Immune modulation and amelioration of joint edema by a PDE -inhibitor and its solvent

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Abstract

Background and aim of the study

PDE inhibitors are possible agents against chronic inflammatory diseases such as rheumatoid arthritis. PDE4 inhibitors reduce the synthesis and release of proinflammatory mediators, cytokines and active oxygen species. The present work aims at investigating the effect of rolipram, as a representative of PDE-4 inhibitors, and its solvent dimethylsulfoxide on edema of arthritic joints and cytokines in adjuvant-induced arthritis (AIA).

Methods

AIA was induced by by intradermal injection of 0.1ml squalene before inoculation of Freund's adjuvant into a different site in the subplantar surface of right hind paw. Volume of edema, in both hind paws, was measured daily from day 0 until day 30 after adjuvant inoculation. Serum samples were taken for TNF-alpha and IL-10 assay.

Results

Prophylactic and therapeutic rolipram significantly ($P<0.05$) inhibited the increase of hind paws volume of arthritic rats in a dose- dependent manner. Interestingly, prophylactic and therapeutic DMSO protocols were effective in inhibiting the increase in right hind paw volume and its

prophylactic administration entirely prevented the change of left hind paw volume. Prophylactic or therapeutic administration of rolipram did not alter significantly the serum level of TNF-α. Serum levels of IL-10 were significantly higher ($P<0.05$) in rat groups given therapeutic rolipram compared to levels in adjuvant non-treated animals.

Conclusion

Systemic use of rolipram and its solvent dimethylsulfoxide significantly ameliorated edema of inflamed joints in rats and increase level of the anti-inflammatory cytokine IL-10. Further studies are needed to prove whether the dramatic antiinflammatory and immunmodulatory effects are owed to rolipram or to its solvent.

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Introduction

Rheumatoid arthritis (RA) is a chronic progressive and disabling T cell- mediated autoimmune disorder of unknown cause. It is a highly inflammatory polyarthritis disease often leading to joint destruction, deformity and loss of function (Pincus and Callahan, 1993). Phosphodiesterase (PDE) inhibitors were known to suppress asthmatic immunopathology caused by chronic inflammatory and immune responses (Giembycz, 2007). At the same time, this approach was also expanded to consider PDE inhibitors as a possible agent against the other chronic inflammatory diseases such as RA, because of the elevation of intracellular level of cyclic AMP in leukocytes which is accompanied by inhibition of production of TNF-alpha (Teixeira et al., 1997).

The PDE 4 family of enzymes is cAMP specific and particularly abundant in neutrophils, T-lymphocytes, macrophages and eosinophils. In these cells, PDE4 inhibitors reduce the synthesis and release of proinflammatory mediators, cytokines and active oxygen species .These effects on immunocompetent cells may explain the anti-inflammatory and bronchodilatatory effects induced by PDE4 inhibitors in animal models of inflammatory diseases (Souness et al., 2000 and Huang et al., 2001).

Rolipram is a representative of PDE 4 inhibitors. Harada et al., (2008) mentioned that the PDE 4 inhibitors cilomilast, roflumilast, and rolipram have curative effects in dermatitis mouse model. Kobayashi et al., (2007) reported that PDE IV inhibitors could have therapeutic effects on pannus formation in rheumatoid arthritis by inhibition of cytokine production by macrophages and synovial fibroblast proliferation.

The present work aims at investigating the anti-inflammatory and immunomodulatory activities of rolipram, as a representative of PDEIs, and its solvent dimethylsulfoxide on edema of arthritic joints and cytokines in adjuvant-induced arthritis (AIA), a model of RA in rats that exhibit several pathological changes similar to those occurring in RA. Experimental RA model, AIA, created by the administration of Freund's adjuvant has been used extensively in studying the roles of autoimmunity and inflammation in the pathogenesis of joint disease.

