(3-cyclopentyloxy-4-methoxyphenyl)-3-methyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-5-ium-5-olate) is a novel 3,4-dialkoxyphenyl derivative developed by Daewoong Pharmaceutical Co. Ltd. as a specific PDE4 inhibitor. The chemical structures of DWP205505 and RP73401 are illustrated in Fig. 1. Both of the chemicals are substituted dialkoxyphenyl derivatives.

Cells

Peripheral blood mononuclear cells (PBMC) were obtained from blood samples of healthy donors by centrifugation

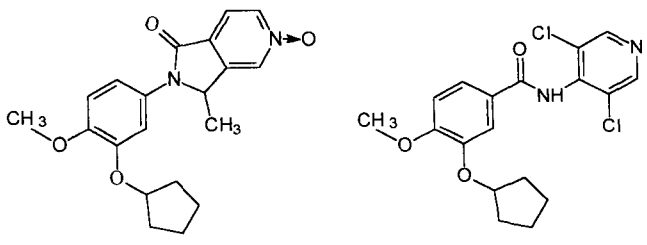

Fig. 1. Chemical structures of DWP205505 and RP73401.

over a cushion of Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden) as described before [18]. Synovial fluids were aspirated from the swollen knee joints of rheumatoid arthritis patients by conventional puncture technique and immediately mixed with 2 fold of phosphate buffered saline (PBS). Synovial fluid and PBS mixture were then centrifuged over a cushion of Ficoll-Hypaque. Cells accumulated at the interphase were collected and called synovial fluid mononuclear cells (SFMC = T cells, B cells, monocyte, etc.). The cells accumulated at the bottom of the tube after centrifugation were treated with red blood cell lysis buffer to obtain synovial fluid precipitated cells (SFPC = macrophage, polymorphonuclear leukocytes, etc.). Cells were maintained in RPMI 1640 containing 10% heat inactivated fetal bovine serum, 1,000 U of penicillin per ml, and 1,000 μg of streptomycin (Sigma Chemical Co. St. Louis, MO, U.S.A.) per ml.

Sandwich ELISA

96-well ELISA plates were coated with capture antibody and the non-specific binding sites were blocked using 1% bovine serum albumin. Frozen kept cell culture supernatant was thawed and added for binding. Biotinylated anti-human TNF-α or IL-10 was used to detect TNF-α and IL-10, respectively. Color reaction was performed using ExtrAvidine®-alkaline phosphatase conjugate (Sigma Chemical Co.) and Sigma 104® phosphatase substrate before reading the absorbance at 405 nm. Serially diluted recombinant human TNF-α and IL-10 were used as standards.

RNA Isolation and cDNA Synthesis

Cells were lysed with RNAzol™ B (Tel-Test Inc. Friendswood, TX, U.S.A.) by following manufacturer's instruction. Complementary DNA (cDNA) was synthesized from RNA by the priming of 2 μg total RNA at 42°C for 60 min in a mixture containing 2.5 μM of oligo (dT) primer, and 1 mM of dNTPs and mouse mammary leukemia virus reverse transcriptase.

Oligonucleotides for PCR Analysis

The primer sequences for human TNF-α were 5'-GAGTGACAAGCCTGTAGCCCATGTTGTAGC and 5'-GCAATGATCCCAAAGTAGACCTGCCCAGACT. The human IL-10 primer sequences were 5'-CATCAAG-GCGCATGTGAAC and 5'-AGAGCCCCAGATCCG-ATTTT. Primer sets specific for the β-actin gene were 5'-ATCATGTTTGAGACCTTCAACACCC and 5'-CATG-GTGGTGCCGCCAGACAG.

Semiquantitation of Cytokine mRNA by PCR Analysis

To compare cytokine mRNA expression in different samples, we used semiquantitative PCR using β-actin as an internal control. For each sample, an aliquot containing approximately 0.2 μg of cDNA was used as a template and amplified by PCR. The PCR reaction mixture contained 0.25 μM of primers, 0.2 μM each dNTP, 2.5 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase. Ethidium bromide-stained PCR products were subjected to electrophoresis and viewed after electrophoresis. The density of PCR product bands was analyzed using a densitometer. After optimizing the PCR condition, amplification was performed for 3 different cycles for each sample. The cycle showing the least detectable PCR product was used for analysis to avoid using PCR conditions within the plateau phase of the amplification. First, the ratio of cytokine to the β-actin band density of each sample was calculated and the value from each sample was compared with that from the control sample. The amount of each cytokine cDNA was presented as % of the control.

