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High Anti-Inflammatory Activity and Low Toxicity of Thalidomide Analogs

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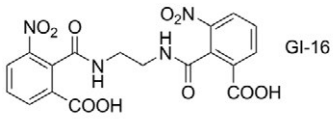
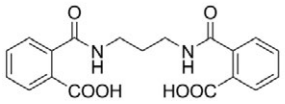
Abstract

Thalidomide is an immunomodulatory agent with anti-inflammatory activity, however it may also cause serious side effects. New compounds derived from thalidomide effective in modulating inflammatory responses and having an improved safety profile is being investigated. In this study, two thalidomide analogs, GI-16 and SC-15, were evaluated using the carrageenan-induced paw swelling and the lipopolysaccharide (LPS)-induced lung inflammation in mice. Acute and sub-chronic toxicity of the compounds were investigated in blood and serum samples of Wistar rats by measurements of hematological and biochemical parameters. Histopathological analyses were conducted to assess inflammatory cell infiltration in heart, liver and kidneys. Our results show that treatment with GI-16 and SC-15 reduced the carrageenan-induced paw edema over a 24 hour period. GI-16 and SC-15 treatments inhibited LPS-induced TNF-α and IL-6 in lung homogenates. In contrast, thalidomide and SC-15 enhanced IL-10 ($p<0.05$). Histopathological analysis showed reduction in LPS–induced lung inflammation after treatment with GI-16 and SC-15. Wistar rats treated with the compounds did not develop any clinical signs of acute or sub-chronic toxicity. No mortality occurred in both control and treated animals and body weight gain over time was similar in all groups. In addition, no significant alterations were detected in enzyme activity of aspartate aminotransferase, alanine aminotransferase or alkaline phosphatase, and no significant alterations were found in glucose, urea, creatinine, total cholesterol or triglyceride levels. GI-16 and SC-15 treatments did not modify hemoglobin, red and white blood cell count, and sections of liver, kidneys and heart tissues showed no pathological alterations under light microscopy. In conclusion, the remarkable *in vivo* anti-inflammatory activity and low toxicity of SC-15 and GI-16 makes them promising drug candidates to treat inflammatory conditions.



GI-16



SC-15

Keywords: Thalidomide analogs; Inflammation; TNF-α; Toxicity

Introduction

Thalidomide is a potent anti-inflammatory drug. Initially used to treat anxiety, insomnia and nausea [1-3], thalidomide has been shown to improve clinical symptoms in a variety of diseases including erythema nodosum leprosum, Crohn disease, rheumatoid arthritis, cancer and some other vascular and inflammatory diseases [2,4-6]. However, thalidomide is only prescribed through a controlled distribution program because of neuropatic effects and teratogenesis [4,6-9]. In order to improve the pharmacokinetic properties of thalidomide and reduce the incidence of side effects, several analogs have been developed with increasingly promising results [1,2,9]. The first studies with thalidomide analogs sought the relationship between chemical structure and biological activity [10]. Structural modifications were carried out in glutarimidics and phtalimidics thalidomide groups [10] and changes in the phtalimidic subunit caused reduction of tumor necrosis factor-alpha (TNF-α) release [2,10-12]. Additionally, thalidomide analogs with open phtalimidic structure not only reduced TNF-α levels, but also decreased interleukin-12 (IL-12) and IL-1β production in lipopolysaccharide (LPS)-stimulated monocytes [2]. In previous study from our laboratory it has been shown that thalidomide analogs containing diamines and open phthalimide structures with the presence of NO_2 group showed high inhibitory *in vitro* activity in key molecules, such as TNFα, IL-12, IFN-γ, IL-6, CXCL9, CXCL10 and CD80 in J774 macrophages. In contrast, some compounds induced an increase in IL-10 production [1,2].

This study aimed to evaluate the *in vivo* effects of two selected compounds derived from thalidomide, namely GI-16 and SC-15,

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characterized by their opened pthalimidic subunits, in attempt to obtain more effective drugs with less toxicity. Here, the anti-inflammatory activity of GI-16 and SC-15 were evaluated *in vivo* using the carrageenan-induced paw swelling and the LPS-induced lung inflammation murine models. In addition, the acute and subchronic toxicity of these compounds on rats were investigated.

