In: Liver Cirrhosis: Causes, Diagnosis and Treatment ISBN 978-1-61209-248-5
Editor: Miranda L. Michelli

Chapter 1

Thalidomide and its Analogs: A Potential Immunomodulatory Alternative for Treating Liver Diseases and Cirrhosis

Eduardo Fernández-Martínez [*]
Laboratory of Medicinal Chemistry and Pharmacology
Cuerpo Académico de Biología de la Reproducción,
Área Académica de Medicina del Instituto de Ciencias de la Salud (I.C.Sa.)
Universidad Autónoma del Estado de Hidalgo (UAEH)

Abstract

Thalidomide is currently used for treating erythema nodosum leprosum, multiple myeloma, angiogenesis, rheumatoid arthritis, graft-versus-host disease, among others. Thalidomide effects are related to its capacity to inhibit the proinflammatory cytokine tumor necrosis factor-α (TNF-α) and, in consequence, causes immunomodulation on other cytokines. During the establishment of some diseases the balance between proinflammatory and antiinflammatory cytokines is disrupted, promoting a pathological state; thus, elevated levels of proinflammatory cytokines mediate several deleterious processes such as inflammation, necrosis, apoptosis and fibrosis. These events are present in acute and chronic degenerative liver diseases such as hepatitis, cholangitis, cirrhosis and hepatocellular carcinoma (HCC). Then, the immunomodulation on cytokines by drugs seems to be a pharmacological target to ameliorate liver damage and cirrhosis. In fact, there are not sufficient drugs for relief or cure of cirrhosis currently; some of these

[*] Address: Calle Dr. Eliseo Ramírez Ulloa no. 400,
Colonia Doctores, Pachuca, Hidalgo, México
C.P. 42090
Telephone: (52) +7717172000 ext. 4510 and 4512
E-mail: efernan@uaeh.edu.mx and tomedyfm@hotmail.com

few are expensive, unstable and palliative or may cause side effects. Novel thalidomide analogs have been synthesized with improved stability and potency as TNF-α inhibitory and immunomodulatory agents, besides low or none teratogenicity. Experimental assessment of thalidomide and its analogs in animal models of liver injury have afforded very hopeful outcomes. Thalidomide and two analogs have evidenced anticholestatic, antinecrotic and antifibrotic activities in bile duct ligation-induced biliary cirrhosis. Another analog protected D-galactosamine/endotoxin-treated mice from liver damage. Thalidomide ameliorated the alcoholic hepatic injury and prevented necrosis, cholestasis and fibrosis induced by CCl_4 in rats. Moreover, this drug salvaged from lethal hepatic necroinflammation and accelerated the recovery from established thioacetamide-provoked cirrhosis in rats. The antiinflammatory, antinecrotic and antifibrotic effects elicited by thalidomide and its analogs are mainly mediated by the inhibition on TNF-α through two different routes, as well as the down-regulation of nuclear factor-κB (NF-κB) signaling pathway and by diminishing adhesion molecules to prevent the progression of liver fibrosis and cirrhosis. Furthermore, thalidomide showed beneficial effects on HCC by decreasing angiogenesis and metastasis in murine models; therefore, diverse clinical phase I/II studies were carried out to evaluate its antitumoral or disease control outcomes. However, thalidomide as a single drug therapy yields very modest benefits, although in most cases this is well tolerated and offers disease stabilization. Different doses and the combination with other chemotherapeutic agents appear to enhance therapeutic effects; the assessment of the new thalidomide analogs in next clinical trials of HCC healing is strongly suggested. Thalidomide and its analogs may be a feasible option for the treatment of liver diseases and cirrhosis.

Introduction

History of Thalidomide

Thalidomide (Tha, α-N-phthalimidoglutarimide, Figure 1) was first synthesized in Germany in 1954 by the pharmaceutical company Chimie-Grünenthal GmbH. The former intended use for Tha was as a mild hypnotic-sedative agent similar to barbiturates but without their addictive or toxic effects (Keller et al., 1956; Somers, 1960). Grünenthal introduced Tha since 1956 in Germany wherein that was approved in 1957 as a safe sedative drug for sales over the counter. Soon thereafter, this drug was marketed in other countries including United Kingdom, the rest of Europe, New Zealand, Australia, Japan and Canada under the brand names such as "Contergan, Distaval, Talimol, Kevadon and Softenon"; however, Tha was never approved in United States because the Food and Drug Administration requested more information from Grünenthal concerning peripheral neuritis reports (Fullerton et al., 1961; Marriott et al., 1999; Teo, 2005). Meanwhile, Tha had been considered as a virtually non-toxic drug, due to its very low acute toxicity in rodent models (Somers, 1960; Williams, 1968); in addition, Tha became a very common sleep-inducing agent with very good antiemetic properties and, consequently, it began to be used by pregnant women for treating the nausea due to morning-sickness in the first trimester of gestation (Marriott et al., 1999; Teo, 2005). The reports of birth defects and deformed babies emerged at the end of 1956 to 1961, thus the strong suspicion regarding teratogenicity by Tha in man grew up; finally, in 1961 Lenz (1961; Lenz, 1992) published the first paper suggesting that Tha was responsible for limb deformities in newborn infants, this suggestion was rapidly confirmed by others as

well as experimentally in rabbits (McBride, 1961; Somers, 1962; Williams, 1968). Due to those terrible cases of amelia and phocomelia, ranging from 10000-12000 children around the world, Tha was withdrawn from the market in November 26, 1961 and during 1962. Nevertheless, by 1965 this agent had evidenced antiinflammatory properties, because Sheskin (1965) administered Tha as a sedative to leprosy patients suffering from erythema nodosum leprosum (ENL, a severe dermatological complication of Hansen´s disease formerly known as leprosy) and found amazing effects, given that clinical signs and symptoms of ENL were attenuated within 48 h. Such discovery established the basis to the future increased interest on Tha mechanism of action and the synthesis of novel safer analogs with non-teratogenic effects, as well as the further applications in the treatment of several inflammatory, degenerative and chronic diseases.

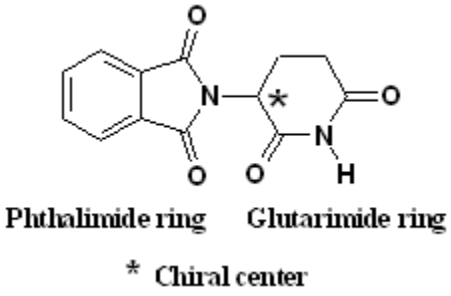

Figure 1 Thalidomide chemical structure.

Thalidomide Chemical Properties

The chemical name of Tha is 2-(2,6-dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione and it is composed by two imide moieties, the phthalimide ring and the glutarimide ring; its empiric formula is $C_{13}H_{10}N_2O_4$, with a molecular weight of 258.2 g/mol. Tha is a white, tasteless crystalline powder with a melting point of 269-271°C. This drug is sparingly soluble in water (6 mg/100mL), methanol, ethanol, acetone, and glacial acetic acid but readily soluble in acetone, dioxane, dimethyl formamide, pyridine, chloroform, and dimethyl sulfoxide. It is insoluble in ether and benzene (Somers, 1960). Its low solubility in water would suggest that its concentration in body fluids at any time would be small (Williams, 1968), so the efforts to get Tha dissolved have led to prepare solutions in alkaline pH that promotes the spontaneous hydrolysis of Tha; in consequence, such solutions contain Tha in addition to its hydrolysis products. The main route of hydrolysis of Tha at pH 6, 7.4 and 8 is cleavage of the phthalimido ring to α-(o-carboxybenzamido)glutarimide; this compound is reasonably stable at these pH values, however, as the pH is increased the bonds of glutarimide ring become susceptible to hydrolysis and, at pH 7.4 and 8 especially, considerable amounts of 2- and 4-phthalimidoglutaramic acids are formed (Schumacher et al., 1965a). It suggests that Tha biological effects may also be shared by its hydrolysis products or its metabolites, thus, this compound may act as a prodrug for one or more of those Tha derivatives (Fabro et al., 1965; Muller et al., 1996). In addition, Tha possesses a single chiral center in the glutarimide ring, an asymmetric carbon that originates the R and S enantiomers; thus, Tha is administered as a racemic mixture of (-)-(S)- and (+)-(R)-enantiomeric forms. It has been demonstrated for Tha

that there is a relationship between quirality and its biological effects (Blaschke et al., 1979); this point will be commented farther on.

Thalidomide and its Analogs: Characteristics and Mechanisms of Action

Nowadays, Tha has showed several mechanisms of action as an antiinflammatory and immunomodulatory drug because of its broad range of inhibitory and stimulatory effects on the immune system (Zwingenberger and Wnendt, 1995; Mujagić et al., 2002). However, so far the most plausible mechanism is the inhibition on the production of the important, pleiotropic, pronecrotic and proinflammatory cytokine tumor necrosis factor-α (TNF-α) (Sampaio et al., 1991), through enhancing the TNF-α mRNA degradation (Moreira et al., 1993). On this way, other authors have proposed that Tha has also immunomodulatory activity by two more routes: a) reducing the number of IgM plaque-forming cells and b) enhancing the secretion of the cytokine interleukin-2 (IL-2) in peripheral blood mononuclear cells (PBMC) (Shannon et al., 1997). Regarding other immunomodulatory effects, it has been observed that Tha has a costimulatory role in the upregulation of T helper 2 (Th2)-type immunity, because Tha increases the production of Th2-type (humoral) cytokines, for example interleukin-4 (IL-4) and interleukin-5 (IL-5); as well as inhibits the production of the Th1-type (cellular) cytokine interferon-γ (IFN-γ) in PBMC (McHugh et al., 1995; Marriott et al., 1999), while in T cells Tha induces a high production of IFN-γ and IL-2 (Corral et al., 1999).

There are other possible immunomodulatory and/or regulatory mechanisms of Tha on diverse endogen mediators of the immune and inflammatory response. It deserves special consideration the first report concerning the inhibition on the activation of the nuclear factor-κB (NF-κB) in HIV-infected primary macrophages (Moreira et al., 1997). NF-κB is a transcription nuclear factor that has received much attention since its discovery in 1986, because of its activation by many different stimuli and its diverse and prominent roles in maintaining the homeostasis, control of disease development, regulation of cell survival and activation of innate and adaptive immune responses, for instance, its activation provokes the production of proinflammatory cytokines including the most potent TNF-α (Sun and Karin, 2008). On this way, there are reports that support that Tha strongly suppresses at different levels the NF-κB activation induced by TNF-α and reactive oxygen species (ROS) such as H_2O_2, besides this inhibition is apparently not cell type specific, although these effects are not seen during the NF-κB activation by other inducers (Majumdar et al., 2002; Kim et al., 2004). This likely mechanism of action has prompted the design and synthesis of NF-κB inhibitors derived from Tha (Carcache de-Blanco et al., 2007).

Cyclooxigenase-2 (COX-2) is the inducible enzyme that catalyzes the synthesis of prostaglandins (PG) which are very well known potent endogen proinflammatory agents and that regulate the cytokines expression. COX-2 is induced by bacterial lipopolysaccharides (LPS) and it has been considered as a pharmacological target for the prevention and treatment of angiogenesis and cancer; indeed, Tha has evidenced promising effects as inhibitor of LPS-induced COX-2 (Fujita et al., 2001). Furthermore, novel Tha analogs have been recently synthesized and/or evaluated as inhibitors of COX-2 (Suizu et al., 2003; Fujimoto et al., 2006).

Other via of regulation by Tha is achieved through diminishing the nitric oxide (NO) production and its multiple biological actions by two possible ways, a) decreasing the TNF-α synthesis, since this cytokine as well as interleukin-1β (IL-1β) and IFN-γ are important mediators of NO production, because they regulate the expression of the inducible nitric oxide synthase (iNOS) (López-Talavera et al., 1996) and b) it has been also demonstrated that Tha possesses weak but significant inhibitory activity on iNOS, what encouraged the synthesis of Tha-related inhibitors of NOS (Shimazawa et al., 2004); moreover, some authors have designed their counterpart, some NO-donating Tha analogs as anticancer agents (Wang et al., 2009).

There is another feasible immunomodulatory mechanism of action of Tha, it has been showed that this drug binds to a pair of proteins identified as isoforms of the α_1-acid glycoprotein (α_1-AGP), suggesting a potential role for α_1-AGP as a mediator of the pharmacological effects of Tha; additionally, Tha analogs do not compete for that binding site (Turk et al., 1996; Niwayama et al., 1998). α_1-AGP is one of the major acute phase proteins in humans, rats, mice and other species; its concentration is elevated in response to systemic tissue injury, inflammation or infection, and these changes in serum protein concentrations have been correlated with increases in hepatic synthesis. The α_1-AGP expression is regulated by cytokines such as TNF-α, IL-6 and IL-1β; although the exact physiological role of α_1-AGP remains still to be completed, this protein is considered as a natural antiinflammatory and immunomodulatory endogen agent (Fournier et al., 2000; Hochepied et al., 2003).