RESULTS

Effect on the PBMC Isolated from Normal People

The effect of the newly synthesized PDE4 inhibitor DWP205505 was compared with that of RP73401. DWP205505 as well as RP73401 did not affect cell viability up to 10 μM concentration during the 24 h culture period (Fig. 2). For the following experiments, both PDE4 inhibitors were used at 1 μM concentration. To test the effect of PDE4 inhibitors on the secretion of TNF-α and IL-10 in normal PBMC, cells were treated with either DWP205505 or RP73401 for 24 h before harvesting the culture media. Secreted TNF-α and IL-10 were assayed with the sandwich ELISA method. Both PDE4 inhibitors showed a tendency of reducing TNF-α release in unstimulated PBMC but the effects were not statistically

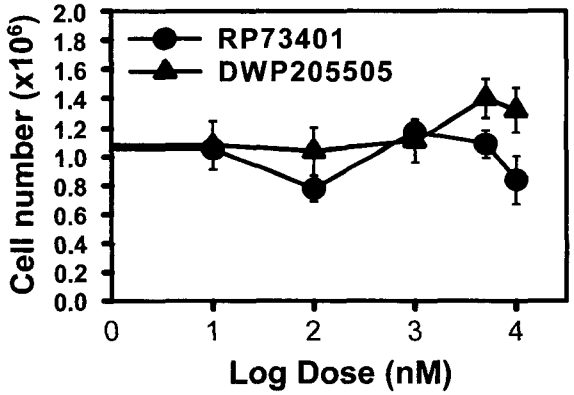

Fig. 2. Effects of PDE4 inhibitors on cell viability. 10^6 PBMC were cultured in a media with increasing doses of DWP205505 or RP73401.

After 24 h, cell viability was assessed by trypan blue exclusion test and results are expressed as mean ± standard error of the mean (SEM) of viable cell numbers. Data were obtained from 4 different PBMC.

significant (Fig. 3). To test the effects of the PDE4 inhibitors on LPS-induced TNF-α secretion, cells were treated with either DWP205505 or RP73401 together with 1 μg/ml LPS. LPS stimulation more than doubled TNF-α secretion and both PDE4 inhibitors significantly reduced TNF-α secretion (Fig. 3). RP73401 seemed more potent than DWP205505. The effects of PDE4 inhibitors on IL-10 secretion in normal PBMC were also tested. LPS itself did not change IL-10 secretion. RP73401 reduced IL-10 secretion slightly in unstimulated PBMC but did not affect that in LPS-stimulated cells. DWP205505 showed no effect on IL-10 secretion in unstimulated or LPS-stimulated PBMC (Fig. 3).

To test the effects of PDE4 inhibitors on TNF-α and IL-10 gene expression in normal PBMC, mRNAs for the two cytokines were assayed by semi-quantitative RT-PCR. Cells were treated and total RNA was extracted for reverse transcription. Obtained cDNAs were PCR amplified using primers specific for TNF-α or IL-10. β-Actin was used as an internal control to correct the efficiency of reverse-transcription as well as the amount and quality of each cDNA. PCR product of the expected size was obtained for each primer set and no unspecifically amplified product was observed (Fig. 4A). At 1 h after LPS

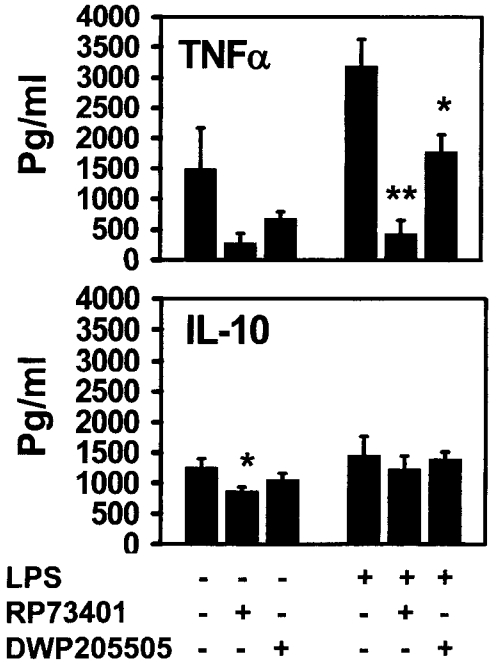

Fig. 3. Effects of PDE4 inhibitors on TNF-α and IL-10 secretion in normal PBMC.

