Acta Derm Venereol (Stockh) 1995; 75: 287–289

Urinary Excretion of Melanocyte Metabolites during Treatment with Chloroquine Phosphate

G. SJÖLIN-FORSBERG[1], C. HANSSON[2], B. KÅGEDAL[3], E.-L. GANNEDAHL[4] and B. BERNE[1]

[1]Department of Dermatology, University Hospital, Uppsala, [2]Department of Occupational Dermatology, University Hospital, Lund, [3]Department of Clinical Chemistry, University Hospital, Linköping and [4]Department of Rheumatology, University Hospital, Uppsala, Sweden

The antimalarial drug chloroquine is also used in the prevention of photodermatoses and in patients with inflammatory connective diseases. The drug binds strongly to melanin. Melanocytic activity can be studied by analysis of the urinary markers of eumelanin (6-hydroxy-5-methoxyindole-2-carboxylic acid, 6H5MI-2-C) and phaeomelanin (5-S-cysteinyldopa, 5-S-CD). To determine whether chloroquine interacts with this activity, we measured the urinary excretion of the two metabolites in 16 patients with either systemic or discoid lupus erythematosus, polymorphic light eruption or rheumatoid arthritis, during a period with and without treatment with chloroquine phosphate. Two control groups consisting of 7 untreated patients and 10 healthy subjects were also included in the study. During medication, there was a significant increase in 5-S-CD excretion, while the excretion of 6H5MI-2-C was not significantly affected. No significant changes in the excretion of any of the two urinary markers were found in the untreated patients, while a non-significant increase in 5-S-CD excretion was seen in the healthy controls at the follow-up. ***Key words: cysteinyldopa; 6-hydroxy-5-methoxyindole-2-carboxylic acid; melanocytic activity; melanin.***

(Accepted January 23, 1995.)

Acta Derm Venereol (Stockh) 1995; 75: 287–289.

G. Sjölin-Forsberg, Department of Dermatology, University Hospital, S-75185 Uppsala, Sweden.

Melanogenesis occurs in the epidermal melanocytes, where a sequence of reactions takes place. For the production of melanin in mammals, tyrosinase is an essential enzyme, catalyzing a hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (dopa) and subsequent oxidation of the latter to dopaquinone. The second of these two reactions can also occur spontaneously without the participation of tyrosinase. Dopaquinone is a branching point from where the melanin synthesis can proceed either along the indolic pathway, resulting in the eumelanins, or along the cysteinyldopa pathway, forming the phaeomelanins (1, 2). The eumelanins are dark brown or black, while the phaeomelanins are lighter and reddish-brown. Between these two extremes several mixed types of melanins can be found (3,4).

Melanocytic activity can be studied by analysis of the urinary excretion of the intermediate metabolites of the two kinds of melanin: in the case of eumelanin the indolic compound 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C) (5) and in that of phaeomelanin the thioether compound 5-S-cysteinyldopa (5-S-CD) (6).

The antimalarial drugs chloroquine and hydroxychloroquine are among the drugs that bind strongly to melanin (7), and they accumulate in tissues with a high melanin content such as the eye (8) and the skin (9).

In dermatology, chloroquine has for many years been used mainly for the prevention of photodermatoses such as polymorphic light eruption (10) and porphyria cutanea tarda (11). Chloroquine is also used therapeutically in certain inflammatory connective tissue diseases, such as rheumatoid arthritis (12).

Against the background of the strong capacity of melanin to bind chloroquine, it seemed of interest to study the interaction, if any, of the drug with melanocytic activity by measuring the urinary melanin metabolites in patients with and without chloroquine treatment.

