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REVIEW

Mechanisms of Action and Potential Therapeutic Uses of Thalidomide

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Thalidomide was first introduced to the market in Germany under the brand name of *Contergan* in 1956, as a "non-barbiturate hypnotic", advocated to ensure a good night's sleep and to prevent morning sickness in pregnancy. It was advertised for its prompt action, lack of hangover, and apparent safety. It has been banned from the market since 1963 after it caused the worldwide teratogenic disaster: babies exposed to thalidomide *in utero* during the first 34-50 days of pregnancy were born with severe life-threatening birth defects. Despite its unfortunate history, thalidomide has attracted scientific interest again because of its recently discovered action against inflammatory diseases and cancer. Its broad range of biological activities stems from its ability to moderate cytokine action in cancer and inflammatory diseases. Early studies examined its anxiolytic, mild hypnotic, antiemetic, and adjuvant analgesic properties. Subsequently, thalidomide was found to be highly effective in managing the cutaneous manifestations of leprosy, being superior to Aspirin in controlling leprosy-associated fever. Recent research has shown promising results with thalidomide in patients with myeloma, myelodysplastic syndrome, a variety of infectious diseases, autoimmune diseases, cancer, and progressive body weight loss related to advanced cancer and AIDS. Here we review the history of its development, pharmacokinetics, metabolism, biologic effects, and the results of clinical trials conducted thus far. Further research in this field should be directed towards better understanding of thalidomide metabolism, its mechanism of action, and the development of less toxic and more active analogs.

Key words: *angiogenesis inhibitors; myelodysplastic syndromes; multiple myeloma; pharmacokinetics; neoplasms; thalidomide*

Among the recent discoveries in cancer therapeutics, the revival of thalidomide ranks as one of the most surprising and intriguing. This sedative with a tragic history of causing abnormalities of fetal limb development has become the subject of intense scientific interest because of its newly discovered activity in treating infectious diseases (1-5), autoimmune diseases (6-9), and cancer (9,10). The drug was synthesized and first marketed in Germany under the brand name *Contergan* in 1956 as a "non-barbiturate hypnotic" with a notable prompt action, lack of hangover, and apparently favorable safety profile. It was banned from commercial use in 1963, after it had been discovered that it exerted teratogenic effects if taken between the 34th and 50th day of pregnancy (10,11). Over 12,000 affected children were born with skeletal abnormalities, an event that led to a major reform of drug approval procedures in the United States and elsewhere. The basis of these fetal abnormalities is unknown, although the drug has subsequently been found to have a broad range of biological effects on cytokine secretion, immune function, angiogenesis, cell adhesion, and cell proliferation (12-17). Which of these mechanisms account for its clinical activities and teratogenic effects remains an unresolved issue. However, its value as a novel therapeutic is unquestioned (18-22).

Its range of effectiveness in infectious and autoimmune diseases extends from its well established value in the management of cutaneous leprosy (3) and the suppression of leprosy-associated fever (11) to the reversal of weight loss associated with acquired immunodeficiency syndrome (AIDS) (23) and cancer (24,25), and encouraging initial trials in the treatment of aphtous ulcers and Behcet's disease, tuberculosis, inflammatory bowel disease, Sjögren's syndrome, rheumatoid arthritis, and other collagen and vascular diseases (26). Recent studies have demonstrated consistent responses in graft-versus-host disease (GVHD) and in cancer, including multiple myeloma, myelodysplasia, Kaposi's sarcoma, and several other solid tumors (27). An abbreviated history of thalidomide is given in Table 1.

Despite its tragic initial experience, thalidomide has become the subject of major interest because of its newly demonstrated clinical value in infectious disease and cancer (2,6,12,25). Thalidomide has attracted the attention of investigators because of its wide range of biological actions. It inhibits angiogenesis (36-38), and as an immunomodulatory agent

Table 1. The (re)discovery of thalidomide

Year	observation	Ref. No.
1953	First synthesis by "Chemie Grünental" in Germany.	1
1956	Introduced to market in Germany under the brand name Contergan.	6
1957	Introduced to European and Canadian markets as a sleeping aid.	7
1958	First reports of peripheral neuritis in some patients on long-term use.	10
1960	By this year used widely in Europe, UK, and Canada to ameliorate nausea in pregnancy.	11
1961	First reports about birth defects and further reports on peripheral neuropathies.	12
1962	Reports of twelve thousand cases of human fetal abnormalities related to use of thalidomide in pregnant women.	1
1963	Thalidomide withdrawn from the market.	11
1965	Reports showing curative effect of thalidomide in patients with erythema nodosum leprosum (erythema nodosum leprosum) and reports showing therapeutic responses of some solid malignant tumors.	3,29
1966	Demonstrated effect against graft-versus-host reaction in animal models.	31
1980	Immunosuppressive and anti-inflammatory thalidomide analogs identified in preclinical setting.	11
1986	Thalidomide showed therapeutic response in patients with graft-versus-host disease.	30
1989	First report on plasma pharmacokinetics and urinary excretion.	33
1990	Active against pharyngeal and esophageal ulcerations in Behcet's syndrome.	34
1991	Inhibits tumor-necrosis factor a production and angiogenesis in experimental systems.	28
1992	Inhibition of replication of human immunodeficiency virus type 1 (HIV 1) in vitro.	32
1996	Synthesis of thalidomide analogs and demonstration of their immunosuppressive and anti-inflammatory efficacy.	35
1998	Food And Drug Administration (FDA) approval for therapeutic use in patients with erythema nodosum leprosum. Activity against HIV-associated Kaposi's sarcoma reported.	26
1999	Antitumor activity of thalidomide in refractory multiple myeloma and possible activity against various solid malignant tumors. Immunomodulatory effects reported. Clinical testing of analogs started.	18