PBMC were cultured for 24 h with 1 μM of DWP205505 or RP73401 before harvesting the culture media. Some cells were stimulated with 1 μg/ml of LPS simultaneously with PDE4 inhibitor treatments. Released cytokines were assayed by the sandwich ELISA method. Results are expressed as mean±SEM of data obtained from 4 different PBMC. * and **: significantly different from control values (untreated with PDE4 inhibitors) at $p<0.05$ or 0.01, respectively.

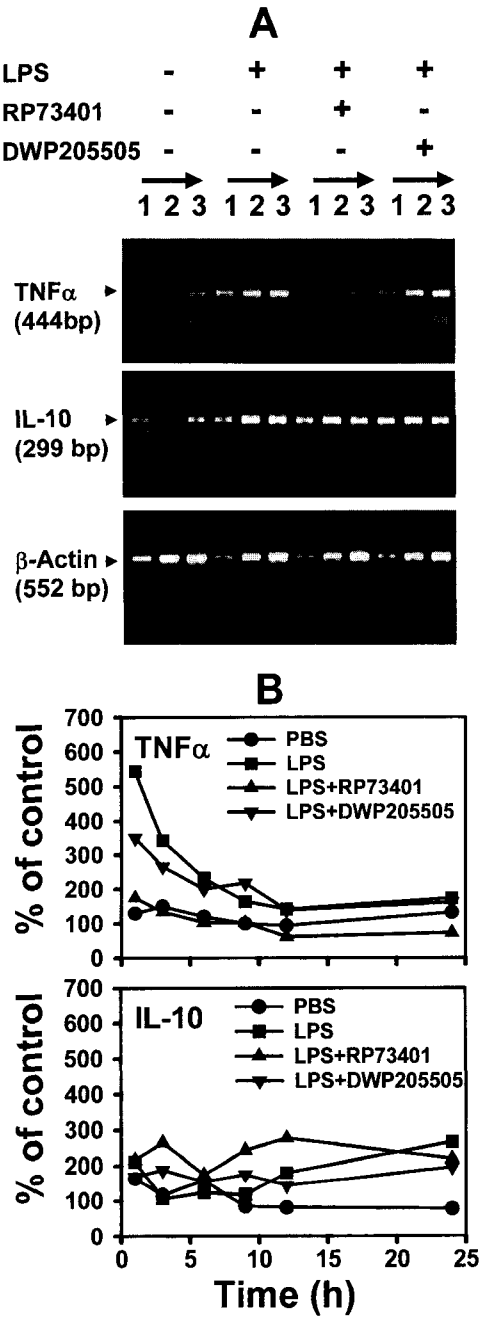

Fig. 4. Effects of PDE4 inhibitors on TNF-α and IL-10 mRNA expression in normal PBMC.

(A) PBMC were cultured in a media containing 1 μM of DWP205505 or RP73401 as indicated. Some cells were stimulated with 1 μg/ml of LPS simultaneously with PDE4 inhibitor treatments. Semi-quantitative RT-PCR was performed for TNF-α and IL-10 using β-actin as an internal control. For each sample, 3 different PCR cycles were used. The PCR cycle which gives the least detectable PCR product was chosen for comparison between samples to avoid reaching the PCR plateau. Results from a representative experiment are shown. (B) PBMC were treated with 1 μM of DWP205505 or RP73401 simultaneously with LPS stimulation. Cells were harvested at the indicated time points and semi-quantitative RT-PCR was performed for TNF-α and IL-10. Results are means from 4 different PBMC samples and expressed as percent of the control value which was obtained from uncultured PBMC.

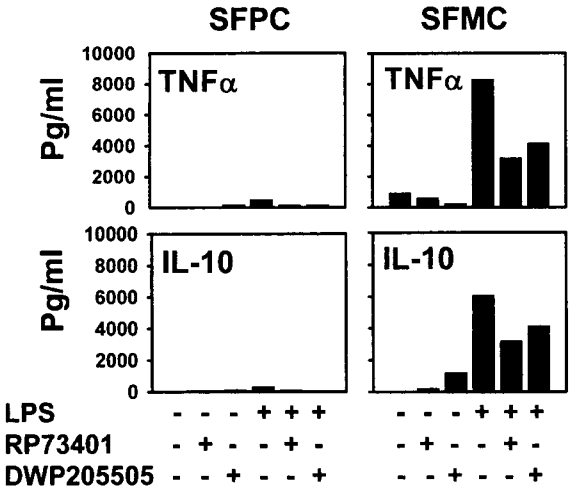

Fig. 5. Effects of PDE4 inhibitors on TNF-α and IL-10 secretion in cells isolated from rheumatoid arthritis synovial fluid.

