British Journal of Pharmaceutical Research
4(8): 992-1003, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-inflammatory, Antiarthritic and Antinociceptive Activities of 3,5-Disubstituted Thiazolidine Derivatives

Diana Jussara do N. Malta[1], Larissa Cardoso C. Araújo[1*], Aline S.G Carrazoni[1], Fernanda Virginia B. Mota[1], Sandra Cabral da Silva[1], Maria Andrea de S. Carmino[1], Maria do Carmo A. Lima[1], Ivan da Rocha Pitta[1] and Teresinha Gonçalves da Silva[1]

[1]Bioassay Laboratory for Drug Screening, Department of Antibiotics, Federal University of Pernambuco (UFPe), PE, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author DJNM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors LCCA, ASGC, FVBM and SCS performed the experiments and performed the statistical analysis. Authors MASC and MCAL synthesized the compounds. Authors IRP and TGS designed the study, wrote the paper, managed the literature searches and managed the analyses of the study. All authors read and approved the final manuscript performed the statistical analysis.

Original Research Article

Received 28th December 2013
Accepted 20th February 2014
Published 12th March 2014

ABSTRACT

Aims: The aim of this study was to investigate the anti-inflammatory, antiarthritic and antinociceptive effects of two thiazolidinedione derivatives: 3-(2-bromo-benzyl)-5-(4-methylsufonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-125) and 3-(2,6-difluoro-benzyl)-5-(4-methylsufonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-192).
Study Design: Study the effects of thiazolidinedione derivatives on the inflammatory process.
Place and Duration of Study: Department of Antibiotics of the Federal University of Pernambuco, Brazil, between March 2010 and February 2012.
Methodology: The carrageenan-induced air pouch in mice was performed and cytokine levels (TNF-α and IL-1β) were determined. Arthritis was induced in female wistar rats by

**Corresponding author: Email: larissa.cca@gmail.com;*

complete Freund's adjuvant (CFA). To determine the antinociceptive activity, acetic acid-induced nociception and hot plate tests in mice were utilized.
Results: Treatment with the compounds reduced leukocyte migration and the release of both TNF-α and IL-1β in the air pouch. Both LPSF/GQ-125 and LPSF/GQ-192 reduced the CFA-induced paw edema and the nociception induced by acetic acid; the hot plate test, however, showed no antinociceptive activity when compared to the control group.
Conclusion: These results indicate that LPSF/GQ-125 and LPSF/GQ-192 exhibit promising anti-inflammatory, antinociceptive and antiarthritic activities related to their ability to inhibit TNF-α and IL-1β production as well as reduction of leukocyte migration.

Keywords: Thiazolidinones; inflammation; air-pouch; cytokines; arthritis.

1. INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that affects both articular and periarticular tissues as well as tendons; however, the anatomical structure most affected is the synovial membrane [1]. RA is the most common autoimmune disease, affecting 1–1.5% of the world population, with the highest incidence occurring in people between 40 and 60 years of age. Due to the aging population, it is estimated that by 2030, the incidence of this disease will increase significantly [2,3]. Research indicates that the innate immune system may be involved in the development of rheumatoid arthritis, which is responsible for the recruitment of inflammatory cells into joint cavities, culminating with the release of factors such as cytokines and prostaglandins, which contribute to the perpetuation of inflammation [4]. Some of the pro-inflammatory cytokines crucial to the development of RA are IL-1β, IL-6 and TNF-α [5].

Currently, several different classes of substances are used in the treatment of RA. Symptom-modifying antirheumatic drugs (SMARDs) are composed of analgesics (opioid and nonopioid) to reduce pain and nonsteroidal anti-inflammatory drugs (NSAIDs) (including cyclooxygenase-2 inhibitors) to lessen pain and stiffness [1]. NSAIDs supplemented with a steroid hormone are the current gold standard for RA treatment, but studies have shown that long-term treatment with NSAIDs may result in serious side effects, such as gastrointestinal ulcers and renal morbidity. Analgesics and NSAIDs neither alter the course of the disease nor prevent joint destruction; therefore, they are not to be used as a stand-alone treatment [6]. Biological antirheumatic agents, such tumor necrosis factor α (TNF-α) blockers, interleukin 1 (IL-1β) blockers, monoclonal antibodies against B cells, T cell co-stimulation blocker and interleukin 6 (IL-6) blockers, have also been used [7].

