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A Quantitative Assay of Porphyrins in Leprosy Patients. A Spectrophotometric Study[1]

P. Pavithran, F. Handa, and K. S. Sidhu[2]

Although synthesized in every cell of the body, there are two main sites of porphyrin synthesis in humans, the erythropoetic bone marrow and the liver ([2]). Leprosy, especially the lepromatous type, is known to affect the bone marrow and the liver ([3, 7, 8]). Diffuse infiltration of the reticuloendothelial system and bone marrow by *M. leprae* could affect porphyrin metabolism. To our knowledge, porphyrin metabolism has not been previously studied in leprosy patients. In order to assess the effects, if any, of leprosy and its treatment with dapsone on porphyrin metabolism, we quantitated porphyrins in the blood, urine, and stools of 40 leprosy patients and compared their porphyrin concentrations to those of normal individuals.

MATERIALS AND METHODS

Forty patients with leprosy attending the Rajindra Hospital, Patiala (Punjab), India were studied. The clinical diagnosis was confirmed by skin scrapings for acid-fast bacilli and histopathological examinations of skin biopsies in every case. Routine examinations of blood, urine, and stools did not reveal any abnormalities. There was no evidence of hemolytic anemia, methemoglobinemia, or photosensitivity in any of these patients. One patient was experiencing erythema nodosum leprosum at the time of the assay.

Thirty patients with a duration of disease ranging from 6 months to 40 years were taking dapsone for periods ranging from 4 months to 36 years. In order to control for any effects of dapsone on porphyrin levels, ten untreated leprosy patients with a duration of disesase ranging from 3 months to 4 years were also studied. The patients were classified according to the Indian Classification ([6]). The patients receiving dapsone received doses ranging from 15 to 600 mg per week with a mean of 370 mg per week; 47% were on more than 300 mg of dapsone per week.

In order to determine if leprosy per se influenced porphyrin metabolism, a comparison was made with porphyrin levels determined by the authors in a large series of normal Punjabis ([1, 4, 5, 9, 10, 11]). Since this comparison involved the results of two independent studies, a statistical formula for comparing independent samples ([13]) was used for analysis.

Porphyrin estimations in blood, urine, and stools were carried out by Rimington's method ([12]). Since porphyrins are photolabile, suitable precautions were necessary. Extractions were made in a dark room from samples collected in amber colored bottles. The principles involved in the quantitative estimations of the porphyrins are briefly outlined below:

Erythrocyte protoporphyrin (EPP) and erythrocyte coproporphyrin (ECP). The porphyrins were extracted, together with much heme, from washed erythrocytes with a mixture of ethyl acetate and acetic acid. The porphyrins were transferred by extraction into 15% hydrochloric acid and from this extract into ether after neutralization with sodium acetate. From the ethereal solution, coproporphyrin was removed with 0.1 N HCl and protoporphyrin removed with 5% HCl. Porphyrin concentrations were then determined spectrophotometrically.

Urinary coproporphyrin (UCP) and urinary uroporphyrin (UUP). Coproporphyrin and any coproporphyrinogen were first extracted with ether containing acetic acid. Coproporphyrinogen was oxidized to por-

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[2] P. Pavithran, M.B.B.S., M.D., Dermatovenereologist, Military Hospital, Delhi, India; F. Handa, M.B.B.S., D.T.D., M.D., Professor and Head, Department of Skin and V.D., Government Medical College, Patiala-147 001 (Punjab) India; K. S. Sidhu, M.Sc., Ph.D., Professor and Head, Department of Chemistry, Punjabi University, Patiala (Punjab) India.