Since Tha possesses valuable therapeutic properties but also teratogenic activity and other side effects, various groups of research in medicinal chemistry and pharmacology around world have synthesized and assessed diverse families of Tha analogs in order to augment the chemical stability, the potency as TNF-α inhibitors and their immunomodulatory efficacy, besides lowering the adverse effects (Corral et al., 1996; Marriott et al., 1998; Muller et al., 1998; Muller et al., 1999; Hashimoto, 2008; Zahran et al., 2008; Man et al., 2009). Some Tha analogs have been synthesized resembling its hydrolysis products (Muller et al., 1996) and others are similar to its hydroxylated metabolites that have been demonstrated to be potent immunomodulators (Yamamoto et al., 2009). Among the many families of novel and promising Tha analogs, there are two of them that are outstanding as potent TNF-α inhibitors; the first group is composed by molecules structurally and functionally very similar to Tha, known as immunomodulatory drugs because of their marked costimulatory properties on T cells promoting the secretion of IL-2 and IFN-γ, but these compounds also inhibit the production of IL-1β, IL-6 and IL-12 as well as greatly increase the synthesis of IL-10, the main antiinflammatory Th2-type cytokine in stimulated PBMC (Corral et al., 1999; Schafer et al., 2003). The most important members of this immunomodulatory group are pomalidomide (CC-4047, Actimid™) and lenalidomide (CC-5013, Revlimid™) (Teo, 2005); both drugs belong to a novel generation of amino-substituted Tha analogs in the phthalimide ring (Muller et al., 1999) (Figure 2).

On the other hand, the second group of effective TNF-α inhibitors was synthesized resembling the structure of Tha hydrolysis products (Corral et al., 1996; Muller et al., 1996), they also increase IL-10 (Marriott et al., 1998; Corral et al., 1999) and possess biological activity as potent phosphodiesterase-4 (PDE-4) inhibitors while Tha is not, therefore, it cannot be excluded that one or more of the Tha metabolites or degradation products may inhibit that enzyme (Muller et al., 1998). PDE-4 is the primary enzyme that catalyzes the

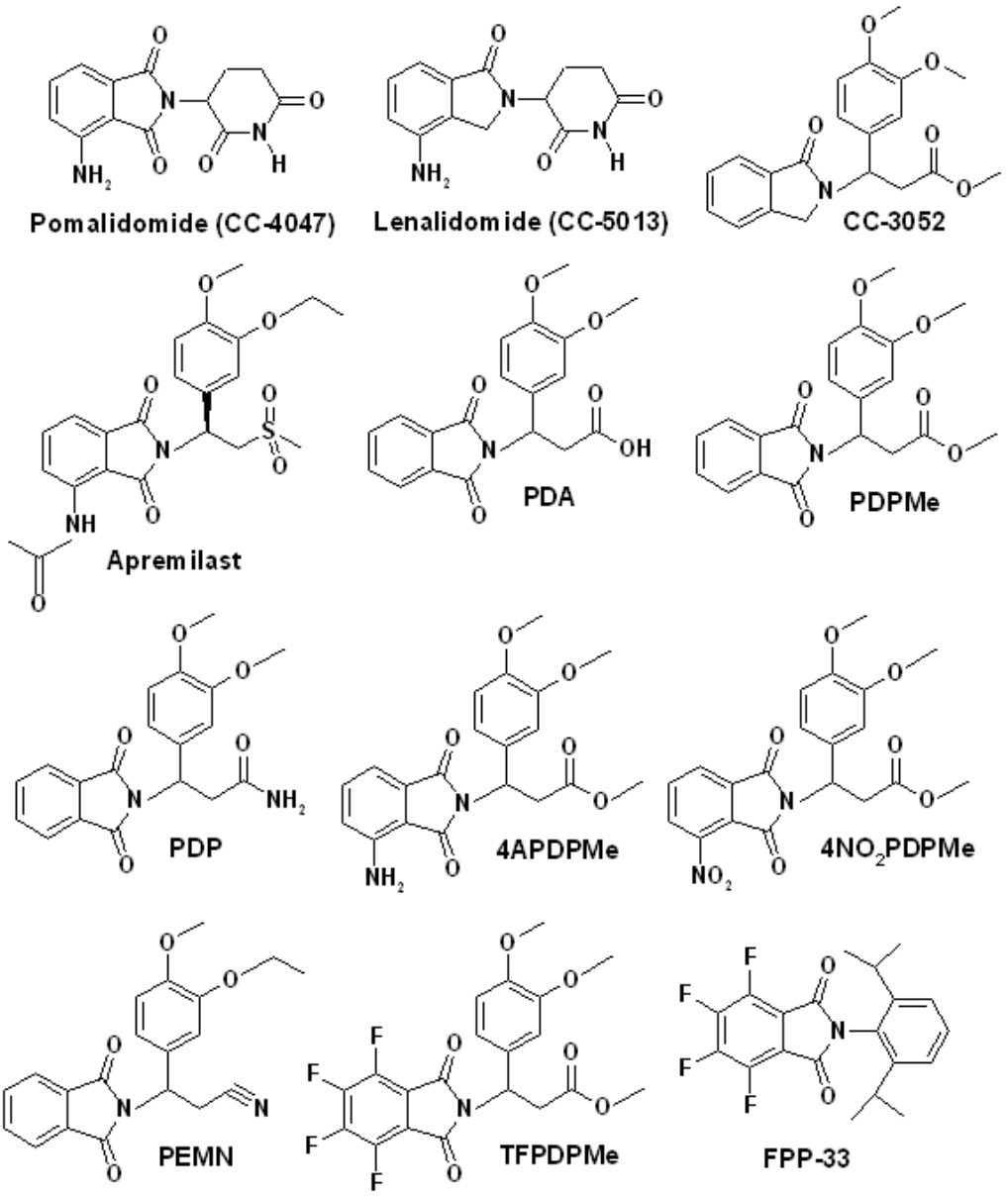

Figure 2 Some structures of thalidomide analogs.

hydrolysis of adenosine 3´,5´-cyclic monophosphate (cAMP), as a result, the inhibitors of PDE-4 are cAMP-elevating agents (Bender and Beavo, 2006). It is well documented that augmented levels of cAMP inhibit the TNF-α production (Cheng et al., 1997; Kast, 2000). Thus, inhibition on PDE-4 has been shown to be an effective via for inhibition of TNF-α production in activated monocytes and PBMC by this group of compounds, especially for perfluorinated Tha analogs (Muller et al., 1998; Niwayama et al., 1998). In addition, stable analogs of cAMP, adenylyl cyclase activators or PDE inhibitors are capable of reducing the activation of NF-κB and in consequence lowering TNF-α as well as other proinflammatory cytokines (Gantner et al., 1997). Some compounds that belong to this group are: PDA, 3-

phthalimido-3-(3,4-dimethoxyphenyl)-propanoic acid; PDP, 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanamide; PDPMe, methyl 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanoate; $4NO_2$PDPMe, methyl 3-(4-nitrophthalimido)-3-(3,4-dimethoxyphenyl)-propanoate; 4APDPMe, methyl 3-(4-aminophthalimido)-3-(3,4-dimethoxyphenyl)-propanoate; TFPDPMe, methyl 3-tetrafluorophthalimido-3-(3,4-dimethoxyphenyl)-propanoate; PEMN, 3-phthalimido-3-(3-ethoxy, 4-methoxyphenyl)-propanitrile (Muller et al., 1996; Muller et al., 1998). Currently, apremilast (CC-10004) is perhaps the most promising drug of this family (Man et al., 2009) (Figure 2).

Concerning the mechanisms of action of Tha and its biological effects, there are reports about the pharmacological differences between the two Tha enantiomers, while (+)-(R)-Tha exhibited significant positive influences on all sedative effects, the (-)-(S)-Tha had a significant effect in the opposite direction, because it has shown the dose-dependent teratogenic activity (Blaschke et al., 1979), besides a superior effect in preventing splenomegaly in chicken embryos (Field et al., 1966) and in immunomodulation (Muller et al., 1999). Other studies have reported that this drug possesses bidirectional immunomodulatory properties, inhibiting or enhancing the TNF-α production depending on the enantiomer administered, R or S (Miyachi et al., 1996), as well as these differential effects have been observed in diverse cellular cultures or by using diverse cytokine inducers or immune stimulatory agents; thus, Tha enhances 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced TNF-α production by human leukemia HL-60 cells, while it inhibits TPA-induced TNF-α production by another leukemia cell line called THP-1, although Tha inhibits TNF-α production by both cell types when they are stimulated with okadaic acid (OA) (Miyachi et al., 1997a; Miyachi et al., 1997b). Furthermore, some research groups are looking for TNF-α bidirectional modulators based on Tha derivatives, for example the compound FPP-33 (Figure 2) (Hashimoto, 2008; Fernández-Braña et al., 2009). Therefore, experimental differences in effectiveness between thalidomide and its analogs might be observed due to variations in potency, stability, metabolism, pharmacokinetics, bioavailability, enantiodependence, cellular type or to a bidirectional cytokine regulation; besides the dose, via of administration, time courses, animal species and schedule of treatment could be other striking factors capable of leading to a variation in the desired pharmacological immunomodulation. Although further studies are required to clarify and optimize immunomodulatory effects, the hopeful outcomes of these compounds on cytokines, at systemic and hepatic level, suggest a wide perspective to use them as immunomodulatory agents in liver diseases; this will be analyzed later on. Tables 1 and 2 summarize some immunomodulatory effects on very important cytokines as well as some possible mechanisms of action of Tha and its most remarkable analogs.

Thalidomide and its Analogs: Pharmacokinetics and Metabolism

The therapeutic dose of Tha as a racemic mixture is normally 100-400 mg/day for multiple myeloma patients and higher doses of 600-1600 mg/day in graft-versus-host disease treatments (Eriksson et al., 1995; Mujagić et al., 2002; Kamikawa et al., 2006). The absolute bioavalability of Tha after oral intake is limited as the drug is slowly absorbed from the gastrointestinal tract, the total absortion of Tha increases proportionally with the increase in

Table 1. Immunomodulatory properties on cytokines of some thalidomide analogs

Analogs	TNF-α IC_{50} (μM)	IL-1β IC_{50} (μM)	IL-6 IC_{50} (μM)	IFN-γ in T cells	IL-10	Times more potent than Tha inhibiting TNF-α
Thalidomide (Tha)	194	↓ Yes	↓ Yes	↑ Yes	↑ Yes	1.00
$4NO_2$PDPMe	64	NA	NA	NA	NA	3.03
PDA	60	↓ Yes	NA	NA	↑ Yes	3.23
PDP	12-13	↓ Yes	NA	↓ Yes	↑ Yes	14.92
PDPMe	2.90	↓ Yes	↓ Yes	NA	Yes	66.89
CC-3052	1.22	↓ ---	↓ ---	NA	↑ Yes	159.01
4APDPMe	0.45	↓ Yes	↓ Yes	NA	↑ Yes	431.11
TFPDPMe	0.26	0.40	0.30	NA	NA	746.15
PEMN	0.12	↓ Yes	↓ Yes	NA	↑ Yes	1616.66
Lenalidomide	0.10	↓ Yes	↓ Yes	↑ Yes	↑ Yes	1940.00
Apremilast	0.077	NA	NA	NA	NA	2519.48
Pomalidomide	0.013	↓ Yes	↓ Yes	↑ Yes	↑ Yes	14923.07

--- No effect or slightly; ↑, Increases its production; ↓, Inhibits its production; NA, not available; IC_{50}, inhibitory concentration-50 (micromolar, μM) on TNF-α production as well as their effects on other cytokines produced by LPS-stimulated peripheral blood mononuclear cells (PBMC), except for IFN-γ in T cells. Tha ↓ IFN-γ in PBMC.