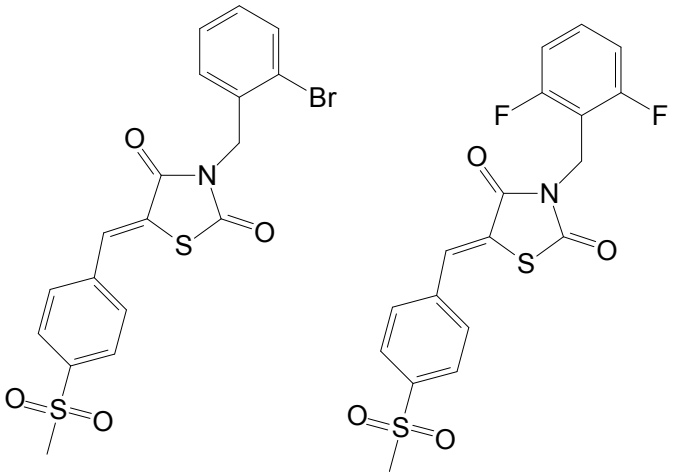
Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. Three types of PPARs have been identified: α, δ/β and γ. These receptors play an important role in glucose and lipid metabolism, production of immunomodulatory cytokines, chemotaxis, cell differentiation, cell proliferation, and survival [8]. Devchand et al. [9] were the first to report that PPARα-deficient mice had an increased inflammatory response induced by both leukotriene B4 and arachidonic acid. There is evidence from *In vitro* and *In vivo* studies demonstrating that PPARα agonists may exert anti-inflammatory activities. PPARγ agonists may also have therapeutic utility in the treatment of other conditions, such as inflammation and cancer [10]. PPARγ is the molecular target of a broad range of both natural and synthetic ligands. These natural ligands include polyunsaturated fatty acids (PUFAs) and prostaglandin derivates (15-

deoxy-Δ prostaglandin-J2 (15d-PGJ) [11,12], as well as components of oxLDL, such as the linoleic acid metabolites 13-HODE and 15-HODE [13]. Synthetic ligands targeting PPARγ include thiazolidinediones, such as ciglitazone, pioglitazone, rosiglitazone, and troglitazone [14]; non-steroidal anti-inflammatory drugs (e.g., indomethacin, fenoprofen, flufenamic acid) [15]; and other PPARγ modulators referred to as selective PPAR modulators [16].

Thiazolidinediones (TZDs) have been the focus of a variety of biological studies. In a study performed by Galli et al. [17], TZDs were identified as having anti-inflammatory action on macrophages as well as being able to inhibit smooth muscle cell proliferation and lymphocyte immunoregulation. Rosiglitazone and 15d-PGJ2, an eicosanoid that is thought to be an endogenous ligand of PPARγ, have been shown to inhibit the edema induced by complete Freund's adjuvant and carrageenan [18-20]. Another study demonstrated that rosiglitazone exerts an anti-inflammatory effect at sites of chronic inflammation and is able to ameliorate tissue damage associated with collagen-induced arthritis [21].

Our group has been conducting studies with thiazolidines-2,4-dione derivatives that have shown both anti-inflammatory and antinociceptive activities [22,23]. The 5-arylidene-3-benzyl-thiazolidine-2,4 dione derivatives have shown considerable anti-inflammatory efficacy compared to rosiglitazone. Docking studies with these compounds indicate that they exhibit specific interactions with key residues located in the PPARγ structure, which corroborates with the hypothesis that these molecules are potential PPAR ligands [23].

The present study aimed to evaluate the anti-inflammatory, antinociceptive and antiarthritic effects of the 3-(2-bromo-benzyl)-5-(4-methylsufonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-125) and 3-(2,6-difluoro-benzyl)-5-(4-methylsufonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-192) (Fig. 1) by observing their effects on the profile of the pro-inflammatory cytokines IL-1β and TNF-α.