SFPC and SFMC were cultured for 24 h with 1 μM of DWP205505 or RP73401 before harvesting the culture media. Some cells were stimulated with 1 μg/ml of LPS simultaneously with PDE4 inhibitor treatments. Released cytokines were assayed by the sandwich ELISA method. Results are expressed as means of data obtained from 2 different SFPC and SFMC.

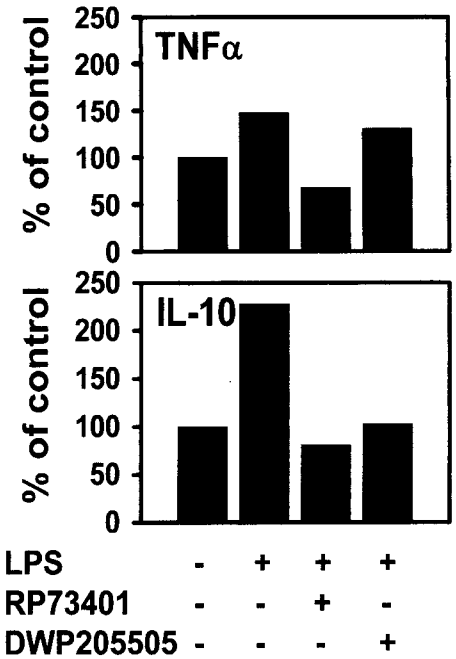

Fig. 6. Effects of PDE4 inhibitors on TNF-α and IL-10 mRNA expression in SFMC.

SFMC were cultured in a media containing 1 μM of DWP205505 or RP73401 as indicated. Some cells were stimulated with 1 μg/ml of LPS simultaneously with PDE4 inhibitor treatments. Semi-quantitative RT-PCR was performed for TNF-α and IL-10 using β-actin as an internal control. Results are means of data obtained from 2 different SFMC samples and expressed as percent of the control value where the cells were treated with PBS (vehicle) only.

stimulation, TNF-α mRNA expression was enhanced over 5 fold and then gradually decreased (Fig. 4B). However, at 24 h after LPS stimulation, the TNF-α mRNA level was still about two fold higher than the control (PBS-treated cells). 1 μM PR73401 completely abolished the effect of LPS on TNF-α mRNA expression. 1 μM DWP205505 partially reduced the effect of LPS on TNF-α mRNA expression (Fig. 4B). IL-10 expression was not changed until 9 h after LPS stimulation and then slightly increased at 12 and 24 h after stimulation (Fig. 4B). DWP205505 and RP73401 did not show noticeable effects on IL-10 mRNA expression in LPS-stimulated cells at any of the time points tested.

Effects on the SFMC Obtained from Rheumatoid Arthritis Patients

Synovial fluid of rheumatoid arthritis patients were centrifuged on Ficoll-Hypaque to obtain SFMC and SFPC. In unstimulated SFPC, there was no detectable TNF-α and IL-10 and the two PDE4 inhibitors had no or minimal effects. When the cells were stimulated with LPS, TNF-α and IL-10 secretions were slightly increased and both PDE4 inhibitors counteracted the effect of LPS (Fig. 5). Unstimulated SFMC released some TNF-α and it was greatly increased by LPS stimulation. Both DWP205505 and RP73401 reduced not only the basal but also LPS-stimulated TNF-α secretion in SFMC (Fig. 5). The effects of the two PDE4 inhibitors on TNF-α and IL-10 mRNA expressions were also tested in SFMC (Fig. 6). LPS enhanced TNF-α mRNA expression about 50% and RP73401 completely abolished the effect of LPS while DWP20505 reduced the effect of LPS slightly. Interestingly, LPS enhanced IL-10 expression over 2 fold in SFMC and both PDE4 inhibitors blocked LPS's effect on IL-10 mRNA expression (Fig. 6).