Materials and Methods

Animals

C57Bl/6 and BALB/c mice (19-25 g) and Wistar rats (250-350 g) at 6-8 week old were obtained from Federal University of Minas Gerais, Belo Horizonte. Animals were kept in appropriate cages allowed free access to sterile chow and water. All procedures were in accordance with the principles of the Brazilian Code for the Use of Laboratory Animals and were approved by the Ethics Committee on the use of laboratory animals of Federal University of Juiz de Fora (N° 0162010).

Thalidomide, SC-15 and GI-16 compounds

Thalidomide was obtained in 100 mg pills from Ezequiel Dias foundation (Belo Horizonte, Minas Gerais, Brazil) and diluted in dimethylsulfoxide (DMSO) at 0.4-2 mg/mL. The final concentration of DMSO used was 3.96%. As previously shown the thalidomide analogs SC-15 and GI-16 were prepared by using very simple synthetic protocols and could be easily scalable for further biological investigations.

In this case, a simple condensation of phthalic anhydride and 3-nitrophthalic anhydride (10 mmol) in methanol (15 mL) with commercially available diamines (5 mmol), following by recristallyzation of the crude reaction mixture, provided the correponding compounds SC-15 and GI-16 in high chemical yields ranging from 83 to 94 % [2]. The analogs were diluted in saline (0.9% NaCl) at 20-50 mg/kg concentrations.The compound SC-15 has three carbon atoms separating the pthalimidic units (spacer) and lacks NO_2 group. In contrast, GI-16 has NO_2 group and two carbon atoms separating the phtalimidic units (Figure 1). These compounds are protected by patent / INPI number 020090036089.

Carrageenan oedema

Carrageenan-induced mice paw swelling is a widely used model to evaluate inflammatory activity. BALB/c mice (n = 6/group) were intraperitoneally (i.p.) treated with one dose of thalidomide, SC-15 or GI-16 at 10 mg/kg or 50 mg/kg in 0.5 mL saline (0.9%). Dexamethasone treatment (10 mg/kg) was used as positive control due to its anti-inflammatory activity [13]. One hour after treatment; the animals were injected with 30 µl of carrageenan (2.5% in saline) into the left footpad. The left hind paw swelling of each animal was measured with the help of a thickness gauge (Starret, Mass, USA). The edema scores were evaluated at 0, 1, 2, 3, 4, 6 and 24 hours after injection with carrageenan. Mice that received only saline were used as negative controls.

Evaluation of LPS-induced pulmonary inflammation

Fifty female C57Bl/6 were divided into five groups of 10 mice each. Mice were i.p. treated with one dose of thalidomide, SC-15, GI-16 or dexamethasone (10-50 mg/kg in 0.5 mL saline). Negative controls were treated with saline. After one hour of treatment, each mouse was intraperitoneally anesthetized with ketamine (10%) and xylazine (2%) and injected intratracheally with LPS (10ug/0.5 mL). After 24 hours, the left lung of each mouse was extracted. One hundred milligrams of lung from each mouse was homogenized using 1 mL of 0.05% tween-20–PBS containing antiproteases (0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM ethylenediaminetetraacetic acid and 20 kallikrein- inhibitor-units of aprotinin A). The samples were then centrifuged for 10 min at 3000 rpm and the supernatants collected and frozen at -80°C until further use.

TNF-α, IL-6 and IL-10 in lung supernatants were measured by indirect standard ELISA, using commercially available antibodies and following the instructions supplied by the manufacturer (BD Biosciences Pharmingen, San Diego, CA). The reaction was stopped with 1M sulfuric acid and reading in a microplate reader (Spectramax 190; Molecular Devices, Sunnyvale, CA) at 450 nm. The amount of cytokines was calculated from the standard curve, for the different concentrations of the recombinant cytokines.

Lung histopathology after LPS stimulation

The right lung of each animal was fixed by immersion in 10% paraformaldehyde in PBS at room temperature. The specimens were routinely processed for paraffin embedding and analyzed as previously described [14]. Cuts of 5 µm were obtained with aid of semi-automated microtome and were captured on common glass slides for histological staining with hematoxylin-eosin. The images were obtained using Olympus BX51 microscope (Olympus Inc., New York, NY, USA) and results recorded by Image Pro Plus software (Media Cybernetics, Inc., Warrendale, PA, USA). The inflammatory lung injury for each animal was evaluated using the semi- quantitative score method. The injury scores were evaluated in 4 graduations: 0 (normal), 1 (mild), 2 (moderate), 3 (severe) and in four categories: interstitial inflammation (presence of tissue damage due to interstitial inflammation), inflammatory cell infiltration (infiltrates of immune cells), congestion (presence of erythrocytes in the analyzed area) and edema (fluid leakage in the zone analyzed). The total lung injury was calculated by the addition of the individual scores for each category [14].