MATERIALS AND METHODS

A group of 22 patients with skin type II and III (1 man and 21 women, mean age 41.4 years) entered the study in 1991. The study was approved by the local ethics committee. Seven of the patients had discoid lupus erythematosus (DLE), 6 polymorphic light eruption (PMLE), 4 rheumatoid arthritis (RA), 3 systemic lupus erythematosus (SLE), 1 subacute cutaneous lupus erythematosus (SCLE) and 1 had reticular erythematous mucinosis (REM). Three of the patients with RA were having concomitant treatment with drugs: 2 with non-steroidal anti-inflammatory drugs and 1 patient had a daily dose of 5 mg of prednisolone during the study. All patients were to be treated with chloroquine phosphate at a daily dose of 250 mg. Six of the patients (1 with DLE, 2 with PMLE, 2 with SLE and the patient with REM) dropped out, as 2 did not return for a second visit, 1 stopped the treatment after 2 weeks because of a suspected adverse drug reaction, and 3 samples from 3 patients had to be excluded during the analysis for technical reasons. In 11 of the 16 remaining patients chloroquine was given during the summer in order to reduce photosensitivity, whereas in the 4 RA patients and the SLE patient medication was given during the winter months. Thirteen patients were asked to collect a 24-h specimen of urine before starting the chloroquine treatment and another specimen after at least 2 months of treatment (steady-state). In 3 patients (1 with PMLE, 1 with RA, 1 with SLE) who were already receiving chloroquine medication, the urine samples were collected during therapy and after a washout period of at least 4 months after discontinuation of the drug. Most of the patients (11/16) were thus treated during the summer, but as 5 of them took chloroquine during the winter months, it seemed of importance to investigate the influence of seasonal variations on the excretion of the melanin metabolites in photosensitive patients. Therefore 11 untreated patients with skin type II and III (4 men and 7 women, mean age 41 years, 4 with DLE, 6 with PMLE, 1 with SLE) were included in the study. The patient with SLE dropped out because of chloroquine treatment, and 3 patients with PMLE dropped out because of initiation of ultraviolet hardening therapy. None of the remaining 7 patients had any medical treatment during the study. They collected a 24-h specimen of urine in the spring (April–May) and another in the early autumn (end of August–September) of 1994. Furthermore, a group of 10 healthy controls with skin type II and III (3 men and 7 women, mean age 38.4 years) were asked to collect identical samples att the same points of time as the untreated patients. All patients in both groups were avoiding the sun as much as possible, but the healthy subjects were allowed sun exposure ad libitum. The specimens were collected in

 ISSN 0001-5555

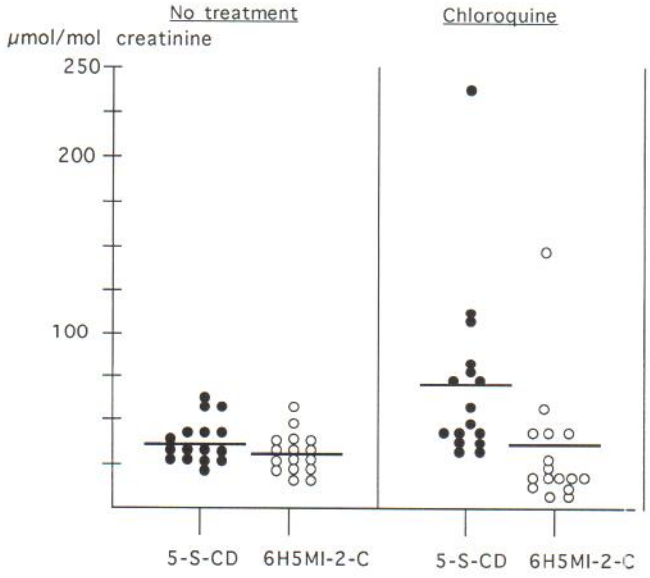

Fig. 1. The individual urinary concentrations of 5-S-cysteinyldopa (5-S-CD), (●) and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C), (○).

plastic bottles containing 50 ml of acetic acid and 1 g of sodium metabisulfite. The total volume was measured and two 10-ml samples of the urine were stored in plastic tubes at −70°C until analysed. The assays of 6H5MI-2-C were performed with a gradient elution HPLC system and fluorometric detection as described earlier (13, 14). The concentration of 5-S-CD was measured according to an automated HPLC method (15, 16).

Statistical analysis

The values are given as means ± SEM. Statistical significance of differences within the groups was assessed by a paired *t*-test and the differences between the groups by a two sample *t*-test for independent samples.

RESULTS

The melanin metabolites 6H5MI-2-C and 5-S-CD were detected in all samples. Fig. 1 shows the concentrations of the two metabolites in μmol/mol creatinine in the patients in the chloroquine-treated group without and during treatment. The urinary excretion of 5-S-CD increased by a mean of 85% during chloroquine therapy (37.8 ± 3.4 μmol/mol creatinine without and 70.1 ± 11.7 during medication; $p = 0.01$). In 4 of the patients the concentrations of 5-S-CD during treatment were high, some in the range of those seen in metastasizing malignant melanoma (6). Two of these patients also showed pathologically high concentrations of the eumelanin metabolite 6H5MI-2-C, during medication, but the values were not as high as those of 5-S-CD. Except in these 2 patients, the urinary excretion of 6H5MI-2-C was not significantly affected during chloroquine treatment (30.8 ± 3.1 without, 33.9 ± 8.3 during treatment) (Fig. 1). One single patient showed an elevation of 6H5MI-2-C above the reference value while not taking chloroquine, but a normal value during steady-state of chloroquine treatment.

