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Changes in the Activity of Blood Plasmin After Administration of 3-Pyridyl Acetic Acid in Leprosy Patients

Correlation of Activity Changes With the Mitsuda Reaction[1]

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This paper presents the results of studies aimed at finding correlations between the clinical picture of leprosy, the lepromin reaction, and the host's plasmin reactivity. Matthews and Trautman (11) drew attention to the fact that leprosy is a disease that should be considered as a model for the study of many hyperglobulinemic states, especially the collagen diseases, in which the causative agent, in contrast to other diseases, is well known.

Leprosy is a dichotomous disease. On the one hand there is the lepromatous case with extensive lesions and abundant bacilli, but no productive cellular recation, and on the other the tuberculoid case with only local lesions, which contain very few bacilli, but show intense cellular reaction in the form of tubercle formation (10).

A third element in the host-parasite interaction is the leprosy reaction, similar to Selye's (16) alarm reaction of the general adaptation system (12). If more were known at the biochemical level about factors determining the predisposition toward the tuberculoid form of the disease or toward the lepromatous form, new prophylactic and therapeutic pathways might be opened; also a point of attack on the diseases mentioned by Matthews and Trautman (11) might be found.

Variations in response to lepromin (Mitsuda reaction) correlate reasonably well with the ability of the host tissues to respond immunologically to the antigens of *M. leprae*. According to Bieling (3) immunity is preceded by a "premunity" in which an "automatic shift" (7) plays a role in the host. Plasmin reactivity changes were also seen in this "shift," and it has been thought that these may be directly related to conditions considered to be of immunologic origin (15, 17). With the use of plasmin reactivity as an indicator for the autonomic shift, variations in plasmin activity were studied after administration of the vasoactive drug 3-pyridyl acetic acid (3-PAA) (1, 6, 14), a homolog of nicotinic acid.

STUDY GROUPS

The experiments were carried out in the République Démocratique du Congo. Forty-three patients (36 adults and 7 children) from Kivuvu, the Leprosarium of the Institut Médical Evangélique at Kimpese, 100 adult patients from the Hôpital de la Rive (National Leprosarium) at Kinshasa, and 11 healthy children of the patients of Kivuvu, were included in this study. The 11 control subjects were obtained for this study by the staff of the Danish Teaching Hospital at Kinshasa. The clinical diagnosis was established by the staff of each hospital. The subjects were divided into groups (Table 1): group I, 11 controls; group II, 23 tuberculoid patients; group III, 16 borderline patients; and group IV, 97 lepromatous patients.

Two tuberculoid patients were in a reactional state and two showed extensive foot ulcerations. Of the lepromatous patients nine were treated with corticosteroids, and seven with chloroquine; 29 were in a reactional state. The rest of the cases were considered to be uncomplicated. The patients were on sulfone therapy except for a few new cases and some of those in a reactional state. The healthy children and child patients were considered separately.

MATERIALS AND METHODS

Owren's veronal buffer solution. (11.7

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TABLE 1. *Variations in euglobulin clot lysis time in healthy subjects and in leprosy. Lysis time before and 15 minutes and 60 minutes after injection of 3-pyridyl acetic acid.*

Clinical diagnosis	No.	Mitsuda reaction	Lysis time					
			Before injection		15 min. after injection		60 min. after injection	
			Range	Mean	Range	Mean	Range	Mean
I. Healthy controls	11	?	30–180	105 ± 49	25–180	87 ± 42	60–145	93 ± 34
II. Tuberculoid leprosy								
a. not in reaction								
1. uncomplicated	19	+	25–190	105 ± 44	10–150	84 ± 30	20–170	83 ± 40
2. with extensive ulcerations	2	+	40– 55		69–90		60–100	
b. in reaction	2	+	40– 55		55– 90		60–110	
III. Borderline leprosy								
a. not in reaction	16	+	40–195	88 ± 64	20–100	51 ± 21	30–135	65 ± 26
IV. Lepromatous leprosy								
a. not in reaction								
A. "not active"	11	4 + 7 −	70–190	136 ± 40	10–150	89 ± 46	25–165	92 ± 48
B. + corticoster.	9	−	60–190	122 ± 48	20–160	77 ± 48	20–120 one 205	104 ± 60
C. + chloroquine	7	−	75–165	100 ± 42	10– 80	55 ± 28	25–140	81 ± 34
D. "more active"	41	−	40–175	95 ± 39	55–190	109 ± 39	35–190	113 ± 43
b. in reaction: A.	12	−	195–540	313 ± 130	75–465	220 ± 125	120–420	198 ± 78
B.	17	−	105–510	229 ± 116	95–960	311 ± 254	225–960	392 ± 204