Acute and sub-chronic toxicity tests

Wistar rats were divided into groups of 8-10 rats each. The acute toxicity test was performed with intraperitoneal (i.p.) administration of a single dose (20 mg/kg) of GI-16 or SC-15 in a 14 day-experiment. Subchronic toxicity was evaluated after administering i.p. doses (every two days) of SC-15 and GI-16 (20 mg/kg) for 28 days. Negative controls received saline or saline plus 10% DMSO. The animals were inspected daily (first day hourly), for general condition and clinical abnormalities. Individual body weight was recorded every four days. Food and water intakes were measured daily. At the end of the exposure period, the rats were sacrificed by deep anesthesia and subjected to hematological and biochemical analysis. In addition, liver, kidneys and heart of each animal were collected for histopathological analysis by hematoxylin and eosin staining.

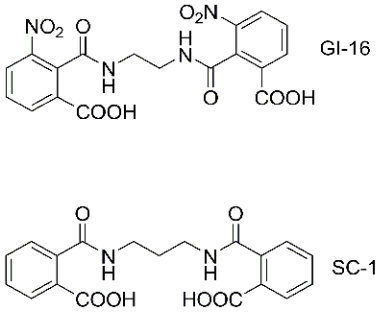

Figure 1: Molecular structure of the thalidomide analogs GI-16 and SC-15.

Biochemical and hematological analysis

Hematological parameters: hemoglobin, percentage and number of erythrocytes, neutrophils, monocytes, granular leukocytes, lymphocytes, platelets and eosinophils were measured using blood samples obtained by cardiac puncture. The samples were placed in tubes with 10% EDTA. Hematological data were obtained through of automated apparatus (Cobas Argos 50® – Roche *Diagnostic System*, Roche Inc., Tallahassee, FLU, USA). The biochemical tests were performed using serum samples from animals, collected in tubes without anticoagulant and centrifuged at 3000 rpm for 15 minutes. The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) (kinetic ultraviolet optimized), alkaline phosphatase (AP) (enzymatic colorimetric assay), glucose (glucose oxidase/peroxidase), creatinine (Jaffe's reaction), triglycerides and cholesterol (enzymatic colorimetric assay), were measured by photometric quantification using automated apparatus (Cobas Mira Plus® - Roche Diagnostic System), and its reactants (Roche Inc., FLU, USA).

Histopathology of the heart, liver and kidneys

Rats were euthanized and heart, liver and kidneys were collected and fixed by immersion in 4% formalin for 20 hours. After dehydration and clearing in baths of alcohol and xylene respectively, tissue fragments were embedded in paraffin. Cuts of 5 μm were obtained with aid of semi-automated microtome and were captured on histological common slides by hematoxylin-eosin staining. The images were obtained using the Olympus BX51 microscope (Olympus Inc., New York, NY, USA) and results recorded by Image Pro Plus software (Media Cybernetics, Inc., Warrendale, PA, USA). Slide analysis focused on central vein and hepatic portal space (two major vascular structures), kidney medulla and cortex (main areas of kidney) and pericardium, myocardium and endocardium (major cardiac layers).

Statistical analysis

Three to six replicates of each condition were performed. Data are reported as mean ± standard error and were analyzed using one-way ANOVA and Tukey post hoc test as appropriate (Graph Pad Prism 5.00 software Inc, San Diego, CA, USA). Statistical significance was set at 5% ($p < 0.05$).

Results

Carrageenan-induced paw swelling

Inflammatory responses to carrageenan injection involve the production of several inflammatory mediators such as histamine, prostaglandins, kinins, nitric oxide and cytokines such as TNF-α [15]. Figure 2 shows that treatment of mice with GI-16 or SC-15 one hour before carrageenan injection caused a significant reduction over 24 h paw edema compared to the saline control group. Both compounds showed reduction of carrageenan-induced swelling similar to thalidomide when administered at 10 mg/kg. At 50 mg/kg, the paw swelling inhibition was higher and similar to those observed with dexamethasone.

