REVIEW

THE POTENTIAL USE OF THALIDOMIDE IN THE THERAPY OF GRAFT-VERSUS-HOST DISEASE — A REVIEW OF CLINICAL AND LABORATORY INFORMATION

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Abstract—This article reviews the historical development of thalidomide as an immunosuppressive agent and the current state of knowledge of thalidomide as an anti-graft-versus-host disease (GVHD) agent. The evidence suggests that metabolites of thalidomide act at an early stage in the antigen recognition–activation pathway of graft T lymphocytes and down regulate normal lymphocyte responses. This effect seems to have beneficial effects in both acute and chronic GVHD, but the optimal mode of use in the clinical setting remains to be determined.

Key words: Thalidomide, graft-versus-host disease.

GRAFT VERSUS HOST DISEASE THERAPY — THE STATE OF THE ART

THE PREVENTION and treatment of graft versus host disease (GVHD) remains a problem of some significance. At the present the basis of prophylaxis and therapy is to attempt to control the immune status of the graft.

ACUTE GVHD

The prevention of acute GVHD has involved the use of three methods. Methotrexate and cyclosporin are immunosuppressive agents now used extensively in GVHD prophylaxis. The third method, using T cell depletion of the graft, is proving to cause problems of its own. Methotrexate was first studied as a prophylactic agent for GVHD in murine [1] and canine [2] models. It proved so successful that it became available for clinical use, and randomized trials comparing methotrexate versus no post-transplant

immunosuppression have not been carried out. However, non-randomized studies have shown that methotrexate reduces the incidence of acute GVHD, though does not eliminate it [3].

The use of cyclosporin, an immunosuppressant that works through the inhibition of IL-2 production and IL-2 receptor expression (for a review on cyclosporin see Shevach [4]), has improved the prophylaxis for GVHD [5]. Cyclosporin and methotrexate appear to be of comparable use in prophylaxis. However the combination of the two results in a reduced incidence of GVHD when compared to either used alone [6].

Whilst these results are encouraging, the hoped-for elimination of acute GVHD has not occurred, and the confirmation that T cells are responsible for the onset of GVHD led to the use of T cell depleted marrow for transplant. A number of studies, have used depletion by a variety of methods, including lectin agglutination [7] and the use of mAbs and complement [8] or toxin-linked mAbs [9]. The rat IgM mAb Campath-1 [10], has been used in combination with complement to remove 99% of T cells from marrow, and significantly reduced incidence of GVHD in 21 consecutive transplants in 18 patients with leukaemia and non-Hodgkin's lymphoma [11]. However, the use of T-depleted marrow has given rise to another problem. In the study of Heit *et al.* [11], the use of Campath-1 resulted in a 13% increase

in graft rejection. Other studies have shown an increase in leukaemic relapse [12] following T cell depletion.

The treatment of GVHD involves a balance between giving sufficient immunosuppression to control graft activity, without giving excessive immunosuppression which would increase the already high risk of developing infection. Corticosteroids and cyclosporin have been the main treatments used, with methyl prednisolone proving very effective [13].

CHRONIC GVHD

The use of combination methotrexate and cyclosporin has proved to be of no benefit in the prevention of chronic GVHD [6], and the prevention of chronic GVHD must lie with the prior prevention of acute GVHD. Established chronic GVHD does, however, respond to low dose prednisolone in combination with azathioprine. This combination prevents the progression of chronic GVHD and significantly reduces the mortality from the condition [14]. However, as with acute GVHD, no treatment has proved to be satisfactory.

The two major problems with existing therapy can be viewed as:

(1) Lack of specificity, and therefore immunosuppressive actions are potentially dangerous for the compromised patient since they increase risk of supervening infection.

(2) The drugs used in prevention and treatment are themselves potentially toxic and therefore possibly life-threatening to the patient.

Research has thus been directed at other drugs with immunosuppressive actions that may be of some benefit in the therapy for GVHD. In this regard, the drug thalidomide has been studied both in animal models and in the clinical setting.