gm. sodium diethylbarbiturate; 14.67 gm. NaCl; 430 ml. 0.1 N HCl; distilled water to 1,000 ml.). The pH was adjusted to 7.35.

Thrombin solution. Fresh solutions were prepared daily by dissolving 3 mgm. Topostasin (Roche) in 2 ml. veronal buffer.

Glassware and equipment. Erlenmeyer flasks, centrifuge tubes, test tubes (8 mm. x 80 mm.), and syringes (luer-lock with 2 gauge needles). The glassware was not siliconed. A waterbath at 38°C was employed. The CO_2 used was commercial grade from cylinder.

Method. Plasmin activity was estimated by the euglobulin clot lysis time, the globulin fraction being isolated according to the method of Kaulla and Schulz ([8]). Fasting plasma was obtained from all patients and controls as follows: Citrated blood was taken before the injection of 3-PAA and 15 and 60 minutes after the intramuscular injection of the equivalent of 150 mgm. nicotinic acid (children 75 mgm. intravenously). Three ml. blood was withdrawn from an antecubital vein with a sphygmomanometer cuff applied at just above diastolic pressure, transferred to a centrifuge tube and centrifuged at 2,200 rpm for three minutes at 0-4°C. With a 1 ml. pipet plasma was added immediately to 15 ml. of ice cold distilled water. The globulin fraction was precipitated by introducing CO_2 gas above the solution and rotating the flask for a three minute period.

TABLE 2. *Variations in euglobulin clot lysis time in healthy subjects and in leprosy (same material as in Table 1).*

Clinical picture	Mitsuda reaction	Lysis time before and/or after injection of 3-PAA of not more than 190 min.		Lysis time before and/or after injection of 3-PAA of more than 190 min.	
		Lysis time not prolonged 15 or 60 min. after injection number	Lysis time prolonged 15 and/or 60 min. after injection number	Lysis time not prolonged 15 and/or 60 min. after injection number	Lysis time prolonged 15 and/or 60 min. after injection number
I. Healthy controls	?	10	1	0	0
II. Tuberculoid leprosy					
a. not in reaction					
1. uncomplicated	+	19	0	0	0
2. with extensive ulcerations	+	0	2	0	0
b. in reaction	+	0	2	0	0
III. Borderline leprosy					
a. not in reaction	+	16	0	0	0
IV. Lepromatous leprosy					
a. not in reaction					
A. "not active"	4 + 7 −	11	0	0	0
B. + corticoster.	−	9	0	0	0
C. + chloroquine	−	7	0	0	0
D. "more active"	−	0	41	0	0
b. in reaction A.	−	0	0	12	0
B.	−	0	0	0	17

The contents were then centrifuged in a conical tube for three minutes at 2,200 rpm, and the tubes were inverted and put in the refrigerator until plasmin activity was determined, with a maximum delay of two hours. The sediment was then diluted in 1.2 ml. buffer; 0.5 ml. of the solution was transferred to each of two lysis tubes, and then 0.1 ml. thrombin solution was added to each. The tubes were then incubated in the waterbath and lysis time was noted.