LPSF/GQ-125 LPSF/GQ-192

Fig. 1. Chemical Structures of LPSF/GQ-125 and LPSF/GQ-192

2. MATERIALS AND METHODS

2.1 Animals

Male mice were used for the tests of acute inflammation; female rats were used for the arthritis model. The rats weighed between 220-250g, and the mice weighed between 25 and 30 g. The animals were provided by the Animal House of the Antibiotics Department of the Federal University of Pernambuco (UFPE) and were kept in a small colony in the animal house at a temperature of 23±2°C, with 12h cycles of light and darkness and access to standard feed (Purina®) and water ad libitum. Before initiation of the experiments, the animals were acclimated to the laboratory environment for at least 60 min. All animals used for the determination of anti-inflammatory and antinociceptive activities were fasted for 8h prior to experimentation. The Animal Studies Committee of the Federal University of Pernambuco approved the experimental protocols (No. 23076.017928/2010-25). The animals were treated according to the ethical principles of animal experimentation of the Brazilian Society of Laboratory Animal Science (SBCAL) and the norms of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2 Chemicals

The derivatives 3-(2-bromo-benzyl)-5-(4-methylsulfonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-125) and 3-(2,6-difluoro-benzyl)-5-(4-methylsulfonyl-benzylidene)-thiazolidine-2,4-dione (LPSF/GQ-192) were synthesized and provided by the Research Center on Therapeutic Innovation of the Federal University of Pernambuco, Brazil. The doses used in this study were chosen according to pilot tests and previous studies performed by our group [23]. Fentanyl was purchased from Cristália Produtos Químicos Farmacêuticos (São Paulo, Brazil), dexamethasone and diclofenac were purchased from Aché Laboratórios Farmacêuticos (São Paulo, Brazil), HEMSTAB EDTA was purchased from Labtest Diagnóstica (São Paulo, Brazil), glacial acetic acid was purchased from VETEC (São Paulo, Brazil) and carrageenan and indomethacin were purchased from Sigma. Mouse TNF-α and Mouse IL-1β ELISA kits were purchased from *e*Bioscience, (San Diego, California, USA). The compounds tested and the standard drugs were dissolved in saline solution containing tween 80 (9:1; v/v).

2.3 Carrageenan-Induced Air Pouch

The anti-inflammatory effects of the compounds were tested on the induction of inflammation by the introduction of carrageenan to air pouches in the dorsal cervical region of mice weighing 25–30 g. A subcutaneous injection of 2.5 ml of sterile air on day 0 followed by a second injection of 2.5 mL of sterile air 3 days later resulted in an air pouch. On day six, the mice were administered vehicle, LPSF/GQ-125 (3 mg/kg), LPSF/GQ-192 (3 mg/kg), diclofenac (10 mg/kg) or rosiglitazone (3 mg/kg) orally. The doses were chosen according to previously published data by our group using similar compounds. One hour after drug administration, inflammation was induced by injecting 1 mL of carrageenan suspension (1% in saline solution) into the air pouch. After 6h, the animals were euthanized in a CO_2 chamber, and the pouches were washed with 3 mL of saline solution containing 3 µM of EDTA. A white blood cell count was performed using an ABX Micros 60 hematology analyzer. The average number of leukocytes from the treated groups was compared to the number of leukocytes from the control group, which was defined as 100%. The exudates

were centrifuged, and the supernatant was stored at -80°C for analysis of TNF-α and IL-1α levels.

2.3.1 Quantification of IL-1β and TNF-α levels

Tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) production was evaluated in the air pouch exudate at 6h after the induction of inflammation. The assay was carried out using a sandwich ELISA kits specific for mice according to the manufacturer's instructions, with a lower detection limit of 10pg/ml.