THALIDOMIDE

Thalidomide (N-phthalidoglutarimide) was first synthesized in 1953 by researchers at Chemie Grunenthal in West Germany, and marketed there from 1956. Following this it was distributed to other countries including the U.K. as a sedative/hypnotic drug. Under the brand name of Distaval® in the U.K., thalidomide was widely prescribed because it seemed to be the 'ideal' drug, showing good activity, combined with the absence of acute toxicity and side effects. Upon long-term use, however, some side-effects became evident, and reports of peripheral neuropathy appeared in the literature [15]. It was, however, the confirmed teratogenic actions of thalidomide, first reported by Lenz & Knapp [16], that

led to the withdrawal of thalidomide from the market. Thalidomide has been unavailable as a mainstream drug since that time, but has been available for defined research purposes.

After the initial shock of the teratogenicity of thalidomide, investigators have used thalidomide in the therapy of many diseases. It was first reported [17] that patients with reactional lepromatous leprosy, who received thalidomide as a sedative, experienced spectacular relief of symptoms. A WHO trial confirmed these results [18]. Since that time, thalidomide has been shown to be effective in a variety of other diseases including chronic discoid lupus erythematosus, Bechet's syndrome, prurigo nodularis and ulcerative colitis (reviewed by Barnhill & McDougall [19]).

The common factor thought to link the disorders mentioned above is that they are all purported to be immunologically mediated. The results have been interpreted as suggesting that thalidomide may have immunosuppressive properties, but the tragedy of thalidomide's teratogenicity, observed after the drug was released onto the market, has seriously inhibited the research into the drug and its mechanism of action.

THALIDOMIDE IN GVHD

It was the possibility that thalidomide may have immunosuppressive actions that led to the interest in the drug as a possible therapeutic agent in GVHD. One early report suggested that thalidomide could at least partially arrest GVHD in a mouse model [20], though the assessment of GVHD by spleen weight is not particularly sensitive. After this report the phenomenon remained uninvestigated until Vogelsang et al. [21] began to study thalidomide in GVHD in a rat model. The Lewis (RT1.1)-ACI (RT1.a) rat major mismatch model (the MHC in the rat is termed the RT1 complex) is an established model that was used in the studies on cyclosporin [22]. Lewis rats are total-body irradiated and then given RT1-incompatible ACI marrow. Within two weeks of transplant, acute GVHD develops, and this is assessed by clinical appearance (erythema of the skin, ears and footpads) and by pathological grading of skin biopsy (Grade II and above). This model has been used to study the potential of thalidomide in both the prophylaxis and treatment of GVHD.

Vogelsang et al. [21] reported that thalidomide, given by gavage at 50 or 100 mg/kg/day for 40 days after clinical and histological onset of acute GVHD, successfully resolved acute GVHD in 22 of 23 animals. In addition, a surprising result was that after stopping thalidomide therapy, there was no

reappearance of GVHD in 19 of the 22 animals. In the remaining three, chronic GVHD developed three weeks later. Chimerism was demonstrated by the acceptance of ACI skin grafts > 100 days post-transplant. Third-party grafts were still rejected. However, in normal Lewis rats, thalidomide did not prolong survival of ACI skin grafts.

In the same study, thalidomide was shown to be of benefit as a prophylactic agent for GVHD. When thalidomide was given on the day of transplant and continued for 40 days, 14 rats out of 22 did not develop GVHD following transplant. The other eight developed a clinical infection with rat corona virus which caused mild GVHD. This responded to thalidomide therapy, and again, in all rats, no GVHD occurred after the drug was stopped. Chimerism was again demonstrated as above. Finally, tolerance was demonstrated by a failure of spleen cells from chimeric animals to respond to recipient or donor lymphocytes. In a more recent series of experiments Vogelsang et al. [23] using lower dose thalidomide, 10 mg/kg, or the same dose plus cyclosporin A, 10 mg/kg, demonstrated the superiority of thalidomide or thalidomide plus cyclosporin over methotrexate or azothiaprine ± steroids.

Vogelsang et al. [24] have also tested compounds structurally related to thalidomide to study the mechanisms of action of thalidomide in GVHD. Phthalimide is structurally the double ring half of the thalidomide molecule and in the same rat model was effective in preventing GVHD (26 out of 26 animals treated), but was unable to control established GVHD (0 out of 4 animals). Aminoglutethamide, the other half of the molecule, was ineffective in prevention and therapy (14 out of 16 animals developed GVHD). A fluorescent derivative of thalidomide, in addition to successfully controlling GVHD in four animals, was capable of inhibiting phytohaemagglutinin (PHA), concanavalin A (Con A) and alloantigen responses of lymphocytes in vitro.

