Available online at www.sciencedirect.com
ScienceDirect
Experimental Eye Research 84 (2007) 553–560
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Preventive and therapeutic anti-inflammatory effects of systemic and topical thalidomide on endotoxin-induced uveitis in rats

Gustavo Büchele Rodrigues [a,1], Giselle Fazzioni Passos [a,1], Gabriella Di Giunta [b], Cláudia Pinto Figueiredo [b], Eduardo Büchele Rodrigues [c], Astor Grumman Jr. [c], Rodrigo Medeiros [a,1], João B. Calixto [a,*]

[a] *Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Bloco D, CCB, Caixa Postal 476, CEP 88049-900, Florianópolis, SC, Brazil*
[b] *Departamento de Anatomia Patológica, Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*
[c] *Serviço de Oftalmologia, Hospital Regional de São José, Florianópolis, SC, Brazil*

Received 4 August 2006; accepted in revised form 14 November 2006
Available online 12 January 2007

Abstract

The present study examined the outcomes of systemic or topical treatment with thalidomide, a compound that possesses anti-inflammatory, immunomodulatory and anti-angiogenic properties, in rats subjected to endotoxin-induced uveitis (EIU). The effects of thalidomide were evaluated on endotoxin-induced leucocyte and protein infiltration and also on the production of interleukin (IL)-1β and tumour necrosis factor (TNF)-α in rat aqueous humour (AqH). Moreover, the actions of thalidomide were assessed on the cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) protein expression in retinal tissue. EIU was produced by a hindpaw injection of lipopolysaccharide (LPS), in male Wistar rats. Thalidomide (5, 25 and 50 mg/kg) was administered orally 1 h before LPS injection. In another set of experiments, to evaluate the therapeutic efficacy, 5% thalidomide was applied topically to both eyes at 6, 12 and 18 h after LPS administration. The oral pre-treatment with thalidomide decreased, in a dose-dependent manner, the number of inflammatory cells, the protein concentration, and the levels of IL-1β and TNF-α in the AqH. Similar results were found in the AqH of rats that received a topical application of thalidomide. Furthermore, oral (50 mg/kg) and local (5%) thalidomide treatment also reduced expression of the pro-inflammatory proteins COX-2 and iNOS in the posterior segment of the eye. Thalidomide exhibited marked preventive and curative ocular effects in EIU in rats, a property that might be associated with its ability to inhibit the production of inflammatory cytokines and the expression of COX-2 and iNOS. This assembly of data provides additional molecular and functional insights into beneficial effects of thalidomide as an agent for the management of ocular inflammation.

Keywords: thalidomide; uveitis; tumour necrosis factor-α; interleukin-1β; cyclooxygenase-2; inducible nitric oxide synthase; inflammation

1. Introduction

Endotoxin-induced uveitis (EIU) is widely accepted as an animal model for the study of acute ocular inflammation (Rosenbaum et al., 1980). EIU is generally considered to be an inflammation of the anterior uvea (iridocyclitis), while some posterior segment changes (in the vitreous and retina) have also been reported (Ruiz-Moreno et al., 1992). Exposure to exogenous bacterial toxins such as lipopolyssacharide (LPS) stimulates ocular resident cells to produce inflammatory cytokines and chemokines. This leads to an infiltration of leucocytes that amplifies the inflammatory reaction through the release of a variety of mediators, such as nitric oxide, prostaglandin-E_2 and cytokines (de Smet and Chan, 2001).

Thalidomide was originally developed in the 1950s to treat pregnancy-induced morning sickness. The discovery of teratogenic effects motivated its withdrawal from the market in 1961

* Corresponding author. Tel.: +55 48 3331 9491; fax: +55 48 3337 5479.
E-mail addresses: calixto@farmaco.ufsc.br, calixto3@terra.com.br (J.B. Calixto).
[1] These authors contributed equally to this work.

0014-4835/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2006.11.009

(Hales, 1999; Raje and Anderson, 1999). In recent years, interest in thalidomide has increased dramatically due to its potent anti-inflammatory, anti-nociceptive, anti-angiogenic and immunomodulatory properties (D'Amato et al., 1994; Raje and Anderson, 1999; Vale et al., 2006). In this regard, the use of thalidomide has expanded to various clinical areas where inflammation is thought to play an important role, such as rheumatoid arthritis (Oliver et al., 1998), leprosy (Sampaio et al., 1993), multiple myeloma (Singhal et al., 1999) and mucocutaneous lesions in Behçet's syndrome (Shek and Lim, 2002). Although the use of thalidomide in ophthalmology is not a common practice, recent reports suggest that thalidomide might have an important effect on ocular inflammatory reactions (Guex-Crosier et al., 1995; Baatz et al., 2001; Parentin et al., 2001). However, the mechanisms through which thalidomide promotes its beneficial actions are poorly understood.

The purpose of the present study was to evaluate the anti-inflammatory effect of systemic (oral) and topical thalidomide administration on several well-described inflammatory markers in EIU.

2. Materials and methods

2.1. Animals

Non-fasted male Wistar rats (6 weeks old, 140–180 g) kept in controlled temperature (22 ± 2 °C) and humidity (60–80%) under a 12-h light/12-h dark cycle (lights on at 06:00 h) were used. All experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, approved by the local Ethics Committee of the Universidade Federal de Santa Catarina.