THE TABLE. *Porphyrin concentrations (mean ± S.D.) in normal Punjabis and leprosy patients. Values are given for erythrocyte protoporphyrin (EPP) and erythrocyte coproporphyrin (ECP) in μg/100 ml of erythrocytes, urinary coproporphyrin (UCP), and urinary uroporphyrin (UUP) in μg/24 hr of urine and fecal coproporphyrin (FCP) and fecal protoporphyrin (FPP) in μg/g dry weight of feces.*

Group of subjects	N	Erythrocytes		Urine		Feces	
		EPP	ECP	UCP	UUP	FCP	FPP
Normal Punjabis	100	25.0 ±13.8	0.22 ±0.66	47.1 ±23.3	3.09 ±3.95	1.33 ±0.66	4.47 ±2.99
Leprosy patients	40	26.7 ±13.6	0.92[a] ±1.74	46.6 ±22.8	1.87[a] ±2.16	1.33 ±0.22	4.53 ±3.18
Untreated	10	40.56[a,b] ±21.30	1.22 ±2.17	45.29 ±21.10	1.20[a] ±2.37	1.38 ±0.75	4.69 ±3.40
Lepromatous and dimorphous only	7	39.17[a,c] ±17.20	1.36 ±2.50	52.38 ±17.70	0.69[a] ±1.29	1.50 ±0.86	5.21 ±3.84
Dapsone treated	30	22.05 ±10.60	0.82[a] ±1.62	47.09 ±23.60	2.09 ±2.14	1.31 ±0.71	4.48 ±3.15
Lepromatous and dimorphous only	16	21.33 ±10.50	1.07 ±2.03	47.79 ±20.00	2.55 ±2.73	1.38 ±0.85	4.60 ±3.13

[a] $p < 0.05$, compared to normal Punjabis, Student's t test.
[b] $p < 0.05$, compared to dapsone treated leprosy patients, Student's t test.
[c] $p < 0.05$, compared to dapsone treated lepromatous and dimorphous patients, Student's t test.

phyrin by shaking the solution with dilute iodine. The total coproporphyrin was then transferred into 5% HCl and determined spectrophotometrically using a formula to correct for absorbing impurities. Uroporphyrin was removed from residual urine and from the washing of the ether phase by adjusting the pH to 1.5 and shaking with cyclohexanone. After the addition of ether, the uroporphyrin was transferred into 5% HCl and its concentration determined spectrophotometrically using a correction formula as in the coproporphyrin determinations.

Fecal coproporphyrin (FCP) and fecal protoporphyrin (FPP). Ether-soluble porphyrins and porphyrinogen were extracted with ether containing acetic acid. Porphyrinogen was oxidized to porphyrin by shaking with a dilute solution of iodine. Coproporphyrin was then transferred into 0.1 N HCl and protoporphyrin into 5% HCl. The concentrations of both were determined spectrophotometrically using a correction formula. Pigments derived from chlorophyll remain in the ether phase.

RESULTS

The findings are given in the Table. The leprosy patients as a whole had significantly increased concentrations of coproporphyrin in their erythrocytes (ECP) and significantly decreased uroporphyrins in their urine (UUP) compared to results obtained in an earlier study in normal Punjabis ([1, 4, 5, 9, 10, 11]).

Erythrocyte protoporphyrins (EPP) were elevated in untreated leprosy patients as a whole, and in the subgroup of untreated lepromatous and dimorphous leprosy patients, compared to the same groups of leprosy patients treated with dapsone.

The 30 dapsone-treated leprosy patients were subdivided on the basis of dapsone dosage. There were no significant differences in porphyrin concentrations between patients receiving more than 300 mg of dapsone per week and those receiving less than this amount. Similarly, there was no significant correlation between dapsone dosage and porphyrin concentrations when the data were analyzed by calculation of a correlation coefficient.