Lung inflammation and LPS-induced cytokine production

Figure 3 shows the effect of GI-16 and SC-15 treatments on LPS-induced lung inflammation and cytokine production. Treatment with the compounds caused a significant reduction in TNF-α at both dose levels, reaching values similar to those obtained by treatment with thalidomide or dexamethasone. IL-6 in lung homogenates was also reduced to thalidomide or dexamethasone values at both doses but only for the GI-16 treated group. SC-15 treatment reduced IL-6 only at high dosage (50 mg/kg). Curiously, the lower dosage used (20 mg/kg) reduced IL-10 in all groups in comparison to the saline control. In contrast, using the high dosage (50 mg/kg), IL-10 levels were increased after thalidomide and SC-15 treatments (Figure 3).

Lung histopathology after LPS stimulation

Lung histopathological analysis showed increased alveolar wall thickness, edema, congestion and inflammatory cell infiltrates (especially neutrophilic) in the LPS- stimulated mice (Table 1, Figure 4A-B). Treatment with SC-15 reduced the lung inflammatory response significantly only at 50 mg/kg (Figure 4F), since edema and moderate to severe cellular infiltration were detected at 20 mg/kg dose (Table 1 and Figure 4E). In contrast, treatment with GI-16 markedly attenuated the inflammatory cell infiltration and improved alveolar wall thickening and interstitial edema, at levels similar to those observed for dexamethasone and thalidomide (Table 1, Figure 4G-H).

Acute and sub-chronic toxicity

In general, no signs of acute or subchronic toxicity were detected immediately or during the 14 day experiment (acute toxicity) or 28 day experiment (subchronic toxicity). No signs of toxicity and no deaths were observed immediately or during the evaluation period. The compounds did not influence thirst, appetite or weight of the tested Wistar rats (data not shown). No significant variation in the biochemical (Suppementary Figures 1 and 2) or hematological

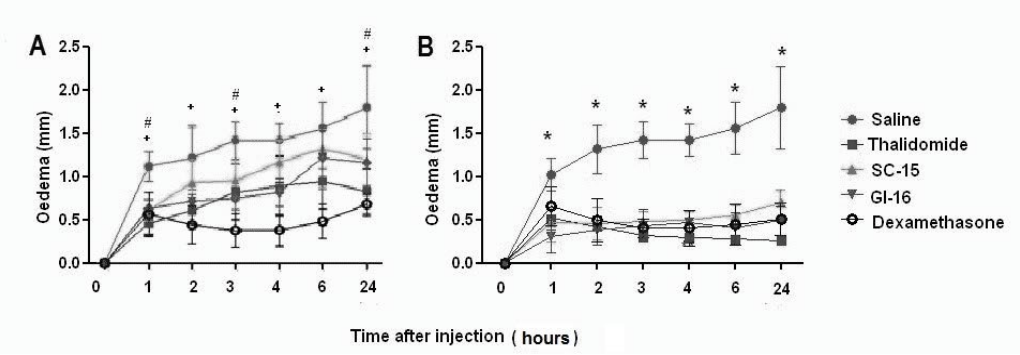

Figure 2: Effect of i.p. injection of GI-16 or SC-15 on carrageenan-induced paw edema in mice. Thalidomide, SC-15 and GI-16 were administered at 10mg/kg (A) or 50mg/kg (B). Controls received vehicle (saline) or dexamethasone (10mg/kg). After one hour, the mice were injected with carrageenan (2.5%) and the left hind 24 h paw swelling evaluated with the help of a thickness gauge (Starret, Mass, USA). The values represent the mean variation in the paw volume + SD (n = 6). * = $p < 0.05$ versus all groups, + = $p < 0.05$ versus GI-16, thalidomide and dexamethasone, # $p < 0.05$ versus SC-15.