it inhibits synthesis and action of tumor necrosis factor α (TNF-α), a lymphokine implicated in cell adhesion, angiogenesis, and cachexia (16,38).

Thalidomide differs from most other anti-cancer agents because of its low level of toxicity (except teratogenicity). Four decades of toxicity data on this drug document few other serious side effects (1,6,7,26). Most likely, it can be safely combined with other anti-cancer drugs (39-41).

Despite the growing interest for this drug and its analogues, we still have only limited data on its metabolism, pharmacology, pharmacokinetics, and mechanism of action.

Structure and Chemical Properties

Chemically, thalidomide is α-N-phthalimidoglutarimide $(C_{13}H_{10}N_2)_4$ with a molecular weight of 258.2 (Fig. 1). It is a derivative of glutamic acid and is structurally related to two other neuropharmaceuticals, an analeptic drug bemegride (α-ethyl-α-methylglutarimide, $C_{18}H_{13}NO_2$) and a sedative and antiepileptic drug glutethimide (β-ethyl-β-phenyl-glutarimide, $C_{15}H_{23}NO_4$). It differs from these two related compounds because it causes a broad range of immunomodulatory and antitumor effects, in addition to sedation (8,9,15-17,22,42,43). It has two ring systems: a left-sided phthalimide and a right-sided glutarimide with an asymmetric carbon atom at position 3′ of the glutarimide ring. It exists in L- and R-isomer forms, representing derivatives of L- and R-glutamic acid. Although some reports suggested that L-isomer was linked to teratogenicity and the R-isomer appeared responsible for sedative purposes (10), these data are not conclusive since enantiomers have not been tested clinically. Furthermore, the isomers are rapidly interconvertible in solution. The imide bonds in the two-ring system of both enantiomers are susceptible to hydrolytic cleavage *in vitro* at pH values greater than 6.

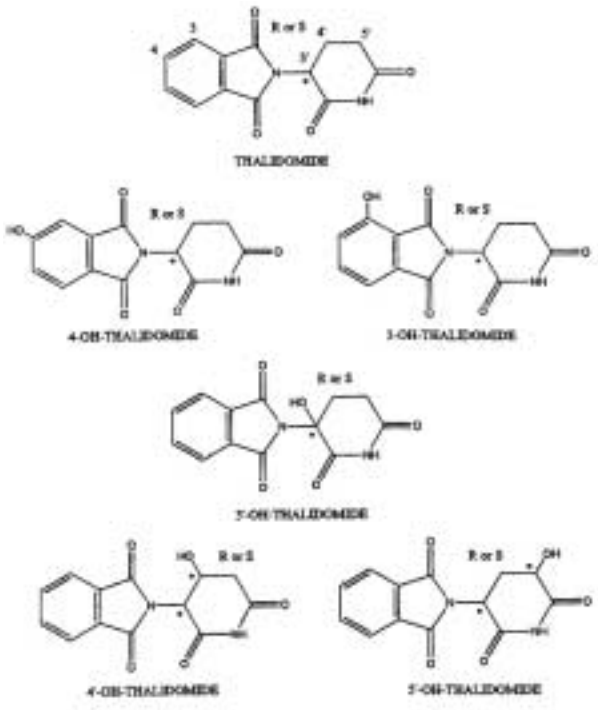

Figure 1. The chemical structure of thalidomide and its major metabolites.

Activity of Thalidomide in Different Diseases

Early studies done in 1953 established the anxiolytic, hypnotic, antiemetic, and adjuvant analgesic properties of thalidomide (44,45). Subsequently, thalidomide was found to be highly effective in suppressing erythema nodosum leprosum (cutaneous manifestation of leprosy) (3,5). Based on its beneficial effects in the treatment of inflammatory dermatoses associated with this specific condition, the drug has been used for the treatment of other inflammatory, autoimmune, and/or dermatological disorders, such as rheumatoid arthritis, inflammatory bowel diseases, lupus erythematosus, pyoderma gangrenosum, tuberculosis, sarcoidosis, Behcet's disease, chronic GVHD, and Sjögren's syndrome (6). Recent research has shown promising results with thalidomide in patients with progressive body weight loss and night sweats related to cancer or AIDS (7,23,25). Most recently,

thalidomide has proved to have antitumor activity in patients with multiple myeloma (19) and myelodysplasia. Also, there are early hints of its activity against human solid tumors including renal cell cancer (21), prostate cancer and melanoma (22), hepatocellular cancer (46), and a variety of other solid tumor malignancies, including Kaposi's sarcoma in HIV-infected patients (47,48).