2.2. Drug preparation

For systemic treatment, thalidomide (Fundação Ezequiel Dias, MG, Brazil) and dexamethasone (Sigma–Aldrich, MO, USA) were diluted in a saline solution (NaCl, 0.9%), in final concentrations of 50 mg/ml and 0.5 mg/ml, respectively. To evaluate the topical effects, thalidomide was prepared in a final concentration of 5% (w/v), in a saline solution containing 0.1% dimethyl sulphoxide (DMSO). All the solutions were filtered using a Millex-AP 25 mm filter unit (Millipore, Carrigtwohill, Ireland).

2.3. Induction of EIU and thalidomide treatment protocols

Rats received an intradermal injection of 0.1 ml of saline containing 200 μg of LPS from *Escherichia coli* (serotype 0111:B4, Sigma–Aldrich) into one hindpaw. Control animals received the same volume of saline intradermally (control group). Thalidomide (5, 25 or 50 mg/kg) was administered orally 1 h prior to LPS injection. Control animals received the same volume of sterile saline solution by oral route (systemic vehicle group, p.o.). As a positive control, animals were systemically treated with dexamethasone (0.5 mg/kg, subcutaneously in the posterior neck region) 4 h before LPS.

To evaluate the therapeutic efficacy in post-inflammatory treatment, animals were anesthetized with isoflurane (1 ml/ml, Abbot Laboratórios do Brasil Ltda., RJ, Brazil) using a vaporizer system (SurgiVet Inc., WI, USA) and then thalidomide (5%) was applied topically (20 μl eye drops) to both eyes at 6, 12 and 18 h after LPS administration. A separate group of animals was treated with saline containing 0.1% DMSO (topical vehicle group). As a positive control, animals received a topical application of dexamethasone 0.1% (Alcon Laboratórios do Brasil) at the same time points.

2.4. Histopathological evaluation

For histological evaluation, rat eyes were collected and fixed in a PBS solution containing 4% paraformaldehyde and 0.2% glutaraldehyde (0.2 ml of 25% stock per 25 ml) for 24 h at room temperature, dehydrated by graded ethanol, and embedded in paraffin. Tissue sections (5 μm) were deparaffinised with xylene and stained with haematoxylin and eosin (H&E). Infiltrating inflammatory cells in the anterior chamber (iris ciliary body, ICB) and in the posterior retinal tissue were counted in a masked fashion. The number of infiltrating inflammatory cells in six sections per eye, of nine eyes from different animals, was averaged and recorded. The experiments were performed on three different experimental days (3 eyes/day).

2.5. Quantification of infiltrating cells and protein concentration in aqueous humour

AqH (20–25 μl/rat) was collected from both eyes, 24 h after LPS treatment, by an anterior chamber puncture using a 29-gauge needle. For cell counting, the AqH sample was diluted in Türk stain solution (1:20), and the cells were counted with a haemocytometer under a light microscope. The number of cells per field was counted manually and the number of cells per μl was obtained by averaging the results of four fields from each sample. The total protein concentration in the AqH samples was measured using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, CA, USA) according to the manufacturer's instructions.

2.6. Quantification of IL-1β and TNF-α levels in AqH

The levels of IL-1β and TNF-α in the AqH obtained from rats with EIU were assessed with a commercially available ELISA kit (R&D Systems, MN, USA) according to the manufacturer's instructions. The AqH from the eyes of 2–3 rats was pooled, and 50 μl was used for a single assay. The ELISA assay was repeated at least once.

2.7. Preparation of retinal tissue

Following AqH collection, the eyes were opened along the ora serrata, and the corneas and lenses were removed. Using

a pair of forceps, the whole neural retina was dissected from the eyecup. Two retinas were gently homogenized in ice-cold 10mM HEPES (pH 7.4) containing 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml pepstatin, 10 μg/ml leupeptin and 0.5 mM dithiothreitol. The homogenate was chilled on ice for 15 min and then vigorously shaken for 15 min in the presence of 0.1% Nonidet P-40. The homogenate was centrifuged at 10,000 × *g* for 30 min, and the resulting supernatant was considered as the cytosolic fraction. This supernatant was stored at −70 °C until use. Protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories).

2.8. Western blotting analysis

Equivalent amounts of proteins were mixed in buffer (Tris 200 mM, glycerol 10%, SDS 2%, β-mercaptoethanol 2.75 mM and bromophenol blue 0.04%) and boiled for 5 min. Proteins were resolved in 10% sodium dodecyl sulphate–polyacrylamide gel by electrophoresis (SDS–PAGE) and transferred onto polyvinylidene difluoride membranes, according to the manufacturer's instructions (Millipore, SP, Brazil). Membranes were saturated by overnight incubation with 10% non-fat dry milk solution and then incubated with anti-iNOS, anti-COX-2 or anti-actin (Santa Cruz Biotechnology, CA, USA). Following washing, the membranes were incubated with adjusted peroxidase-coupled secondary antibodies. Immunocomplexes were visualized using an ECL chemiluminescence detection system (GE Healthcare, SP, Brazil).

2.9. Statistical analysis

All results are expressed as mean ± S.D. The results were analysed by one-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons, and values of $P < 0.05$ were considered significant.