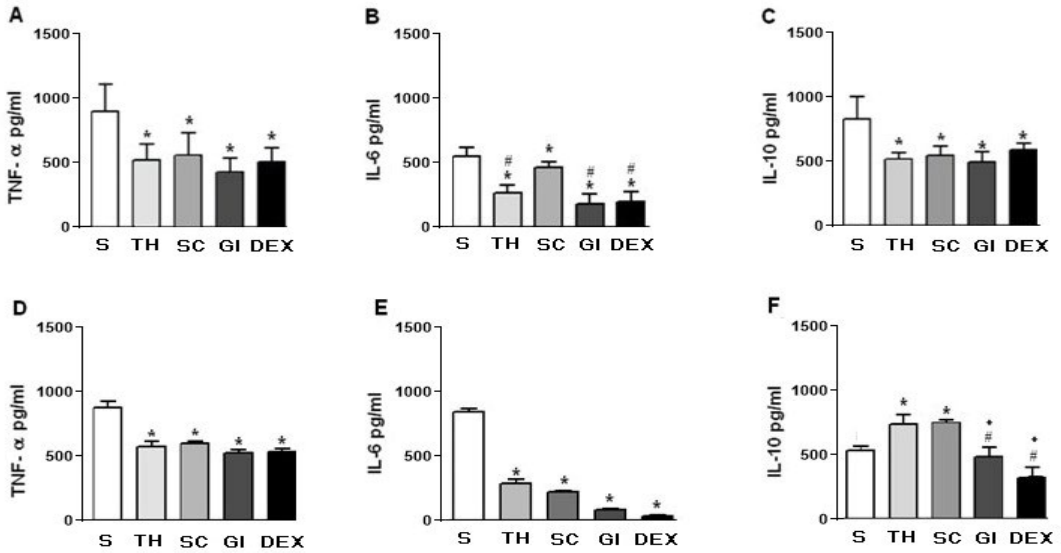

Figure 3: Effect of i.p. injection of GI-16 and SC-15 on LPS-induced TNF-α, IL-6 and IL-10 in lung. Thalidomide (T), SC-15 (SC) and GI-16 (GI) were administered at 20mg/kg (A, B and C) or 50mg/kg (D, E, F). Controls received saline (S) or dexamethasone (DEX, 10mg/kg). One hour after treatment, the animals received intratracheal inoculation with LPS (200µg/ml) and, after 24 hours, TNF-α, IL-6 and IL-10 levels in left lung homogenate supernatants were measured by ELISA. The values represent the mean + SD (n = 6). * = $p < 0.05$ versus saline, # = $p < 0.05$ versus SC-15, += $p < 0.05$ versus thalidomide.

Category	Groups[a]								
	Saline		Thalidomide		SC-15		GL-16		Dexa
	d1	d2	d1	d2	d1	d2	d1	d2	d2
Interstitial inflammation	3[b]	3[b]	1-2	1	2-3	2	1-2	1	1
Inflammatory cell infiltration	3	3	1-2	1	2-3	2	1-2	1	1
Congestion	3	3	1-2	1	2-3	2	1-2	1	1
Oedema	3	3	1-2	1	2-3	2	1-2	1	1
Total Inflammatory score	12	12	4-8	1	8-12	2	4-8	4	4

a-C57Bl/6 mice were i.p. treated with different doses (d1=20 mg/kg and d2=50 mg/kg) of thalidomide, SC-15 and GI-16. Dexamethasone (10 mg/kg) was used as control. One hour after treatment, the animals were inoculated with 50 mL LPS (200 µg/ml). After 24 hours, the right lung of each animal was isolated for histological staining with hematoxylin-eosin.
b-Inflammatory injury score was determined as described in methodology.

Table 1: Scores of LPS-induced lung inflammation in mice treated with thalidomide, SC-15 and GI-16.

parameters (Suppementary Figures 3-6) was detected in the studied groups. Histopathological analysis of the heart, liver and kidneys of GI-16 and SC-15 treated animals did not show any sign of cell damage or infiltrative inflammation (Supplementary Figures 7 and 8).

Discussion

The most salient findings of this work are that mice treated with the thalidomide analogs GI-16 and SC-15 have a significant decrease in carrageenan- induced paw edema and reduction in LPS–induced lung inflammation, showing lower levels of TNF-α and IL-6 in lungs. Moreover, rats administered with GI-16 and SC-15 did not develop any clinical signs of acute or subchronic toxicity. The robust anti-inflammatory activity *in vivo* and low toxicity of SC-15 and GI-16 makes them very promising drug candidates for the treatment of inflammatory conditions.

The present results are in agreement with previous results from our laboratory showing that thalidomide analogs having two separated open pthalimidic units containing diamines as spacer have the ability to inhibit LPS-induced TNF-α production in J774.A1 macrophages as well as in human PBMC [1,2]. The differences between GI-16 and SC-15 in controlling inflammatory cytokines may be explained by their structural configuration. GI-16 and SC-15 are chemically similar, except that GI-16 has NO_2 group in its composition. It has been suggested that NO_2 addition results in amine metabolites that contribute to greater anti- inflammatory action [2]. Our results are also in agreement with studies using thalidomide analogs belonging to pyridinyl-N-(alkyl) phthalimides and 4-amino-5- cyano-2,6-diarylpyrimidine derivatives, where a significant inhibition on carrageenan-induced paw edema inflammation was associated with decreased TNF-α production [12,16]. Similar results were obtained using other analogs of thalidomide, *N*-phenyl-sulfonamide and phthalimide isosters *N*-phenyl-phthalimide amides, where lower neutrophil recruitment was associated with inhibiton of TNF-α [10].