Anti-myeloma Activity

Several phase I/II studies in heavily pretreated patients showed that thalidomide has substantial and, in some patients, remarkable activity against myeloma (Table 2).

The largest and most thoroughly analyzed trial, a phase I study done at the University of Arkansas Cancer Research Center, where doses were increased to the maximum of 800 mg/day, reported 32% of heavily pretreated drug-refractory myeloma patients responding to thalidomide (18). Response was assessed on the basis of a reduction of the myeloma protein in serum or Bence-Jones protein in urine, which lasted for at least 6 weeks. The serum or urine paraprotein level declined by at least 90% in 8 patients, and between 50% and 90% in 13 patients, whereas additional 6 patients had a minor response. Median duration of treatment was 2.7 months, median survival rate was 4.8 months, and many patients continue under follow-up. Disease progressed in 68% of patients. Results in other studies vary with respect to response rates and response duration, but confirm that at least one-third of drug resistant patients derive benefit from the drug (49-55) Thalidomide and glucocorticoids appear to be synergistic in cell culture, raising the possibility of their combined use in earlier stages of disease (51).

Activity of Thalidomide against Myelodysplasia

There is an evidence for the involvement of cytokines in the development of myelodysplastic syndrome (56-58). Some of the immunomodulatory properties of thalidomide can be attributed to the inhibition of monocyte/macrophage cytokine secretion, which provides the rationale for its use in cytokine-driven disease. Data from clinical trials using thalidomide in myelodysplastic syndrome are limited, but positive outcomes have been noticed among certain populations of these patients (Table 3).

The responses were mostly partial, but in two studies done with 200-800 mg daily oral dosage

Table 2. Current results of thalidomide trials in patients with myeloma

Author[a] (reference)	Dose range (mg/day)	No. of patients	Response rate[b] (%)		Stable disease (%)	Median duration (months)	
			complete	partial		treatment	response
Singhal et al (18)	200-800	84	2.4	30.0	64.0	2.7	–
Juliusson et al (19)	200-800	19	–[c]	47.0	21.0	–	6
Rajkumar et al (49)	200-800	16	–	25.0	37.5	10	3
Hideshima et al (50)	100-800	44	2.3	25.0	36.0	6	6
Kneller et al (51)	200-800	17	–	64.0	29.4	3.9	1
Sabir et al (52)	200-800	10	20.0	50.0	30.0	–	9
Shima et al (53)	100-800	13	7.7	54.0	–	–	–
Weber et al (54)	200-800	44	–	25.0	–	3	–
Brian et al (55)	50-400	33	3.0	24.0	–	2	5

[a]Three other studies reported activity of thalidomide in multiple myeloma but criteria for response either were not given, differed from above, or the patient series analyzed was too small.
[b]Criteria of response: complete – 90% or greater decrease in abnormal protein from blood or urine; partial – 50-90% decrease of abnormal protein from blood or urine.
[c]Not reported.

Table 3. Activity of thalidomide against myelodysplasia

Author (ref.)	Disease[a] (No. of patients)	Dose range (mg/day)	Response[b] (%)		Stable disease	Median duration of treatment (weeks)	Side effects	Comment
			complete	partial				
Raza et al (20) (N = 31)	RA (n = 6) RARS (n = 18) RAEB (n = 6) CMML (n = 1)	100-400	–[c]	41	–	12	constipation, fatigue, fluid retention	best response in refractory anemias (CRA, RARS)
Strupp et al (59) (N = 34)	RAEBT (n = 5) RAEB (n = 4) CMML (n = 3) RARS (n = 6) RA (n = 16)	200-400	4	9	10	10	fatigue, skin rash	best response in RARS,RAEB,RAEBT, and CMML
Estey et al (60)[a,d] (N = 74)	AML (n = 52) RAEB (n = 11) RAEBT (n = 11)	400-600	–	42	–	24	fatigue	study terminated because it did not achieve 20% improvement in respiration rate
Thomas et al (61) (N = 27)	AML (n = 10) RAEB (n = 6) RAEBT (n = 2) CMMLT (n = 1) Other (n = 8)	200-800	17	–	in 50% of CML pts.	7.5	neurotoxicity, fatigue, skin rash, infection	hematological improvement in 11% of patients with AML

[a]Abbreviations: AML – acute myeloid leukemia, ANNL – acute nonlymphocytic leukemia, CML – chronic myeloid leukemia, CMML – chronic myelomonocytic leukemia, CMMLT – chronic myelomonocytic leukemia in transformation, RA – refractory anemia, CRA – chronic refractory anemia, MDS – myelodysplastic syndrome, RARS – RA with ringed sideroblasts, RAEB – RA with excess of blasts, RAEBT – RAEB in transformation.
[b]Criteria of response: complete – normalization of all blood counts for at least four months of duration; partial – increase in hemoglobin by 2.0 g/dL, and/or a 50% reduction in packed red blood cell transfusions, increase in platelet count by 30,000/L, or increase in absolute neutrophil count by 500/L.
[c]Not reported.
[d]Combined with liposomal daunorubicin and ara-c.

range of thalidomide, 17-22% of complete responses were reported (59-61). Patients with refractory anemia or refractory anemia with ringed sideroblasts in the early phase of disease demonstrated a better response than those with more advanced disease (20). Responses included the correction of anemia and/or increased platelet and neutrophil counts. Patients with low cytokine and apoptosis levels seemed to have benefited from the treatment with thalidomide. The mean duration of treatment was 14 weeks, but from the multiple myeloma experience it appears that therapy should be continued for up to 25 weeks. Among responders, the median time to achieve response was 29 days (range, 4 days to 6 months). The most frequent side effects were constipation, fatigue, and fluid retention, whereas neurotoxicity was avoided to the large extent by pyridoxine prophylaxis.

