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Anti-inflammatory effect of thalidomide dithiocarbamate and dithioate analogs

Roba Talaat [a], Waheba El-Sayed [a], Hussein Agwa [b], Amira Gamal-Eldeen [c], Shaden Moawia [a], Magdy Zahran [b,*]

[a] Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Sadat City, Egypt
[b] Chemistry Department, Faculty of Science, Menoufiya University, Shebin El-Koam, Egypt
[c] Cancer Biology Laboratory, Center of Excellency for Advanced Sciences, National Research Centre, Dokki, Cairo, Egypt

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ABSTRACT

Thalidomide has anti-inflammatory, immunomodulatory, and anti-angiogenic properties. It has been used to treat a variety of cancers and autoimmune diseases. This study aimed to characterize anti-inflammatory activities of novel thalidomide analogs by exploring their effects on splenocytes proliferation and macrophage functions and their antioxidant activity. MTT assay was used to assess the cytotoxic effect of thalidomide analogs against splenocytes. Tumor necrosis factor (TNF-α) and nuclear factor kappa B (NF-κB-P65) were determined by enzyme-linked immunosorbent assay (ELISA). Nitric oxide (NO) was estimated by colorimetric assay. Antioxidant activity was examined by ORAC assay. Our results demonstrated that thalidomide dithioate analog 2 and thalidomide dithiocarbamate analog 4 produced a slight increase in splenocyte proliferation compared with thalidomide. Thalidomide dithiocarbamate analog 1 is a potent inhibitor of TNF-α production, whereas thalidomide dithiocarbamate analog 5 is a potent inhibitor of both TNF-α and NO. Analog 2 has a pronounced inhibitory effect on NF-κB-P65 production level. All thalidomide analogs showed prooxidant activity against hydroxyl (OH·) radical. Analog 1 and thalidomide dithioate analog 3 have prooxidant activity against peroxyl (ROO·) radical in relation to thalidomide. On the other hand, analog 4 has a potent scavenging capacity against peroxyl (ROO·) radical compared with thalidomide. Taken together, the results of this study suggest that thalidomide analogs might have valuable anti-inflammatory activities with more pronounced effect than thalidomide itself.

1. Introduction

Inflammation is a physiologic process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and/or wounding [1–2]. Dysregulated inflammation plays a major role in resulting chronic inflammation that can contribute to diseases such as arthritis, heart attacks, Alzheimer's disease, and cancer [3]. Inflammatory responses play decisive roles at different stages of tumor development [4]. Inflammation also affects immune surveillance and responses to therapy [4]. Several anti-inflammatory drugs have been found to reduce tumor incidence when used as prophylactics, as well as to slow down progression and reduce mortality when used as therapeutics [5]. These drugs include nonsteroidal anti-inflammatory drugs (such as cyclooxygenase (COX_2) inhibitors), and anti-inflammatory steroids (such as dexamethasone) [6].

Thalidomide has potent anti-inflammatory effects through inhibition of tumor necrosis factor (TNF-α) and suppression of nuclear factor kappa B (NF-κB) activation in response to inflammatory agents [7,8]. Thalidomide has also demonstrated its efficacy in several diseases such as various inflammatory diseases including rheumatoid arthritis, Crohn's disease, and Behcet's disease [9–10]. Furthermore, thalidomide has become associated with a range of immunomodulatory actions [11].

In our previous studies, a series of novel isosteric thalidomide analogs with a distinct and clear potent antitumor activity were designed and synthesized. These thalidomide analogs lead to increase of Fas-L expression, decrease in Ki67 and vascular endothelial growth factor (VEGF) staining in tumor cells which suggests an inhibition of tumor proliferation rate, angiogenic process associated with tumor growth, decrease in lactate dehydrogenase (LDH), intracellular adhesion molecule (ICAM-1) expression

* Corresponding author at: Faculty of Science, Department of Chemistry, Menoufia University, Shebin El-Koam 2323, Egypt. Fax: +20 24159770.
E-mail address: magdyzahran53@gmail.com (M. Zahran).

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and nitric oxide (NO) [12,13]. Moreover, we found that the N-substitution of thalidomide dithiocarbamate and dithioate analogs increased cytotoxic activity and inhibited NF-κB production selectivity on Hep-G2 cells. They had a potent inhibitory effect on proangiogenic mediators accompanied by elevation of antiangiogenic factors on Hep-G2 and MCF-7 cells [14].

In continuation with these studies and based on the previously documented anti-inflammatory and immunomodulatory properties of thalidomide, this work was designed in a trial to find out the ability of new thalidomide dithiocarbamate and dithioate analogs to improve its immunomodulatory potential and anti-inflammatory activities.

2. Materials and methods

2.1. Thalidomide and its sulfur analogs

Thalidomide and its dithiocarbamate and dithioate analogs (Scheme 1) were synthesized according to the previously reported [12] method. They were individually dissolved in their vehicle, dimethyl sulfoxide (DMSO; 99.9%, HPLC grade, Sigma, USA), to get stock solutions of 10 mg/ml.