Table 2. Possible molecular mechanisms of action of thalidomide and some analogs

Analogs	PDE-4 IC_{50} (μM)	Binding to α1-AGP	TNF-α mRNA Degradation
Thalidomide	>500	Yes	↑ Yes
$4NO_2$PDPMe	NA	NA	NA
PDA	NA	NA	No
PDP	9.40	NA	No
PDPMe	2.50	NA	No
CC-3052	3.00	NA	No
4APDPMe	NA	NA	NA
TFPDPMe	4.70	No	No
PEMN	0.13	NA	NA
Apremilast	0.074	NA	NA
Lenalidomide	>100	NA	NA
Pomalidomide	>100	NA	NA

IC_{50}, inhibitory concentration-50 (micromolar, μM) on phosphodiesterase-4 (PDE-4) enzyme activity; ↑, increases its degradation; NA, not available; α1-acid glycoprotein (α1-AGP).

dosage from 200 to 1200 mg given once or twice daily. However, peak plasma concentrations increase in a less than proportional manner and the time to peak plasma concentration is delayed, indicating that Tha poor aqueous solubility affects the rate of dissolution and absorption after oral intake. In healthy men, peak plasma levels (maximal concentration, C_{max}) of 0.8-1.4 µg/mL were obtained in a mean of 4.4 h following a single 200 mg oral dose (Mujagić et al., 2002; Richardson et al., 2002a). In mice, fed with a diet containing 0.03 % w/w of Tha, this drug reached a mean plasma concentration of 0.8 µg/mL (Shannon et al., 1981) which is an equivalent concentration of 0.9 µg/mL quantified in the blood of a man following a single oral dose of 100 mg of Tha (Faigle et al., 1962; Shannon et al., 1997); furthermore, concentrations as high as 5.0 µg/mL of Tha in the blood have been reported when patients were given 200 mg four times a day (Vogelsang, et al., 1992). A study in eight healthy male volunteers, whom received once an oral dose of 200 mg of Tha, reported a volume of distribution of 120.7 L, plasma C_{max} of 1.15 µg/mL, time to maximal plasma concentration (t_{max}) at 4.39 h, a mean absortion half-life of 1.7 h and a mean elimination half-life at 8.7 h in a monocompartament model with renal excretion of 0.6 % of initial dose; that elimination half-life is about three times longer than that observed in animals, besides that the total body clearance rate is relatively slow 10.41 L/h (Chen et al., 1989). Other authors have reported pharmacokinetic parameters for Tha in multiple myeloma (MM) patients in order to offer more accuracy in data in that disease, for example, after a single dose of 200 mg of Tha the C_{max} was 1.39 µg/mL, t_{max} at 4.8 h, area under curve (AUC) 81.0 µmol·h/L and the elimination half-life at 7.6 h; all those parameters were also compared with data from mice and rabbits administered with Tha (Chung et al., 2004). Differences among races should be taken into account when Tha is administered for the treatment of any illnesses because the pharmacokinetic parameters may be affected as well as lower or higher effective doses would be required (Kamikawa et al., 2006).

In spite of the efforts to separate the R and S Tha enantiomers in order to get also both pharmacological activities apart without interference on each other, it has been observed that Tha enantiomers racemize in vitro (Knoche and Blaschke, 1994) as well as after the administration of one pure enantiomer it is racemized in vivo; the pharmacokinetic parameters have been reported, the mean rate of chiral inversion of (+)-(R)-Tha and (-)-(S)-Tha in blood at 37 °C were 0.30 and 0.31/h, respectively. In addition, rate constants of degradation were 0.17 and 0.18/h as well as mean rates constants for in vivo inversion were 0.17/h (R to S) and 0.12/h (S to R), predominating (+)-(R)-Tha at equilibrium; also, mean elimination rate constants were 0.079/h (R) and 0.24/h (S). Furthermore, a considerable faster rate of elimination of the (-)-(S)-Tha was seen and the mean apparent terminal half-life of the Tha enantiomers was 4.7 h (Eriksson et al., 1995). The interconversion of enantiomers under quasi-physiological conditions has been observed inclusive with Tha analogs, as the case of pomalidomide. The S-isomer of pomalidomide, which is more effective as immunomodulatory agent than the R-isomer, undergoes a rapid racemization in 2 h, suggesting that there is no advantage in developing the single isomer (Teo et al., 2003). Pharmacokinetic data for lenalidomide is still in development although in healthy volunteers the oral absorption is rapid, reaching the C_{max} between 0.625 and 1.5 h post-dose. Food reduces the C_{max} by 36 %, its pharmacokinetics is linear and the AUC of 0.8 to 1.2 µmol·h/L increases proportionately with dose. In patients suffering from MM the C_{max} occurs between 0.5 and 4 h and renal half-life of elimination is around 3.1 to 4.2 h (Richardson et al., 2002b).

Regarding the family of Tha analogs inhibitors of PDE-4, the only related data to pharmacokinetics are the half-lives reported in human plasma at 37 °C that reflects the drug stability (Corral et al., 1996), for example, as the case of PDP its half-life is around 8 h, for PDPMe approximately 3 h and an analog which resembles to the 4APDPMe that has a half-life of about 4 h. The most stable Tha analog is CC-3052, which exhibits increased stability in human plasma with a half-life of around 17.5 h versus 1.5 h for Tha (Marriott et al., 1998). Apremilast pharmacokinetic parameters were reported in female rats orally administered with 10 mg/kg, such as C_{max} of 1100 ng/mL, AUC of 1400 ng·h/mL, half-life of 5 h with 64 % absorbed (Man et al., 2009). Differences in half-lives may reflect the solubility and stability of each compound in organism and therefore their bioavailability; then, this is a very important factor because some analogs could be metabolized or eliminated from organism at different rates without reaching a significant immunomodulatory effect, a dose adjustment may be required accordingly.

When Tha is administered orally to animals, only a small amount of the unchanged drug is excreted in the urine and the major portion of the compound is broken down and excreted as at least 20 transformation products (Schumacher et al., 1965b); in dogs, the little unchanged Tha is excreted in feces preferentially. The biotransformation of Tha can occur by non-enzymatic hydrolysis, just by spontaneous rupture catalyzed by alkaline pH, as it has been mentioned above. Also, Tha is metabolically labile because of its biotransformation by hepatic P450 enzyme-mediated hydroxylation, specifically CYP2C (Ando et al., 2002); two major metabolites of Tha are 5-hydroxyTha and 5´-hydroxyTha to form then a multitude of metabolites that have been isolated (Lepper et al., 2006). The hydrolysis products have been detected in diverse species as well as the proportion of hydroxylated metabolites is higher in mice than in rats and rabbits while these are almost undetectable in healthy volunteers and MM patients (Chung et al., 2004). Hence, the possibility should be considered that the teratogenic side effect or/and other multiple pharmacological activities might be due to the interspecies differences in its pharmacokinetics and metabolites, including their enantiomers, rather than Tha itself (Chung et al., 2004; Yamamoto et al., 2009). Lenalidomide is eliminated unchanged through kidneys (two thirds of dose) in healthy volunteers and it has been thought it that undergoes a higher hepatic metabolism than Tha.

Current Uses for Thalidomide and Some Analogs

Thanks to the discovery by Sheskin (1965) that Tha possessed striking effects on the patients suffering from the cutaneous manifestations of ENL, in July 16, 1998, thalidomide reemerged, since the Food and Drug Administration of United States approved its use for the treatment of such disease (Annas and Elias, 1999). A short time later after 1965, Tha was successfully tried in the graft-versus-host reaction in rats and man (Field et al., 1966; Vogelsang et al., 1992); inclusive, there are current reports on this Tha application (Ratanatharathorn et al., 2001). Based on its immunomodulatory properties that evoke an antiinflammatory effect, Tha has been tested in clinical studies for treating MM wherein it has shown high efficacy (Venon et al., 2009); in addition, its analog lenalidomide gained the FDA approval in June 2006 for treating relapsed and refractory MM (Chen et al., 2009; Magarotto and Palumbo, 2009). Tha inhibitory effects on angiogenesis (D'Amato, et al., 1994) opened its potential use for the treatment of many diverse diseases depending on that process, such as

cancer solid tumors (Richardson et al., 2002a) as well as for the use of Tha analogs in the management of those ailments (Marriott et al., 1999; Teo, 2005). Rheumatoid arthritis is a chronic and inflammatory autoimmune-related disease; Tha and its analogs have also been tried in this illness with hopeful results (Gutierrez-Rodriguez et al., 1989; Oliver et al., 1999). Tha and lenalidomide showed to be a successful tool in the treatment of Behçet's disease (Direskeneli et al., 2008; Green et al., 2008). Furthermore, thalidomide has beneficial properties to controlling the aphthous ulcers and cachexia associated with HIV/AIDS (Jacobson et al., 1997; Marriott et al., 1999). Crohn's disease is an autoimmune inflammatory bowel disease in which Tha has been reported to be an effective treatment for patients with refractory episodes; additionally, there are current trials to evaluate Tha analogs in such illness (Mansfield et al., 2007; Gordon et al., 2009).

As Tha and its analogs are clinically or experimentally being tried in many chronic, degenerative and inflammatory diseases, there is a potential risk of producing teratogenic outcomes in possible pregnant patients being administered with these drugs, hence, it has been created the system for thalidomide education and prescribing safety (STEPS) that is a strict as well as comprehensive program to control and monitor access to Tha or its analogs (Zeldis et al., 1999). Although Tha and some analogs display teratogenic effects it is important to keep in mind that their main therapeutic applications are for the treatment of severe, chronic and degenerative diseases wherein patients must not get pregnant (Annas and Elias, 1999).

Cytokines and Liver Damage

Immunomodulation on Cytokines: Restoring the Proinflammatory and Antiinflammatory Disequilibrium

Cytokines are proteins or peptides, some of them glycosylated, produced by cells, mainly immune cells, in response to a variety of inducing stimuli, with growth differentiation and activation functions that regulate the nature of the immune responses or that influence the behavior of other different cell types. Cytokines are involved in nearly every facet of immunity and inflammation, from induction of the innate immune response to the generation of cytotoxic T cells and the development of antibodies by the humoral immune system, as well as they coordinate the functions of the immune system with the rest of body. The combination of cytokines that are produced in response to an immune insult determines which arm of the immune system will be activated. Consequently, cytokines are most distinguished for their activities associated with inflammation, immune reactivity, tissue injury or repair and organ dysfunction. At present, at least 70 candidate cytokines are known or genetically predicted, they are grouped as interferons (IFNs), interleukins (ILs), colony-stimulating factors (CSFs), transforming growth factors (TGFs) and tumor necrosis factors (TNFs) (Clemens, 1991; Simpson et al., 1997; Rose-John, 2002; Kidd, 2003; Steinke and Borish, 2006). The distinctive pattern of effect of each cytokine depends on concentration-dependent binding to specific receptors on the surface of the target cells and subsequent activation of cellular machinery at the cell membrane level, or in some cases, at the level of the nucleus

and genetic machinery (Kidd, 2003); in this regard, two excellent reviews are recommended (Grötzinger, 2002; Ishihara and Hirano, 2002).

One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) cell types activity. The Th1/Th2 hypothesis arose from 1986 research suggesting mouse T-helper cells expressed differing cytokine patterns and other functions. This hypothesis was adapted to human immunity, with Th1 and Th2 cells directing different immune response pathways. Th1 cells drive the type-1 pathway of cellular immunity to fight viruses and other intracellular pathogens or eliminate cancerous cells. Th2 cells drive the type-2 pathway of humoral immunity, allergic responses and up-regulate antibody production to fight extracellular organisms such as elimination of parasites; overactivation of either pattern can cause disease, and either pathway can down-regulate the other. However, such hypothesis cannot explain several immune actions because of human cytokine activities rarely fall into exclusive pro-Th1 or -Th2 patterns. Th1 cells produce several characteristic cytokines, most notably IFN-γ, IL-2 and IL-12 whereas Th2 cells produce a set of cytokines, most notably IL-4 and IL-5. IL-10, formerly assumed to be the major means by which Th2 cells down-regulate Th1 cells, is also produced in comparable amounts by other cell types (Farrar et al., 2002; Kidd, 2003).

As commented above, cytokines are categorized into Th1 and Th2-type cytokines, and it seems that Th1 cytokines behave as proinflammatory mediators involved in the pathogenesis of several diseases, included liver injury; for example, IL-12 and IFN-γ are known to be representative proinflammatory Th1 cytokines and play a crucial role in the host defense against bacterial infection in liver (Seki et al., 2000; Masubuchi et al., 2009), as well as the most important proinflammatory cytokines TNF-α, IL-6 which also possesses antiinflammatory activities, and IL-1β (Rizzardini et al., 1998; Cartmell et al., 2000). Additionally, numerous cytokines have predominantly antiinflammatory effects, including IL-1Ra, transforming growth factor-β (TGF-β) although this is a profibrogenic cytokine in liver as it will be discussed later, IL-22, and the most important antiinflammatory cytokine IL-10 (Louis et al., 2003; Steinke and Borish, 2006).