Table I shows the basal concentrations and the differences in concentration at the follow-up of 5-S-CD and 6H5MI-2-C in the patients. When comparing those who were treated with chloroquine during the summer months with the untreated patients (who left their samples during the same period of time), it can be seen that the concentration of 5-S-CD was significantly increased in the treated group, while in the untreated group no significant change was found at the end of the summer ($p = 0.03$). The differences in the concentrations of 6H5MI-2-C, on the other hand, showed no significant changes in any of the groups during the study (Table I). In the healthy controls a non-significant increase of 5-S-CD (24%) and a non-significant decrease of 6H5MI-2-C (26%) were found at the follow-up.

DISCUSSION

This study has shown that chloroquine medication affects the melanin precursor 5-S-CD, resulting in a markedly increased urinary excretion of this phaeomelanin marker. The excretion of the eumelanin marker 6H5MI-2-C, on the other hand, was hardly affected. Some high values of 5-S-CD, in the range of those seen in melanoma patients, were noted (6). In untreated patients, the excretion of both compounds was clearly within the normal range (5, 13).

The urinary excretion of 5-S-CD has previously been found to be affected by different stimuli. In healthy subjects it varies with the season of the year, being highest in the summer, intermediate in the spring and autumn, and lowest in the winter (17). An increase in melanocyte activity similar to that seen during the summer can be observed after exposure to artificial UV irradiation. During PUVA treatment, for example, a marked increase in

Table I. *Basal concentrations and differences in concentration at the follow-up of 5-S-CD and 6H5MI-2-C (μmol/mol creatinine, means ± SEM)*

	Basal concentrations			Differences in concentration at the follow-up	
	5-S-CD	6H5MI-2-C		5-S-CD	6H5MI-2-C
All patients ($n = 23$)	47.4 ± 4.5	33.4 ± 3.9	chloroquine-treated ($n = 16$)	+32.3 ± 11.7 ($p = 0.01$)	+2.9 ± 8.4 (NS)
			untreated ($n = 7$)	−4.3 ± 7.6 (NS)	−0.3 ± 8.6 (NS)
All patients summer period ($n = 18$)	49.8 ± 5.6	35.3 ± 4.8	chloroquine-treated ($n = 11$)	+43.2 ± 15.6 ($p = 0.02$)	+5.3 ± 12.1 (NS)
			untreated ($n = 7$)	−4.3 ± 7.6 (NS)	−0.3 ± 8.6 (NS)

the urinary excretion of both 5-S-CD and 6H5MI-2-C has been found (5).

These previous findings of seasonal and UV-induced changes in the 5-S-CD metabolism have to be taken into consideration when evaluating the results of the present study, in which chloroquine treatment in 11/16 patients was given only during the summer months. In these patients the drug was given for the purpose of preventing photosensitivity, implying that they were careful to avoid sun exposure and to use sun screens. To further elucidate the possible influence of UV exposure, an untreated group of patients and a group of healthy subjects were asked to collect samples in the spring and early autumn. No increase in 5-S-CD excretion was found in the untreated patients at the follow-up (Table I). The healthy controls who lived a normal outdoor life during the summer showed a moderate increase in the 5-S-CD concentration at the follow-up ($p = 0.2$).

All the patients had pale skin at the follow-up at the end of the summer, except for 2 in the treated group and one in the untreated group, who were more pigmented at the revisit than when entering the study. The 2 patients in the treated group were among those with pathological 5-S-CD values during treatment. Interestingly, the most pigmented of these 2, who showed the highest value of 5-S-CD (Fig. 1), in contrast to the other patients, also displayed a great increase in 6H5MI-2-C urinary excretion during chloroquine treatment. The fact that in most patients during chloroquine treatment the 6H5MI-2-C concentrations were normal, while the 5-S-CD concentrations increased, is evidence against UV-induced stimulation of the melanogenesis, since a simultaneous increase in the two melanocyte metabolites has been observed during PUVA therapy (5).

However, 5-S-CD, in contrast to 6H5MI-2-C, has been found to reflect not only the tyrosinase activity in the melanosomes but also a non-specific oxidation of dopa outside the melanosomes (18). Rorsman & Tegner (19) reported an increase in 5-S-CD excretion in patients with erythematous reactions after PUVA, UVA or UVB treatment. According to Stierner et al. (20), the highest excretion values of 5-S-CD after UVB irradiation are found in patients with skin type II, who are more prone to develop erythematous reactions than patients with darker skin types. Our patients represented skin types II and III, but only 1 of them reported an erythematous reaction after UV exposure more than a month before the steady-state sampling.

