INTERNATIONAL JOURNAL OF LEPROSY Volume 39, Number 2
Printed in the U.S.A.

Thalidomide Activity in Experimental Arthus and Anaphylactic Reactions[1, 2]

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Lepra reaction is characterized by *erythema nodosum leprosum* (ENL) fever, lymphadenopathy, neuritis, arthralgia, and other symptoms which constitute a serious clinical aspect of the disease in many patients with lepromatous leprosy. These reactions, though sometimes present in untreated leprosy, usually occur during the course of treatment with sulfones, when the Morphologic Index (MI) indicates that the number of viable bacteria is low ([10]). The etiology of these reactions has never been adequately demonstrated, but it has been proposed that these are perhaps reactions to immediate hypersensitivity, similar to Arthus or serum-sickness reactions, occurring when the bacilli or their degradative products are liberated, combine with circulating antibody, and fix complement ([14-16]).

Following the work of Sheskin in 1965 ([13]), numerous authors have demonstrated the extraordinary efficacy of thalidomide in the alleviation of the reactional symptoms of leprosy. The mechanism by which this drug acts in the lepra reaction is unknown. The suggestion that the teratogenic effect of thalidomide might in part be based on the suppression of the immune response has stimulated a number of investigations concerning its effect in skin-graft rejection and antibody production ([5, 6]), and a brief report on its effect on delayed and immediate hypersensitivities ([9]). Most of these reports indicate that its role in modifying these immune phenomena would seem to be a minor one.

The present study concerns the effect of thalidomide on the expression of reactions of immediate hypersensitivity (including active and passive Arthus and anaphylactic reactions) in guinea-pigs. This is the initial part of a broader study of the role of immune phenomena in lepra reaction and the possible immunologic mechanism of thalidomide action in this disease.

MATERIALS AND METHODS

Thalidomide administration. Thalidomide was obtained in pill form from Chemie Grunenthal GmbH, Germany. In early experiments, the desired amount was ground and suspended in phosphate-buffered saline, pH 7.2 (PBS). The volume necessary to provide 25 mg/kg of weight per guinea-pig was injected intraperitoneally once daily; the suspensions were kept in the refrigerator (4°C) for three or four days.

In subsequent experiments, only the amount of thalidomide needed for each feeding was suspended in PBS just before use. This was given by mouth twice daily, using a 1 cc syringe. Each animal was given 50 mg/kg body weight at each feeding. Drug administration was continued until all final readings had been made.

Active Arthus reactions. Guinea-pigs were sensitized by injecting 5 mg of egg albumin in complete Freund's adjuvant into two subcutaneous inguinal sites, 0.5 cc of suspension in each site. After 10 to 15 days, reactions were elicited by the intracutaneous injection of 0.5 mg of egg albumin, dissolved in 0.1 cc of PBS. Groups of these animals were then given thalidomide for 10 days; Arthus reactions were elicited as above, and the size of these reactions was compared with the size before treatment

1 Received for publication 30 December 1970.
2 This work was supported in part by Grant No. 5R01 AI04216-05 from the Public Health Service, National Institutes of Health, USA, made to the Venezuelan Association for Dermatologic Investigation.
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and with a group of untreated sensitized controls.

Guinea-pigs which had been sensitized by repeated injections of lepromin, and which gave Arthus-like reactions as well as delayed reactions to this antigen, were skin-tested with standard lepromin. Beginning one week later, thalidomide was given to these animals orally for a period of 10 days. They were skin-tested again with 0.1 cc of standard lepromin, and the size of the reactions was compared with the size of the original reaction.

Reverse passive Arthus reactions. Sera were collected from five guinea-pigs which had been actively sensitized with egg albumin. Each serum was tested for precipitating antibody by a ring test, and the sera which gave strong reactions were pooled.

The reverse passive Arthus reactions were elicited as follows: 8 mg of egg albumin, dissolved in PBS, were injected intravenously into each recipient. After 30 to 40 minutes, 0.2 cc of undiluted pooled antiserum was injected intracutaneously. The reactions were measured at 6 and 24 hours.

Passive cutaneous anaphylaxis. Normal guinea-pigs were given two daily feedings of thalidomide for a period of eight days. These animals, as well as controls, were injected intracutaneously with seven two-fold dilutions of serum from guinea-pigs sensitized to egg albumin, using dilutions from 1:250 to 1:16,000; and in an eighth site with PBS. After four hours, 5 mg of egg albumin in 0.5 cc of PBS and 0.5 cc of 1% Evans blue dye were injected intravenously. The reaction sites were examined after 15 to 30 minutes, and the reactions were measured.