Materials and Methods

Animals

The experimental study was carried out using adult female albino rats of the Sprague-Dawely strain weighing between 160-200 grams. The animals were acclimatized in a light- and temperature- controlled room (23±1°C) with a 12-12 hr dark-light cycle. The rats were fed with commercial pelleted rat feed and water was given ad libitum. Food was placed on the floor of the cage to facilitate access, as the pain which accompanies adjuvant-induced arthritis renders the rats immobile and unable to use their hind limbs to obtain food from the cover mesh of the cage. The experimental protocol was approved by the local ethical committee.

Reagents and Drugs

Complete Freund's adjuvant (CFA) was purchased from Difco laboratories, Detroit, Michigan, USA. Squalene was purchased from MP Biomedicals, Inc. Rolipram was dissolved in 1% diluted DMSO.

Experimental Induction of arthritis

Rat model of AIA, induced by the administration of Freund's adjuvant, has been used extensively in studying the roles of autoimmunity and inflammation in the pathogenesis of joint disease. It exhibits several pathological changes similar to those occurring in RA (Weichmann, 1989).

Preliminary experiments showed that signs of arthritis did not appear in the contralateral non-injected hind paws after CFA inoculation. Also, the use of squalene, a known adjuvant for induction of arthritis (Carlson et al.,2000), alone failed to induce arthritis in the contralateral hind paw. So, the method of induction of adjuvant arthritis by Trentham et al (1977) was modified by intradermal injection of 0.1ml squalene before inoculation of CFA into a different site in the subplantar surface of right hind paw to increase the sensitivity of rats to CFA. Squalene was also used by others to potentiate the effect of CFA (Santos and Tipping,1994). Each animal in all groups was injected with 0.1ml squalene and 0.1 ml CFA except animals of control non-adjuvant group. The day of inoculation was regarded as day 0 while day 16 was the day in which edema in the contralateral, non-injected, hind paw was observed.

Volume of hind paws edema was measured daily from day 0 until day 30 after adjuvant inoculation. At the end of the study, the animals were sacrificed and the blood was collected. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes and serum samples were stored at -80°C until assayed for TNF-alpha and IL-10. To assess the secondary immune reaction, specimens of left ankle joint tissues were also examined for histopathology.

Investigation of the effect of rolipram and its solvent dimethylsulfoxide on adjuvant arthritis in rats

Two groups (I&II) of 6 animals each served as control non-adjuvant and adjuvant non-treated arthritic rats received saline intraperitoneally (i.p.) daily. Other animals were randomly allocated into two treatment protocols (prophylactic or therapeutic). Each treatment protocol contains 6 groups of 6 animals each. Drug treatment was started on day 5 till day 14 in prophylactic

protocol and on day 16 till day 25 in therapeutic protocol. Groups IV, V and VI in each protocol received i.p. rolipram alone in doses of 4.5,3 and 1.5 mg/kg/d respectively. Rats of groups III were given orally 1 ml of dimethylsulfoxide (1% diluted in water).

Measurement of paw volume changes

For each rat in the previously described experimental groups, volumes of hind paws were measured before and daily (till day 30 after disease induction) after adjuvant inoculation by using water displacement plethysmometry (David et al., 2001). The water displacement produced by the immersion of rat's hind paw in the water chamber of the plethysmometer induced a change in the conductance of the platinum electrode. The changes of volumes of hind paws, from those of day 0, were calculated.

Measurement of cytokines

Animals were sacrificed on day 30 after disease induction and samples of blood were taken to separate sera from control ,adjuvant non-treated and SNP-treated arthritic rats .Serum levels of TNF- alpha and IL-10 were determined using enzyme-linked immunosorbent assay (ELISA) kits from (Bender Medsystems,Vienna, Austria). Antibodies specific for rat TNF- alpha and IL-10 were coated onto the wells of the microtiter strips and the samples including standards of known rat TNF-alpha and IL-10 were pipetted into the wells, incubated and washed. Intensity of the color was determined at (450) nm with a correction wave length of (630) nm.

Statistical Analysis

The results are presented as the mean ±standard error. Hind paws volumes and serum levels of cytokines, measured in different treatment groups, were compared with control groups by one way analysis of variance (ANOVA) and Student's t-tests for significance.