2.4 Adjuvant-Induced Chronic Arthritis

To evaluate the effects of thiazolidinone derivatives on chronic inflammation, the adjuvant-induced arthritis model as described by Newbould was used with modifications [24,25]. The compounds LPSF/GQ-125 (3 mg/kg), LPSF/GQ-192 (3 mg/kg) or the reference drug diclofenac (10 mg/kg) were administered orally, whereas the animals in the control group received vehicle [saline: tween 80 (9:1)]. The doses were administered once daily for 14 consecutive days. On the third day of treatment, 25 µL of Freund's complete adjuvant (FCA) (Sigma, St. Louis, Missouri, USA) was injected into the subplantar tissue in the right hind paw of each rat. The paw edema was measured on a plethysmometer (Model 7150, Ugo Basile Co., Varese, Italy) daily from the third to the fourteenth day and on the twenty-first day.

2.5 Writhing Test

The writhing test was performed as described by Koster et al. [25] with minor modifications. Animals were administered LPSF/GQ-125 (3 mg/kg), LPSF/GQ-192 (3 mg/kg) or diclofenac (10 mg/kg) orally. After an hour, 1% acetic acid (0.1 mL/10 g) was injected into the peritoneal cavity of the animals. Ten minutes later, the mice were observed, and the level of writhing during a 20 minute period was recorded. The antinociceptive effect was expressed as the reduction in the number of writhing incidences for those animals pretreated with compounds LPSF/GQ-125, LPSF/GQ-192 or diclofenac compared to the control group.

2.6 Hot Plate Test

Mice were individually placed on a hot plate with the temperature adjusted to 55±1°C. Latency in terms of discomfort reactions (i.e., paw licking or jumping) was determined both before and after the administration of the drugs. The latency to the response was recorded, and the maximum amount of time permitted on the hot surface was 30 seconds. An analgesic effect was defined as an increase in the withdrawal latency. The reaction time was recorded at 1h, 2h and 3h after the treatment with LPSF/GQ-125 (3 mg/kg, p.o.), LPSF/GQ-192 (3 mg/kg, p.o.) or fentanyl (0.2 mg/kg, subcutaneous). The test was performed according to previously described methods [26].

2.7 Statistical Analysis

All results were expressed as the mean values ± standard deviation for each experimental group. Statistical analysis between the groups was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni’s test. For a confidence interval of 95%, P

values less than 0.05 ($P < 0.05$) as determined with Graph Pad Prism software (version 5.0) were considered to be statistically significant.

3. RESULTS

3.1 Carrageenan-Induced Air Pouch

As shown in Table 1, both compounds inhibited leukocyte migration compared to the control group. LPSF/GQ-192 was more effective in inhibiting cell migration, with percentages similar to diclofenac (77%) and rosiglitazone (82%), drugs belonging to the same class of compounds.

Table 1. Effects of LPSF/GQ-125 and LPSF/GQ-192 upon cell migration on inflammation induced by carrageenan in the murine air pouch model

Compound	Dose (mg/kg)	Nº of PMNL /mL (x10^6)	Inhibition (%)
LPSF/GQ-125	3	4.26±0.5*	69.7
LPSF/GQ-192	3	4.08±1.5*	73.9
Rosiglitazone	3	2.71±0.5*	82.6
Diclofenac	10	3.56±1.0*	77.0
Control	-	15.64±1.9	-

*Each value represents the mean ± SD of at least six animals. *P <0.05. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group*

3.2 Quantification of IL-1β and TNF-α Levels in Air Pouches

The data show that the dose of 3 mg/kg of either compound inhibited TNF-α levels compared to the control group. Both compounds also decreased the production of IL-1β (Table 2).

Table 2. Effects of LPSF/GQ-125, LPSF/GQ-192, rosiglitazone, indomethacin and diclofenac upon TNF-α and IL-1β, levels on inflammation induced by carrageenan in the air pouch model

Treatment	Dose (mg/kg)	TNF-α (pg/mL)	IL-1β (pg/mL)
Control	-	1,096±87	871±15
LPSF/GQ-125	3	416±10*	692±40*
LPSF/GQ-192	3	240±25*	339±30*
Rosiglitazone	3	426±21*	676±23*
Diclofenac	10	1,001±98*	935±11