Activity of Thalidomide against Solid Tumors

Because thalidomide was shown to be an inhibitor of angiogenesis in experimental *in vitro* models using endothelial cells (15), it entered several phase I/II trials against other cancers and is currently being evaluated for the treatment of a variety of malignancies. Results reported thus far are summarized in Table 4.

The first oncologic studies with thalidomide were reported in 1965, using daily doses between 300 mg and 2 g (67). Seventy-one patients with various malignancies were treated and a renal cancer patient with lung metastases responded (25). Since that initial trial, several other have been initiated and produced responses in patients with prostate cancer (36-68%) (21), Kaposi's sarcoma (17-37%) (68), renal cell cancer (8-17%) (22), recurrent high grade gliomas (6-15%) (62), hepatocellular cancer (5%) (46), breast cancer (53%) (63), and other various solid tumors (36-62%) (28). There was no objective response in squamous cell cancer of the head and neck (64) and non-small cell lung cancer (69) (Table 4). Combinations of thalidomide and cytotoxic drugs were tested, but the response rates have been difficult to evaluate. It is unclear whether thalidomide made a positive contribution.

Activity of Thalidomide against Graft-Versus-Host Disease

Because of its immunosuppressive properties thalidomide has been studied in bone marrow allotransplant patients for the suppression of chronic GVHD unresponsive to other therapies (Table 5). There have been 150 patients reported, and the dose of thalidomide ranged from 100 mg to 600 mg. A complete response was obtained in 32% and a partial response in 27% of patients. Most studies included patients who previously failed treatment with cyclosporine, azathioprine, and/or corticosteroids (30,31,70,71).

Activity of Thalidomide against Kaposi's Sarcoma

Preliminary laboratory studies have suggested that thalidomide may have a potential in the treatment of AIDS patients (4,15). It significantly reduced

Table 4. The use of thalidomide in solid tumor malignancies

Malignancy (ref.)	Dose range (mg)	No. of patients	Response rates (%)[a] partial	minor	Stable disease	Median time to progression (weeks)	Adverse reactions
Prostate cancer (21)	200	50	36[b]	–[c]	–	–	constipation, dizziness, edema, fatigue, neuropathy
	up to 1,200	13	68[b]	–	–	–	
Melanoma (22)	100	17	–	–	24	20	–
Renal cell cancer (22)	100	18	17	–	17	–	lethargy, grade 2, neuropathy, skin rashes
	400-1,200	15	8	8	24	–	
Ovarian cancer (22)	100[d]	19	–			–	–
Hepatocellular cancer (40)	400-1,000	21	5	5	48	8	somnolence, skin rash grade 3-4
High grade glioma (62)	800-1,200	36	6	6	33	10	constipation, drowsiness
	100-500	37	15	–	32	–	
	300 mg/m^2[e]	46	12	–	70	24	
Breast cancer (63)	100	12	–			–	–
	100-300	7	43	–	14	–	
	400	7	study is still going on			–	
Squamous cell cancer of the head and neck (64)	200-1,200	17	– 94% discontinued due to progression				–
Non small cell lung cancer (60)	200-1,000	9	–			–	fatigue, myalgia, constipation, grade 1 neuropathy
Miscellaneous solid tumor malignancies (61)	200-2,400	58	62[f]	10% discontinued due to toxicity		–	somnolence, rash, constipation, fatigue, changes in mental status

[a]Criteria of response: for gliomas – those proposed by McDonald et al (65); for prostate cancer – complete: disappearance of circulating prostate-specific antigen (PSA) from the peripheral blood for at least six months; partial: decline in PSA concentration in peripheral blood for more than 50% for at least six months; other authors reporting on other solid tumors used criteria generally compatible with the International Union Against Cancer (UICC, ref. 66): 1. Complete response: disappearance of all clinical and laboratory signs of the disease for at least four weeks. 2. Partial response: at least 50% reduction in tumor size as the sum of the products of the longest perpendicular diameters of all indicator lesions. 3. Progressive disease: the appearance of new lesions or an increase in at least 25% in the sum of the product of the longest perpendicular diameters of measurable lesions; minor response – regression of measurable or indicator lesions for less than 50%.
[b]Decline in PSA greater than 50%.
[c]Data were not reported or were erratic, or cryteria of response were not conclusive, or not mentioned at all, or no objective response was observed.
[d]Ten patients had combination therapy.
[e]Combined with carboplatin.
[f]Combined with CAF (cyclophosphamide, doxorubicin, 5-fu).