DISCUSSION

Porphyrin, the purple pigment, is an integral part of many enzymes and is known for its numerous photodynamic properties. The porphyrin-iron complex, heme, plays a vital role in human cells. Porphyrin is syn-

thesized in the body from basic building units of glycine and succinyl coenzyme-A, which combine together in the presence of ALA synthetase, pyridoxal, and ferrous ions, to form α-amino-beta-ketoadipic acid, which decarboxylates to δ-amino-levulinic acid (d-ALA). Two molecules of d-ALA combine to form one molecule of porphobilinogen (PBG). Transformation of PBG to uroporphyrinogen I occurs in the presence of PBG deaminase. Uroporphyrinogen isomerase modifies the action of PBG deaminase so that uroporphyrinogen III results. The enzyme uroporphyrinogen decarboxylase decarboxylates both the I and III series and produces coproporphyrin. Coproporphyrin is then converted to protoporphyrin which in turn is incorporated with ferrous ion to form heme.

Leprosy patients had significantly elevated concentrations of coproporphyrins in their erythrocytes and significantly decreased concentrations of uroporphryins in their urine compared to normal Punjabis. These changes seemed to be due to the disease process since the most marked changes occurred in untreated cases, particularly untreated lepromatous and dimorphous patients, in both parameters. Although these tendencies are statistically significant and would appear valid, their cause is not clear. It should be pointed out that the data were variable, and most of the values fell in the normal range.

Dapsone appeared to have no adverse effect on porphyrin metabolism since dapsone treated patients did not develop raised porphyrin levels in the blood, urine, or stools. In other words, the dose and duration of treatment with dapsone did not have a porphyrinogenic effect. Indeed, erythrocyte protoporphyrin values were elevated in untreated leprosy patients compared to patients receiving dapsone.

The present findings suggest that leprosy patients, particularly untreated leprosy patients, have increased protoporphyrins and increased coproporphyrins in their erythrocytes and excrete decreased amounts of uroporphyrins in their urine compared to normal individuals. Urinary coproporphyrin excretion appears normal as do fecal coproporphyrin and fecal protoporphyrin levels.

These results are inconclusive as to their clinical significance. One may speculate that elevations of immediate precursors of heme in erythrocytes, protoporphyrins, and coproporphryins may be related to the anemia of untreated leprosy. The data would suggest that these abnormalities tend to return to normal with anti-leprosy chemotherapy. On the other hand, the decreased urinary uroporphyrin excretion in leprosy patients is puzzling in the face of normal urinary coproporphyrin excretion since both uroporphyrins and coproporphyrins are products of uroporphyrinogens. Both require oxidations and occur readily, catalyzed by light and the porphyrins that are formed. The latter requires an intermediate step of decarboxylation of the acetate groups on the uroporphyrinogens catalyzed by uroporphyrinogen decarboxylase. Such a pattern cannot be readily explained on the basis of an abnormality involving a single metabolic step and would imply that leprosy may induce a variety of biochemical abnormalities in porphyrin metabolism.

SUMMARY

Quantitative estimations of porphyrin in the blood, urine, and feces of 30 leprosy patients under treatment with dapsone, ten untreated cases, and 100 normal subjects were done by Rimington's method.

Dapsone had no adverse effect on porphyrin metabolism because none of the cases of leprosy under study developed statistically significantly raised porphyrin levels in the blood, urine, and stools.

Although erythrocyte coproporphyrin levels were significantly higher in leprosy patients than controls and urinary uroporphyrin levels significantly lower, most values fell within the normal range. These differences did not appear to have any clinical significance, and their cause remains unknown.

RESUMEN

Usando el método de Rimington, se hicieron determinaciones cuantitativas de porfirina en sangre, orina y heces, en 30 pacientes con lepra en tratamiento con dapsona, en 10 pacientes sin tratamiento y en 100 individuos sanos.

La dapsona no tuvo ningún efecto adverso sobre el metabolismo de las porfirinas porque ninguno de los

pacientes estudiados tuvo niveles elevados de porfirina en sangre, orina o heces, que fueran estadísticamente significativos. Los niveles elevados de protoporfirina eritrocítica pueden deberse a un efecto hemolítico de la droga.