In conclusion, during chloroquine treatment an increase in the urinary excretion of 5-S-CD was found. By using two control groups we excluded the possibility that this increase would be related to sun exposure only. The fact that only 5-S-CD excretion increased but not the excretion of 6H5MI-2-C indicates that this increase is not related to tyrosinase activity, but rather to non-specific dopa oxidation outside the melanocyte, i.e. chloroquine does not seem to interact with melanocytic activity.

ACKNOWLEDGEMENTS

Roland Pettersson is acknowledged for his assistance with the statistical analyses. This study has been supported by grants from the Swedish Cancer Society (Project No. 2357-B94-09XCC) and from the Welander-Finsen Foundation.

REFERENCES

1. Jimbow K, Quevedo WC Jr, Fitzpatrick TB, Szabó G. Biology of melanocytes. In: Fitzpatrick TB, et al., eds. Dermatology in general medicine. New York: McGraw-Hill Inc., 1993: 261–289.
2. Prota G, ed. Melanins and melanogenesis. London: Academic Press, Inc., 1992: 153–184.
3. Rorsman H, Agrup G, Hansson C, Rosengren A-M, Rosengren E. Detection of pheomelanins. Pigment Cell 1979; 4: 244–252.
4. Ito S, Fujita K. Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography. Anal Biochem 1985; 144: 527–536.
5. Hansson C, Wirestrand L-E, Aronsson A, Rorsman H, Rosengren E. Urinary excretion of 6-hydroxy-5-methoxyindole-2-carboxylic acid and 5-S-cysteinyldopa during PUVA treatment. Photodermatol 1985; 2: 52–57.
6. Agrup G, Agrup P, Andersson T, Hafström L, Hansson C, Jacobsson S, et al. 5 years' experience of 5-S-cysteinyldopa in melanoma diagnosis. Acta Derm Venereol (Stockh) 1979; 59: 381–388.
7. Larsson B, Tjälve H. Studies on the mechanism of drug-binding to melanin. Biochem Pharmacol 1979; 28: 1181–1187.
8. Perez R, Mansour AM, Rubin M, Zvaifler NJ. Chloroquine binding to melanin; characteristics and significance. Arthritis Rheum 1964; 7: 337.
9. Lindquist NG, Ullberg S. The melanin affinity of chloroquine and chlorpromazine studied by whole body autoradiography. Acta Pharmacol Toxicol 1972; 31 (Suppl 2): 1–32.
10. Cahn MM, Levy EJ, Schaffer B. The use of chloroquine diphosphate (Aralen) and quinacrine (Atabrine) hydrochloride in the prevention of polymorphous light eruptions. J Invest Dermatol 1954; 22: 93–96.
11. Swanbeck G, Wennersten G. Treatment of porphyria cutanea tarda with chloroquine and phlebotomy. Br J Dermatol 1977; 97: 77.
12. Bell CL. Hydroxychloroquine sulphate in rheumatoid arthritis: long-term response rate and predictive parameters. Am J Med 1983; 75: 46–51.
13. Hansson C. 6-Hydroxy-5-methoxyindole-2-carboxylic acid in normal human urine. Acta Derm Venereol (Stockh) 1984; 64: 185–190.
14. Kågedal B, Lenner L, Årstrand K, Hansson C. The stability of 5-S-cysteinyldopa and 6-hydroxy-5-methoxy-2-carboxylic acid in human urine. Pigment Cell Res Suppl 1992; 2: 304–307.
15. Hansson C, Kågedal B, Källberg M. Determination of 5-S-cysteinyldopa in human urine by direct injection in coupled column high performance liquid chromatography. J Chromatogr 1987; 420: 146–151.
16. Kågedal B, Källberg M, Årstrand K, Hansson C. Automated high-performance liquid chromatographic determination of 5-S-cysteinyldopa-3,4-dihydroxyphenylalanine in urine. J Chromatogr 1989; 473: 359–370.
17. Rorsman H, Agrup G, Falck B, Rosengren A-M, Rosengren E. Exposure to sunlight and urinary excretion of 5-S-cysteinyldopa. In: Riley V, ed. Pigment cell, vol 2. Basel: Karger, 1976: 284–289.
18. Ekelund M, Carstam R, Hansson C, Rorsman H. Urinary excretion of 5-S-cysteinyldopa and 6-hydroxy-5-methoxyindole-2-carboxylic acid: differences between pigmented and albino mice. Acta Derm Venereol (Stockh) 1985; 65: 437–439.
19. Rorsman H, Tegner E. Biochemical observations in UV-induced pigmentation. Photodermatol 1988; 5: 30–38.
20. Stierner U, Rosdahl I, Augustsson A, Kågedal B. Urinary excretion of 5-S-cysteinyldopa in relation to skin type, UVB-induced erythema, and melanocyte proliferation in human skin. J Invest Dermatol 1988; 91: 506–510.