When an etiological agent is present in the organism this causes a disruption on the normal antiinflammatory/proinflammatory cytokines equilibrium, often up-regulating excessively the proinflammatory cytokines and as a consequence down-regulating the antiinflammatory ones; if this event is strong enough in an acute manner or chronically persistent, this may set the basis for a disease. Thus, the immunomodulation is intended to reestablish the balance of cytokines into the normal homeostatic levels, without depleting any of them or exacerbating others. Th1/Th2 regulation is exceedingly complex, but its importance is unquestionable, particularly in the study of diverse diseases and autoimmune disorders. This is an active area of research for the design of immunomodulatory therapies proposed either to dampen overreactive responses or to strengthen weak ones. Magic bullets and master switches may be rare commodities in this area; nevertheless, defining all the mechanisms controlling these processes and the use of immunomodulatory compounds such as Tha and its derivatives will help make rational therapies to manipulate Th1/Th2 balance during diseases (Farrar et al., 2002).

Cytokines Role in Deleterious Processes of Liver Damage

Cytokines have been implicated in the pathogenesis and progression of chronic liver disease (CLD). Indeed, the cellular source and biological target of cytokines are not restricted to cells of the immune system, as in the liver endothelial cells, stellate cells (Ito cells, fat-storing cells or activated myofibroblasts), hepatic resident macrophages called Kupffer cells (may be the most important source in liver) and hepatocytes are capable of producing and responding to a number of different cytokines. Additionally, the liver is an important organ in the metabolism of cytokines. All cells normally resident in the liver have the capacity to produce cytokines, which by stimulating surrounding cells (paracrine effect) or themselves (autocrine effect) leads to a further cytokine production and an amplification of an inflammatory response. While some cytokines are released by resting cells in liver, the concentrations and variety of cytokines released are considerably increased following stimulation by a variety of inducers, such as bacterial endotoxin or lipopolysaccharides (LPS), viruses, chemical agents, cancer, liver ischaemia-reperfusion and alcohol consumption (Simpson et al., 1997). Hepatic uptake of circulating cytokines is inhibited by alcohol and this may contribute to the elevated levels of TNF-α and IL-6 observed in such patients, in fact, cytotoxic cytokines likely induce liver cell death by both necrosis and apoptosis in alcoholic liver disease. Anticytokine therapy has been highly successful in attenuating cell injury/death in a variety of toxin-induced models of liver injury, including alcohol-related liver injury (McClain et al., 1999).

There are many hepatic disorders or diseases wherein the effect of cytokines has been proved as a key part of those deleterious processes, as well as during liver injury and inflammation; furthermore, cytokines also are implicated in the normal hepatic function and metabolism as well as in liver regeneration (Simpson et al., 1997; Galun and Axelrod, 2002; Gao, 2005). Particularly, the proinflammatory and pronecrotic TNF-α functions as two edged sword in the liver, this cytokine is required for normal hepatocyte proliferation during liver regeneration. It functions both as a comitogen and to induce the transcription factor NF-κB which has antiapoptotic effects. On the other hand, TNF-α is the mediator of cholestasis and hepatotoxicity in many animal models, including those involving the toxins concanavalin-A and LPS. TNF-α has also been implicated as an important pathogenic mediator in patients with alcoholic liver disease and viral hepatitis (Green et al., 1996; Bradham et al., 1998).

In experimental animal models, such as the bile duct ligation (BDL) in rats that induces a stable and frank secondary biliary cirrhosis by chronic cholestasis, the plasma and liver levels of proinflammatory/antiinflammatory cytokines and NO are modified when compared to normal rats; those changes are related to biochemical markers of cholestasis, necrosis and fibrosis (Fernández-Martínez et al., 2006). Whereas in clinical studies, the serum levels of IL-1β, IL-6, TNF-α, IFN-γ, and C-reactive protein (CRP) have been found elevated in patients with CLD. Cirrhotic patients with CLD show higher serum levels in IL-1β, IL-6, TNF-α, and CRP than noncirrhotic cases. Elevated concentrations of cytokines represent a characteristic feature of CLD regardless of underlying disease (Tilg et al., 1992). Regarding the cytokine gene expression in cirrhotic and noncirrhotic human liver, data suggest that TGF-β has a predominant role in liver fibrosis. Whereas, IL-1β, 6, 8, TNF-α, and IFN-γ, appear to participate in the pathogenesis of the mild to severe inflammatory phenomena seen in alcoholic and post-hepatitis C liver cirrhosis, respectively. Also, data indicate that neither

IFN-γ nor IL-10, at least at the levels observed in post-hepatitis C liver cirrhosis, are able to counteract the fibrotic/inflammatory processes (Llorente et al., 1996).

Summarizing there is increasing evidence that several cytokines mediate the deleterious hepatic inflammation, apoptosis, and necrosis of liver cells, as well as the characteristic chronic processes cholestasis and fibrosis, previous to the end stage cirrhosis (Tilg, 2001). Throughout CDL the relationship among chronic hepatocellular damage, liver inflammation and cirrhosis has not been clearly defined, but cytokines could be the common link between these complex pathological outcomes (Llorente et al., 1996).

Thalidomide Effects on Liver Damage and Cirrhosis

Thalidomide Effects on Liver Damage

Alcoholic liver disease is maybe one of the most important hepatopathies around the world. In this regard, the effect of Tha has been studied in an animal model of alcohol-induced liver damage; the sensitization of Kupffer cells to LPS and the overproduction of TNF-α are critical for progression of alcoholic liver injury. The treatment with ethanol for 8 weeks caused marked steatosis, necrosis, and inflammation in the liver. These pathologic parameters were diminished markedly by treatment with Tha. In a 4-week ethanol group, the LPS-induced liver damage was aggravated and Kupffer cells were sensitized to LPS. Coadministration of thalidomide with ethanol prevented the sensitization of those cells completely. Furthermore, thalidomide abolished the LPS-induced increase in CD14 (this is a functional LPS receptor on macrophages/monocytes and neutrophils) expression and intracellular calcium concentration $[Ca^{2+}]i$ elevation in the macrophages. Moreover, thalidomide reduced the LPS-induced TNF-α production by Kupffer cells by decreasing TNF-α mRNA. Thus, Tha prevented alcoholic liver injury through suppression of TNF-α production and abolishment of Kupffer cells sensitization (Enomoto et al., 2002; Enomoto et al., 2004)

Activation of Kupffer cells by LPS plays a pivotal role in the onset of pathophysiological events that occur during endotoxemia and septic shock, as well as $[Ca^{2+}]i$ is involved in LPS-stimulated cytokine production, as the case of TNF-α which is mostly produced by Kupffer cells. TNF-α plays a key role in the initiation and progression of multiple organ failure syndrome induced by septic shock as well as in the cholestasis provoked by sepsis (Van Amersfoort et al., 2003; Moseley, 2004). Enomoto and coworkers (2003) determined whether Tha could prevent LPS-induced liver injury, they found that LPS caused focal necrosis with neutrophil infiltration in the liver. Moreover, LPS dramatically increased ALT/AST. These pathologic parameters and increases of serum transaminases were diminished markedly by Tha. In isolated Kupffer cells, LPS-induced increases in [Ca2+]i and TNF-α production were suppressed by treatment with Tha. To further explore the mechanism by which Tha directly abrogated Kupffer cell sensitivity to LPS, they determined the effect of Tha in vitro on LPS-induced [Ca2+]i response and TNF-α production. With the addition of Tha to the culture media before LPS, these parameters were suppressed. They concluded that Tha prevents LPS-induced liver injury via mechanisms dependent on the suppression of TNF-α production from

Kupffer cells. The immunomodulatory effects of Tha have been evidenced in other two models of sepsis, the first of Escherichia coli sepsis in vivo in rat as well as in in vitro by using human monocytes (Giamarellos-Bourboulis et al., 2003), and the second in sepsis by multidrug-resistant Pseudomonas aeruginosa (Giamarellos-Bourboulis et al., 2005); in those studies Tha inhibited the microbial-induced NO, TNFα and IL-1β but not IL-6, as well as increased the survival rate in septic rats.

Concerning the evaluation of Tha analogs in animal models of liver injury as hepatoprotective drugs, there are three current reports. Thiele and coworkers (2002) synthesized and assessed the immunomodulatory and hepatoprotective properties of a Tha analog named TFBA, which was found to be an TNF-α, IL-6 and IL-10 inhibitor in isolated and stimulated monocytes; this drug is not either a PDE-4 inhibitor or costimulator of T cells. When TFBA was administered to mice with hepatic injury by galactosamine/LPS, it diminished the ALT activity, TNF-α production but not IL-6; however this drug increased the hepatoprotective IL-10. Furthermore, a serial of Tha analogs have been synthesized and evaluated as immunomodulatory agents in liver and plasma in an acute model of LPS-induced septic challenge in rat. Animal groups were twice administered with Tha or its analogs in an equimolar dose. Two hours after last dose, rats were injected with saline or LPS. The cytokines TNF-α, IL-6, -1β and -10 were quantified and studied in plasma and liver. After two hours of LPS-induction, different patterns of measured cytokines were observed with Tha analogs administration evidencing their immunomodulatory effects in both tissues. Interestingly, some analogs decreased significantly plasma and hepatic levels of LPS-induced proinflammatory TNF-α and others increased plasma concentration of antiinflammatory IL-10. Tha analogs also showed slight effects on the remaining proinflammatory cytokines in both tissues. Differences among immunomodulatory effects of analogs might be related to potency, mechanism of action, and half lives (Fernández-Martínez et al., 2004). Finally, hepatic glycogen metabolism is altered by NO during endotoxic shock and the previously tested serial of Tha analogs immunomodulate the endotoxin-induced cytokines which regulate the NO release. Therefore, the short-term effects of those Tha analogs were assessed on the hepatic glycogen store and on the plasma and hepatic NO in an acute model of endotoxic challenge in rat. Endotoxin caused inverse dose-dependent effects increasing plasma NO and lowering hepatic glycogen. Tha analogs showed short-term regulatory effects on glycogen, some of them increased it. Plasma NO was almost unaffected by analogs but hepatic NO was strikingly modulated. Analogs slightly up-regulated the liver IFN-γ (a known NO-coinducer) and two of them increased it significantly. Due to their interesting effects the Tha analogs may be used as a pharmacological tool due to their short-term regulatory effects on glycogen and NO during endotoxic shock, since drugs that increase glycogen may improve liver injury in early sepsis (Fernández-Martínez et al., 2008).

Thalidomide Effects on Fibrosis, Cirrhosis and its Complications

Cholestasis is defined as a disorder of cholepoiesis and bile secretion as well as mechanical or functional stoppage of the bile flow in intrahepatic or extrahepatic bile ducts, with bile components passing into the blood. Persistent cholestasis with concomitant inflammatory and connective tissue reactions as well as all forms of chronic cholangitis may lead to irreversible cholestasis and, after long-term, to biliary fibrosis (excessive

accumulation of collagen) with preserved liver structure or to the last phase known as biliary cirrhosis (Kuntz and Kuntz, 2006). Secondary biliary cirrhosis induced in rat by BDL is a widespread experimental model (Kountouras et al., 1984). Tha and two analogs, PDP and PDA, have been assessed as hepatoprotective agents during BDL (Fernández-Martínez et al., 2001; Fernández-Martínez et al., 2009). Tha showed a poor improvement on biochemical markers of liver damage when compared to those afforded by PDP. That analog protected from liver injury since it diminished alkaline phosphatase (AP, cholestasis marker) and alanine aminotransferase (ALT, indicator of necrosis) activities, as well as bilirubins (indicator of cholestasis) and prevented collagen accumulation significantly; while thalidomide showed only modest beneficial effects on bilirubins and ALT. PDP diminished the increase in plasma TNF-α levels induced by BDL, while thalidomide not only failed to inhibit this cytokine, but slightly increased it. PDA is a water soluble analog that also improved cholestasis, necrosis and fibrosis outcomes in the BDL model. Histopathology showed remarkable liver damage amelioration due to Tha and even better for both analogs. The effectiveness of these Tha analogs has been related to its potency as TNF-α and PDE-4 inhibitors, therefore, inhibiting proinflammatory cytokines results in decreased necrosis, cholestasis and fibrosis in biliary secondary cirrhosis (Tilg, 2001).