human immunodeficiency virus (HIV-1) replication both in mononuclear cells from the human peripheral blood and in laboratory cell lines (4), and it inhibited proliferation of endothelial cells *in vitro* (15). Since Kaposi's sarcoma is a tumor derived from endothelial cells, several phase I or phase I/II trials have been performed in patients with AIDS–related Kaposi's sarcoma (Table 6). Altogether 62 patients have been treated and their median age was 39 years. The dose of thalidomide ranged from 100-1000 mg/day, given mostly before sleep. There were 34% partial responses, and the disease was stable in an additional 38% of patients. Median duration of treatment was 4.2 months, and duration of response 4.8 months. In a study, 8 patients dropped out because of toxicity. Major toxic effects were drowsiness and peripheral neuropathy (47,48,74,75).

Activity in Other Diseases

Thalidomide has been designated an orphan drug by the Food and Drug Administration (FDA) for the treatment of erythema nodosum leprosum and reactional lepromatous leprosy. It has also been approved by the FDA for the treatment of HIV-associated wasting syndrome, prevention and treatment of severe recurrent aphtous stomatitis in immunocompromised patients, treatment of clinical manifestations of mycobacterial infection caused by *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, treatment of Crohn's disease, and treatment of primary brain tumors (26). In nonmalignant diseases, thalidomide was given in a 100-400 mg/day dose range, and produced 66-75% of responses in patients with erythema nodosum leprosum, 16% in patients with Behcet's disease, 55% in patients with HIV-associated diarrhea, and 9% in patients with HIV-associated aphtous stomatitis (77). There is also evidence of activity against ankylosing spondilitis, refractory rheumatoid arthritis, and sarcoidosis (78).

Mechanism of Action

Despite of the complexity of thalidomide metabolism and the potential contribution of its numerous metabolites, our current understanding of the mechanism of action is limited to studies of the parent compound (79). At least two properties, anti-angiogenesis and immune modulation (8,9,16,17), represent the leading hypotheses regarding its anti-tumor activity. In fact, these two effects may be closely related through the effects of thalidomide on cytokine secretion.

Antiangiogenic Activity

Thalidomide inhibits angiogenesis in several experimental assay systems, such as *in vivo* suppression of vessel proliferation in the rabbit micropocket assay (13), and *in vitro* against rat and human vascular endothelial cells in culture (15,80). It suppresses TNF-α and interferon γ (IFN-γ) secretion, both of which upregulate endothelial cell integrin expression, a process crucial for a new vessel formation (9). It inhibits secretion of basic fibroblast growth factor (bFGF), an angiogenic factor secreted by human tumors (62,81,82). Whether any or all of these effects account for its antitumor activity is unknown.

Immune Modulation

Thalidomide has a broad range of inhibitory and stimulatory effects on the immune system. It inhibits the migration of both immune and phagocytic cells in experimental systems. For example, it blocks leukocyte chemotaxis and phagocytosis, an effect associated with decreasing integrin beta-chain production (9,42,43,82). It reduces tumor-associated macrophage infiltration possibly through suppressing expression of endothelial cell adhesion molecules (7). In experimental animals, it promotes the switch to a Th^2 immune response, enhances the production of interleukins (IL) 4 and 5, and decreases helper T-cell production. In humans, thalidomide treatment is as-

Table 5. The activity of thalidomide against chronic graft-versus host disease (GVHD)

Author (ref.)	Previous treatment	No. of patients	Dose of thalidomide (mg)	Responders (%) (complete + partial)[a]
Parker et al (70)	Prednisone Cyclosporine	80	100 × 4/day; escalation to 200 and 300 × 4/day	20
Mehta et al (71)	Cyclosporine Azathyoprine steroids Tacrolimus	6	12.5-25 mg/kg	33
Vogelsang et al (30)	Cyclosporine lymphocyte-depleted marrow	44	200 × 4/day	64
Rovelli et al (72)	Cyclosporine Corticosteroids	14	50 × 2-3/day; escalation to 800/day	71
McCharty et al (31)	–[b]	6	200-600	33

[a]Criteria of response: complete – disappearance of all clinical and laboratory signs of GVHD; partial – 50% or more improvement in clinical symptoms and laboratory parameters (73).
[b]Data not reported.

Table 6. Effects of thalidomide in patients with Kaposi's sarcoma

Author (ref.)	No. of patients	Age	Dose of thalidomide (mg/day)	Response rate (%)[a]		Median duration (months)	
				complete + partial	stable disease	treatment	response
Little et al (47)	20	29-49	200-1,000	47	11.7	6.3	7.3
Fife et al (48)	17	33-48	100	35	–	2	–
Politi et al (74)	12	27-50	200-600	17	58	until toxicity	5.5
Yarchoan et al (75)	13	–[b]	200-1,000	36	45	–	9

[a]Criteria of response: AIDS Clinical Trials Group method (76).
[b]Data were not reported or were erratic, or criteria of response were not conclusive, or not mentioned at all.