This study suggests that the active part of the thalidomide molecule in GVHD is the phthalimide ring. In addition, the in-vitro results suggest an inhibitory action on lymphocyte activation signals. Further work has shown an additive or synergistic effect of thalidomide and cyclosporin in the prophylaxis of GVHD [25].

Clinical work with thalidomide in GVHD is in its infancy. Anecdotal evidence began appearing in the literature in 1988, when Lim et al. [26] reported control of established acute GVHD with thalidomide at 400 mg, increasing to 800 mg per day, and combined with prednisolone. Saurat et al. [27] claimed to successfully treat chronic GVHD with 300 mg per day for 6 months. However, after this time the drug

had to be withdrawn when paraesthesia developed in both feet. A report from Sweden [28] showed that in patients with supervening infections, thalidomide may fail to halt the progress of GVHD. Two reports of successful therapy for chronic GVHD in children have also appeared in the literature [29, 30]. Vogelsang and her colleagues have reported that they have commenced a clinical trial using thalidomide in GVHD [25].

MECHANISMS OF ACTION

Studies on the immunosuppressive action of thalidomide are extremely contradictory, and little recent work has been undertaken. Hellmann et al. [31] found that thalidomide prolonged the survival of skin homografts transplanted across MHC barriers in mice. They later showed that thalidomide appeared to decrease the number of 'immunoblasts' in local lymph nodes after skin homografts [32]. However, other workers have failed to find any effects of thalidomide in the same system [33, 34]. Thalidomide was found to have no effect on Types I, III and IV hypersensitivity reactions [35]. Coulson et al. [36] showed that thalidomide derivatives could inhibit the production of transformed cells in MLC, whereas they did not inhibit the response to PHA. This may imply that thalidomide derivatives may act early in the antigen-recognition pathway. However, it must be accepted that the experiments designed to study the immunosuppressive actions of thalidomide have not reached any firm conclusions.

Studies directed at the teratogenicity of thalidomide may provide some additional information. In the presence of hepatic microsomal drug-metabolizing system, thalidomide metabolites, but not thalidomide itself, inhibited the attachment of cells to Con A-coated plates [37], suggesting an interference with cell–cell interaction and/or cell activation. Using the same system, thalidomide metabolites were shown to be directly toxic to human lymphocytes [38], though thalidomide itself was ineffective.

PROPOSED ACTION IN GVHD

The important points from the research outlined above need to be summarized and considered in the context of GVHD:

In-vitro work suggests that:

(a) Thalidomide itself may not be the active compound, but may require metabolism to active derivatives, probably in the liver.

(b) The active compound appears to act at an early stage in the recognition–activation pathway of

lymphocytes to prevent responses to antigenic or mitogenic stimuli.

(c) This action is probably at a surface receptor in the lymphocyte membrane, suggested by the relatively rapid effects of the compound.

(d) The effect on the cell is somehow to down-regulate normal responses to antigenic stimulus — this effect could either be a specific interference with a particular messenger system or a direct toxicity effect on the cell. (There is evidence for the latter mechanism.)

In-vivo data indicate that:

(a) Thalidomide or metabolite(s) can prevent and cure clinical GVHD, both acute and chronic.

(b) In contrast to other immunosuppressants, thalidomide appears to act in a permanent way: GVHD is controlled after therapy has been stopped.

Taken together, the hypothesis that can be tentatively made is that an active metabolite of thalidomide, produced by the liver, acts on graft T cells via a surface receptor to produce a permanent non-responsive state. In the situation of the newly transplanted recipient, this down-regulation will prevent the occurrence or progression of any graft-versus-host reactions.

CONCLUSIONS

Graft-versus-host disease still remains the major complicating factor of allogeneic bone marrow transplantation, and it is clear that a great deal more work needs to be done at the cellular level to establish the exact pathogenic mechanisms which occur. However, thalidomide appears to offer promise as a new therapy for GVHD. Research must be done to study more closely the mechanisms of action of this versatile drug, but controlled clinical trials of thalidomide will be needed to establish efficacy in the clinical arena.

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