Carbon tetrachloride (CCl_4)-induced liver damage is a very used animal model, wherein cytokines are involved too (Recknagel et al., 1989; DeCicco et al., 1998); chronic administration of this hepatotoxicant produces cirrhosis similar to that induced by alcoholism. In that model Tha has showed promising effects in several papers. Muriel and coworkers (2003) reported by first time the effect of Tha during the chronic intoxication with CCl_4 that induced 33.3% mortality, while Tha cotreatment reduced it to 13.3%. The serum activities of ALT, γ-glutamyl transpeptidase (GGTP, marker of cholestasis) and AP increased 3, 2 and 4-fold by CCl_4 treatment; Tha completely prevented elevation of these enzymes. Hepatic lipid peroxidation increased about 20-fold and glycogen was abolished in CCl_4-cirrhotic rats; Tha completely diminished the former and partially the later. Histology showed that CCl_4-treated rats receiving Tha had minor histological alterations and thinner bands of collagen. The antifibrotic effect estimated by collagen was partial but significant. Thus, Tha ameliorated the cirrhosis induced by CCl_4. Similar results were obtained by other authors using this model, in addition, they have found that Tha might exert an effect on the inhibition of oxidative stress via down-regulation of NF-κB signaling pathway to prevent the progression of liver cirrhosis; it may be due to the oxidative stress parameters such as superoxide dismutase, glutathione peroxidase and malondialdehyde as well as the expression of NF-κBp65, TGF-β (the most potent profibrogenic cytokine) and the tissue inhibitor of metalloproteinase-1 (TIMP-1, this protein inhibits the collagenase activity) were significantly diminished in CCl_4-cirrhotic rats treated with Tha (Lv et al., 2006a). In addition, some authors have proposed that Tha is able to prevent or to reverse, in an established CCl_4-induced cirrhosis model, the liver damage and fibrosis by down-regulation of NF-κB activation because of the inhibition on the degradation of the inhibitor of NF-κB (IκB) as well as NF-κB-induced adhesion molecules like intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, these adhesion molecules are very important in the process of recruitment and migration of inflammatory cells provoked by endothelial activation in cirrhosis; furthermore, as a consequence, levels of hyaluronic acid, laminin, procollagen-III and collagen-IV are diminished by the antifibrotic effects of Tha (Lv et al., 2006b; Paul et al., 2006; Lv et al., 2007; Xiao et al., 2007).

Tha has shown to be a very good hepatoprotective as well as antifibrotic agent in two more models of cirrhosis. Yeh and coworkers (2004) demonstrated the hepatoprotective and antifibrotic effects of Tha in the model of thioacetamide-induced cirrhosis, given that this drug improved the survival rate, reduced the expression of TNF-α, TGF-β, TIMP-1 and TIMP-2 in rats; moreover, they verified in vitro that the Kupffer cells isolated from Tha-treated rats had suppressed the production of TNF-α and TGF-β. Furthermore, similar antifibrotic effects reported for Tha have been studied in the model of dimethylnitrosamine-induced cirrhosis. In such experiment Tha also diminished in vivo the fibrosis scores of cirrhotic livers from intoxicated rats, the content of collagen as well as the NF-κB positive cells were reduced, and that the mRNA expressions of TGF-β, collagen 1α2, TNF-α and iNOS genes were attenuated by Tha. The same authors tried Tha on rat hepatic stellate cells (HSC-T6) in vitro, they found that Tha inhibited in a concentration-dependent manner the NF-κB transcriptional activity induced by TNF-α, incluiding IκB kinase-α (IKKα) and IκB phosphorylation, as well as lowered the TGF-β-induced collagen deposition in these stellate cells (Chong et al., 2006). For further reference regarding the NF-κB activation and its regulation in liver as well as the relationship TGF-β/liver fibrosis four excellent reviews are recommended (Fallowfield et al., 2006; Gieling et al., 2008; Iimuro and Brenner, 2008; Sun and Karin, 2008).

In addition to the hopeful results obtained by Tha as discussed for experimental models of fibrosis and cirrhosis, it rises interesting to comment briefly about the experimental and clinical effects of Tha on the portal hypertension, that is a clinical syndrome very common in cirrhotic patients, whom later may develop gastroesophageal variceal bleeding (Sanyal et al., 2008). The first report concerning this issue was in an animal model of portal hypertension, wherein rats were administered with Tha; this drug inhibited the TNF-α synthesis, reduced the NO production, blunted the development of hyperdynamic circulation and decreased the portal pressure (López-Talavera et al., 1996). Other two experimental approaches have been recently done by using cirrhotic BDL-rats; fist, the chronic administration of Tha improved the portal-systemic collateral vascular responsiveness to arginine-vasopressin in cholestatic rats, which was partially related to the inhibition of the cytokine vascular endothelial growth factor (VEGF) (Chang et al., 2009), and second, Tha decreased portal venous pressure as well as the intrahepatic resistance by reducing the hepatic thromboxane-A_2, a potent vasoconstrictor (Yang et al., 2009). An open pilot study in patients with stable alcoholic-cirrhosis and esophageal varices were administered with Tha or with pentoxifylline (immunomodulatory drug and PDE inhibitor); Tha, but not pentoxiphylline, reduced the hepatic venous pressure as well as TNF-α levels, that suggested future controlled clinical trials (Austin et al., 2004). Finally, a patient study case of intractable bleeding from hypertensive gastropathy secondary to extrahepatic portal vein obstruction was managed successfully by Tha (Karajeh et al., 2006).

Thalidomide in Experimental and Clinical Hepatocellular Carcinoma (HCC)

One of the first approaches to use Tha in liver diseases was perhaps its application in primary biliary cirrhosis (PBC), an idiopathic autoimmune disease with many clinical and pathological similarities to other illnesses wherein Tha had been successful; thus, a double-blind placebo controlled pilot study was performed, nonetheless, there were no improvements

in liver function tests or in liver histology. A number of patients treated with Tha reported an improvement in pruritus. That study suggested that Tha was unlikely to be affective in PBC (McCormick et al., 1994). Later, after the renaissance of Tha for the treatment of MM, the next obvious step was using this drug in several clinical trials for the treatment of HCC, which is the fifth most common cancer in the world and the third cause of cancer-related mortality. Cirrhosis is the main risk factor underlying HCC, and there is a clear association between chronic infections with hepatitis B and C virus, excessive alcohol consumption, cirrhosis and HCC; however, HCC also occurs in a noncirrhotic liver and the need of a systemic therapy is urgent (Witjes et al., 2009). Experimentally, Tha alone has significantly evidenced to inhibit angiogenesis and metastasis of HCC in the nude mice model, besides its inhibitory effects on TNF-α (Zhang et al., 2005a). Also, Tha in combination with paclitaxel (Zhang et al., 2005b) or with doxorubicin (Yang et al., 2005) afforded synergistic and significant necrosis and growth inhibitory effects on tumors, reduced angiogenesis and metastasis. These experimental results encouraged the Tha assessment in clinical trials.

Tha effects in clinical evaluations for treating HCC have yielded varied outcomes. Some authors have documented very promising effects, since individual case studies (Quek et al., 2006) or several patients in phase II clinical studies have responded to Tha therapy alone or in combination with other drugs (Wang et al., 2004; Demeria et al., 2007) and with radiotherapy (Hsu et al., 2006). Although Tha as a single treatment might not have very good results, because of the modest effects obtained in most cases (3-6 %), for example reducing the tumors size and/or achieving the stabilization of the disease for a longer period (Lin et al., 2005; Chuah et al., 2007); consequently, authors strongly recommend the use of Tha in combination with other chemotherapeutic agents, including the new immunomodulatory Tha analogs, to treat early small but not large tumors (Walder, 2005; Pinter et al., 2008). Generally Tha side effects are reported as well tolerated for patients, although few publications claim that this drug is not well tolerated when it is administered with other adjuvant agents (Cappa et al., 2005; Schwartz et al., 2005). As a unique case, it has been reported the successful treatment with oral Tha of a male patient (52 years old) who was suffering from hepatic epithelioid hamangioendothelioma (HEH) and lung metastasis, that cancer type is a very rare vascular tumor of the liver with an unpredictable malignant potential; Tha inhibited the growth and progression of the tumor (Mascarenhas et al., 2004).

Adverse Effects by Thalidomide: Hepatotoxicity

As commented above, Tha is a relatively safe drug compared with other known teratogens due to its very low acute toxicity, LD_{50} of >5000 mg/kg in mice and rabbits (Somers, 1960; Williams, 1968). The low toxicity of Tha has been observed in man, as 20 cases of accidental or intentional overdosage had been reported and all recovered eventfully (Somers, 1960), it may be possible that the absence of toxicity is due to the limited absorption. Regardless that, the common side effects during treatment with Tha are sedation, constipation, fatigue, skin rash and peripheral neuropathy (Singhal et al., 1999); less frequently bradycardia, hypotension and hypothyroidism have been described during prolonged treatments, but often well tolerated by patients, as it has been commented for Tha administration in MM and HCC clinical trials.

However, recent case reports concerning extremely rare but existing Tha hepatotoxicity in patients have been published. Post marketing surveillance of Tha since reintroduction in 1998 has identified one case where the cause of death was thought to be directly due to treatment with Tha (Clark et al., 2001). One case of Tha-induced fulminant hepatic failure which proved to be fatal in a 64-year-old woman has also been described (Hamadani et al., 2007). Almost the rest of cases have also been elder patients (57-79 years old) and just one younger woman (36) undergoing refractory MM with some complications as hypertension, diabetes, renal failure or leukemia and one with stable chronic hepatitis C; none of them had previously manifested hepatic alterations as well as their liver function tests in plasma were normal before beginning Tha treatment (Trojan et al., 2003; Dabak and Kuriakose, 2009; Levesque and Bradette, 2009). In addition, there are documented cases of hepatotoxicity but due to lenalidomide treatment in MM patients (Hussein, 2005; Hussain, 2007). Two patients with advanced HCC whom were under Tha therapy developed tumor lysis syndrome (TLS), similar to other two patients that received Tha for the treatment of MM (Spencer et al., 2003; Lee et al., 2006). Most cases returned to normal or quasinormal plasma values of liver damage biochemical markers (ALT, AST, AP and bilirubins), after Tha dosage cessation. Therefore, it becomes increasingly important to identify patient groups that may be particularly susceptible to specific adverse drug effects and to identify conditions under which specific adverse events may be more likely to occur. Oncology patients may represent a patient population with increased susceptibility to Tha-associated adverse effects (Clark et al., 2001).

It is necessary to comment that in no one of the diverse experiments in animal models of liver damage herein reviewed, and performed by several authors, Tha or its analogs administered in acute or chronic manner have showed by themselves either any sign of hepatotoxicity or a negative modification on the liver injury indicators during their evaluation, as well as they have not affected the normal plasma and liver cytokine profiles in control groups; what is more, even when some models are really drastic or severe (CCl_4, BDL, LPS, dimethylnitrosamine and thioacetamide) Tha or its analogs have resulted hepatoprotective agents, that has also been assessed by histopathology. There is only one report about exacerbation of acetaminophen hepatotoxicity by Tha, nevertheless authors did not find any significant influence on normal plasma levels of liver damage markers by the only administration of Tha (Kröger et al.; 1995).

Conclusion

In spite of the current medical advances and the development of new expensive therapies for the treatment of liver diseases and their last stage cirrhosis, nowadays there are not enough nor successfully effective and secure systemic drugs to guarantee a much better quality of life or the cure to the patients suffering from those illnesses (Muriel and Rivera-Espinoza, 2008). Nevertheless, immunomodulation seems to be a complex but very promising pharmacological approach to reestablish the cytokine imbalance during the liver damage and cirrhosis, this in order to manipulate or possibly to control the deleterious and beneficial processes that those mediators of inflammation regulate and, as a consequence, to maintain their levels into the normal liver and systemic homeostasis. Therefore, on the basis of all the reports herein

reviewed, including pro and con analyzed, the use of Tha and its analogs is suggested as potential hepatoprotective immunomodulatory drugs, although it is very clear that further and deeper basic as well as clinical research is necessary to detail the ratio risk/benefit.

References

Ando, Y; Fuse, E; Figg, WD. Thalidomide metabolism by the CYP2C subfamily. *Clin Cancer Res*. 2002;8:1964-73.

Annas, GJ; Elias, S. Thalidomide and the Titanic: reconstructing the technology tragedies of the twentieth century. *Am J Public Health*, 1999;89:98-101.

Austin, AS; Mahida, YR; Clarke, D; Ryder, SD; Freeman, JG. A pilot study to investigate the use of oxpentifylline (pentoxifylline) and thalidomide in portal hypertension secondary to alcoholic cirrhosis. *Aliment Pharmacol Ther*. 2004;19:79-88.