The term 'control' refers to animals that received only an intradermal injection of saline into the hindpaw. All other groups received an intradermal injection of LPS into the hindpaw. The results from systemic thalidomide- and dexamethasone-treated animals were compared to those of the systemic vehicle-treated animals (saline, p.o.). The results from topical thalidomide- and dexamethasone-treated animals were compared to those of the topical vehicle-treated animals (0.1% DMSO in saline).

3. Results

3.1. Effect of thalidomide on histological changes in the EIU

By histology, no infiltrating cells were detected in the eyes of animals that received an intradermal injection of saline into the hindpaw (control animals, $n = 9$). Conversely, the EIU induced by inoculation of LPS was characterized by the infiltration of inflammatory cells into the whole eye, mainly into the anterior chamber (ICB), but also into the posterior segment of the eye (retina) (Fig. 1). On average, 60 ± 10 inflammatory cells per ocular section (predominantly neutrophils) were detected 24 h after LPS administration ($n = 9$, $P < 0.001$ compared to control animals). Systemic pre-treatment with thalidomide (50 mg/kg, p.o., 1 h) or with dexamethasone (0.5 mg/kg, s.c., 4 h) significantly reduced the number of inflammatory cells in the whole eye, in comparison to systemic vehicle-treated animals ($n = 9$, both $P < 0.001$). On average, 15 ± 5 and 5 ± 1 inflammatory cells per ocular section were detected after the systemic treatment with thalidomide or dexamethasone, respectively. No statistical difference was found between thalidomide- and dexamethasone-treated groups ($P > 0.05$).

The therapeutic efficacy of 5% thalidomide was also tested. When applied topically after LPS treatment, 5% thalidomide significantly reduced the number of inflammatory cells in the whole eye in comparison to the topical vehicle-treated animals (12 ± 6 cells per ocular section, $n = 9$, $P < 0.001$). As shown in Fig. 1C, a similar inhibition was observed following topical treatment with 0.1% dexamethasone, with 2 ± 2 cells per ocular section ($n = 9$, $P < 0.001$). No statistical difference was found between the thalidomide and dexamethasone groups ($P > 0.05$).

3.2. Effect of thalidomide on cellular infiltration and protein concentration in AqH

No infiltrating cells were detected in the AqH in control animals. In contrast, the number of inflammatory cells that infiltrated the AqH 24 h after LPS treatment was significantly higher (50 ± 8 × 10^5 cells/ml, $n = 6$, $P < 0.001$ compared to control animals). The number of inflammatory cells was dose-dependently reduced in animals pre-treated orally with thalidomide, with a mean 50% inhibitory dose (ID_{50}) value of 7.0 (4.6–11.0) mg/kg (Fig. 2A). At the dose of 50 mg/kg, thalidomide almost completely blocked the cellular infiltration induced by LPS ($n = 6$, $P < 0.001$ compared to systemic vehicle-treated animals). No statistical difference was found between thalidomide and dexamethasone groups ($P > 0.05$).

Little protein was detected in the AqH of control animals (0.7 ± 0.2 mg/ml, $n = 6$). The protein concentration in AqH was 27 ± 2 mg/ml in rats 24 h after LPS injection ($P < 0.001$ compared to control animals), and the systemic pre-treatment with thalidomide reduced, in a dose-dependent manner, the protein leakage (Fig. 2B). The calculated mean ID_{50} value for this effect was 35.5 (21.4–58.2) mg/kg. At the dose of 50 mg/kg, the calculated inhibition caused by thalidomide was 64 ± 8% ($n = 6$, $P < 0.001$ compared to systemic vehicle-treated animals). A similar inhibition was observed following systemic treatment with dexamethasone ($n = 6$, $P < 0.001$ compared systemic vehicle-treated animals).

Fig. 3 shows the effects of topical therapeutic treatment (following LPS injection) with 5% thalidomide on inflammatory cell count and protein concentration in AqH. Thalidomide treatment caused a significant reduction of cellular infiltration in comparison to topical vehicle-treated animals (8 ± 6 × 10^5 cells/ml, inhibition of 81 ± 4%, $n = 6$, $P < 0.001$). Moreover,