Antibody production. Guinea-pigs were given thalidomide by mouth, 50 mg/kg twice daily, for seven days. Then 5 mg of egg albumin were given intravenously every third day in a series of three injections while continuing the administration of thalidomide. Systemic anaphylaxis occurred with the third injection, so the animals were bled, and their sera were examined for antibody by the Ouchterlony method and with dilutions in passive cutaneous anaphylaxis.

RESULTS

Table 1 presents the results obtained when sensitized guinea-pigs were given thalidomide orally for 10 days, and then active Arthus reactions were elicited. The average diameter in the test group sensitized to egg albumin was somewhat smaller after 10 days of treatment. However, the drop in average diameter was comparable in the untreated control group, indicating that this drop was probably due to testing when the level of sensitivity had already begun to decline, and not to the administration of the drug.

A control group of animals giving an

TABLE 1. *Effect of thalidomide, 50 mg./kg. orally twice daily, on the size of active Arthus reactions in guinea-pigs sensitized to lepromin or egg albumin.*

Group	Number	Size of original reaction at 24 hr.[a]	Size of reaction at 24 hr. after treatment for 10 days
Egg albumin Test	1	32 × 2	23 × 2½
	2	33 × 3½	28 × 2½
	3	26 × 2	26 × 3
	4	31 × 2	32 × 2½
	5	38 × 2	30 × 2½
	Average	32 mm.	28 mm.
Control	1	31 × 3	28 × 2
	2	28 × 3	20 × 3
	3	26 × 1½	25 × 2
	4	31 × 1½	18 × 1½
	Average	29 mm.	23 mm.
Lepromin Test	1	20 × ½	21 × 1
	2	36 × ½	37 × 1½
	3	25 × ¾	20 × 1
	4	20 × ¾	22 × 1
	Average	25 mm.	25 mm.

[a] Diameter and increase of skin thickness in mm. (latter determined by $\frac{\text{thickness of skin fold reaction site}}{\text{thickness of skin fold normal site}} \div 2$)

Arthus-like reaction to lepromin was not included because repeated skin testing of these animals had previously demonstrated that the average reaction size remained quite constant. In this group, thalidomide had no demonstrable effect on the size of the reactions. It should be noted that this reaction to lepromin is not due to nonspecific irritation; normal animals give reactions of 4 to 8 mm when tested with the same preparation.

The results of thalidomide administration on the expression of reverse passive Arthus reactions to egg albumin are presented in Table 2. The average reaction size in the test group is somewhat smaller than that of the untreated control group, particularly at 24 hours, but this difference is not statistically significant.

As indicated in Table 3, pretreatment of guinea-pigs with thalidomide either orally or by intraperitoneal injection did not diminish their responsiveness in the elicitation of passive cutaneous anaphylaxis reactions.

Although we did not do quantitative antibody determinations, induction of antibody production by intravenous injection of egg albumin was not demonstrably affected in guinea-pigs treated with thalidomide (Table 4). All of the sera which gave positive passive cutaneous anaphylaxis reactions with the 1:100 dilution also gave precipitin lines in double diffusion tests.

TABLE 2. *Passive Arthus reactions in guinea-pigs receiving thalidomide orally.*

Group	Number	Reaction size	
		6 hours	24 hours
Test	1	37 × 2½	40 × ½
	2	30 × 1	20 × ⅛
	3	33 × 1½	35 × ½
	4	30 × 2	45 × ¾
	5	38 × 2	20 × ⅛
	Average	34 mm.	32 mm.
Control	1	38 × 2	36 × ½
	2	32 × 1½	37 × ¼
	3	33 × 2½	37 × 1
	4	37 × 1½	36 × ½
	5	38 × 2	40 × ¼
	Average	36 mm.	37 mm.

TABLE 3. *Passive cutaneous anaphylaxis in guinea-pigs pretreated with thalidomide.*

Group	Guinea-pig	Highest positive dilution of serum from egg albumin sensitized guinea-pigs
25 mg./kg. daily, IP		
Test	1	1:8,000
	2	1:8,000
	3	1:8,000
	4	1:8,000
	5	1:4,000
Control	1	1:8,000
	2	1:8,000
	3	1:8,000
	4	1:4,000
	5	1:8,000
50 mg./kg. twice daily oral		
Test	1	1:4,000
	2	1:2,000
	3	1:8,000
	4	1: 500
	5	1:2,000
Control	1	1:4,000
	2	1:2,000
	3	1:8,000
	4	1:2,000
	5	1:2,000