It is well known that thalidomide analogs have the ability to control IL-6 production [1,17]. Lenalidomide, used to treat myeloma, showed inhibitory effect on IL-6, an important survival signal in bone marrow stromal cells [17]. In this study, SC-15 and GI-16 showed significant inhibitory activity on IL-6 production. The mechanisms that regulate the TNF-α and IL-6 production need to be investigated and might involve: 1) inhibition of NF-kB activation [18], 2) induction of apoptosis [19], 3) events involving PDE 4 inhibition [20], 4) binding to alpha 1-acid glycoprotein with high specificity [21], 5) mechanisms involving mRNA regulation [22].

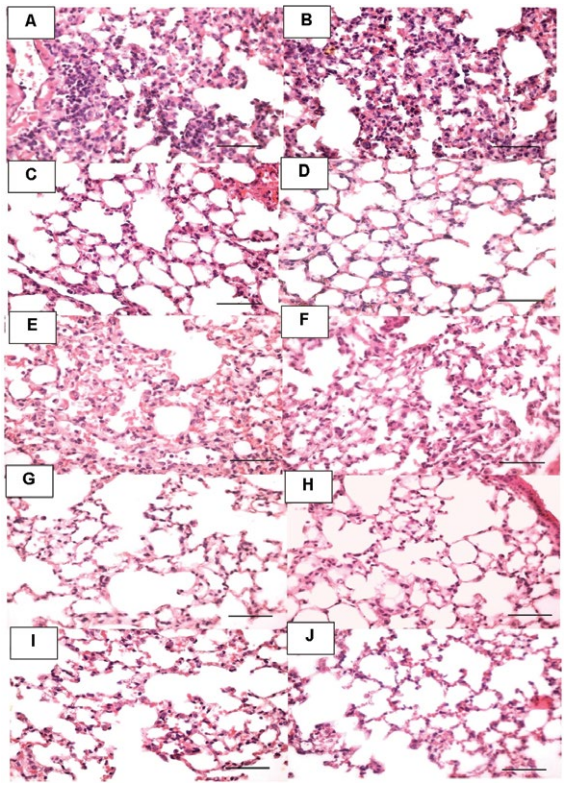

Figure 4: Photomicrograph of lung parenchyma of mice 24 hours after intratracheal administration of LPS. Mice were treated with 20mg/ml (C,E,G) or 50mg/ml (D,F,H) of thalidomide (C-D), SC-15 (E-F) or GI-16 (G-H). Controls received saline (A-B) or dexamethasone (I-J, 10mg/kg). One hour after treatment, the animals were i.t. stimulated with LPS (200µg/ml). After 24 hours, lung slices were stained with hematoxylin and eosin. Bars = 50µm (A-H).

Interestingly, treatment with both thalidomide and SC-15 increased IL-10 production. Corral and cols. [23] observed higher IL-10 production in LPS- stimulated human PBMC cultures treated with phtalimidic analogs. The authors have also demonstrated that treatment with anti-IL-10 did not modify TNF-α production, indicating that IL-10 was not involved in the inhibition of TNF-α. In contrast, treatment with thalidomide resulted in reduced production of IL-10 mRNAs in epithelial tissue biopsy of patients with ENL and in the lungs of mice infected with *M. tuberculosis* [24]. The immunomodulatory activities of thalidomide are not fully understood and deserve further investigations.

Treatment with GI-16 at both doses diminished pro-inflammatory cytokines in LPS-stimulated lungs resulting in a mild local inflammatory infiltration and slight vascular congestion. On the other hand, SC-15 treatment only reduced lung inflammation when used at the higher dose. In agreement with our findings, thalidomide has been responsible to reduce the proinflammatory cytokines and favored the control of cellular inflammatory infiltration in lungs, causing clinical improvements in patients with sarcoidosis, hypersensitive pneumonia and idiopathic pulmonary fibrosis [25]. Treatment with thalidomide analogs from 2- phenoxy-phthalimide derivatives containing substituents on aromatic ring resulted in approximately 60% reduction in neutrophil influx and lower TNF-α levels in lungs of mice challenged with LPS intranasally [11]. Treatment with compound SC-15 in order to control inflammation in the lungs at 20 mg/kg failed. Perhaps, this result might be associated with the high IL-6 levels. High IL-6 production, combined with increased IL-1, IL-17 and IL-8 release, favor the recruitment and activation of macrophages and neutrophils that cause inflammation and tissue damage [26]. Increasing the dose of SC-15 to 50 mg/kg, caused a reduction of IL-6 production and increased IL-10 levels, while maintaining TNF-α at lower levels. We may speculate that the increased production of IL-10 by thalidomide and SC-15 may be partially responsible for the protection from deleterious LPS effects. It is important to mention that the lung histopathology differences between the treatment of GI-16 and SC-15 might be associated with other pro-inflammatory mediators.