*The value represents the mean ± SD of at least six animals. *P <0.05. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.*

3.3 Adjuvant-Induced Chronic Arthritis

The edema formed after the injection of the adjuvant was measured daily using a plethysmometer. The results are shown in Fig. 2. Pretreatment with either LPSF/GQ-125 or LPSF/GQ-192 had a therapeutic effect represented by reduction of edema in adjuvant-induced chronic arthritis, with LPSF/GQ-192 exerting a more pronounced effect. The animals treated with the derivatives showed similar effects to those receiving diclofenac. During the

treatment, the animals treated with LPSF/GQ-125 or LPSF/GQ-192 had lower edema values on the eighth day of the test. The compound LPSF/GQ-192 showed the highest percentage of inhibition on the eighth day of treatment (30.9%), whereas LPSF/GQ-125 inhibited the paw edema by 24.6% on the thirteenth day of treatment.

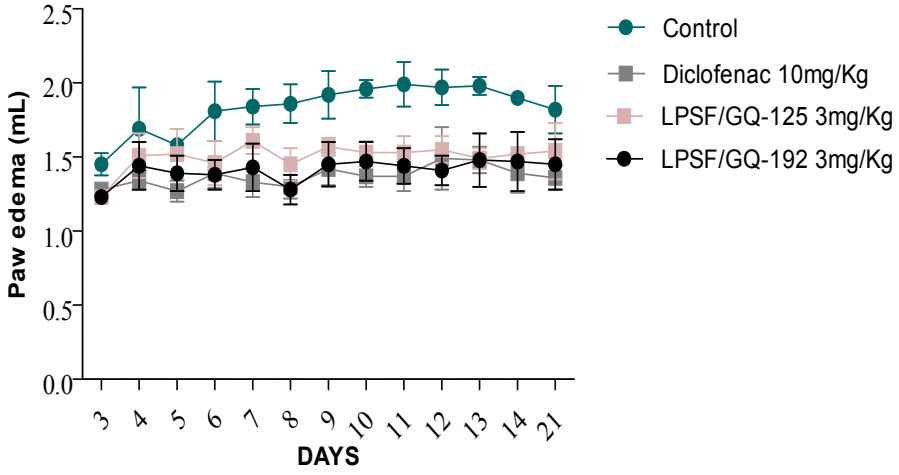


Fig. 2. Effect of LPSF/GQ-125 and LPSF/GQ-192 on the development of hind paw edema in rats induced by FCA

Test drugs: significant from normal control, P <0.05. Significance was determined using two-way ANOVA followed by Bonferroni's post-test

3.4 Writhing Test

The effects of LPSF/GQ-125 or LPSF/GQ-192 on acetic acid-induced writhing behavior in mice are shown in Table 3. The acetic acid injection (1%; 0.1 mL/10 g) induced a significant amount of writhing in the control group. Treatment with both compounds significantly reduced the number of writhing instances in mice compared to the control group, with the derivative LPSF/GQ-125 showing statistically similar results compared to diclofenac treatment.

Table 3. Antinoceptive effect of LPSF/GQ-125 and LPSF/GQ-192 by acetic acid-induced abdominal constriction

Compound	Dose (mg/kg)	Writhing (nº/20 min)	Inhibition %
Control	-	83.5±4.8	-
LPSF/GQ-192	3	40.4±7.8*	51.6
LPSF/GQ-125	3	34.4±5.1*#	58.8
Diclofenac	10	26.8±4.9*	68.0

*Values expressed as mean ± S.D. The number of animal used for each group was six. *P <0.05. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group. *Statistically significant from control group. # No statistically significant from standard group*

3.5 Hot Plate Test

The compounds tested showed no activity in animals during any of the time points observed in the hot plate test in comparison with the control group (Table 4).