Aunque los niveles de coproporfirina erithrocítica fueron significativamente mayores en los pacientes que en los controles, y los niveles de uroporfirina urinaria significativamente menores, la mayoría de los valores cayeron dentro del rango normal. Estas diferencias no parecen tener ningún significado clínico y su causa permanece desconocida.

RÉSUMÉ

Chez 30 malades de la lèpre en traitement par la dapsone, 10 cas non traités, et 100 sujets normaux, on a procédé à des estimations quantitatives de la porphyrine du sang, de l'urine et des selles, par la méthode Rimington.

Il n'a été observé aucun effet adverse sur le métabolisme de la porphyrine, car aucun des malades atteints de lèpre repris dans cette étude n'a montré des taux de porphyrine significativement élevés, dans le sang, dans l'urine, ou dans les selles. Une élévation de la protoporphyrine des érythrocytes pourrait être due à un effet hémolytique du médicament.

Quoique les taux de coproporphyrine dans les érythrocytes étaient significativement plus élevés chez les malades de la lèpre que chez les témoins, et que par ailleurs les taux urinaires d' uroporphyrine étaient significativement abaissés, la plupart des valeurs observées se situaient dans les limites normales. Ces différences ne paraissent pas avoir de signification clinique, et leurs causes restent inexpliquées.

REFERENCES

1. ADARSH, C., HANDA, F. and SIDHU, K. S. A quantitative assay of erythrocyte proto- and coproporphyrins in 100 normal Punjabis—A spectrophotometric study. Thesis for M.D. examination, Punjabi University, Patiala, India, 1975.
2. CAIRNS, R. J. *Textbook of Dermatology*. Rook, G. A., *et al.*, eds. Oxford: Blackwell Scientific Publications, 1972, p. 1833.
3. COCHRANE, R. G. and DAVEY, T. F., eds. *Leprosy in Theory and Practice*. Bristol: John Wright and Sons Ltd., 1964, p. 429.
4. DASS, K. D., HANDA, F. and SIDHU, K. S. A quantitative assay of faecal copro- and protoporphyrins in 100 normal Punjabis—A spectrophotometric assay. Thesis for M.D. examination, Punjabi University, Patiala, India, 1975.
5. DASS, K. D., HANDA, F. and SIDHU, K. S. A quantitative assay of faecal porphyrins in 100 normal Punjabis—A spectrophotometric assay. Indian J. Dermatol. Venereol. **44** (1978) 334–337.
6. DHARMENDRA. The Indian Classification. In: *Leprosy*. Vol. 1. Dharmendra, ed. Bombay: Kothari Medical Publishing House, 1978, pp. 330–340.
7. DHARMENDRA. *Notes on Leprosy*. 2nd ed. Delhi; Ministry of Health, 1967, p. 78.
8. PRAVESH NIGAM, MUKHIJA, R. D., GOYAL, B. M., JOSHI, L. D. and SAMUEL, K. G. Study of histofunctional complex of liver in leprosy. Indian J. Dermatol. Venereol. **42** (1976) 212–222.
9. RAJ KUMAR GARG, HANDA, F. and SIDHU, K. S. A quantitative assay of urinary copro- and uroporphyrins in 100 normal Punjabis—A spectrophotometric study, Thesis for M.D. examination, Punjabi University, Patiala, India, 1973.
10. RAJ KUMAR and HANDA, F. Relations of age, sex, body weight, diet and urinary coproporphyrin excretion in 100 normal Punjabis. Indian J. Dermatol. Venereol. **40** (1974) 223.
11. RAJ KUMAR and HANDA, F. Relations of age, sex, body weight, diet and urinary uroporphryin excretion in 100 normal Punjabis. Indian J. Dermatol. Venereol. **42** (1976) 225.
12. RIMINGTON, C. Association of Clinical Pathologists Broadsheet. **70** (Revised Broadsheet **36**) August 1971.
13. SNEDECOR, G. W. and COCHRAN, W. G. *Statistical Methods*. 6th ed. Oxford: IBH Publishing Co., 1967, p. 114.