RESULTS

The results are summarized in Tables 1 and 2. Considerable variability of fibrinolysis time was observed both in healthy subjects and patients. In healthy subjects and uncomplicated leprosy the fibrinolysis time varied from 20 to 190 minutes before the injection of 3-PAA and from five to 190 minutes after the injection of 3-PAA. In the complicated conditions of lepromatous leprosy, i.e., the so-called reactional states, these values always exceeded 190 minutes, before and/or after the injection. On division of the leprosy patients into the groups mentioned and comparison of the means of the values, significant differences were observed only in the lepromatous patients in reaction. A statistical comparison of the means of the groups by the t-distribution method gave the following results:

P-values were calculated in the different groups, comparing lysis times before the injection and 15 and 60 minutes after the injection of 3-PAA. Here values of 0.1 to 0.01 were observed. P-value calculations for the differences between the groups showed $P<0.001$ for each of groups I (controls), II (tuberculoid leprosy), III (borderline leprosy), and IV-a (lepromatous leprosy not in reaction) *vs* group IV-b (lepromatous leprosy in reaction, A as well as B). These values were obtained on comparing lysis times before the injection of 3-PAA, as well as 15 and 60 minutes after the injection. The differences of the means of the other groups were not significant. Thus lepromatous patients in reaction demonstrated significant reduction in euglobulin clot lysis time in comparison with both nonlepromatous subjects and lepromatous patients not in reaction. Thus the results of Meyers ([13]), who used other methods, were confirmed. Injection of 3-PPA did not interfere with this relationship.

An interesting picture was presented on consideration of the variations occurring after the injection of 3-PAA, when the effect of the biologic variability of fibrinolysis time on mean and variance calculations was eliminated by considering only decrease ("normal response") or increase ("reverse response") after the injection of 3-PAA. Thus groups were distinguished correlating rather well with the clinical picture and the Mitsuda reaction. These correlations may be related to the activity of the disease and predisposition to reactional states.

The healthy controls all showed a decrease of fibrinolysis time ("normal response") after the injection of 3-PAA (Tables 1 and 2, group I). One subject, seemingly in good health, showed an increase in fibrinolysis time; three months later a carcinoma of the intestine was found. The tuberculoid and borderline patients (groups II and III) showed the same kind of reaction to the injection as the healthy subjects, except in the case of two patients in a reactional state and two patients with extensive foot ulcerations, who demonstrated a prolonged lysis time following injection of 3-PAA ("reverse response"). The most interesting results were found in lepromatous patients. In 41 uncomplicated lepromatous patients fibrinolytic activity was decreased after the injection of 3-PAA (group IV-a-D); seven presented the "normal response" (group IV-a-A). Eight under treatment with corticosteroids in moderate doses (increasing doses 1-25 mgm. daily during three weeks, decreasing doses 25-1 mgm. daily during next three weeks), or with chloroquine (4 weeks, 500 mgm. daily) showed a "normal response" (group IV-a-B and-C). One patient who had received 25 mgm. of corticosteroids daily during four weeks, showed, after the injection of 3-PAA, a "reverse response" and a fibrinolysis time of 205 minutes. The lepromatous patients in reaction had a fibrinolysis time exceeding 190 minutes before and/or after the injection of 3-PAA. In 11 patients the fibrinolysis time was shortened

TABLE 3. *Variations in euglobulin clot lysis time in healthy and leprosy children.*

No.	Sex	Age (yrs)	Controls and type of leprosy	Fernandez reaction	Mitsuda reaction	Lysis time in minutes			Affected relatives	Type of leprosy in relatives
						Before inj.	15 min. after inj.	60 min. after inj.		
A- 1	f	8	healthy	4 mm	15 mm + ulcer	72	72	70	father	lepromatous
A- 2	m	6	healthy	4 mm	14 mm + ulcer	108	46	67	father	lepromatous
A- 3	f	8	healthy	6 mm	13 mm + ulcer	83	60	97	father	lepromatous
A- 4	m	4	healthy	7 mm	12 mm + ulcer	68	50	54	father	lepromatous
									brother	lepromatous
A- 5	m	8	healthy	4 mm	12 mm	105	70	80	mother	borderline
A- 6	f	10	healthy	4 mm	10 mm	65	65	37	father	lepromatous
A- 7	m	5	healthy	5 mm	8 mm	75	63	98	grandfather	lepromatous
									mother	borderline
A- 8	m	7	healthy	4 mm	7 mm	27	20	22	father	lepromatous
									brother	lepromatous
A- 9	m	6	healthy	4 mm	6 mm	76	68	76	father	lepromatous
A-10	f	6	healthy	8 mm	5 mm	75	61	56	father	lepromatous
									mother	borderline
A-11	m	6	healthy	5 mm	5 mm	75	60	54	mother	borderline
B- 1	f	9	borderl.	4 mm	3 mm	51	50	50	mother	lepromatous
B- 2	f	12	indeterm.	4 mm	4 mm	68	54	44	father	lepromatous
B- 3	f	12	borderl. tub.	3 mm	5 mm	51	41	19	father	lepromatous
B- 4	f	13	borderl.	3 mm	8 mm	86	83	80	mother	borderline
C- 5	f	7	indeterm.	4 mm	neg	29	29	35	father	lepromatous
C- 6	m	10	lepromat.	3 mm	neg	28	40	43	mother	lepromatous
C- 7	f	8	lepromat.	4 mm	neg	30	32	36	father	lepromatous