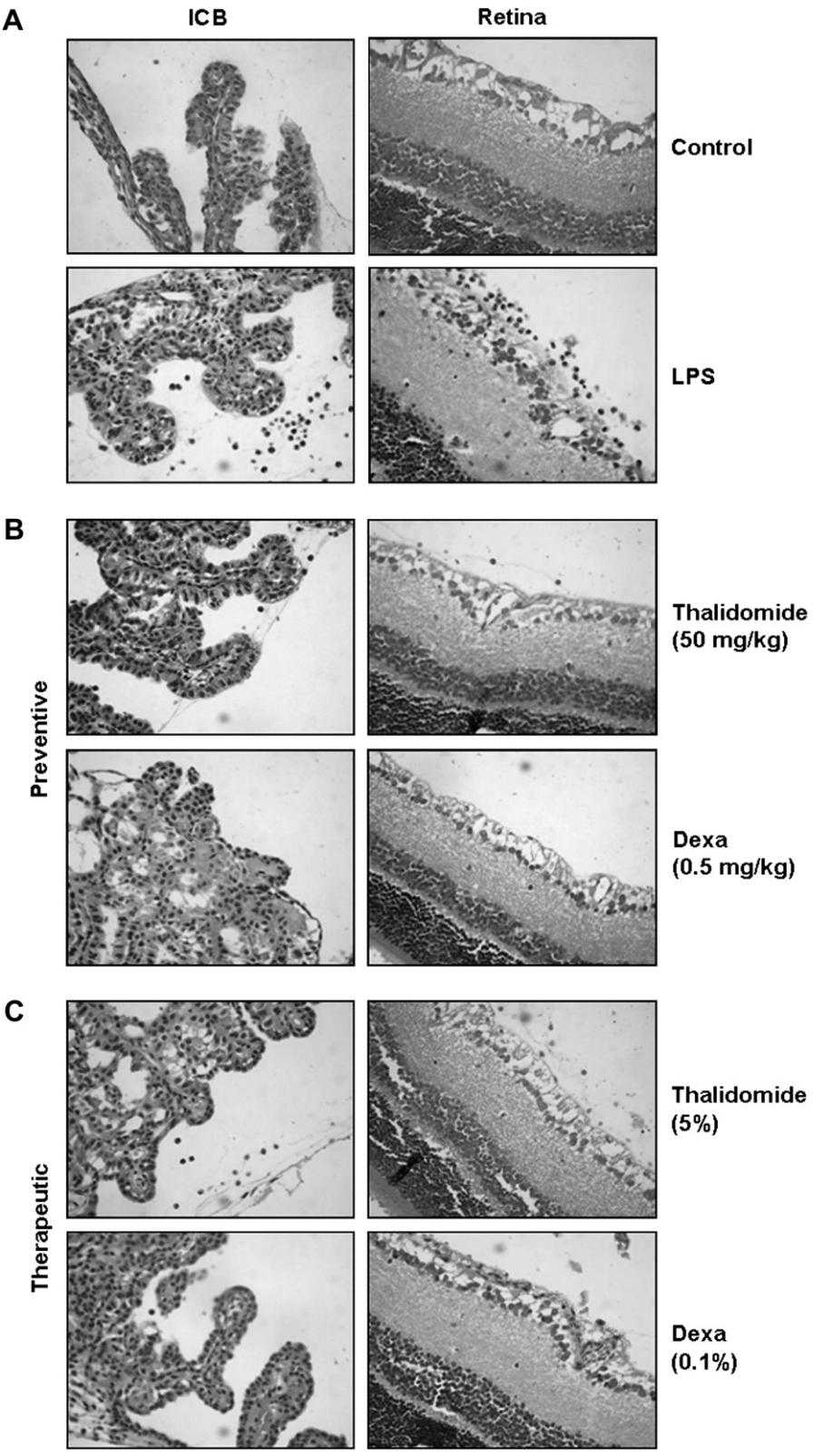

Fig. 1. Histological changes in the iris-ciliary body (ICB) and retina at 24 h after LPS injection. (A) Histological changes in the eye induced by hindpaw injection of saline (control) or LPS (200 μg/paw) plus vehicle. (B) Effect of preventive treatment with thalidomide (50 mg/kg, p.o., 1 h prior to LPS) or dexamethasone (0.5 mg/kg, s.c., 4 h prior to LPS). (C) Therapeutic effect of thalidomide (5%, 6–18 h after LPS) or dexamethasone (0.1%, 6–18 h after LPS) in rats with EIU. H&E staining, original magnification 40×.

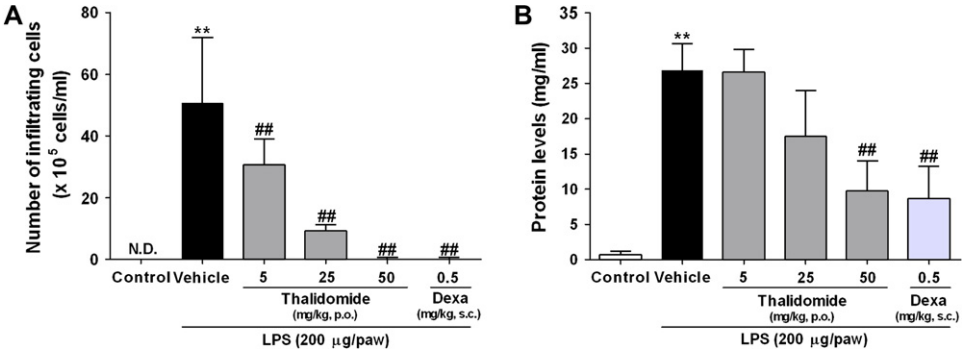

Fig. 2. Preventive effect of thalidomide on cell migration or protein levels in the aqueous humour after LPS injection. (A) Effect of thalidomide (5–50 mg/kg, p.o., 1 h prior to LPS) or dexamethasone (0.5 mg/kg, s.c., 4 h prior to LPS) on (A) cell migration and (B) plasma exudation induced by LPS injection (200 μg/paw, 24 h) in the AqH of rats. Each column represents the average of 6 animals and the vertical bars the S.D. $^{**}P < 0.01$; $^{\#\#}P < 0.01$. N.D., not detected.