Treatment with GI-16 or SC-15 at single dose (14-day experiment) or on alternate days (28-day experiment) did not induce any clinical toxic signs in Wistar rats, immediately or during treatment. No morbidity or mortality of the treated animals was observed. There was no significant biochemical or hematological changes induced by the use of GI-16 or SC-15. None of the compounds caused damage, inflammation, or cellular/tissue changes in heart, liver and kidneys of the Wistar rats tested. Previous research had already pointed to a low incidence of toxicity and side events triggered by treatment with thalidomide analogs [17]. Lenalidomide and actimide have high ability to suppress inflammatory cytokines and strong antiangiogenic action, which explains its high efficacy in treatment of cancers and autoimmune diseases like severe rheumatoid arthritis [17]. These compounds were well tolerated by their users with scarcely detectable incidence of somnolence, neuropathy and myelosuppression over 4 months treatment in elderly patients [17]. These studies demonstrated that thalidomide analogs may express recognized efficacy with lower side effects occurrence.

The development of thalidomide analogs also aims the prevention or reducing rates of teratogenesis [27]. Teratogenicity is the most severe side effect associated with thalidomide use [28]. The intake of 100 mg of thalidomide during 20 to 40 days during pregnancy may result in severe deformities such as phocomelia, marrow hypoplasia, failures in neural tube formation, bone abnormalities and other fetal malformations [28]. Pthalimidic groups are associated with thalidomide teratogenicity, which is usually due to low vascular formation and inhibition of enzymes such as cereblon, responsible for the development of fetal limbs in the first two months of pregnancy [4,7,28]. Lenalidomide and pomalidomide exerted teratogenic action in zebrafish and human cells by inhibiting protein cereblon [7,29,30]. Interestingly, some studies have reported that thalidomide analogs that exhibit removal of teratogenic chiral center, as observed in SC-15 and GI-16, had reduced occurrence of teratogenicity [31]. Nevertheless, further studies are necessary to determine whether SC-15 or GI-16 treatment may cause birth defects.

Conclusions

The potent antiinflammatory activity *in vivo* and low toxicity of SC-15 and GI-16 thalidomide analogs makes them very promising antiinflammatory drug candidates. These compounds can be easily synthesize in large scale since they were produce by using very simple protocols, further studies are needed to verify its potential teratogenicity.

Acknowledgments/Disclosures

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References

1. de Almeida MV, Teixeira FM, de Souza MV, Amarante GW, Alves CC, et al.

(2007) Thalidomide analogs from diamines: Synthesis and evaluation as inhibitors of TNF-alpha production. Chem Pharm Bull (Tokyo) 55: 223-226.