2.2. Animals

Three-month-old male CD1 mice (completely normal free of any pathogen) were purchased from Schistosome Biological Supply Program, Theodor Bilharz Research Institute, Giza, Egypt, and housed in Animal House of National Research Center (NRC), Egypt. Animals were housed in room maintained at 20 ± 1 °C with an alternating 12 h light–dark cycles. They were allowed to access to food and water *ad labium* throughout the acclimatization and experimental periods. All experimental protocols described in this study were in accordance with the rules and regulation of the Animal Ethics Rules, NRC, Egypt. Animals were allowed to acclimatize to the conditions for one week before the experiment.

2.3. Splenocyte proliferative activity

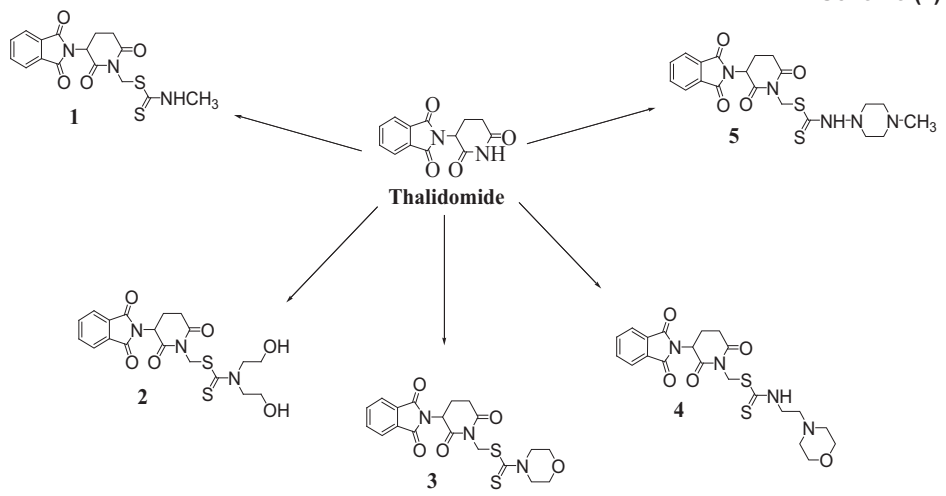
Normal mice were sacrificed and their spleens were removed under completely aseptic conditions [15]. Single cell suspensions were prepared by forcing splenic tissue through stainless steel mesh and red blood cells were lysed using Ammonium–Chlorid e–Potassium (ACK) lysing buffer (Sigma Chemical Co., St. Louis, MO, USA). The cells were then washed twice (by centrifugation for 10 min at 2000×*g*) and resuspended in incomplete RPMI-1640 medium (RPMI-1640 medium was supplemented with L-glutamine (200 mM), penicillin (100 U/ml), streptomycin (100 lg/ml), and HEPES buffer (1 M)). The viability of the cells was measured using Trypan blue stain (Sigma) and was ~90%. The cells were adjusted at a concentration of 0.5×10^5 cells/well in complete phenol-red free RPMI-1640 medium supplemented with 10% heat inactivated mycoplasma- and virus-free fetal bovine serum (FBS). The cells were cultured in triplicates in a volume of 180 µl/well into flat-bottom 96-well tissue culture plates (Griener Labortechnik, Kremsmunster, Austria). Splenocytes were incubated with various concentrations of thalidomide and different thalidomide analogs (12.5, 25, and 50 µg/ml) [14] for four (96 h) successive days at 37 °C in humidified air containing 5% CO_2 (NuAire). The effect of thalidomide analogs on splenocytes proliferation was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyltetrazolium bromide MTT assay [16]. The yellow tetrazolium salt of MTT is reduced by mitochondrial dehydrogenases in metabolically active cells to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent. At the final day of culture, 40 µl/well of MTT (5 mg/ml in PBS) was added to the culture and incubated at 37 °C for additional 4 h. MTT crystals were solubilized by adding 180 µl/well of acidified isopropanol (0.04 N HCl in absolute isopropanol) and incubated for 24 h at 37 °C. The absorbance at 570 nm was measured (FLUOstar OPTIMA; BMG Labtech GmbH, Offenburg, Germany). The data are expressed as the mean percentage of viable cells as compared to the respective control cultures treated with the solvent. All culture material was obtained from Cambrex BioScience (Copenhagen, Denmark) unless otherwise mentioned.

2.4. Macrophage function assays

2.4.1. Supernatant preparation

Single cell suspension was prepared from spleens of normal male CD1 mice (pool of 3 mice) as previously mentioned. The adherent monocytes (MQ) were removed by two cycles of splenocytes to plastic Petri dishes (100 mm, Griener). For the first cycle, splenocytes were suspended in 15 ml complete RPMI-1640 and dispensed as 5 ml/Petri dish and then incubated

Scheme (1)



Scheme 1. Thalidomide and its sulfur analogs 1–5.

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