Mean values	before injection	15 minutes after inj.	60 minutes after inj.
Healthy children	75 ± 22	58 ± 14	65 ± 22
Borderlin. + 1 indeterminate	64 ± 17	57 ± 18	48 ± 25
Lepromat. + 1 indeterminate	29 ± 1	34 ± 4	38 ± 4

after the injection, while in 15 patients the fibrinolysis time was prolonged (group IV-b-A and B).

The lepromin tests were always positive in the patients diagnosed as tuberculoid and borderline leprosy, and weakly positive in a few lepromatous (atypical) patients. In the other cases the lepromin tests gave negative results.

To get more detailed information the plasmin pattern in seven children with leprosy and 11 healthy children was examined. The results are presented in Table 3. The healthy children all showed a positive Mitsuda reaction. The plasmin pattern was "normal" in nine and "reverse" in two of these children. In the leprosy children the values were similar to those found in adult patients.

DISCUSSION

In preliminary experiments with other vasoactive drugs (prostigmine, carbaminoylcholinechloride, acetylcholine, and nicotinic acid) comparable results were obtained. 3-PAA gave promising results with respect to changes in fibrinolysis time, and the patients never complained of unpleasant sensations. Changes in streptokinase-activated fibrinolysin were similar to those seen in unactivated fibrinolysin after the injection of 3-PAA; the antifibrinolysin activity did not change.

In agreement with the findings of Billimoria, *et al.* (4), who used whole blood, only comparatively slight diurnal and day-to-day variations of fibrinolytic activity were found. In the uncomplicated cases the plasmin activity pattern, i.e., shortened or prolonged lysis time after the injection of 3-PAA, did not change during the time of observation. Repeated studies at three month intervals over a two year period were performed on individual patients. No essential changes in the plasmin pattern were found. In three patients undergoing reaction the lysis time before the injection increased steadily as the reaction progressed, and the original pattern was altered when the lysis time had reached about 190 minutes, since the lysis time after injection of 3-PAA in these patients was not increased proportionally. As the reaction diminished, the original pattern was gradually reestablished. It would be interesting in such cases to compare the 17-ketosteroid excretion with the plasmin pattern, since in tuberculosis activation of the process is preceded by a decrease in urinary 17-ketosteroids (18). In this respect the values found in patients under corticosteroid treatment are noteworthy. Obviously the dose of the corticosteroids is of primary importance. A high dose of corticosteroids altered the plasmin pattern to that seen in reactional states. It should be noted that none of the patients in group IV-a-A was in an active disease state at the time of study and had not been so for an extended period of time. Four of them had a Mitsuda reaction measuring 2-3 mm. In none of these cases was a biopsy available.

Generally a positive Mitsuda reaction demonstrating an ability to respond immunologically, was related to a decreased lysis time after the injection of 3-PAA. A negative Mitsuda reaction, indicating an inadequate immunologic response, was related to a prolonged lysis time after the injection of 3-PAA. Treatment with corticosteroids and probably also chloroquine, seemed to interfere with this correlation. A lysis time of more than 190 minutes, under the conditions of these studies, might be considered as an indication of a state of existing or impending reaction.

The correlations between Mitsuda reaction, clinical picture, and plasmin pattern were significant. Statistical analysis of patients giving a negative Mitsuda reaction and prolonged lysis time after the injection of 3-PAA on the one hand, and positive Mitsuda reaction and decreased lysis time after the injection of 3-PAA, on the other, gave $P<0.001$. This relationship held even when the patients under corticosteroid and chloroquine treatment were included in the calculations.