Results

Effect of rolipram on changes of paw volume (see tables 1A&B):

Starting from day 1 after inoculation of CFA , volumes of the right hind paw of non-treated control rats (group II) exhibited significant increases ($P<0.05$) compared with that of non-adjuvant control animals (group I). The change in the volume of the right paw was 1.38±0.15ml

on day 5 and increased on day 30 to 1.57±0.1ml On the other hand, little change was noticed in the volume of left non-injected hind paw before day 16. The increase in volume of the left hind paw was peaked on day 20; it was 0.3±0.03 ml then slightly decreased on the subsequent days to become 0.26±0.04ml on day 30 after adjuvant inoculation.

The increase of right hind paw volume of arthritic rats was significantly ($P<0.05$) inhibited by prophylactic as well as therapeutic rolipram alone in a dose- dependent manner. The changes of right hind paw volume of groups treated with 4.5, 3 and 1.5 mg/kg/d rolipram were 0.35±0.01ml, 0.66±0.01ml and 0.88±0.02ml respectively in prophylactic protocol and 0.45±0.01ml, 0.53±0.02ml and 0.55±0.01ml respectively in therapeutic protocol. The later changes were significantly lower ($p<0.05$) compared with those of vehicle-treated arthritic rats (0.96±0.03ml and 0.66±0.01ml). Prophylactic rolipram entirely prevented the change of left hind paw volume. However, the protective effect of the therapeutic rolipram was less than that of prophylactic rolipram.

Compared with adjuvant arthritic control rats, prophylactic and therapeutic DMSO protocols were effective in inhibiting the increase in right hind paw volume and its prophylactic administration entirely prevented the change of left hind paw volume.

Serum TNF-alpha and IL-10 in adjuvant arthritic rats

As shown in Table (2) serum TNF-alpha level was insignificantly and slightly greater in adjuvant arthritic non-treated control rats (group II) than that of control non-adjuvant saline-treated animals on day 30 after adjuvant inoculation (31.04±1.4 picograms, $P>0.05$ in group II compared with 30.7 ±2.3 picograms in saline-treated rats, group I). Serum IL-10 level was significantly lower ($P<0.05$) in adjuvant non-treated control rats, 171.6±34 picograms, $P<0.05$ compared with 345.6±64.4 in saline- treated rats). TNF-alpha levels in sera , obtained on day 30 after adjuvant inoculation, of arthritic rats treated with 3 mg/kg/d rolipram, either prophylactically or therapeutically (groups V) were insignificantly altered. Serum levels of IL-10 were insignificantly higher ($P>0.05$) in rats given prophylactic rolipram alone (382 ±97.04) while they were significantly higher ($P<0.05$) in rat groups given therapeutic rolipram

(454.2±150.2) However, IL-10 levels in the aforementioned groups were insignificantly higher (P>0.05) compared those of saline-treated non adjuvant arthritic animals (group I).

Table 1 A: Effect of prophylactic administration of rolipram on the change of left hind paws volume (ml) of adjuvant arthritic rats

Drug treatment	change of left hind paw volume (ml)			
	Day 5	Day 9	Day 14	Day 30
Saline-treated non adjuvant rats (group I)	0	0	0	0
Adjuvant non- treated arthritic rats (group II)	0	0	0.04±0.01	0.26±0.04
Vehicle-treated (1% DMSO) adjuvant arthritic rats (group III)	0	0	0*****	**0***
Rolipram-treated (4.5mg/kg/d) arthritic rats (group IV)	0	0	0*****	0*****
Rolipram-treated (3mg/kg/d) arthritic rats (group V)	0	0	0*****	0*****
Rolipram-treated (1.5mg/kg/d) arthritic rats (group VI)	0	0	0*****	0

Table 1B: Effect of therapeutic administration of rolipram on the change of left hind paws volume (ml) of adjuvant arthritic rats