DISCUSSION

The etiology of lepra reactions has never been clarified, but several possibilities exist. The conditions in which the reactions occur seem characteristic of the conditions encountered in allergic reactions of the immediate hypersensitivity type. Reactions are most severe in persons who have lesions rich in bacilli, where most of the bacilli are nonviable as judged by the MI. These conditions are conducive to the liberation of mycobacterial antigen ([10]). In addition, most lepromatous patients have appreciable amounts of precipitating antimycobacterial antibody ([8, 11]). Histologically, infiltration by polymorphonuclear leucocytes is a frequent observation in ENL

TABLE 4. *Induction of antibody production in guinea-pigs treated with thalidomide, as tested by PCA reactions.*

Group	Guinea-pig	PCA reactions (mm.)				
		Not diluted	1:10	1:50	1:100	1:500
Test	1	21	19	—	8	0
	2	17	15	16	0	0
	3	16	15	—	8	0
	4	13	12	13	0	0
Control	1	16	14	13	0	0
	2	17	16	—	6	0
	3	21	15	14	0	0
	4	16	15	—	8	0

lesions ([12]). Reduced complement levels have been reported during reactions, presumably as the result of fixation by an antigen-antibody reaction ([4]). Fixed complement and immunoglobulin deposits have been demonstrated in ENL lesions ([16]). Many of the symptoms of lepra reactions are characteristic of the symptoms observed in both clinical and experimental hypersensitive states.

Bonomo *et al.* ([1]), Matthews and Trautman ([7]), and other authors have reported the presence of antibodies and/or reactions characteristic of autoimmune disease in leprosy. These observations include positive tests for rheumatoid factor, antinuclear and LE-cell factors, and antithyroglobulin antibodies. This suggests the possibility that lepra reactions may have an autoimmune component, perhaps because the presence of large numbers of bacilli might alter the antigenic composition of normal tissue, but there is no experimental evidence to support this possibility.

Finally, primary toxicity of dead bacilli or their degradation products may be a factor in the etiology of lepra reactions.

Thalidomide, which is strikingly and rapidly effective in the treatment of lepra reactions, does not seem to be very active in modifying immunologic responses. However, since most of the reported experiments have been done with other objectives in mind, we began a study in some detail of the possible modification of one or more of the various types of immune response which might be involved in lepra reactions.

The present study did not demonstrate any modification of active or reverse passive experimental Arthus or anaphylactic reactivity in guinea-pigs, using quantities of thalidomide at least 15 times greater, on a weight basis, than those used in human beings to control lepra reactions. There was no gross modification in the induction of antibody production. We have previously reported that continuous administration of thalidomide for six to eight months in human beings does not appreciably alter the level of circulating antimycobacterial antibody ([15]).

The possibility exists that the metabolism of thalidomide in guinea-pigs is quite distinct from its metabolism in human beings. It is perhaps worth noting that the amounts of thalidomide used in this study did not produce any obvious sedation, weight loss, or other obvious physical changes in the experimental animals. However, blood levels of thalidomide after oral administration of 60 mg/kg were appreciably higher than the levels measured in human blood after administration of therapeutic doses of the drug.

It might be suggested that suspensions of thalidomide are not active, but in most of the experiments reported here, the suspen-

sions were administered within 5 to 10 minutes after their preparation. Similarly-prepared suspensions, when given to lepromatous patients, were completely effective in eliminating reactional symptoms ([2]).

SUMMARY

The effect of thalidomide in the expression of active and reverse passive Arthus reactions, cutaneous anaphylaxis, and the elicitation of antibody production was studied in guinea-pigs. In spite of the use of quantities of thalidomide much greater than those used in the treatment of lepra reactions in human beings, no appreciable diminution in these reactions was observed.

RESUMEN

Se estudió, en cobayos, el efecto de la talidomida en la expresión de reacciones de Arthus activa y pasiva invertida, anafilaxia cutánea y en la producción de anticuerpos. A pesar de que se utilizaron cantidades de talidomida mucho mayores que las que se utilizan en el tratamiento de la reacción leprosa en seres humanos, no se observó una reducción apreciable de estas reacciones.

RÉSUMÉ

On a étudié chez les cobayes la manière dont la thalidomide révèle les réactions d'Arthus, d'active et passive (reverse passive), l'anaphylaxie cutanée, ainsi que son action pour déclencher la production d'anticorps. Malgré que des quantité de thalidomide beaucoup plus élevées que celles généralement employées dans le traitement de la réaction lépreuse chez des humains aient été utilisées pour cette étude, on n'a pu observer aucune diminution appréciable dans l'intensité de ces réactions.

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