Table 4. Effects of the LPSF/GQ-125 and LPSF/GQ-192 on the latency time of mice exposed to the hot plate test

Compounds Dose (mg/kg)		Latency (s)			
		0	60	120	180
Control	-	9.30±0.49	9.70±1.42	8.84±1.31	8.58±1.48
LPSF/GQ-125	3	7.18±1.27	8.96±0.85	7.64±1.38	9.88±1.82
LPSF/GQ-192	3	7.26±0.75	8.64±0.71	6.78±0.88	6.52±1.74
Fentanyl	0.2	9.54±1.72	18.10±0.60 *	11.30±0,90*	8.80±1.10

*Each value represents the mean ± SD of at least six animals. *P <0.05. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.*

4. DISCUSSION

The air pouch model is used to mimic symptoms of rheumatoid arthritis. The induction of inflammation by carrageenan into the subcutaneous air pouch forms a membrane that presents similar characteristics to the inflamed synovial membrane of patients with rheumatoid arthritis [27]. It is, therefore, a model used for screening potential drug candidates to treat arthritis. Our study of the murine air pouch model induced by carrageenan showed that the thiazolidinedione derivatives LPSF/GQ-125 and LPSF/GQ-192 exerted pronounced anti-inflammatory effects by reducing both cell migration and the levels of the major proinflammatory cytokines TNF-α and IL-1β. The results reported here corroborate the results of previous studies performed by our research group [23].

As these results suggested the antiarthritic activity of these compounds, the next step was to evaluate these compounds in the CFA-induced arthritis model. The treatment with the derivatives resulted in a reduction in the manifestation of some clinical signs of arthritis, such as bone deformities and edema, with LPSF/GQ-192 exerting a more pronounced effect.

Adjuvant-induced arthritis in rats is used as a convenient model for preclinical studies of drugs affecting human arthritis as well as studies of nonsteroidal drugs and diseases-modifying anti-rheumatic drugs [28,29]. This model of arthritis in animals is similar to rheumatoid disease in humans, including histopathological changes in cellular infiltration, hypersensitivity and swelling of the affected joint [30]. There is evidence that suggests that either reducing or eliminating the production of TNF-α [31] and IL-1β are important for the treatment of arthritis [32]. In rheumatoid arthritis, TNF-α and IL-1β participate in the initiation and perpetuation of inflammation. Inhibition of TNF-α leads to a reduction of IL-6 levels, and inhibition of IL-1β production slows the destruction of cartilage and bone joints [33]. TNF-α stimulates the development of osteoclasts and promotes the recruitment of leukocytes into the joint through upregulation of adhesion molecules (such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, and E-selectin) as well as through endothelial layer permeability increases [34]. Furthermore, TNF-α stimulates the activation of matrix metalloproteinase's, which may promote bone erosion [35], and inhibits the production of tissue inhibitors of metalloproteinases (TIMPs) by synovial fibroblasts [36]. In

this study, there is evidence of involvement of cytokines TNF-α and IL-1β in antiarthritic effect presented by compounds LPSF/GQ-125 and LPSF/GQ-192.

RA is a severe and debilitating chronic inflammatory disease that affects various locations (most commonly synovial joints and periarticular tissues), and is characterized by pain and aggressive deformities [1]. Therefore, an effective drug for RA should reduce the inflammatory process and combat the pain. To evaluate the antinociceptive effects of LPSF/GQ-125 and LPSF/GQ-192, we used the writhing and the hot plate tests. The writhing model works by injecting acetic acid, which releases endogenous mediators that stimulate the nociceptive neurons causing (on the peritoneal level) increased levels of PGE_2 and $PGF_{2\alpha}$, serotonin, and histamine, as well as the release of bradykinin and cytokines, such as TNF-α and IL-8 [37,38]. The writhing induced by acetic acid is primarily used for the screening of synthetic and natural compounds to determine the presence of central and/or peripheral antinociceptive activity because this model is sensitive to both nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids [39]. In this model, LPSF/GQ-125 and LPSF/GQ-192 were able to inhibit nociception. The model was not specific and should include a test to confirm the activity profile of the compounds. The hot plate test is an adequate model for studying central activity and is selective for certain drugs, such as opioid-derived analgesics. This test evaluates the predominant activity of drugs that act on the central nervous system, such as fentanyl and morphine, both of which increase the latency time of the nociceptive response from the hot plate [40,41]. Acute treatment with LPSF/GQ-125 or LPSF/GQ-192 did not inhibit the nociceptive response in the hot plate test, suggesting that these compounds do not cause changes in the central nociceptive response. These data are in agreement with previously published data, where there is no evidence that thiazolidinediones promote nociceptive effects related to CNS [41].