Drug treatment	change of left hind paw volume (ml)			
	Day 15	Day 20	Day 25	Day 30
Saline-treated non adjuvant rats (group I)	0	0	0	0
Adjuvant non- treated arthritic rats (group II)	0.12±0.01	0.3±0.03	0.26±0.04	0.26±0.04
Vehicle-treated (1% DMSO) adjuvant arthritic rats (group III)	0.14±0.02	0.12±0.001	0.11±0.001*	0.1±0.002*
Rolipram-treated (4.5mg/kg/d) arthritic rats (group IV)	0.14±0.02	0.08±0.002	0.05±0.002* **º**	0.04±0.001* **º**
Rolipram-treated (3mg/kg/d) arthritic rats (group V)	0.14±0.02	0.12±0.01	0.11±0.01*****	0.07±0.001*** º**
Rolipram-treated (1.5mg/kg/d) arthritic rats (group VI)	0.16±0.02	0.15±0.01	0.13±0.02*****	0.08±0.001*****

Values represent the mean±SE.* p<0.05 vs. groups II, º p<0.05 vs. groups III,† p<0.05 vs. groups V, ANOVA.

Table 2: Effect of rolipram on serum levels of TNF-α and IL-10 in adjuvant arthritic rats

Group	Drug treatment	Serum levels (picograms)	
		TNF- α	IL-10
I	Saline – treated (non- adjuvant)	30.7±2.3	345.6±64.4
II	Adjuvant arthritic (non-treated)	31.04±1.4	171.6±34†
V	Rolipram-treated [3mg/kg/d] (Prophylactic protocol)	27.5±1.5	382±97.04
V	Rolipram-treated [3mg/kg/d] (Therapeutic protocol)	30.1±1.8	454.2±150.2*

Samples were taken from rats on day 30 after adjuvant inoculation. Values represent the mean±SE. * P <0.05 for groups V,VIII and IX vs. group II, † P <0.05 for group II vs. group I,ANOVA.

Discussion

It has been reported that both nonselective as well as PDE4 specific inhibitors were effective in ameliorating autoimmune disease in different experimental autoimmune encephalomyelitis models (Sommer et al., 1995) and collagen-induced arthritis models (Nyman et al., 1997). However, the therapeutic utility of PDE4 inhibitors and their new structural classes to suppress inflammation have not been disclosed till now due to lack of tolerability (Span, 2008; Giembycz, 2008). Several recent studies reported that PDE4 inhibitors possesed anti-inflammatory activities due to their ability to reduce the synthesis and release of proinflammatory mediators, cytokines and active oxygen species. Mendes et al., 2009 found that cilostazol, a PDE4 inhibitor, and pentoxifylline decreased angiogenesis, inflammation, and fibrosis in sponge-induced intraperitoneal adhesion in mice. Paintlia et al., 2008 reported that rolipram, a PDE 4 inhibitor, suppressed the severity of experimental autoimmune encephalomyelitis when it was combined with lovastatin. Also, Harada et al., 2008 mentioned that the PDE 4 inhibitors cilomilast, roflumilast, and rolipram had curative effects in dermatitis mouse model.

Findings of our work provided evidence for an anti-inflammatory effect of rolipram. It was observed that rolipram therapy, either prophylactic or therapeutic, significantly led to marked suppression of adjuvant arthritis in rats depending on the dose administered. The therapeutic efficacy of rolipram was evidenced by decreased hind paw volumes of arthritic rats. These results were in remarkably good agreement with previous studies demonstrating an inhibitory effect of rolipram in other models of arthritis in mice (Ross et al.,1997; Yamaki et al.,2004, 2005) and rats (Francischi et al.,1997;Laemont et al., 1999; Nyman et al., 1997; Sekut et al.1995). YM-393059, an attractive phosphodiesterase 7 and 4 inhibitor, was investigated by Yamamoto et al.,2007 for the treatment of rheumatoid arthritis in several animal models and it potently inhibited proinflammatory cytokine production and ameliorated mouse collagen-induced arthritis. On the contrary, McCluskie et al., (2006) reported that PDE 4 inhibitors, roflumilast and piclamilast, possessed both pro- and anti-inflammatory properties.