Bender, AT; Beavo, JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev*. 2006;58:488-520.

Blaschke, G; Kraft, HP; Fickentscher, K; Köhler F. Chromatographic separation of racemic thalidomide and teratogenic activity of its enantiomers. *Arzneimittelforschung*. 1979;29:1640-2.

Bradham, CA; Plümpe, J; Manns, MP; Brenner, DA; Trautwein, C. Mechanisms of hepatic toxicity I. TNF-induced liver injury. *Am J Physiol*. 1998;275(3 Pt 1):G387-92.

Cappa, FM; Cantarini, MC; Magini, G; Zambruni, A; Bendini, C; Santi, V; Bernardi, M; Trevisani, F. Effects of the combined treatment with thalidomide, megestrol and interleukine-2 in cirrhotic patients with advanced hepatocellular carcinoma. A pilot study. *Dig Liver Dis*. 2005;37:254-9.

Carcache de-Blanco, E; Pandit, B; Hu, Z; Shi, J; Lewis, A; Li, PK. Inhibitors of NF-κB derived from thalidomide. *Bioorg Med Chem Lett*. 2007;17:6031-5.

Cartmell, T; Poole, S; Turnbull, AV; Rothwell, NJ; Luheshi, GN. Circulating interleukin-6 mediates the febrile response to localised inflammation in rats. *J Physiol*. 2000;526 Pt 3:653-61.

Chang, CC; Wang, SS; Huang, HC; Lee, FY; Lin, HC; Lee, JY; Chen, YC; Lee, SD. Chronic thalidomide administration enhances vascular responsiveness to vasopressin in portal-systemic collaterals of bile duct-ligated rats. *J Chin Med Assoc*. 2009;72:234-42.

Chen, C; Reece, DE; Siegel, D; Niesvizky, R; Boccia, RV; Stadtmauer, EA; Abonour, R; Richardson, P; Matous, J; Kumar, S; Bahlis, NJ; Alsina, M; Vescio, R; Coutre, SE; Pietronigro, D; Knight, RD; Zeldis, JB; Rajkumar, V. Expanded safety experience with lenalidomide plus dexamethasone in relapsed or refractory multiple myeloma. *Br J Haematol*. 2009;146:164-70.

Chen, TL; Vogelsang, GB; Petty, BG; Brundrett, RB; Noe, DA; Santos, GW; Colvin, OM. Plasma pharmacokinetics and urinary excretion of thalidomide after oral dosing in healthy male volunteers. *Drug Metab Dispos Biol Fate Chem*. 1989;17:402-5.

Cheng, JB; Watson, JW; Pazoles, CJ; Eskra, JD; Griffiths, RJ; Cohan, VL; Turner, CR; Showell, HJ; Pettipher, ER. The phosphodiesterase type 4 (PDE4) inhibitor CP-80,633 elevates plasma cyclic AMP levels and decreases tumor necrosis factor-alpha (TNFalpha) production in mice: effect of adrenalectomy. *J Pharmacol Exp Ther*. 1997;280:621-6.

Chong, LW; Hsu, YC; Chiu, YT; Yang, KC; Huang, YT. Anti-fibrotic effects of thalidomide on hepatic stellate cells and dimethylnitrosamine-intoxicated rats. *J Biomed Sci*. 2006;13:403-18.

Chuah, B; Lim, R; Boyer, M; Ong, AB; Wong, SW; Kong, HL; Millward, M; Clarke, S; Goh, BC. Multi-centre phase II trial of thalidomide in the treatment of unresectable hepatocellular carcinoma. *Acta Oncol*. 2007;46:234-8.

Chung, F; Lu, J; Palmer, BD; Kestell, P; Browett, P; Baguley, BC; Tingle, M; Ching, LM. Thalidomide pharmacokinetics and metabolite formation in mice, rabbits, and multiple myeloma patients. *Clin Cancer Res*. 2004;10:5949-56.

Clark, TE; Edom, N; Larson, J; Lindsey, LJ. Thalomid (Thalidomide) capsules: a review of the first 18 months of spontaneous postmarketing adverse event surveillance, including off-label prescribing. *Drug Saf*. 2001;24:87-117.

Clemens, MJ. Cytokines: Introduction to cytokines. In: Read AP, Brown T editors. Medical perspectives series. Oxford, UK: Ed. Bioscientific publishers; 1991; 1-20.

Corral, LG; Haslett, PAJ; Muller, GW; Chen, R; Wong, LM; Ocampo, CJ; Patterson, RT; Stirling, DI; Kaplan, G. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α. *J Immunol*. 1999;163:380-6.

Corral, LG; Muller, GW; Moreira, AL; Chen, Yuxi; Wu, M; Stirling, D; Kaplan, G. Selection of novel analogs of thalidomide with enhanced tumor necrosis factor-α inhibitory activity. *Mol Med*. 1996;2:506-15.

Dabak, V; Kuriakose, P. Thalidomide-induced severe hepatotoxicity. *Cancer Chemother Pharmacol*. 2009;63:583-5.

D'Amato, RJ; Loughnan, MS; Flynn, E; Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA*, 1994;91:4082-5.

DeCicco, LA; Rikans, LE; Tutor, CG; Hornbrook, KR. Serum and liver concentrations of tumor necrosis factor α and interleukin-1β following administration of carbon tetrachloride to male rats. Toxicol Lett 1998;98:115-21.

Demeria, D; Birchall, I; Bain, VG. Dramatic reduction in tumour size in hepatocellular carcinoma patients on thalidomide therapy. *Can J Gastroenterol*. 2007;21:517-8.

Direskeneli, H; Ergun, T; Yavuz, S; Hamuryudan, V; Eksioglu-Demiralp, E. Thalidomide has both anti-inflammatory and regulatory effects in Behcet's disease. *Clin Rheumatol*. 2008;27:373-5.

Enomoto, N; Takei, Y; Hirose, M; Ikejima, K; Kitamura, T; Sato, N. Thalidomide prevents alcoholic liver injury in rats through inhibition of Kupffer cell sensitization. *Comp Hepatol*. 2004;3 Suppl 1:S37.

Enomoto, N; Takei, Y; Hirose, M; Ikejima, K; Miwa, H; Kitamura, T; Sato, N. Thalidomide prevents alcoholic liver injury in rats through suppression of Kupffer cell sensitization and TNF-alpha production. *Gastroenterology*, 2002;123:291-300.

Enomoto, N; Takei, Y; Hirose, M; Kitamura, T; Ikejima, K; Sato, N. Protective effect of thalidomide on endotoxin-induced liver injury. *Alcohol Clin Exp Res*. 2003;27 Suppl:2S-6S.

Eriksson, T; Björkman, S; Roth, B; Fyge, Å; Höglund, P. Stereospecific determination, chiral inversion in vitro and pharmacokinetics in humans of the enantiomers of thalidomide. *Chirality*, 1995;7:44-52.

Fabro, S; Schumacher, H; Smith, RL; Stagg, RBL; Williams, RT. The metabolism of thalidomide: some biological effects of thalidomide and its metabolites. *Brit J Pharmacol*. 1965; 25:352-62.

Faigle, JW; Kerberle, HR; Schmid, K. The metabolic fate of thalidomide. *Experientia*, 1962;18:389.

Fallowfield, JA; Kendall, TJ; Iredale, JP. Reversal of fibrosis: no longer a pipe dream? *Clin Liver Dis*. 2006;10:481-497.

Farrar, JD; Asnagli, H; Murphy, KM. T helper subset development: roles of instruction, selection, and transcription. *J Clin Invest*. 2002;109:431-5.

Fernández-Braña, M; Acero, N; Añorbe, L; Muñoz-Mingarro, D; Llinares, F; Domínguez, G. Discovering a new analogue of thalidomide which may be used as a potent modulator of TNF-alpha production. *Eur J Med Chem*. 2009;44:3533-42.

Fernández-Martínez, E; Morales-Ríos, MS; Pérez-Álvarez, V; Muriel, P. Effects of thalidomide and 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanamide on bile duct obstruction-induced cirrhosis in the rat. *Drug Dev Res*. 2001;54:209-18.

Fernández-Martínez, E; Morales-Ríos, MS; Pérez-Álvarez, V; Muriel, P. Immunomodulatory effects of thalidomide analogs on LPS-induced plasma and hepatic cytokines in the rat. *Biochem Pharmacol*. 2004;68:1321-9.

Fernández-Martínez, E; Pérez-Álvarez, V; Tsutsumi, V; Shibayama, M; Muriel, P. Chronic bile duct obstruction induces changes in plasma and hepatic levels of cytokines and nitric oxide in the rat. *Exp Toxicol Pathol*. 2006;58:49-58.

Fernández-Martínez, E; Pérez-Hernández, N; Muriel, P; Pérez-Álvarez, V; Shibayama, M; Tsutsumi, V. The thalidomide analog 3-phthalimido-3-(3,4-dimethoxyphenyl)-propanoic acid improves the biliary cirrhosis in the rat. *Exp Toxicol Pathol*. 2009;61:471-9.

Fernández-Martínez, E; Wens-Flores, I; Moreno-G, M; Ortiz, MI; Muriel, P; Pérez-Álvarez, V. Short-term effects of thalidomide analogs on hepatic glycogen and nitric oxide in endotoxin-challenged rats. *Gen Physiol Biophys*. 2008;27:203-10.

Field, EO; Gibbs, JE; Tucker, DF; Hellmann, K. Effect of thalidomide on the graft versus host reaction. *Nature*, 1966;211:1308-10.

Fournier, T; Medjoubi-N, N; Porquet, D. Alpha-1-acid glycoprotein. Biochim *Biophys Acta*, 2000;1482:157-71.

Fujimoto, H; Noguchi, T; Kobayashi, H; Miyachi, H; Hashimoto, Y. Effects of immunomodulatory derivatives of thalidomide (IMiDs) and their analogs on cell-differentiation, cyclooxygenase activity and angiogenesis. *Chem Pharm Bull*. 2006;54:855-60.

Fujita, J; Mestre, JR; Zeldis, JB; Subbaramaiah, K; Dannenberg, AJ. Thalidomide and its analogues inhibit lipopolysaccharide-mediated Iinduction of cyclooxygenase-2. *Clin Cancer Res*. 2001;7:3349-55.

Fullerton, PM; Kremer, M. Neuropathy after intake of thalidomide (Distaval). *Brit Med J*. 1961;2:855-8.

Galun, E; Axelrod, JH. The role of cytokines in liver failure and regeneration: potential new molecular therapies. *Biochim Biophys Acta*, 2002;1592:345-358.

Gantner, F; Küsters, S; Wendel, A; Hatzelmann, A; Schudt, C; Tiegs, G. Protection from T cell-mediated murine liver failure by phosphodiesterase inhibitors. *J Pharmacol Exp Ther*. 1997;280:53-60.

Gao, B. Cytokines, STATs and liver disease. *Cell Mol Immunol*. 2005;2:92-100.

Giamarellos-Bourboulis, EJ; Bolanos, N; Laoutaris, G; Papadakis, V; Koussoulas, V; Perrea, D; Karayannacos, PE; Giamarellou, H. Immunomodulatory intervention in sepsis by multidrug-resistant Pseudomonas aeruginosa with thalidomide: an experimental study. *BMC Infect Dis*. 2005;5:51.

Giamarellos-Bourboulis, EJ; Poulaki, H; Kostomitsopoulos, N; Dontas, I; Perrea, D; Karayannacos, PE; Giamarellou, H. Effective immunomodulatory treatment of Escherichia coli experimental sepsis with thalidomide. *Antimicrob Agents Chemother*, 2003;47:2445-9.

Gieling, RG; Burt, AD; Mann, DA. Fibrosis and cirrhosis reversibility - molecular mechanisms. *Clin Liver Dis*. 2008;12:915-937.

Gordon, JN; Prothero, JD; Thornton, CA; Pickard, KM; Di Sabatino, A; Goggin, PM; Pender, SL; MacDonald, TT. CC-10004 but not thalidomide or lenalidomide inhibits lamina propria mononuclear cell TNF-α and MMP-3 production in patients with inflammatory bowel disease. *J Crohn's Colitis*, 2009;3:175-82.

Green, J; Upjohn, E; McCormack, C; Zeldis, J; Prince, HM. Successful treatment of Behçet's disease with lenalidomide. *Br J Dermatol*. 2008;158:197-8.

Green, RM; Beier, M; Gollan, JL. Regulation of hepatic bile salt transporters by endotoxin and inflammatory cytokines in rodents. *Gastroenterology*, 1996;111:193-8.

Grötzinger, J. Molecular mechanisms of cytokine receptor activation. Biochim *Biophys Acta*, 2002;1592:215-23.