5. CONCLUSION

In summary, the results reported in this paper suggest that the efficacy of the compounds tested is associated with their ability to reduce TNF-α and IL-1β levels, possibly by acting on PPARs. Further studies are needed to investigate this signaling pathway. This work has shown that LPSF/GQ-125 and LPSF/GQ-192 have anti-inflammatory, antiarthritic and antinociceptive properties, with the antinociceptive effect related to inflammatory pain instead of CNS mechanisms. These compounds appear to be more effective in regulating acute inflammation by modulating the innate immune system through a decrease in the levels of major inflammatory cytokines.

CONSENT

The present study did not involve patients.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as the ethical principles of the Brazilian Society of Laboratory Animal Science (SBCAL). All experiments have been examined and approved by the committee for ethics in Animal Research of the UFPE (process number 23076.017928/2010-25).

ACKNOWLEDGEMENTS

This work was supported by the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) in Brazil. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Smith HS, Smith AR, Seidner P. Painful rheumatoid arthritis. Pain Physician. 2011;14(5):E427-58.
2. Abell JE, Hootman JM, Zack MM, Moriarty D, Helmick CG. Physical activity and health related quality of life among people with arthritis. J Epidemiol Community Health. 2005;59(5):380-85.
3. Khurana R, Berney SM. Clinical aspects of rheumatoid arthritis. Patophysiology. 2005;12(3):153-65.
4. Müller-Ladner U, Pap T, Gay RE, Neidhart M, Gay S. Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. Nat Clin Pract Rheumatol. 2005;1(2):102-10.
5. Choy E, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med. 2001;344(12):907-16.
6. Pincus T, Marcum SB, Callahan LF. Longterm drug therapy for rheumatoid arthritis in seven rheumatology private practices. II. Second line drugs and prednisone. J Rheumatol. 1992;19(12):1885–94.
7. Bansback NJ, Regier DA, Ara R, Brennan A, Shojania K, Esdaile JM, et al. An overview of economic evaluations for drugs used in rheumatoid arthritis: focus on tumour necrosis factor-alpha antagonists. Drugs. 2005;65(4):473-96.
8. Ward JE, Tan X. Peroxisome proliferator activated receptor ligands as regulators of airway inflammation and remodelling in chronic lung disease. PPAR Res. 2007;2007(1):14983.
9. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. Nature. 1996;384(6604):39-43.
10. Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: From orphan receptors to drug discovery. J Med Chem. 2000;43(4):527-50.
11. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ et al. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci U S A. 1994;91(15):7355–59.
12. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell. 1995;83(5):803–12.
13. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell. 1998;93(2):229-40.

14. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem. 1995;270(22):12953-56.
15. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem. 1997;272(6):3406–10.
16. Balint BL, Nagy L. Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases. Endocr Metab Immune Disord Drug Targets. 2006;6(1):33–43.
17. Galli A, Crabb DW, Ceni E, Salzano R, Mello T, Svegliati-Baroni G, et al.: Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation *In vivo* and *In vitro*. Gastroenterology. 2002;122(7):1924-40.
18. Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue K et al. 15-deoxy-delta(12,14)-PGJ(2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest. 2000;106(2):189-97.
19. Taylor BK, Dadia N, Yang CB, Krishnan S, Badr M. Peroxisome proliferator-activated receptor agonists inhibit inflammatory edema and hyperalgesia. Inflammation. 2002;26(3):121-27.
20. Cuzzocrea S, Pisano B, Dugo L, Ianaro A, Maffia P, Patel NS et al. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute inflammation. Eur J Pharmacol. 2004;483(1):79-93.
21. Cuzzocrea S, Mazzon E, Dugo L, Patel NS, Serraino I, Di Paola R et al. Reduction in the evolution of murine type II collagen-induced arthritis by treatment with rosiglitazone, a ligand of the peroxisome proliferator-activated receptor gamma. Arthritis Rheum. 2003;48(12):3544-56.
22. Uchôa FT, Silva TG, Lima MCA, Galdino SL, Pitta IR, Costa TD. Preclinical pharmacokinetic and pharmacodynamic evaluation of thiazolidinone PG15: an anti-inflammatory candidate. J Pharm Pharmacol. 2009;61(3):339-45.
23. Barros CD, Amato AA, Oliveira TB, Iannini KBR, Silva AL, Silva TG et al. Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-diones as PPARγ ligands. Bioorg Med Chem. 2010;18(11):3805-11.
24. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J Pharmacol Chemother. 1963;2(1):127-36.
25. Henderson B, Edwards J, Pettipher E. Mechanisms and Models in Rheumatoid Arthrits. 1st ed. San Diego: Academic Press; 1995.
26. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Fed Proc. 1959;18(1):412-16.
27. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacology. 1968;13(3):222-57.
28. Sedgwick AD, Lees P. Studies of eicosanoid production in the air pouch model of synovial inflammation. Agents Actions. 1986;18(3-4):429-39.
29. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med. 1956;91(1):95-01.
30. Whitehouse MW. Adjuvant arthritis 50 years on: The impact of the 1956 article by C. M. Pearson, Development of arthritis, periarthritis and periostitis in rats given adjuvants. Inflamm Res. 2007;56(4):133-38.
31. Billiau A, Matthys P. Modes of action of Freund's adjuvant in experimental models of autoimmune disease. J Leukoc Biol. 2001;70(6):846-60.
32. Sitônio MM, Carvalho Júnior CH, Campos IA, Silva JB, de Lima MCA, Góes AJ, et al. Anti-inflammatory and anti-arthritic activities of 3, 4-dihydro-2, 2-dimethyl-2 H-naphthol[1,2-b]pyran-5, 6-dione (β-lapachone). Inflamm Res. 2013;62(1):107-13.

33. Mcculloch CA, Downey GP, El-Gabalawy H. Signalling Platforms that Modulate the Inflammatory Response: New Targets for Drug Development. Nat Rev Drug Discov. 2006;5(10):864-76.
34. Joosten LA, Radstake TR, Lubberts E, Van den Bersselaar LA, van Riel PL, van Lent PL, et al. Association of interleukin-18 expression with enhanced levels of both interleukin-1beta and tumor necrosis factor alpha in knee synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum. 2003;48(2):339-47.
35. Majka DS, Deane KD, Parrish LA, Lazar AA, Barón AE, Walker CW, et al. Duration of preclinical rheumatoid arthritis related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Ann Rheum Dis. 2008;67(6):801–07.
36. Mclnnes IB, O'Dell JR. State-of-the-art: Rheumatoid arthritis. Ann Rheum Dis. 2010;69(11):1898-06.
37. Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, Goldring SR. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum. 200;43(2):250–58.
38. Deraedt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol. 1980;61(1):17-24.
39. Ribeiro RA, Vale ML, Thomazzi SM, Pascholato ABP, Poole S, Ferreira SH, Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 2000;387(1):111-18.
40. Fischer LG, Santos D, Serafin C, Malheiros A, Monache FD, Monache GD, et al. Further antinociceptive properties of extracts and phenolic compounds from *Plinia glomerata* (Myrtaceae) leaves. Biol Pharm Bull. 2008;32(2):235-39.
41. Levy RA, Proudit HK. The analgesic action of baclofen [beta-(4-chlorophenyl)-gamma-aminobutyric acid]. J Pharmacol Exp Ther. 1977;202(2):437-45.
42. Oliveira ACP, Bertollo CM, Rocha LTS, Jr. Nascimento EB, Costa KA, Coelho MM. Antinociceptive and antiedematogenic activities of fenofibrate, an agonist of PPAR alpha, and pioglitazone, an agonist of PPAR gamma. Eur J Pharmacol. 2007;561(1-3):194-01.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=440&id=14&aid=3946