Although the data presented in Table 3 were obtained from a limited number of individuals, they indicate that the "reverse response" may exist in the healthy state. Probably the findings reported here should be considered as from a fortuitously selected group of subjects who contracted leprosy. But as the "reverse response" exists in

healthy persons also, a constitutional factor is suggested, with a large range of possibilities in which decrease and increase of plasmin activity following an injection of a certain amount of nicotinic acid homolog is only one facet.

On the basis of the findings reported here, investigations were made treating patients of group IV-b-A with oral nicotinic acid, 100 mgm. twice daily. One patient developing a reactional state, as diagnosed by change in the plasmin pattern, also received this treatment. Encouraging results were obtained; in the patient undergoing reaction the treatment appeared to arrest the progress of the reaction.

It may be noted here that the results of plasmin stimulation are many and varied. Plasmin is able to digest the peptide linkage of muco-polysaccharide-protein complexes. In the aorta it increases the permeability of the vessel wall ([9]). As plasmin activity itself is probably not directly involved in anaphylaxis ([2, 5]), Klynstra's ([9]) results may point to the way in which plasmin stimulation might influence the course of a reactional state.

The results in leprosy reported here indicate that the method employed may serve to help find a means of approach to treatment and prophylaxis for hyperglobulinemic conditions. It would be of interest to employ the method described here on healthy subjects, especially identical twins, in order to determine if correlations exist between the experimental findings and the eventual development of or resistance to disease (e.g., leprosy).

SUMMARY

Correlations were found between the reaction pattern of plasmin, as determined by the euglobulin clot lysis time before and after injection of 3-pyridyl acetic acid, on the one hand, and the clinical picture of leprosy and the Mitsuda reaction on the other. In uncomplicated leprosy the fibrinolysis time did not exceed 190 minutes. Tuberculoid and borderline leprosy were correlated with a positive Mitsuda reaction and with a decrease of fibrinolysis time after the injection of 3-PAA. Lepromatous leprosy was correlated with a negative Mitsuda reaction and an increase of fibrinolysis time after the injection of 3-PAA. However, fibrinolysis time in lepromatous patients in reaction always exceeded 190 minutes either before or after the injection of 3-PAA. Both increases and decreases in fibrinolysis time were noted following the injection.

A method to prevent reactional states in leprosy is discussed. The importance of changes in fibrinolytic activity in the treatment and prophylaxis of other related pathologic conditions is also considered.

RESUMEN

Se encontró una correlación entre el cuadro de reacción de plasmina, determinado por el tiempo de lisis del coágulo de euglobulina antes y después de la inyección del ácido 3-pyridyl acético (3-PAA) por una parte, y el cuadro clínico de la lepra y la reacción de Mitsuda por otra. En lepra no complicada el tiempo de fibrinolisis no excedió de 190 minutos. Lepra tuberculoide y borderline estuvieron relacionadas con una reacción de Mitsuda positiva y con una disminución del tiempo de fibrinolisis después de la inyección de 3-PAA. La lepra lepromatosa estuvo correlacionada con una reacción de Mitsuda negativa y con un aumento del tiempo de fibrinolisis después de la inyección de 3-PAA. Sin embargo, el tiempo de fibrinolisis en enfermos lepromatosos en fase de reacción siempre excedió 190 minutos tanto antes como después de la enyección de 3-PAA. Tanto el aumento como la disminución del tiempo de fibrinolisis se notaron a continuación de la inyección.

Se discute un método para prevenir los estados reaccionales en lepra. La importancia de los combios de la actividad fibrinolítica en el tratamiento y profilaxis de otros estados patológicos relacionados es tamblién considerada.