Gutiérrez-Rodríguez, O; Starusta-Bacal, P; Gutiérrez-Montes, O. Treatment of refractory rheumatoid arthritis the thalidomide experience. *J Rheumatol*. 1989;16:158-63.

Hamadani, M; Benson, DM Jr; Copelan, EA. Thalidomide-induced fulminant hepatic failure. *Mayo Clin Proc*. 2007;82:638.

Hashimoto, Y. Thalidomide as a multi-template for development of biologically active compounds. *Arch Pharm*. (Weinheim) 2008;341:536-47.

Hochepied, T; Berger, FG; Baumann, H; Libert, C. Alpha(1)-acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. *Cytokine Growth Factor Rev*. 2003;14:25-34.

Hsu, WC; Chan, SC; Ting, LL; Chung, NN; Wang, PM; Ying, KS; Shin, JS; Chao, CJ; Lin, GD. Results of three-dimensional conformal radiotherapy and thalidomide for advanced hepatocellular carcinoma. *Jpn J Clin Oncol*. 2006;36:93-9.

Hussain, S; Browne, R; Chen, J; Parekh, S. Lenalidomide-induced severe hepatotoxicity. *Blood*, 2007;110:3814.

Hussein, MA. Lenalidomide: patient management strategies. *Semin Hematol*. 2005;42:S22-5.

Iimuro, Y; Brenner, DA. Matrix metalloproteinase gene delivery for liver fibrosis. *Pharm Res*. 2008;25:249-58.

Ishihara, K; Hirano, T. Molecular basis of the cell specificity of cytokine action. *Biochim Biophys Acta*, 2002;1592:281-96.

Jacobson, JM; Greenspan, JS; Spritzler, J; Ketter, N; Fahey, JL; Jackson, JB; Fox, L; Chernoff, M; Wu, AW; MacPhail, LA; Vasquez, GJ; Wohl, DA. Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. *N Engl J Med*. 1997;336:1487-93.

Kamikawa, R; Ikawa, K; Morikawa, N; Asaoku, H; Iwato, K; Sasaki, A. The pharmacokinetics of low-dose thalidomide in Japanese patients with refractory multiple myeloma. *Biol Pharm Bull*. 2006;29:2331-4.

Karajeh, MA; Hurlstone, DP; Stephenson, TJ; Ray-Chaudhuri, D; Gleeson, DC. Refractory bleeding from portal hypertensive gastropathy: a further novel role for thalidomide therapy? *Eur J Gastroenterol Hepatol*, 2006, 18, 545-8.

Kast, RE. Tumor necrosis factor has positive and negative self regulatory feed back cycles centered around cAMP. Int J Immunopharmacol 2000;22:1001-6.

Keller, H; Kunz, W; Muckter, H. N-phthalyl-glutamic acid imide: experimental studies on a new synthetic product with sedative properties. *Arzneimittelforschung*, 1956;6:426-30.

Kidd, P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev*. 2003;8:223-46.

Kim, YS; Kim, JS; Jung, HC; Song, IS. The effects of thalidomide on the stimulation of NF-kappaB activity and TNF-alpha production by lipopolysaccharide in a human colonic epithelial cell line. *Mol Cells*, 2004;17:210-6.

Knoche, B; Blaschke, G. Investigations on the in vitro racemization of thalidomide by high-performance liquid chromatography. *J Cromatography*, 1994, 666, 235-40.

Kountouras, J; Billing, BH; Scheuer, PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. *Br J Exp Pathol*. 1984;65:305-11.

Kröger, H; Klewer, M; Grätz, R; Dietrich, A; Ockenfels, H; Miesel, R. Exacerbation of acetaminophen hepatotoxicity by thalidomide and protection by nicotinic acid amide. *Gen Pharmacol*. 1995;26:1243-7.

Kuntz, E; Kuntz, HD. Hepatology: principles and practice. Germany: Springer Medizin Verlag Heidelberg; 2006.

Lee, CC; Wu, YH; Chung, SH; Chen, WJ. Acute tumor lysis syndrome after thalidomide therapy in advanced hepatocellular carcinoma. *Oncologist*, 2006;11:87-8.

Lenz, W. Kindliche misbildungen nach medikament-einnehmen während de gravidität. *Dtsch Med Wochenschr*. 1961; 86:2555-6.

Lenz, W. A personal perspective on the thalidomide tragedy. *Teratology*, 1992;46:417-8.

Lepper, ER; Smith, NF; Cox, MC; Scripture, CD; Figg, WD. Thalidomide metabolism and hydrolysis: mechanisms and implications. *Curr Drug Metab*. 2006;7:677-85.

Levesque, E; Bradette, M. Hepatotoxicity as a rare but serious side effect of thalidomide. *Ann Hematol*. 2009;88:183-4.

Lin, AY; Brophy, N; Fisher, GA; So, S; Biggs, C; Yock, TI; Levitt, L. Phase II study of thalidomide in patients with unresectable hepatocellular carcinoma. *Cancer*, 2005;103:119-25.

Llorente, L; Richaud-Patin, Y; Alcocer-Castillejos, N; Ruiz-Soto, R; Mercado, MA; Orozco, H; Gamboa-Domínguez, A; Alcocer-Varela, J. Cytokine gene expression in cirrhotic and non-cirrhotic human liver. *J Hepatol*. 1996;24:555-63.

López-Talavera, JC; Cadelina, G; Olcowski, J; Merrill, W; Groszmann, RJ. Thalidomide inhibits tumor necrosis factor α, decreases nitric oxide synthesis, and ameliorates the hyperdynamic circulatory syndrome in portal hypertensive rats. *Hepatology*, 1996;23:1616-21.

Louis, H; Le Moine, O; Goldman, M; Devière, J. Modulation of liver injury by interleukin-10. *Acta Gastroenterol Belg*. 2003;66:7-14.

Lv, P; Luo, HS; Zhou, XP; Paul, SC; Xiao, YJ; Si, XM; Liu, SQ. Thalidomide prevents rat liver cirrhosis via inhibition of oxidative stress. *Pathol Res Pract*. 2006a;202:777-88.

Lv, P; Paul, SC; Xiao, Y; Liu, S; Luo, H. Effects of thalidomide on the expression of adhesion molecules in rat liver cirrhosis. Mediators Inflamm 2006b; DOI: 10.1155/MI/2006/93253.

Lv, P; Luo, HS; Zhou, XP; Xiao, YJ; Paul, SC; Si, XM; Zhou, YH. Reversal effect of thalidomide on established hepatic cirrhosis in rats via inhibition of nuclear factor-κB/inhibitor of nuclear factor-κB pathway. *Arch Med Res*. 2007;38:15-27.

Magarotto, V; Palumbo, A. Evolving role of novel agents for maintenance therapy in myeloma. *Cancer J*. 2009;15:494-501.

Majumdar, S; Lamothe, B; Aggarwal, BB. Thalidomide suppresses NF-kappa B activation induced by TNF and H_2O_2, but not that activated by ceramide, lipopolysaccharides, or phorbol ester. *J Immunol*. 2002;168:2644-51.

Man, HW; Schafer, P; Wong, LM; Patterson, RT; Corral, LG; Raymon, H; Blease, K; Leisten, J; Shirley, MA; Tang, Y; Babusis, DM; Chen, R; Stirling, D; Muller, GW. Discovery of (S)-N-[2-[1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl] acetamide (apremilast), a potent and orally active phosphodiesterase 4 and tumor necrosis factor-alpha inhibitor. *J Med Chem*. 2009;52:1522-4.

Mansfield, JC; Parkes, M; Hawthorne, AB; Forbes, A; Probert, CS; Perowne, RC; Cooper, A; Zeldis, JB; Manning, DC; Hawkey, CJ. A randomized, double-blind, placebo-controlled trial of lenalidomide in the treatment of moderately severe active Crohn's disease. *Aliment Pharmacol Ther*. 2007;26:421-30.

Marriott, JB; Muller, G; Dalgleish, AG. Thalidomide as an emerging immunotherapeutic agent. *Immunol Today*, 1999;20:538-40.

Marriott, JB; Westby, M; Cookson, S; Guckian, M; Goodbourn, S; Muller, G; Shire, MG; Stirling, D; Dalgleish, AG. CC-3052: A water-soluble analog of thalidomide and potent inhibitor of activation-induced TNF-α production. *J Immunol*. 1998;161:4236-43.

Mascarenhas, RC; Sanghvi, AN; Friedlander, L; Geyer, SJ; Beasley, HS; Van Thiel, DH. Thalidomide inhibits the growth and progression of hepatic epithelioid hemangioendothelioma. *Oncology*, 2004;67:471-5.

Masubuchi, Y; Sugiyama, S; Horie, T. Th1/Th2 cytokine balance as a determinant of acetaminophen-induced liver injury. *Chem Biol Interact*. 2009;179:273-9.

McBride, WB. Thalidomide and congenital abnormalities. *Lancet*, 1961;2:1358.

McClain, CJ; Barve, S; Deaciuc, I; Kugelmas, M; Hill, D. Cytokines in alcoholic liver disease. *Semin Liver Dis*. 1999;19:205-19.

McCormick, PA; Scott, F; Epstein, O; Burroughs, AK; Scheuer, PJ; McIntyre, N. Thalidomide as therapy for primary biliary cirrhosis: a double-blind placebo controlled pilot study. *J Hepatol*. 1994;21:496-9.

McHugh, SM; Rifkin, IR; Deighton, J; Wilson, AB; Lachmann, PJ; Lockwood, CM; Ewan, PW. The immunosuppressive drug thalidomide induces T helper cell type 2 (Th2) and concomitantly inhibits Th1 cytokine production in mitogen- and antigen-stimulated human peripheral blood mononuclear cell cultures. *Clin Exp Immunol*. 1995;99:160-7.

Miyachi, H; Azuma, A; Hioki, E; Iwasaki, S; Hashimoto, Y. Enantio-dependence of inducer-specific bidirectional regulation of tumor necrosis factor (TNF)-alpha production: potent TNF-α production inhibiting. Bioorg Med Chem Lett 1996;6:2293-8.

Miyachi, H; Azuma, A; Ogasawara, A; Uchimura, E; Watanabe, N; Kobayashi, Y; Kato, F; Kato, M; Hashimoto, Y. Novel biological response modifiers: phthalimides with tumor necrosis factor-α production-regulating activity. J Med Chem 1997a;40:2858-65.

Miyachi, H; Ogasawara, A; Azuma, A; Hashimoto, Y. Tumor necrosis factor-alpha production-inhibiting activity of phthalimide analogues on human leukemia THP-1 cells and a structure-activity relationship study. Bioorg Med Chem 1997b;5:2095-102.

Moreira, AL; Corral, LG; Ye, W; Johnson, B; Stirling, D; Muller, GW; Freedman, VH; Kaplan, G. Thalidomide and thalidomide analogs reduce HIV type 1 replication in human macrophages in vitro. AIDS Res Hum Retroviruses 1997;13:857-63.

Moreira, AL; Sampaio, EP; Zmuidzinas, A; Frindt, P; Smith, KA; Kaplan, G. Thalidomide exerts its inhibitory action tumor necosis factor-α by enhancing mRNA degration. *J Exp Med*, 1993, 177, 1675-80.

Moseley, RH. Sepsis and cholestasis. Clin Liver Dis 2004;8:83-94.

Mujagić, H; Chabner, BA; Mujagić, Z. Mechanisms of action and potential therapeutic uses of thalidomide. Croat Med J 2002;43:274-85.

Muller, GW; Chen, R; Hauang, SY; Corral, LG; Wong, LM; Patterson, RT; Shire, MG; Chen, Y; Kaplan, G; Stirling, DI. Amino-substituted thalidomide analogs: potent inhibitors of TNF-α production. Bioorg Med Chem Lett 1999;9:1625-30.

Muller, GW; Corral, LG; Shire, MG; Wang, H; Moreira, A; Kaplan, G; Stirling, DI. Structural modifications of thalidomide produce analogs with enhanced tumor necrosis factor inhibitory activity. J Med Chem 1996; 39: 3238-40.

Muller, GW; Shire, MG; Wong, LM; Corral, LG; Patterson, RT; Chen, Y; Stirling, DI. Thalidomide analogs and PDE4 inhibition. Bioorg Med Chem Lett 1998;8:2669-74.

Muriel, P; Fernández-Martínez, E; Pérez-Álvarez, V; Lara-Ochoa, F; Ponce, S; García, J; Shibayama, M; Tsutsumi, V. Thalidomide ameliorates carbon tetrachloride induced cirrhosis in the rat. Eur J Gastroenterol Hepatol 2003;15:951-7.