5% thalidomide was also capable of reducing the protein leakage induced by LPS injection in the AqH (12 ± 2 mg/ml, inhibition of $48 \pm 9\%$, $n = 6$, $P < 0.001$ compared to topical vehicle-treated animals). Topical treatment with 0.1% dexamethasone also significantly reduced cellular infiltration and protein leakage in comparison to topical vehicle-treated animals (inhibitions of 77 ± 2 and $81 \pm 4\%$, respectively; both $P < 0.001$). A significant difference in the reduction of LPS-induced protein leakage was found between thalidomide and dexamethasone topical treatments ($P < 0.05$, Fig. 3B).

3.3. Effect of thalidomide on IL-1β and TNF-α levels in AqH of eyes with EIU

The results depicted in Fig. 4 indicate that TNF-α was not detectable in the control animals, whereas low levels of IL-1β were observed in these animals (100 ± 49 pg/ml, $n = 3$). As expected, up-regulated levels of IL-1β and TNF-α were detected in the AqH of LPS-treated rats (1120 ± 251 and 224 ± 39 pg/ml, respectively; $n = 3$, both $P < 0.001$ compared to control animals). When systemically pre-administered, thalidomide prevented, in a dose-dependent manner, the increase of both cytokines in response to LPS. The mean ID_{50} values for thalidomide against IL-1β and TNF-α were 35.2 (26.0–59.3) and 17.3 (8.4–34.1) mg/kg, respectively. At the dose 50 mg/kg, thalidomide completely blocked the increase in IL-1β and TNF-α levels ($n = 3$, $P < 0.001$ compared to systemic vehicle-treated animals). A similar effect was found in the systemic dexamethasone-treated group ($n = 3$, $P < 0.001$ compared to systemic vehicle-treated animals).

The topical therapeutic treatment with 5% thalidomide was able to significantly reduce the increase in the IL-1β and TNF-α levels in AqH (Fig. 5, inhibitions of 78 ± 13 and $96 \pm 4\%$, respectively; both $P < 0.001$ compared to topical vehicle-treated animals). Likewise, both cytokines were almost completely reduced in AqH from eyes treated topically with 0.1% dexamethasone ($n = 3$, $P < 0.001$ compared to topical vehicle-treated animals).

3.4. Effect of thalidomide on COX-2 and iNOS expression in the retina

The data in Fig. 6 indicate that the control animals had a detectable expression of both COX-2 and iNOS proteins. The LPS-treatment resulted in a remarkable up-regulation of both COX-2 and iNOS protein expression. Systemic treatment with thalidomide caused a suppression of both COX-2 and iNOS expression (Fig. 6). A similar result was observed in the systemic dexamethasone-treated group.

As depicted in Fig. 7, topically applied 5% thalidomide partially reduced the induction of COX-2 and iNOS protein

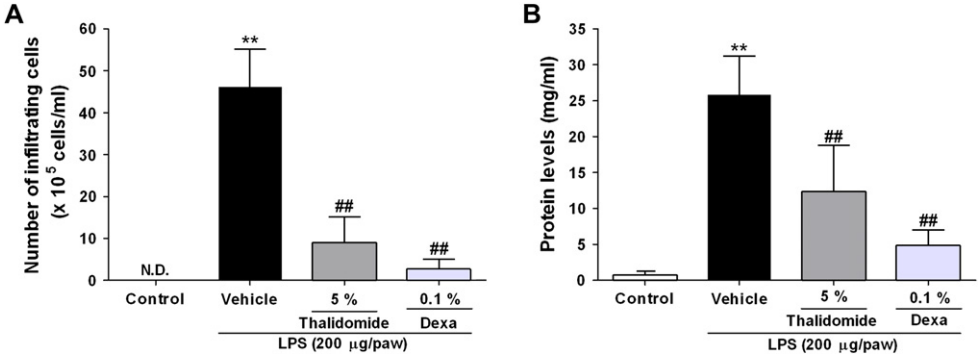

Fig. 3. Therapeutic effect of thalidomide on cell migration or protein levels in aqueous humour of LPS-treated rats. Effect of thalidomide (5%, 6–18 h after LPS) or dexamethasone (0.1%, 6–18 h after LPS) on (A) cell migration and (B) plasma exudation induced by LPS injection (200 μg/paw, 24 h) in the AqH of rats. Each column represents the average of 6 animals and vertical bars the S.D. $^{**}P < 0.01$; $^{\#\#}P < 0.01$. N.D., not detected.