2. Mazzoccoli L, Cadoso SH, Amarante GW, de Souza MV, Domingues R, et al. (2012) Novel thalidomide analogues from diamines inhibit pro-inflammatory cytokine production and CD80 expression while enhancing IL-10. Biomed Pharmacother 66: 323-329.
3. Hashimoto Y1 (2002) Structural development of biological response modifiers based on retinoids and thalidomide. Mini Rev Med Chem 2: 543-551.
4. Ito T, Ando H, Suzuki T, Ogura T, Hotta K, et al. (2010) Identification of a primary target of thalidomide teratogenicity. Science 327: 1345-1350.
5. Malleo G, Mazzon E, Genovese T, Di Paola R, MuiÃ C, et al. (2008) Effects of thalidomide in a mouse model of cerulein-induced acute pancreatitis. Shock 29: 89-97.
6. Melchert M, List A (2007) The thalidomide saga. Int J Biochem Cell Biol 39: 1489-1499.
7. Lopez-Girona A, Mendy D, Ito T, Miller K, Gandhi AK, et al. (2012) Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia 26: 2326-2335.
8. Paravar T, Lee DJ (2008) Thalidomide: mechanisms of action. Int Rev Immunol 27: 111-135.
9. Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, et al. (1999) Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J Immunol 163: 380-386.
10. Lima LM, Fraga CAM, Barreiro EJ (2001) O Renascimento de um Fármaco: Talidomida. Quim Nova 24: 683-688.
11. Machado AL, Lima LM, Araujo JX, Fraga CA, Koatz VL, et al. (2005) Design, synthesis and antiinflammatory activity of novel phthalimide derivatives, structurally related to thalidomide. Bioorg Med Chem Lett15:1169-1172.
12. Collin X, Robert J, Wielgosz G, Le Baut G, Bobin-Dubigeon C, et al. (2001) New anti-inflammatory N-pyridinyl(alkyl)phthalimides acting as tumour necrosis factor-alpha production inhibitors. Eur J Med Chem 36: 639-649.
13. Xu Z, Zhou J, Cai J, Zhu Z, Sun X, et al. (2012) Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. J Inflamm (Lond) 9: 2.
14. Simons RK, Maier RV, Chi EY (1991) Pulmonary effects of continuous endotoxin infusion in the rat. Circ Shock 33: 233-243.
15. Rocha AC, Fernandes ES, QuintÃ£o NL, Campos MM, Calixto JB (2006) Relevance of tumour necrosis factor-alpha for the inflammatory and nociceptive responses evoked by carrageenan in the mouse paw. Br J Pharmacol 148: 688-695.
16. Falcao EP, de Melo SJ, Srivastava RM, Catanho MT, Do Nascimento SC (2006) Synthesis and antiinflammatory activity of 4-amino-2-aryl-5-cyano-6-{3- and 4-(N-phthalimidophenyl)} pyrimidines. Eur J Med Chem 41: 276-282.
17. Aragon-Ching JB, Li H, Gardner ER, Figg WD (2007) Thalidomide analogues as anticancer drugs. Recent Pat Anticancer Drug Discov 2: 167-174.
18. Keifer JA, Guttridge DC, Ashburner BP, Baldwin AS Jr (2001) Inhibition of NF-kappa B activity by thalidomide through suppression of IkappaB kinase activity. J Biol Chem 276: 22382-22387.
19. Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, et al. (2000) Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood 96: 2943-2950.
20. Moreira AL, Corral LG, Ye W, Johnson B, Stirling D, et al. (1997) Thalidomide and thalidomide analogs reduce HIV type 1 replication in human macrophages in vitro. AIDS Res Hum Retroviruses 13: 857-863.
21. Turk BE, Jiang H, Liu JO (1996) Binding of thalidomide to alpha1-acid glycoprotein may be involved in its inhibition of tumor necrosis factor alpha production. Proc Natl Acad Sci U S A 93: 7552-7556.
22. Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, et al. (1993) Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. J Exp Med 177: 1675-1680.
23. Corral LG, Muller GW, Moreira AL, Chen Y, Wu M, et al. (1996) Selection of novel analogs of thalidomide with enhanced tumor necrosis factor alpha inhibitory activity. Mol Med 2: 506-515.
24. Moreira AL, Tsenova-Berkova L, Wang J, Laochumroonvorapong P, Freeman S, et al. (1997) Effect of cytokine modulation by thalidomide on the granulomatous response in murine tuberculosis. Tuber Lung Dis 78: 47-55.
25. Ye Q, Chen B, Tong Z, Nakamura S, Sarria R, et al. (2006) Thalidomide reduces IL-18, IL-8 and TNF-alpha release from alveolar macrophages in interstitial lung disease. Eur Respir J 28: 824-831.
26. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, et al. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441: 235-238.
27. Marriott JB, Westby M, Cookson S, Guckian M, Goodbourn S, et al. (1998) CC-3052: a water-soluble analog of thalidomide and potent inhibitor of activation-induced TNF-alpha production. J Immunol 161: 4236-4243.
28. Faver IR, Guerra SG, Su WP, el-Azhary R (2005) Thalidomide for dermatology: a review of clinical uses and adverse effects. Int J Dermatol 44: 61-67.
29. Zhu YX, Braggio E, Shi CX, Bruins LA, Schmidt JE, et al. (2011) Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood 118: 4771-4779.
30. Gordon GB, Spielberg SP, Blake DA, Balasubramanian V (1981) Thalidomide teratogenesis: evidence for a toxic arene oxide metabolite. Proc Natl Acad Sci U S A 78: 2545-2548.
31. Parman T, Wiley MJ, Wells PG (1999) Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. Nat Med 5: 582-585.