sociated with multiple changes in cytokine levels and cellular cytokine secretion. It stimulates IL-2 and IL-12 production in HIV-infected patients (83,84), suppresses IFN-γ production in macrophages (85), but stimulates IFN-γ production in lipopolisacharide-stimulated polimorphonuclear cells in healthy individuals (86) and blocks TNF-α production in patients with erythema nodosum leprosum (5). In addition, two indirect anti-tumor effects of thalidomide have been recognized: inhibition of secretion of IL-6, a cytokine secreted by the bone marrow stroma essential for survival and proliferation of myeloma cells, and stimulation of secretion of IL-12, a potent inhibitor of angiogenesis and stimulator of IFN-γ synthesis. The broad nature of its action raises the possibility that at least part of its anti-tumor effects could be dependent on these or other as yet unrecognized effects on cytokines or specific immune cell subpopulations.

Finally, thalidomide or its metabolites may have direct anti-tumor effects. In cell culture, thalidomide suppresses the proliferation of human myeloma cells, but only at very high and probably pharmacologically irrelevant concentrations (100 μmol/L) (50). Thalidomide analogues have at least 100-fold greater potency in directly inhibiting tumor cell growth, but thalidomide metabolites have not been clinically tested yet. Until its metabolism is better understood, the possibility of direct cytotoxic action cannot be ruled out.

Mechanism of Antitumor Action

The precise biologic mechanism whereby thalidomide exerts its antineoplastic effect remains to be determined. Perhaps its most interesting property is the ability to block the growth of blood vessels. Angiogenesis is a central property of tumors and a prognostic factor for survival in carcinomas of the breast (87,88), esophagus (89), lung (90), and prostate (91). The density of tumor vasculature also correlates with increased metastases, recurrences, and overall worse prognosis for carcinomas of the bladder (32,92), colon (93), stomach (94), and melanoma (95).

Bone marrow microvessel density in hematological malignancies makes a correlation to tumor vascularization. In some studies, increased marrow microvessel density in childhood acute lymphocytic leukemia (96), multiple myeloma (49,50), and myeloid metaplasia (51,52) correlated with poor prognosis.

In a rabbit cornea micropocket assay *in vivo* (13) and in rat aorta cell and human endothelial cell cultures *in vitro* (15,81) thalidomide inhibited vessel growth stimulated by bFGF. It also inhibited vascular endothelial growth factor (VEGF) activity in a mouse and rabbit corneal vascularization model (37,97). Thalidomide may also indirectly inhibit angiogenesis through its inhibition of macrophage/monocyte function since these cells are potent inducers of endothelial cell growth (38,98). Whether thalidomide, or its metabolite, is responsible for the anti-angiogenic activity is unclear. One experiment in which the drug was incubated with microsomes suggested that the anti-angiogenic effect of thalidomide might be a result of its metabolic activation by cytochrome P450 (99). Since most malignant tumors depend on angiogenesis to proliferate and metastasize, this could be the major mechanism of its beneficial action in patients with myeloma and solid tumors.

The effects of thalidomide on myeloma may result from the inhibition of cytokine pathways that control myeloma cell growth and viability. IL-6 is secreted by bone marrow stroma and macrophages, as well as by some myeloma cells. It supports myeloma growth *in vitro* and facilitates CD-44 mediated contact through beta-integrins and fibronectins to bone marrow stroma. Attachment to stroma by myeloma cells stimulates production of VEGF and bFGF, both potent angiogenic factors, as well as a number of other cytokines (TNF-α, IL-1_{β}, and IL-10), which further promote proliferation of myeloma cells. Thalidomide blocks IL-6 secretion by myeloma cells, thus interrupting the cascade of cytokine secretion and proliferation.

Pharmacokinetics

The pharmacokinetics of thalidomide has been studied in healthy adult volunteers, older prostate cancer patients, patients with leprosy, and to some extent in HIV-infected patients (Table 7).

The absolute bioavailability of thalidomide administered as the commercially available racemic mixture after oral intake is limited as the drug is slowly absorbed from the gastrointestinal tract. The total absorption of thalidomide increases proportionally with the increase in dosage from 200 mg to 1,200 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 thalidomide's poor aqueous solubility affects the rate of dissolution and absorption after oral intake. In healthy men, peak plasma levels of 0.8-1.4 μg/mL were obtained in a mean of 4.4 h (range, 1.9-6.2 h) following a single 200-mg oral dose. Peak plasma levels of 3.6 μg/mL would be achieved at steady-state with administration of thalidomide at the above dose every 6 h (99). Volume of distribution for thalidomide was 120.69±45.36 L. Plasma concentration vs time curves fit a one-compartment model with first-order absorption and elimination; absorption half-life was 1.7±1.05 h, and

Table 7. Pharmacokinetic parameter values for thalidomide (33,99,100)

Subjects			Dose	Peak plasma	Time range	Absorption	Elimination half	Volume of
type	No.	Age	(mg)	concentration (mg/mL)	to peak (h)	half time ±SD (h)	time ±SD (h)	distribution ±SD (L)
Healthy male volunteers	8	21-43	200	1.15±0.2	3.12-5.56	1.70±1.05	8.70±4.11	120.69±45.4
Prostate cancer patients	13	55-80	200	1.15-3.79	2.01-7.09	1.50±0.45	6.52±3.81	66.9±34.27
Prostate cancer patients	11	55-80	1200	2.41-8.41	1.35-7.12	3.34±0.89	18.25±14.08	165.81±84.18
Leprosy patients	6	–[a]	400	3.44	2.9-5.7	–	6.86	46.4