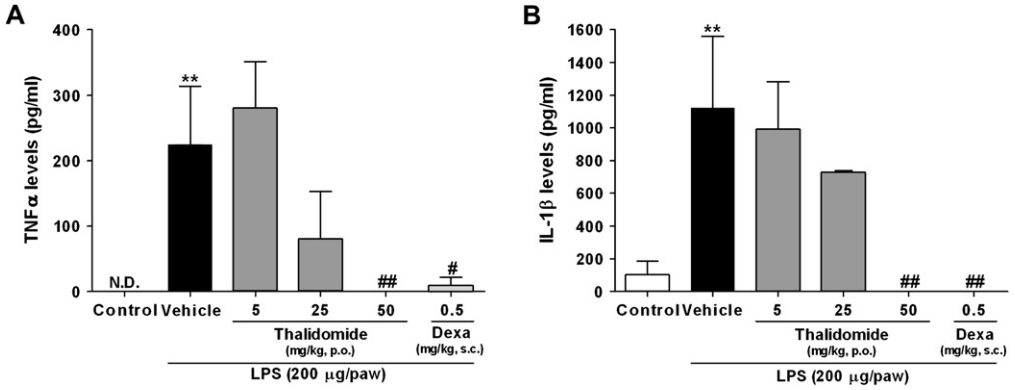

Fig. 4. Preventive effect of thalidomide on TNF-α and IL-1β levels in the aqueous humour of LPS-treated rats. Effect of thalidomide (5–50 mg/kg, p.o., 1 h prior to LPS) or dexamethasone (0.5 mg/kg, s.c., 4 h prior to LPS) treatment on the increase of (A) TNF-α and (B) IL-1β levels induced by the LPS injection (200 μg/paw, 24 h) in the AqH of rats. Each column represents the average of 3 experiments and vertical bars the S.D. $^{**}P < 0.01$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$. N.D., not detected.

expression in the retinal tissue 24 h after LPS injection. Additionally, 0.1% dexamethasone also reduced the effect of LPS on COX-2 and iNOS induction (Fig. 7).

4. Discussion

The current results provide clear functional and molecular evidence indicating that thalidomide attenuates endotoxin-induced uveitis in the rat eye at different levels. Of interest, both systemic and topical schedules of treatment revealed that thalidomide had preventive and therapeutic effects on the EIU. The anti-inflammatory effects of thalidomide might be associated with the suppression of IL-1β and TNF-α cytokine production in the anterior segment of the eye. Additionally, thalidomide showed an important effect on the inflammatory cascade in the posterior segment of the eye by preventing the expression of COX-2 and iNOS, as well as by decreasing the number of infiltrating inflammatory cells in the retinal tissue. Such data allow us to suggest a potential role for this pharmacological agent in the clinical therapy of posterior uveitis, in addition to the previously found effect on anterior segment inflammation.

It is well recognized that systemic or local injection of LPS elicits several immunological effects such as endotoxic shock, inflammation and endotoxin tolerance through massive production of pro-inflammatory cytokines, chemokines and adhesion molecules (Beutler and Rietschel, 2003). Likewise, in the ocular tissue, endotoxin induces breakdown of the blood-aqueous barrier that leads to plasma protein extravasation and cellular infiltration into the AqH (De Vos et al., 1992; Hoekzema et al., 1992). It has been shown previously that increasing doses of thalidomide decrease proteins and cells in the anterior chamber in the EIU model (Guex-Crosier et al., 1995; Baatz et al., 2001). Guex-Crosier et al. (1995) have shown that very high doses of thalidomide (300 and 400 mg/kg; i.p.) significantly reduce anterior chamber inflammation, whereas the 150 mg/kg dose had no influence on inflammatory changes. In a more recent study, a single intraperitoneal dose of thalidomide (80 mg/kg) significantly reduced the leucocyte–endothelium adhesion in iris vessels, as well as the cell count and protein concentration in the AqH of rats in EIU (Baatz et al., 2001). Extending this previous evidence, our results demonstrate that relatively low doses of thalidomide, given orally or topically, significantly suppress the development of ocular inflammation in rats with EIU. Systemic pre-treatment with thalidomide strikingly reduced both cellular infiltration and protein leakage in the AqH. Also, an anti-inflammatory effect was achieved even when thalidomide was administered

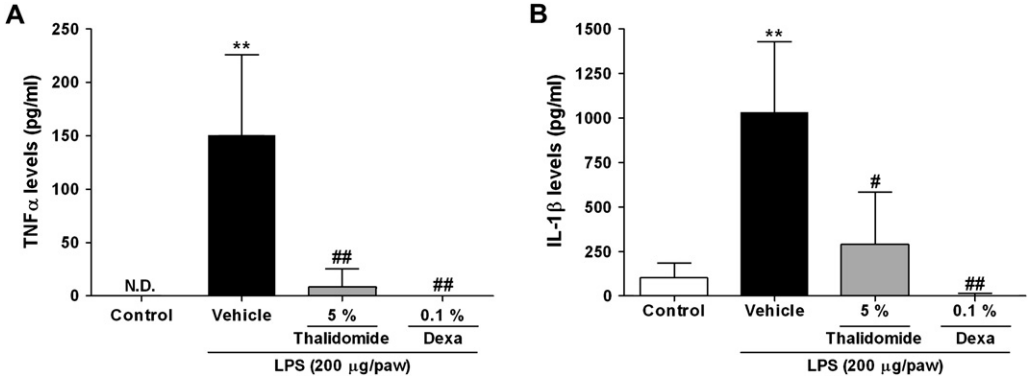

Fig. 5. Therapeutic effect of thalidomide on the TNF-α and IL-1β levels in the aqueous humour of LPS-treated rats. Effect of thalidomide (5%, 6–18 h after LPS) or dexamethasone (0.1%, 6–18 h after LPS) treatment on the increase of (A) TNF-α and (B) IL-1β levels induced by the LPS injection (200 μg/paw, 24 h) in the AqH of rats. Each column represents the average of 3 experiments and vertical bars the S.D. $^{**}P < 0.01$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$. N.D., not detected.