DISCUSSION

The activity of DWP205505, a novel PDE4 inhibitor, was evaluated in the cells isolated from normal blood and rheumatoid arthritis synovial fluid. Comparisons were made with another PDE4 inhibitor, RP73401. Both DWP205505 and RP73401 inhibited LPS-induced TNF-α secretion from PBMC and SFMC. Interestingly, RP73401 was more potent than DWP205505 in PBMC but the two PDE4 inhibitors showed comparable potencies in SFMC. Similar discrepancy of potencies between rolipram and RP73401 were observed when two different cells, monocytes and eosinophils, were used [16, 19]. These might be due to the fact PDE4 is not just one enzyme but is comprised of 4 distinct subtype enzymes

(PDE4A~D) which are differentially regulated and expressed in different cells [20]. If DWP205505 and RP73401 selectively inhibit a certain PDE4 subtype, different potencies of the two PDE4 inhibitors are expected in cells isolated from two different sources. Another possible explanation is that PDE4 may adopt different conformations in different cell types, as several investigators have suggested [21, 22], resulting in different sensitivity to the inhibitory actions of the two PDE4 inhibitors.

The exact mechanism of the suppression of TNF-α secretion by increased cAMP is not clearly understood yet. cAMP may down regulate TNF-α mRNA expression through cAMP-responsive transcription factors [16] or by attenuating NF-κB activity which plays a role in LPS-induced TNF-α expression [23]. Kambayashi *et al.* reported that rolipram inhibited TNF-α secretion without affecting TNF-α mRNA expression in part by augmenting IL-10 production in LPS-stimulated mouse peritoneal macrophages [17]. In this study, both DWP205505 and RP73401 slightly increased IL-10 release while reducing TNF-α secretion from unstimulated SFMC. However, in LPS-stimulated SFMC, DWP205505 and RP73401 inhibited the secretion of both TNF-α and IL-10. In addition, IL-10 as well as TNF-α mRNA expression was also inhibited by the two PDE4 inhibitors in LPS-stimulated SFMC. Thus, DWP205505 and RP73401 seem not to exert their effects through production of IL-10 in our experimental system especially when the cells were stimulated with LPS. Rather, both of the PDE4 inhibitors could have inhibited TNF-α secretion partly by reducing TNF-α mRNA expression.

When the cells isolated from the synovial fluid of rheumatoid arthritis patients were studied, SFMC containing monocytes, T lymphocytes, and B lymphocytes produced TNF-α in both unstimulated and LPS-stimulated conditions. In contrast, SFPC containing mainly polymorphonuclear leukocytes secreted little or no TNF-α with or without LPS stimulation. This suggests that monocytes and T lymphocytes accumulated in the synovial fluid of rheumatoid arthritis joints, could produce TNF-α resulting in tissue damage. PDE4 inhibitors could be beneficial for rheumatoid arthritis patients by reducing TNF-α production which aggravates tissue inflammation leading to tissue destruction.

Most PDE4 inhibitors synthesized to date, such as rolipram and RP73401, have both beneficial effects and side effects. New PDE4 inhibitors retaining anti-inflammatory activity while having reduced capacity to cause nausea and emesis could have a wider therapeutic window. The newly developed PDE4 inhibitor, DWP205505, warrants further study to evaluate its beneficial and side effects on the inflammatory disease caused by the over-production of TNF-α.