RÉSUMÉ

On a observé des corrélations entre le profil de réaction de la plasmine, tel qu'il est déterminé par le temps de lyse du caillot de globuline avant et après injection d'acide 3-pyridyl acétique (3-PAA), d'une part, et d'autre part l'image clinique de la lépre et le résultat de la réaction de Mitsuda. Dans les cas de lèpre non compliquée, le temps de fibrinolyse ne dépasse pas 190 minutes. La lépre tuberculoïde, de même que la lèpre border-line, était associée à une réaction de Mitsuda positive, ainsi qu'à une diminution du temps de

fibrinolyse après l'injection de 3-PAA. La lèpre lépromateuse était associée à une réaction de Mitsuda négative, ainsi que à une augmentation du temps de fibrinolyse après l'injection de 3-PAA. Néanmoins, chez les malades lépromateux souffrant de réaction, le temps de fibrinolyse dépassait toujours 190 minutes, que ce soit avant ou aprés l'injection de 3-PAA. A la suite de l'injection, on a noté aussi bien des augmentations que des diminutions dans les temps de fibrinolyse.

On discute une méthode destinée à prévenir l'apparition d' états réactionnels dans la lèpre. On considère également l'importance des modifications survenant dans l'activité fibrinolytique au cours du traitement et de la prophylaxie d'autres conditions pathologiques proches de la lèpre.

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REFERENCES

1. Antonini, F. M. and Sordi, A. The serum lipid lowering effect of nicotinic acid and 3-pyridylacetic acid and duodenal heparinoid and their possible mechanism of action. *In* Drugs Affecting Lipid Metabolism. Garattini, S. and Paoletti, R., Eds. Amsterdam, London, New York. Elsevier Publishing Co. (1961) 392-411.
2. Austen, K. F. and Humphry, J. H. *In vitro* studies of the mechanism of anaphylaxis. Immunology **3** (1963) 1-96.
3. Bieling, R. Resistenz und Immunitat. *In* Handbuch der allgemeinen Pathologie, Vol. VII, No. 1 (1956) 601-673.
4. Billimoria, J. D., Drysdale, J., James, D. C. O. and Maclagan, N. F. Determination of fibrinolytic activity of whole blood. Lancet **2** (1959) 471-475.
5. Burdon, K. L., McGovern, J. P., Barkin, G. B. and Meyers, W. M. Fibrinolysis and anaphylaxis in the guinea pig. J. Allergy **32** (1961) 55-62.
6. Gibelli, A. Sacchetti, G., Mainieri, L. and Soardi, F. Attivazione della fibrinolisi da acido 3-pyridil acetico e da acido nicotinico nell'uomo. Acta Vitam. **16** (1962) 107-119.
7. Hoff, F. Non-specific resistance and non-specific therapy. Stanford Med. Bull. **17** (1959) 133-141.
8. von Kaulla, K. N. and Schulz, R. L. Methods for the evaluation of human fibrinolysis; studies with two combined technics. American J. Clin. Path. **29** (1958) 104-112.
9. Klynstra, F. B. Gaubius Institute of the University of Leyden, Leyden (The Netherlands) Personal communication, 1967.
10. Lowe, J. The leprosy bacillus and the host reaction to it. Leprosy Rev. **26** (1955) 15-24.
11. Matthews, L. and Trautman, J. R. Clinical and serological profiles in leprosy. Lancet **2** (1955) 915-917.
12. Muir, E. Lepra reaction and the general adaptation syndrome. Leprosy Rev. **33** (1962) 240-251.
13. Meyers, W. M. Plasma heparin-precipitable fraction in leprosy patients. Internat. J. Leprosy **36** (1968) 192-202.
14. Nicola, P. de. Diffuse intravascular clotting. Ed. Schattauer Verlag. Stuttgart, 1966, pp. 289-295.
15. Rodriguez-Erdmann, F. Studies on the pathogenesis of the generalized Shwartzman reaction. Thromb. Diath. Haem. **12** (1964) 452-483.
16. Selye, H. The physiology and pathology of the exposure to stress. Acta Inc. Montreal, 1950-1957, pp. 10-12.
17. Stamm, H. Einführung in die Klinik der Fibrinolyse. Ed. S. Karger, Basel, New York, 1962, pp. 113-118 and 267-341.
18. Zimmerman, W. and Bruhl, P. Die Rolle der Steroidhormonen im Infektionsablauf. Zeitschr.f. Hyg. **149** (1963) 194-205.