Investigation of analgesic effect of PDE-4 inhibitors on adjuvant-induced pain in a rat model of rheumatoid arthritis

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Abstract

Background and aim of the work: Rheumatoid arthritis (RA) is an autoimmune disorder of unknown cause. It is a highly inflammatory polyarthritis disease often leading to joint destruction, deformity and loss of function. There is conflicting data about the effect of PDEIs on pathogenesis of RA. This work aims at investigation of the potential analgesic effect of rolipram as representative of PDEIs on adjuvant-induced pain in a model of RA in rats that exhibit several pathological changes similar to those occurring in RA.

Methods: In the present study, we used rat model of adjuvant-induced arthrits (AIA), a model of RA in rats that exhibit several pathological changes similar to those occurring in RA in human, by subplantar administration of Freund's adjuvant into hind paws of rats. Pain threshold to pressure on hind paws was measured daily from day 0 until day 30 after adjuvant inoculation.

Results: Rolipram therapy, either prophylactic or therapeutic, significantly leads to marked suppression of adjuvant arthritis in rats depending on the dose administered. Hyperalgesia of adjuvant arthritic rats was significantly reduced in rolipram-treated animals compared to non-treated group. Interestingly, the present work demonstrated that hyperalgesia was significantly attenuated by DMSO treatment.

Conclusion: The results presented in this study show that rolipram, a PDE 4 inhibitor displayed anti-hyperalgesic actions in adjuvant arthritic rats in a dose-dependant manner. Further studies are needed to prove analgesic efficacy of rolipram to exclude effect of the solvent DMSO exhibited in this study.

{Citation: Romany Helmy Thabet, Maher Salamah Raja Alanazi, Ibrahim Abdulhakim Alduraywish, Abdulaziz Saud Saad Alhuwaymil, Hanadi Awad Salman Alanazi. Investigation of analgesic effect of PDE-4 inhibitors on adjuvant-induced pain in a rat model of rheumatoid arthritis. American Journal of Research Communication, 2016, 4(2): 1-11} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Rheumatoid arthritis (RA) is a 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 have been regarded as promising drugs to be used in asthma therapy because these compounds 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). 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 has not been disclosed till now due to lack of tolerability (Span, 2008; Giembycz, 2008).

Rolipram is a PDE 4 inhibitor. It is being researched as a possible alternative to current antidepressants (Washtel, 1983; Bobon et al., 1988). Recent studies show that rolipram may have antipsychotic effects (Maxwell et al.,2004; Kanes et al., 2006).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

recently, Mendes et al., (2009) found that cilostazol, a PDE4 inhibitor, and pentoxifylline decrease angiogenesis, inflammation, and fibrosis in sponge-induced intraperitoneal adhesion in mice. Paintlia et al.,(2008) reported that rolipram suppresses the severity of experimental autoimmune encephalomyelitis when it is combined with lovastatin. Also, 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.

It is obvious from the aforementioned data that there is conflicting data about the effect of PDEIs on pathogenesis of RA. The present work examined the potential analgesic effect of rolipram as representative of PDEIs 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 purchased from Sigma chemical, St.Louis, USA. Rolipram was dissolved in 1% diluted Dimethyl sulfoxide (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.

Pain threshold to pressure on hind paws was measured daily from day 0 until day 30 after adjuvant inoculation.

Design of experimental groups

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 DMSO (1% diluted in water). For each rat in the previously described experimental groups, the following evaluation parameters were daily measured till day 30 after disease induction:

Analgesimetry

Using a Ugo basile analgesimeter (Ugo Basile Biological Research Apparatus, Italy), a crescent pressure (in grams) was applied separately to the posterior paws until the animal displayed a reaction that consisted of withdrawing the paw and/or vocalizing (Andersen and

Tufik, 2000). The slide of the device moved at the speed of 16mm per second. The force on the paw was at rate of 16 grams per second, so a distance of 11.5mm means 115 grams. The pain threshold to pressure (gm) on hind paws of rats was measured. The following formula was used to calculate the percentage of change of pressure (gm) on hind paws on day 30 for each animal:

$$\frac{\text{(Pressure on day 30 – pressure before adjuvant injection on day 0) X 100}}{\text{Pressure before adjuvant injection on day 0}}$$

Statistical Analysis

The results are presented as the mean ±standard error. Percentage of change of pressure (gm) on hind paws was compared with control groups by one way analysis of variance (ANOVA) and Student's t-tests for significance.

Results

Adjuvant inoculation into control rats (group II) was accompanied by hyperalgesia as evidenced by lowering of the pain threshold to pressure (gm) on hind paws. The animals presented a reduction of pain threshold until the end of experiments on day 30. On day 30 after adjuvant inoculation, the percentage of reduction of pressure (gm) on right and left hind paws were 54.9 ±0.3 and 62±0.4 respectively as compared with pressure at day 0 before adjuvant injection. Prophylactic and therapeutic administration of rolipram alone in groups IV, V and VI markedly ($P<0.05$) decreased hyperalgesia of the arthritic rats by increasing the pain threshold to pressure on both hind paws (by decreasing the percentage of reduction of pressure). On day 30, the percentages of decrease of pressure on right hind paws of these groups were 3.5±0.1, 4.2±0.1 and 6.2±0.1 in prophylactic protocol and 4.1±0.02, 7±0.1 and 8.2±0.01 in therapeutic protocol respectively (Tables.1and 2).