Acknowledgments

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REFERENCES

1. Beutler, B. 1995. TNF, immunity and inflammatory disease: Lessons of the past decade. *J. Invest. Med.* **43:** 227–235.
2. Hopkins, S. J. and A. Meager. 1988. Cytokines in synovial fluid: II. The presence of tumour necrosis factor and interferon. *Clin. Exp. Immunol.* **73:** 88–92.
3. Chu, C. Q., M. Field, M. Feldmann, and R. N. Maini. 1991. Localization of tumor necrosis factor α in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheu.* **34:** 1125–1132.
4. Feldmann, M., F. M. Brennan, and R. N. Maini. 1996. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* **14:** 397–440.
5. Feldmann, M., F. M. Brennan, D. Chantry, C. Haworth, M. Turner, E. Abney, G. Buchan, K. Barrett, D. Barkley, A. Chu, M. Field, and R. N. Maini. 1990. Cytokine production in the rheumatoid joint: Implications for treatment. *Ann. Rhum. Dis.* **49:** 480–486.
6. William, J., L. Koopman, and W. Moreland. 1998. Rheumatoid arthritis: Anticytokine therapies on the horizon. *Ann. Int. Med.* **128:** 231–233.
7. Elliott, M. J., R. N. Maini, M. Feldmann, A. Long-Fox, P. Charles, P. Katsikis, F. M. Brennan, J. Walker, H. Bijl, J. Ghrayeb, and J. N. Woody. 1993. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis Rheu.* **36:** 1681–1690.
8. Endres, S., H. J. Fulle, B. Shinha, D. Stoll, C. A. Dinarello, R. Gerzer, and P. C. Weber. 1991. Cyclic nucleotides differentially regulate the synthesis of tumor necrosis factor alpha and interleukin 1β by human mononuclear cells. *Immunology* **72:** 56–60.
9. Beavo, J. A., M. Conti, and R. J. Heaslip. 1994. Multiple cyclic nucleotide phosphodiesterases. *Mol. Pharmacol.* **46:** 399–405.
10. Palfreyman, M. N. and J. E. Souness. 1996. Phosphodiesterase type IV inhibitors. *Prog. Med. Chem.* **33:** 1–52.
11. Mauro, M. T., R. W. Gristwood, N. Cooper, and P. G. Hellewell. 1997. Phosphodiesterase (PDE) 4 inhibitors: Anti-inflammatory drugs of the future? *Trends Pharmacol. Sci.* **18:** 164–170.
12. Semmler, J., H. Wachtel, and S. Endres. 1993. The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor alpha production by human mononuclear cells. *Int. J. Immunopharmacol.* **15:** 409–413.
13. Sekut, L., D. Yarnall, S. A. Stimpson, L. S. Noel, R. Bateman-Fite, R. L. Clark, M. F. Brackeen, J. A. Menius Jr. and K. M. Connolly. 1995. Anti-inflammatory activity of phosphodiesterase (PDE)-IV inhibitors in acute and chronic models of inflammation. *Clin. Exp. Immunol.* **100:** 126–132.

14. Sommer, N., P. A. Loschmann, G. H, Northoff, M. Weller, A. Steinbrecher, J. P. Steinbach, R. Lichtenfels, R. Meyermann, A. Riethmuller, and A. Fontana. 1995. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis. *Nature Med.* **1:** 244–248.
15. Raeburn, D., S. L. Underwood, S. A. Lewis, V. R. Woodman, C.H. Battram, A. Tomkinson, S. Sharma, R. Jordan, J. E. Souness, and S. E. Webber. 1994. Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. *Br. J. Pharmacol.* **113:** 1423–1431.
16. Souness, J. E., M. Griffin, C. Maslen, K. Ebsworth, L. C. Scott, K. Pollock, M. N. Palfreyman, and J. A. Karlsson. 1996. Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNF alpha generation from human monocytes by interacting with a 'low-affinity' phosphodiesterase 4 conformer. *Br. J. Pharmacol.* **118:** 649–658.
17. Kambayashi, T., C. O. Jacob, D. Zhou, N. Mazurek, M. Fong, and G. Strassmann. 1995. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF-alpha and IL-6 release by endotoxin-stimulated macrophages. *J. Immunol.* **155:** 4909–4916.
18. Fruehling, S., S. K. Lee, R. Herrold, B. Frech, G. Laux, E. Kremmer, F. A. Grasser, and R. Longnecker. 1996. Identification of latent membrane protein 2A (LMP2A) domains essential for the LMP2A dominant-negative effect on B-lymphocyte surface immunoglobulin signal transduction. *J. Virol.* **70:** 6216–6226.
19. Souness, J. E. and L. C. Scott. 1993. Stereospecificity of rolipram actions on eosinophil cyclic AMP-specific phosphodiesterase. Biochem. J. **291:** 389–395.
20. Muller, T., P. Engels, and J. R. Fozard. 1996. Subtypes of the type 4 cAMP phosphodiesterases: Structure, regulation and selective inhibition. *Trends Pharmacol. Sci.* **17:** 294–298.
21. Barnette, M. S., C. D. Manning, L. B. Cieslinski, M. Burman, S. B. Christensen, and T. J. Torphy. 1995. The ability of phosphodiesterase IV inhibitors to suppress superoxide production in guinea pig eosinophils is correlated with inhibition of phosphodiesterase IV catalytic activity. *J. Pharmacol. Exp. Ther.* **273:** 674–679.
22. Souness, J. E. and S. Rao. 1997. Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterases. *Cell Signal.* **9:** 227–236.
23. Wang, P., P. Wu, M. I. Siegel, R. W. Egan, and M. M. Billah. 1995. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J. Biol. Chem.* **270:** 9558–9563.