[a]Not reported.

elimination half-life of parent compound was 8.7 ± 4.11 h, about three times longer than that observed in animals (100). Total body clearance rate is relatively slow: 10.41 ± 2.04 L/h. Information on distribution of thalidomide in humans is not available. Administration of radiolabeled drug into animals results in an even distribution of radioactivity except for slight enhancement in kidneys, liver, biliary tissue, white matter of CNS, and peripheral nerve trunks. The exact route of elimination in humans is not known but less than 0.6 ± 0.22% of drug is excreted in urine as unchanged drug in the first 24 h, suggesting a dominant non-renal route of excretion (100).

Metabolism

Two routes of thalidomide degradation seem likely. One is hydrolysis of the four amide bonds, which are labile in aqueous solution. The other is enzymatic P450 mediated hydroxylation of the phthalimide and possibly the glutaramide ring. The precise pattern of metabolites and the potential contribution to the biological action of thalidomide are uncertain. Studies with canine hepatic microsomes in the presence of nicotinamidadenindinucleotidephosphate (NADPH) suggest that metabolites are formed via hepatic degradation (99). After single oral administration to humans two metabolites were isolated from urine: 3-hydroxyphthalimic acid and 4-phthalimidoglutarimic acid (101). Thalidomide does not induce or inhibit its own metabolism. When the drug was administered to healthy women at a dosage of 200 mg/day for 18 days, similar pharmacokinetics were observed on the first and last day of dosage (27).

Thalidomide undergoes rapid spontaneous hydrolysis in aqueous solutions *in vitro* at pH 6.0 or greater to form three primary products: 4-phthalimidoglutarimic acid, 2-phthalimidoglutarimic acid, and α-(o–carboxylbenzamido)-glutarimide. When thalidomide is administered orally to animals, only a small amount of the unchanged drug is excreted in the urine (102). The major portion of the compound is broken down and excreted as transformation products. After administration of the drug to rats and rabbits it was possible to isolate 4-phthalimidoglutarimic acid from their urine. After drug had been given to humans, 3-hydroxyphthalimic acid and 4-phthalimidoglutarimic acid were isolated from urine. Also, a fluorescent compound, considered to be 3-hydroxyphthalimic acid, was detected (33). Considering five positions for thalidomide hydroxylation five primary metabolites could be expected in humans (4-OH-thalidomide, 3-OH-thalidomide, 3′-OH-thalidomide, 4′-OH-thalidomide, and 5′-OH-thalidomide, Fig. 1), and a cascade can follow each of them.

Less than 15% of thalidomide is present in plasma 24 h after an oral dose. On the basis of these data and non-polar properties of the drug, it has been speculated that protein binding of the drug in plasma is high.

It is important to mention that antiangiogenic activity was not possible *in vitro* without addition of liver microsomes. Therefore, the parent compound does not possess this activity. It is attributed to one or more of its metabolites and even more probable, to one of epoxide intermediary metabolites (33).

In vitro studies have suggested that metabolites are formed via hepatic metabolism (70) involving the cytochrome P450 family, and only the parent compound is enzymatically modified. However, aromatic hydroxylation is an enzymatic reaction and, therefore, species-specific.

Toxicity

The primary side effects of thalidomide are drowsiness and constipation. Overdosage may cause prolonged sleep. Thalidomide has been reported to enhance the sedative effects of barbiturates, chlorpromazine, and reserpine, and may potentiate somnolence caused by alcohol (103). Drugs known to be associated with peripheral neuropathy, such as antiretroviral agents (didanosine, zalcitabine) and microtubular cytoskeleton inhibitors (paclitaxel, vinca alkaloids), should be used with caution in patients receiving thalidomide (104). Other adverse effects of thalidomide are somnolence, nausea, peripheral neuropathy, skin rash, and neutropenia (Table 8). With long-term use, peripheral neuropathy may become significantly bothersome. Sedation can be ameliorated by taking medication in the evening before bedtime, but may restrict dose escalation in some patients. Constipation at higher doses (above 200 mg/day) may also limit dose escalation and requires prophylactic use of stool softeners and laxatives in many patients.

Table 8. Major side effects of thalidomide (105-108)

Side effect	ENL[a] (%)	HIV[b] (%)	Other (%)	Comment
Somnolence and drowsiness	40	35	32	most common side effect
Nausea and constipation	21	40	30	associated with prolonged use
Peripheral neuropathy	1	8	5	can become irreversible if thalidomide is not discontinued
Skin rash	42	56	16	more common in AIDS[c] and BMT[d] patients
Neutropenia	29	9	18	
Respiratory (cough, bronchial lung edema, pneumonia)	12	26	–[e]	mostly in terminally ill AIDS[c] patients
Musculoskeletal	8	11	23	associated with prolonged use

[a]Erythema nodosum leprosum.
[b]Human immunodeficiency virus.
[c]Acquired immunodeficiency syndrome.
[d]Bone marrow transplantation.
[e]Data not reported.