The present work demonstrated that joint inflammation was significantly attenuated by DMSO treatment as evidenced by reduced paw swelling. However, there was a significant difference between rolipram- and DMSO-treated groups. This is in line with the observation of Santos and Tipping, 1994 that the reactive oxygen species (ROS) scavenger DMSO, inhibited all indices of arthritis in a dose-dependent fashion providing an evidence for ROS scavenging as the mechanism of attenuation of injury in adjuvant arthritis of rats. Colucci et al.,(2008) mentioned that oral administration of DMSO produced anti-inflammatory effects on zymosan-induced edema in the mouse paw, whereas local administration potentiated the inflammatory action exerted by zymosan. Simons et al.,(2009) reported that topical diclofenac in DMSO vehicle was an effective treatment option for knee osteoarthritis with efficacy similar to, but tolerability better than oral diclofenac.

Several potential mechanisms may underpin the anti-arthritic actions of rolipram. Inhibition of leukotriene B4 (LTB4) (Griswold et al.,1993), interferon-gamma (IFNγ) (Sommer et al., 1995; Essayan et al., 1997), tumor necrosis factor-alpha (TNF- α) (Pettipher et al.,1996; Singh et al.,1997), interleukin-4 (IL-4) and interleukin-5 (IL-5) (Essayan et al.,1997), increase of interleukin-10 (IL-10) release and suppression of T-lymphocyte function, as well as direct, protective effects on cartilage and bone (Souness and Foster,1998), are all potential mechanisms by which the anti-inflammatory effects of roliparm are mediated. Inhibition of (TNF- α) is

especially important because of the pivotal role this cytokine plays in inflammatory processes by attracting leukocytes (Pettipher et al.,1996; Kiely et al.,1995), activating endothelial cells (Sekut et al.,1995; Shimmer et al.,1995) and contributing to edema (Sekut et al.,1995).

The present study demonstrated that prophylactic or therapeutic administration of rolipram did not alter significantly the serum level of TNF-α. Regarding IL-10, our study demonstrated a significantly augmenting effect of treating adjuvant arthritic rats with rolipram, from day 16 to day 25 after disease induction in a dose of 3 mg/kg/d given orally. Serum levels of IL-10 in the aforementioned groups were significantly higher compared to levels in adjuvant non-treated animals. This confirmed the anti-inflammatory activity of IL-10 in adjuvant- induced arthritis in rats and in agreement with other previous studies. Hisadome et al., (2000) reported that a novel antirheumatic drug, Y-39041, has an anti-arthritic effect through not only TNF- α and interlukin-6 suppression but also interleukin-10 augmentation. Eigler et al., (1998) demonstrated that cAMP- elevating agents like rolipram enhance lipopolysaccharide- induced IL-10 synthesis and suppress TNF- α production. Also, Kambayashi et al., (2001) reported that cAMP-elevating agents, especially PDE inhibitors, increase IL-10 and inhibit TNF- α and IL-12 production and these drugs shift the immune response towards a Th2 phenotype. Autoimmune disease models which are Th1- mediated such as collagen-induced arthritis (Nyman et al., 1997) have been successfully treated with PDE 3 and 4 inhibitors. On the contrary, Jimenez et al., (2001) have found that the specific inhibition of PDE4 by rolipram reduces the production of several cytokines such as IL-5, IL-10, TNF- α and IL-2 but poorly affects IFN-γ and T-cell proliferation in response to activation by anti- CD3. Other reports indicated that PDE inhibitors, such as pentoxifylline and rolipram, also inhibited IL-4, IL-5 and IL-10 secretion by T cells (Chan et al., 1993; Essayan et al., 1995; Crocker et al., 1996; Foissier et al., 1996).

In conclusion, our results presented in this study revealed that systemic use of rolipram and its solvent dimethylsulfoxide significantly ameliorated edema of inflamed joints in rats and increase level of the anti-inflammatory cytokine IL-10. Further studies are needed to prove whether the dramatic antiinflammatory and immunmodulatory effects are owed to rolipram or to its solvent.

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