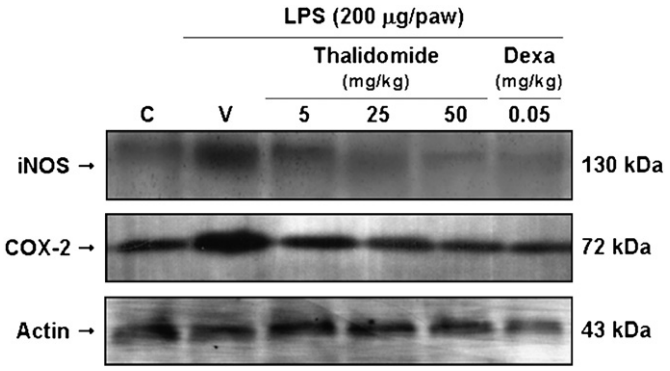

Fig. 6. Preventive effect of thalidomide on COX-2 and iNOS up-regulation induced by LPS in the retina. Effect of thalidomide (5–50 mg/kg, p.o., 1 h prior to LPS) or dexamethasone (0.5 mg/kg, s.c., 4 h prior to LPS) treatment on the increase of COX-2 and iNOS expression induced by the LPS injection (200 μg/paw, 24 h) in the retina of rats. Figures are representative of 3 independent experiments performed on different experimental days. C, control; V, vehicle.

topically 6 h after LPS-injection, indicating an important aspect of its therapy.

Reports regarding the effects of cytokines on EIU-pathogenesis have indicated that some cytokines, namely IL-1β and TNF-α, are involved in the initiation of uveitis (de Smet and Chan, 2001). Of interest, most anti-inflammatory properties of thalidomide have been associated with its ability to inhibit the production and release of a wide range of pro-inflammatory cytokines and growth factors (Raje and Anderson, 2002). Thalidomide inhibits the release and action of TNF-α (Sampaio et al., 1991) and prevents the increase of TNF-α synthesis following the stimulation of human monocytes by LPS (Moreira et al., 1993). Supporting these ideas, our data showed that systemic or topical administration of thalidomide, as well as dexamethasone, dose-dependently decreased LPS-induced TNF-α and IL-1β release.

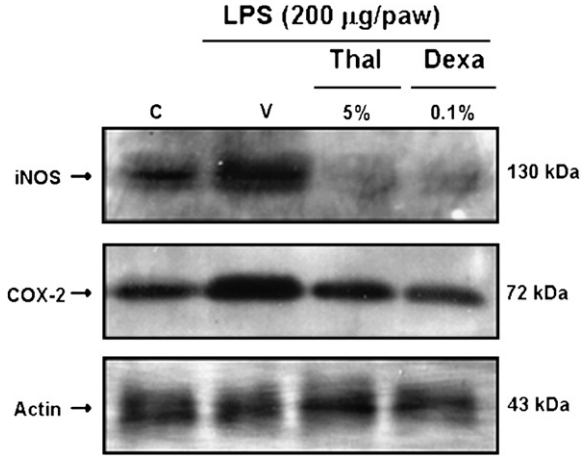

Fig. 7. Therapeutic effect of thalidomide on COX-2 and iNOS up-regulation induced by LPS in the retina. Effect of thalidomide (5%, 6–18 h after LPS) or dexamethasone (0.1%, 6–18 h after LPS) treatment on the increase of COX-2 and iNOS expression induced by the LPS injection (200 μg/paw, 24 h) in the retina of rats. Figures are representative of 3 independent experiments performed on different experimental days. C, control; V, vehicle.

It has been reported that inflammatory responses are mediated by multiple molecular mechanisms. Two of the most prominent effects include the production of nitric oxide by iNOS and the formation of prostaglandins via the up-regulation of COX-2 (Moncada, 1999; Turini and DuBois, 2002). Neither of these enzymes are generally constitutive under normal conditions, but they may be over-expressed following some inflammatory stimuli, including LPS and cytokine treatments (Appleton et al., 1996). The results of the present study clearly indicate that the hindpaw LPS treatment of Wistar rats resulted in a marked increase of COX-2 and iNOS expression in the retinal tissue. Furthermore, the systemic pre-treatment with thalidomide was capable of producing a dose-dependent reduction of the expression of both proteins. Notably, the topical treatment with thalidomide promoted an inhibition in the expression of both COX-2 and iNOS proteins. Taken together, our results suggest that thalidomide promoted a satisfactory inhibition of the inflammatory reaction in the posterior segment of the eye due to the decreased expression of COX-2 and iNOS proteins in the retinal tissue. Although the topical administration does not provide high drug concentrations to the retina, some evidence supports the notion that the drug might reach small but relevant vitreous and retinal levels in animal models (Siefert et al., 1999). However, in our study the amount of thalidomide reaching the retina following the topical application of a 5% solution is not known. Some chemical properties of thalidomide such as lipophilicity and molecule size, as well as pharmacokinetic factors including movement in the liquids or cell membrane permeability, could facilitate the passage of thalidomide into intraocular tissues.

In summary, we have provided evidence that systemic and topical thalidomide is able to significantly reduce the ocular inflammatory responses including the cellular infiltration, protein extravasation, and cytokine production in the AqH. Furthermore, we have also reported that thalidomide at the same doses and schedules of treatment significantly reduces the COX-2 and iNOS protein expression in the retina in the EIU model. Based on our results and previous investigations we propose that thalidomide exerts its anti-inflammatory properties in EIU through regulation of the expression of inflammatory proteins, probably by inhibiting the activation of transcriptional factors such as nuclear factor-κB (NF-κB). Further investigation should provide additional biochemical data to elucidate the precise ocular anti-inflammatory mechanisms of thalidomide. Collectively, the present findings suggest that thalidomide might constitute a relevant therapeutic alternative for the treatment of ocular inflammatory diseases.