Therapeutic and prophylactic administration of rolipram was also effective in reducing the hyperalgesia of left hind paw induced by adjuvant inoculation. Percentages of decrease of pressure were 5.2±0.01, 6.1±0.04, 8.2±0.02 in groups IV, V and VI respectively.

Prophylactic and therapeutic DMSO protocols reduced hyperalgesia of right and left hind paws of arthritic rats compared with control adjuvant arthritic group. However, rolipram in

higher doses (4.5 and 3 mg/kg/d) were significantly more effective in reducing hyperalgesia compared with DMSO.

Table.1: Effect of prophylactic administration of rolipram on the % of change to pressure (gm) on the right hind paw of adjuvant arthritic rats

Drug treatment	% of change to pressure (gm) on the right hind paw			
	Day 5	Day 9	Day 14	Day 30
Saline-treated non adjuvant rats (group I)	4.6±0.01	0	3.5±0.1	2.3±0.1
Adjuvant non- treated arthritic rats (group II)	39.4±0.2	33.8±0.3	26.7±0.4	54.9±0.3
Vehicle-treated (1% DMSO) adjuvant arthritic rats (group III)	42.5±0.1	29.8±0.1	18.6±0.1	7.5±0.1*
Rolipram-treated (4.5mg/kg/d) arthritic rats (group IV)	38.5±0.1	13.5±0.1	11.9±0.1* º	3.5±0.1* º
Rolipram-treated (3mg/kg/d) arthritic rats (group V)	37±0.3	22.2±0.1	12.3±0.2* º	4.2±0.1* º
Rolipram-treated (1.5mg/kg/d) arthritic rats (group VI)	41.5±0.1	28.3±0.2	17.2±0.1*	6.2±0.1*

Table.2: Effect of therapeutic administration of rolipram on the % of change to pressure (gm) on the right hind paw of adjuvant arthritic rats

Drug treatment	% of change to pressure (gm) on the right hind paw			
	Day 15	Day 20	Day 25	Day 30
Saline-treated non adjuvant rats (group I)	3.5±0.1	4.6±0.1	8±0.1	2.3±0.1
Adjuvant non- treated arthritic rats (group II)	29±0.4	44±0.2	46±0.2	54.9±0.3
Vehicle-treated (1% DMSO) adjuvant arthritic rats (group III)	8±0.1	4±0.1	19.2±0.1	13.1±0.1*
Rolipram-treated (4.5mg/kg/d) arthritic rats (group IV)	24±0.1	14.5±0.2	11.1±0.1* º	4.1±0.02* º
Rolipram-treated (3mg/kg/d) arthritic rats (group V)	19±0.1	15±0.1	13±0.1* º	7±0.1* º
Rolipram-treated (1.5mg/kg/d) arthritic rats (group VI)	20±0.1	16±0.1	14.1±0.1* º	8.2±0.01* º

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

Discussion

Adjuvant arthritis in rats is an experimental model that shares many features with human RA, such as swelling, cartilage degradation, and loss of joint function. It has been used for many years for evaluation of anti- arthritic / anti-inflammatory agents (Watnich, 1975). In this model, rats develop a chronic swelling in multiple joints, with influx of inflammatory cells, erosion of joint cartilage and bone destruction after inoculation of CFA. In our study, signs and symptoms of rheumatoid arthritis did not appear in contralateral non-injected hind paw after CFA inoculation. So, the method of induction of adjuvant arthritis was modified by intradermal inoculation of squalene in addition to CFA into the subplantar surface of the right hind paw, to increase sensitivity of arthritic rats to CFA. Squalene was also used by others to potentiate the effect of CFA (Santos and Tipping, 1994).

PDE inhibitors have been regarded 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). 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 previous studies reported that PDE4 inhibitors possessed 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.

In the present study, we carefully evaluated the therapeutic potential of the PDE IV inhibitor, rolipram in various doses in treatment of adjuvant-induced arthritis in rats. 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. Hyperalgesia of adjuvant arthritic rats was significantly reduced in rolipram-treated animals compared with non-treated group. Most recently, Kim and his colleagues observed that rolipram relieved paclitaxel- induced hyperalgesia n rats (Kim et al., 2015). 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 lower clinical scores and 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. More recently, 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.

The present work demonstrated that DMSO treatment, prophylactic or therapeutic, alleviated hyperalgesia of arthritic rats to mechanical pressure on hind paws. This observation is consistent with the study of Colucci et al.,(2008) who reported that orally administered DMSO displayed anti-nociceptive effects but to thermal (hot plate and tail-flick test) and chemical (formalin test) stimuli.

In conclusion, our results revealed that systemic use of rolipram and its solvent DMSO significantly ameliorated hyperalgesia in adjuvant-induced arthritis in rats.

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