The most common side effect in patients with HIV and erythema nodosum leprosum (38%) appears to be a skin rash, which is reported as the third most frequent side effect (Table 8). Frequently reported neutropenia is more common in AIDS and bone marrow transplant patients than in other patients, and requires no further treatment after discontinuation of thalidomide. Peripheral neuropathy is the most serious complication of thalidomide treatment; it was reported in 1-30% of patients with myeloma. The incidence probably depends on the nature of the patient

population observed and their past treatment history. It is usually associated with a prolonged use of thalidomide (more than 6 months) and with a cumulative dose of more than 50 g. Most patients recover spontaneously after withdrawal of thalidomide, but neuropathy can become irreversible if the drug is not discontinued. Some of the side effects were observed either exclusively or more frequently in patients with AIDS-related Kaposi's sarcoma than in other patient populations. For example, fever is reported only in that group. Although it is sporadically observed in other groups, depression is most frequently seen in the AIDS group. Musculoskeletal problems have been reported in 14% of the patients. Respiratory problems were reported only with erythema nodosum leprosum, and HIV patients. In general, thalidomide offers the possibility of long-term therapy with relatively low toxicity (Table 9).

Table 9. Overview of indications and contraindications for thalidomide

Food and Drug Administration (FDA) approved indication:
moderate to severe erythema nodosum leprosum (27)
Literature-supported oncological and AIDS indications:
refractory multiple myeloma (11,18)
refractory chronic graft-versus host disease (30,31,72)
AIDS-related cachexia (23,102)
AIDS-related mucocutaneous ulcers (106,107)
Potential oncology uses (still requires additional clinical research):
AIDS-related Kaposi's sarcoma (47,48,74,75)
plasma cell leukemia (39)
miscellaneous advanced solid tumors (ie, breast, prostate, melanoma, CNS) (6,22,23,25,27,64)
Potential oncology uses (phase I/II controlled clinical trials):
cancer cachexia (108)
severe profuse uncontrollable night sweats (24,109)
antiangiogenic and cardioprotective anthracycline-based combination therapy (40,41)
combination therapy with chemotherapy and/or radiation (39,40)
Contraindications:
pregnant and potentially becoming pregnant women (10,27)
patients hypersensitive to thalidomide (11)
allogeneic bone marrow transplant (AlloBMT) recipients without chronic graft-versus-host disease (110)
toxic epidermal necrolysis (106)

Conclusion

Thalidomide has become one of the major subjects of scientific interest because of its newly discovered activity against infectious diseases and certain types of tumors. It has a broad range of indications, including its well established value in the management of cutaneous inflammatory complications of leprosy (erythema nodosum leprosum), activity against other inflammatory diseases, and the reversal of weight loss associated with AIDS and cancer. In addition, thalidomide showed activity against myeloma, myelodysplastic syndrome, glioma, and prostate cancer (Table 9), but these findings require confirmation, given the notorious difficulty in judging clinical responses in both types of solid tumors.

Although there are many phase I/II trials currently going on and some remarkable results in deleterious diseases reported (myeloma and myelodysplastic syndromes), there still is a great need for larger series of patients and more properly reported studies since some of them did not use clear-cut criteria to judge a therapeutic response.

The metabolism of thalidomide and its metabolites and hydrolytic cleavage products is poorly understood. There are several potential routes of degradation and any of these metabolites could be responsible for its various biological effects. Because of the complexity of its metabolism, our current understanding of the mechanism of action is limited to the studies of the parent compound.

Thalidomide has a broad range of inhibitory and stimulatory effects on the immune system. It suppresses monocyte/macrophage function and thereby TNF-α and IFN-γ secretion, both of which upregulate endothelial cell integrin expression, a process crucial for new vessel formation, but these as well as other immunological effects and their relationships need to be clarified. The major oncologic interest is concentrated on multiple myeloma. At least two properties of thalidomide, anti-angiogenesis and immune modulation, represent the leading hypotheses regarding its anti-tumor action and especially against myeloma.

Detailed pharmacological studies would significantly aid our understanding of the mechanism of action of the drug, and the role of its degradation products, as would studies of its mechanism of action in animal models. The possibility that it may be the first active angiogenic drug lends additional importance to these pharmacological studies. Broader clinical testing of thalidomide is justified only after pharmacokinetics and metabolism are better understood. The trials should probably include decadron and perhaps chemotherapy, in addition to thalidomide in myeloma patients. The initiation of clinical trials of analogues with greater anti-angiogenic and cytotoxic potency has stimulated further interest in this unusual class of drugs.

As a sedative with an unacceptable side-effect profile in a selected population (women in their third trimester of pregnancy), thalidomide was denied a license in many countries. Almost 50 years later, it has been given a limited license, allowing its use in life-threatening conditions where other drugs have failed. As such, thalidomide is an issue of risk/benefit ratio, and its future as an orphan drug, or a possible therapeutic and market success, despite promising results, is quite uncertain.

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