Acknowledgements

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Programa de Apoio aos Núcleos de Excelência (PRONEX), all from Brazil. G.B.R is an undergraduate medical student receiving a grant from CNPq. R.M. and G.F.P. are PhD students in pharmacology receiving grants from CNPq.

References

Appleton, I., Tomlinson, A., Willoughby, D.A., 1996. Induction of cyclooxygenase and nitric oxide synthase in inflammation. Adv. Pharmacol. 35, 27–78.

Baatz, H., Tonessen, B., Prada, J., Pleyer, U., 2001. Thalidomide inhibits leukocyte-endothelium interaction in endotoxin-induced uveitis. Ophthalmic Res. 33, 256–263.

Beutler, B., Rietschel, E.T., 2003. Innate immune sensing and its roots: the story of endotoxin. Nat. Rev. Immunol. 3, 169–176.

D'Amato, R.J., Loughnan, M.S., Flynn, E., Folkman, J., 1994. Thalidomide is an inhibitor of angiogenesis. Proc. Natl. Acad. Sci. USA 91, 4082–4085.

de Smet, M.D., Chan, C.C., 2001. Regulation of ocular inflammation—what experimental and human studies have taught us. Prog. Retin. Eye Res. 20, 761–797.

De Vos, A.F., Hoekzema, R., Kijlstra, A., 1992. Cytokines and uveitis, a review. Curr. Eye Res. 11, 581–597.

Guex-Crosier, Y., Pittet, N., Herbort, C.P., 1995. The effect of thalidomide and supidimide on endotoxin-induced uveitis in rats. Graefes. Arch. Clin. Exp. Ophthalmol. 233, 90–93.

Hales, B.F., 1999. Thalidomide on the comeback trail. Nat. Med. 5, 489–490.

Hoekzema, R., Verhagen, C., van Haren, M., Kijlstra, A., 1992. Endotoxin-induced uveitis in the rat. The significance of intraocular interleukin-6. Invest. Ophthalmol. Vis. Sci. 33, 532–539.

Moncada, S., 1999. Nitric oxide: discovery and impact on clinical medicine. J.R. Soc. Med. 92, 164–169.

Moreira, A.L., Sampaio, E.P., Zmuidzinas, A., Frindt, P., Smith, K.A., Kaplan, G., 1993. Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. J. Exp. Med. 177, 1675–1680.

Oliver, S.J., Cheng, T.P., Banquerigo, M.L., Brahn, E., 1998. The effect of thalidomide and 2 analogs on collagen induced arthritis. J. Rheumatol. 25, 964–969.

Parentin, F., Da Pozzo, S., Lepore, L., Perissutti, P., 2001. Thalidomide effectiveness for bilateral chronic idiopathic anterior uveitis ina three-year-old child. Ophthalmologica 215, 70–73.

Raje, N., Anderson, K., 1999. Thalidomide—a revival story. N. Engl. J. Med. 341, 1606–1609.

Raje, M., Anderson, K.C., 2002. Thalidomide and immunomodulatory drugs as cancer therapy. Curr. Opin. Oncol. 14, 635–640.

Rosenbaum, J.T., McDevitt, H.O., Guss, R.B., Egbert, P.R., 1980. Endotoxin-induced uveitis in rats as a model for human disease. Nature 286, 611–613.

Ruiz-Moreno, J.M., Thillaye, B., de Kozak, Y., 1992. Retino-choroidal changes in endotoxin-induced uveitis in the rat. Ophthalmic Res. 24, 162–168.

Sampaio, E.P., Sarno, E.N., Galilly, R., Cohn, Z.A., Kaplan, G., 1991. Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. J. Exp. Med. 173, 699–703.

Sampaio, E.P., Kaplan, G., Miranda, A., Nery, J.A., Miguel, C.P., Viana, S.M., Sarno, E.N., 1993. The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. J. Infect. Dis. 168, 408–414.

Shek, L.P., Lim, D.L., 2002. Thalidomide in Behcet's disease. Biomed. Pharmacother. 56, 31–35.

Siefert, B., Pleyer, U., Muller, M., Hartmann, C., Keipert, S., 1999. Influence of cyclodextrins on the in vitro corneal permeability and in vivo ocular distribution of thalidomide. J. Ocul. Pharmacol. Ther. 15, 429–438.

Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeddis, J., Barlogie, B., 1999. Antitumor activity of thalidomide in refractory multiple myeloma. N. Engl. J. Med. 341, 1565–1571.

Turini, M.E., DuBois, R.N., 2002. Cyclooxygenase-2: a therapeutic target. Annu. Rev. Med. 53, 35–57.

Vale, M.L., Cunha, F.A., Brito, G.A., Benevides, V.M., Ferreira, S.H., Poole, S., Ribeiro, R.A., 2006. Anti-nociceptive effect of thalidomide on zymosan-induced experimental articular incapacitation. Eur. J. Pharmacol. 536, 309–317.