Muriel, P; Rivera-Espinoza, Y. Beneficial drugs for liver diseases. J Appl Toxicol 2008;28:93-103.

Niwayama, S; Loh, C; Turk, BE; Liu, JO; Miyachi, H; Hashimoto, Y. Enhanced potency of perfluorinated thalidomide derivatives for inhibition of LPS-induced tumor necrosis factor-α production is associated with a change of mechanism of action. Bioorg Med Chem Lett 1998;8:1071-6.

Oliver, SJ; Freeman, SL; Corral, LG; Ocampo, CJ; Kaplan, G. Thalidomide analogue CC1069 inhibits development of rat adjuvant arthritis. Clin Exp Immunol 1999;118:315-21.

Paul, SC; Lv, P; Xiao, YJ; An, P; Liu, SQ; Luo, HS. Thalidomide in rat liver cirrhosis: blockade of tumor necrosis factor-α via inhibition of an inhibitor of nuclear factor-κB. Pathobiology 2006;73:82-92.

Pinter, M; Wichlas, M; Schmid, K; Plank, C; Müller, C; Wrba, F; Peck-Radosavljevic, M. Thalidomide in advanced hepatocellular carcinoma as antiangiogenic treatment approach: a phase I/II trial. Eur J Gastroenterol Hepatol 2008;20:1012-9.

Quek, R; Lim, ST; Ong S. Hepatocellular carcinoma - Pathological complete response to oral capecitabine, megestrol and thalidomide. Acta Oncol 2006;45:95-7.

Ratanatharathorn, V; Ayash, L; Lazarus, HM; Fu, J; Uberti, JP. Chronic graft-versus-host disease: clinical manifestation and therapy. Bone Marrow Transplant 2001;28:121-9.

Recknagel, RO; Glende, EA Jr; Dolak, JA; Waller, RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989;43:139-54.

Richardson, P; Hideshima, T; Anderson, K. Thalidomide: emerging role in cancer medicine. Annu Rev Med 2002a;53:629-57.

Richardson, PG; Schlossman, RL; Weller, E; Hideshima, T; Mitsiades, C; Davies, F; LeBlanc, R; Catley, LP; Doss, D; Kelly, K; McKenney, M; Mechlowicz, J; Freeman, A; Deocampo, R; Rich, R; Ryoo, JJ; Chauhan, D; Balinski, K; Zeldis, J; Anderson, KC. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. Blood 2002b;100:3063-7.

Rizzardini, M; Zappone, M; Villa, P; Gnocchi, P; Sironi, M; Diomede, L; Meazza, C; Monshouwer, M; Cantoni, L. Kupffer cell depletion partially prevents hepatic heme oxygenase 1 messenger RNA accumulation in systemic inflammation in mice: role of interleukin 1beta. Hepatology 1998;27:703-10.

Rose-John, S. Cytokines come of age. Biochim Biophys Acta 2002;1592:213-4.

Sampaio, EP; Sarno, EN; Galilly, R; Cohn, ZA; Kaplan, G. Thalidomide selectively inhibits tumor necrosis factor-α production by stimulated human monocytes. *J Exp Med*, 1991, 173, 699-703.

Sanyal, AJ; Bosch, J; Blei, A; Arroyo, V. Portal hypertension and its complications. Gastroenterology 2008;134:1715-28.

Schafer, PH; Gandhi, AK; Loveland, MA; Chen, RS; Man, HW; Schnetkamp, PP; Wolbring, G; Govinda, S; Corral, LG; Payvandi, F; Muller, GW; Stirling, DI. Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs. J Pharmacol Exp Ther 2003;305:1222-32.

Schumacher, H; Smith, RL; Williams, RT. The metabolism of thalidomide: the spontaneous hydrolysis of thalidomide in solution. Brit J Pharmacol 1965a; 25:324-37.

Schumacher, H; Smith, RL; Williams, RT. The metabolism of thalidomide: the fate of thalidomide and some of its hydrolysis products in various species. Brit J Pharmacol 1965b; 25:338-51.

Schwartz, JD; Sung, M; Schwartz, M; Lehrer, D; Mandeli, J; Liebes, L; Goldenberg, A; Volm, M. Thalidomide in advanced hepatocellular carcinoma with optional low-dose interferon-alpha2a upon progression. Oncologist 2005;10:718-27.

Seki, S; Habu, Y; Kawamura, T; Takeda, K; Dobashi, H; Ohkawa, T; Hiraide, H. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+ T cells in T helper 1 immune responses. Immunological reviews 2000;174:35-46.

Shannon, EJ; Miranda, RO; Morales, MJ; Hastings, EC. Inhibition of the novo antibody synthesis by thalidomide as a relevant mechanism of action in leprosy. Scand J Immunol 1981;13:17-25.

Shannon, EJ; Morales, MJ; Sandoval, F. Immunomodulatory assays to study structure-activity relationships of thalidomide. Immunopharmacol 1997;35:203-12.

Sheskin, J. Thalidomide in the treatment of lepra reactions. Clin Pharmacol Ther 1965;6:303-6.

Shimazawa, R; Sano, H; Tanatani, A; Miyachi, H; Hashimoto, Y. Thalidomide as a nitric oxide synthase inhibitor and its structural development. Chem Pharm Bull 2004;**52**:498-9.

Simpson, KJ; Lukacs, NW; Colletti, L; Strieter, RM; Kunkel, SL. Cytokines and the liver. J Hepatol 1997;27:1120-32.

Singhal, S; Mehta, J; Desikan, R; Ayers, D; Roberson, P; Eddlemon, P; Munshi, N; Anaissie, E; Wilson, C; Dhodapkar, M; Zeddis, J; Barlogie, B. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med 1999;341:1565-71.

Somers, GF. Pharmacological properties of thalidomide (α-phthalimidoglutarimide), a new sedative hypnotic drug. Brit J Pharmacol 1960;15:111-6.

Somers, GF. Thalidomide and congenital abnormalities. Lancet 1962;1:912-3.

Spencer, A; Robert, A; Bailey, M; Schran, H; Lynch, K. No evidence for an adverse interaction between zoledronic acid and thalidomide: preliminary safety analysis from the Australasian Leukemia and Lymphoma Group MM6 Myeloma Trial. Blood 2003;102:383.

Steinke, JW; Borish, L. 3. Cytokines and chemokines. J Allergy Clin Immunol 2006;117:S441-5.

Suizu, M; Muroya, Y; Kakuta, H; Kagechika, H; Tanatani, A; Nagasawa, K; Hashimoto Y. Cyclooxygenase inhibitors derived from thalidomide. Chem Pharm Bull 2003;51:1098-102.

Sun, B; Karin, M. NF-κB signaling, liver disease and hepatoprotective agents. Oncogene 2008;27:6228-44.

Teo, SK. Properties of thalidomide and its analogues: implications for anticancer therapy. AAPS J 2005;7:E14-9.

Teo, SK; Chen, Y; Muller, GW; Chen, RS; Thomas, SD; Stirling, DI; Chandula, RS. Chiral inversion of the second generation IMiD™ CC-4047 (ACTIMID™) in human plasma and phosphate-buffered saline. Chirality 2003;15:348-51.

Thiele, A; Bang, R; Gütschow, M; Rossol, M; Loos, S; Eger, K; Tiegs, G; Hauschildt, S. Cytokine modulation and suppression of liver injury by a novel analogue of thalidomide. Eur J Pharmacol 2002;453:325-34.

Tilg, H. Cytokines and liver diseases. Can J Gastroenterol 2001;15:661-8.

Tilg, H; Wilmer, A; Vogel, W; Herold, M; Nölchen, B; Judmaier, G; Huber, C. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992;103:264-74.

Trojan, A; Chasse, E; Gay, B; Pichert, G; Taverna, C. Severe hepatic toxicity due to thalidomide in relapsed multiple myeloma. Ann Oncol 2003;14:501-2.

Turk, BE; Jiang, H; Liu, JO. Binding of thalidomide to α_1-acid glycoprotein may be involved in its inhibition of tumor necrosis factor α production. Proc Natl Acad Sci USA 1996;93:7552-6.

Van Amersfoort, ES; Van Berkel, TJ; Kuiper, J. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. *Clin Microbiol Rev*. 2003;16:379-414.

Venon, MD; Roccaro, AM; Gay, J; Moreau, AS; Dulery, R; Facon, T; Ghobrial, IM; Leleu, X. Front line treatment of elderly multiple myeloma in the era of novel agents. *Biologics*. 2009;3:99-109.

Vogelsang, GB; Farmer, ER; Hess, AD; Altamonte, V; Beschorner, WE; Jabs, DA; Corio, RL; Levin, LS; Colvin, OM; Wingard, JR; Santos, GW. Thalidomide for the treatment of chronic graft-versus-host disease. *New Eng J Med*. 1992;326:1055-8.

Wadler, S. Thalidomide for advanced hepatocellular carcinoma, is this a real alternative? *Cancer*, 2005;103:1-4.

Wang, TE; Kao, CR; Lin, SC; Chang, WH; Chu, CH; Lin, J; Hsieh, RK. Salvage therapy for hepatocellular carcinoma with thalidomide. *World J Gastroenterol*. 2004;10:649-53.

Wang, T; Zhang, YH; Kong, XW; Lai, YS; Ji, H; Chen, YP; Peng, SX. Synthesis and biological evaluation of nitric oxide-donating thalidomide analogues as anticancer agents. *Chem Biodivers*. 2009;6:466-74.

Williams, RT. Thalidomide: a study of biochemical teratology. *Arch Environ Health*, 1968;16:493-502.

Witjes, CD; Verhoef, C; Verheul, HM; Eskens, FA. Systemic treatment in hepatocellular carcinoma; 'A small step for man...'. *Neth J Med*. 2009;67:86-90.

Wnendt, S; Finkam, M; Winter, W; Ossig, J; Raabe, G; Zwingenberger, K. Enantioselective inhibition of TNF-α release by thalidomide and thalidomide analogues. *Chirality*, 1996;8:390-6.

Xiao, YJ; Luo, HS; Lv, P. Effects of thalidomide on adhesion molecules in rat liver fibrosis. *World Chinese J Digestol*. 2007;15:3372-6.

Yamamoto, T; Shibata, N; Sukeguchi, D; Takashima, M; Nakamura, S; Toru, T; Matsunaga, N; Hara H; Tanaka, M; Obata, T; Sasaki, T. Synthesis, configurational stability and stereochemical biological evaluations of (S)- and (R)-5-hydroxythalidomides. *Bioorg Med Chem Lett*. 2009;19:3973-6.

Yang, YM; Du, GJ; Lin, HH. Experimental study of thalidomide for treatment of murine hepatocellular carcinoma. Di Yi Jun Yi Da Xue Xue Bao (J First Mil Med Univ) 2005;25:925-8.

Yang, YY; Huang, YT; Lin, HC; Lee, FY; Lee, KC; Chau, GY; Loong, CC; Lai, CR; Lee, SD. Thalidomide decreases intrahepatic resistance in cirrhotic rats. *Biochem Biophys Res Commun.* 2009;380:666-72.

Yeh, TS; Ho, YP; Huang, SF; Yeh, JN; Jan, YY; Chen, MF. Thalidomide salvages lethal hepatic necroinflammation and accelerates recovery from cirrhosis in rats. *J Hepatol*. 2004;41:606-12.

Zahran, MA; Salem, TA; Samaka, RM; Agwa, HS; Awad, AR. Design, synthesis and antitumor evaluation of novel thalidomide dithiocarbamate and dithioate analogs against Ehrlich ascites carcinoma-induced solid tumor in Swiss albino mice. *Bioorg Med Chem*. 2008;16:9708-18.

Zeldis, JB; Williams, BA; Thomas, SD; Elsayed, ME. S.T.E.P.S.: a comprehensive program for controlling and monitoring access to thalidomide. *Clin Ther*, 1999, 21, 319-30.

Zhang, ZL; Liu, ZS; Sun, Q. Effects of thalidomide on angiogenesis and tumor growth and metastasis of human hepatocellular carcinoma in nude mice. *World J Gastroenterol*. 2005a;11:216-20.

Zhang, ZL; Liu, ZS; Sun, Q. Anti-tumor effect of thalidomide and paclitaxel on hepatocellular carcinoma in nude mice. *Chin Med J*. (Engl) 2005b;118:1688-94.

Zwingenberger, K; Wnendt, S. Immunomodulation by thalidomide: systematic review of the literature and of unpublished observations. *J Inflamm*. 1995-1996;46:177-211.