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Increased accumulation of chloroquine and desethylchloroquine in homozygous sickle cells

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The effect of haemoglobin genotype on the level of chloroquine in the erythrocytes of homozygous sickle-cell (SS), normal (AA), and heterozygous (AS) subjects was investigated in vivo *and* in vitro. *Two hours after a single oral dose of chloroquine its level in plasma was consistently lower in SS than in AA subjects. In contrast, its level in the erythrocytes was higher in SS than in AA subjects. Desethylchloroquine, a metabolite of chloroquine, was detected only in the erythrocytes of SS blood but was present in both the plasma and erythrocytes of AA blood. For the* in vitro *test, a 5% suspension of erythrocytes was incubated for 1 hour with a 2.06 μmol/l solution of chloroquine. The mean chloroquine distribution ratio (μmol chloroquine per kg erythrocytes: μmol chloroquine per litre medium) was 31.0, 3.5, and 2.7 for SS, AA, and AS erythrocytes, respectively. The results of the study indicate that haemoglobin genotype appears to influence the level of chloroquine in erythrocytes.*

Identification of chloroquine-resistant malaria *in vivo* requires evidence of intake of the drug and its absorption by the infected case. This is best achieved by determining the concentration of chloroquine in blood (*1*). Currently, however, there is no consensus as to which component of blood should be analysed; some workers have determined the level of chloroquine in serum (*2*), while others have reported the level in whole blood (*3*). The concentrations of chloroquine in these components differ, and it is therefore difficult to compare data reported by different investigators and to interpret the possible relation between drug concentration in a given component and clinical effect. In general, however, in healthy individuals the ratio of the level of chloroquine in erythrocytes to that in plasma rarely exceeds 6, but the ratio for those who are diseased or have other special conditions has not yet been adequately determined.

In areas where malaria is endemic those with sickle-cell disease regularly use antimalarial drugs, and this paper reports the distribution of chloroquine and its metabolite desethylchloroquine in the blood of subjects with sickle-cell anaemia (SS) and sickle-cell trait (AS) as well as normal individuals (AA).

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MATERIALS AND METHODS

Accumulation of chloroquine in erythrocytes was determined after both *in vivo* and *in vitro* exposure to the drug. The prior consent of all subjects was obtained.

In vivo *test*

Vital data on the participants in the study are shown in Table 1. All nine subjects lived in Nigeria. Subject A was hospitalized with sickle-cell crisis, whereas the others were in good health.

A few minutes before administration of chloroquine, 2 ml of venous blood was collected from eight of the participants (subject A was excluded). The blood samples were stored refrigerated in heparinized plastic centrifuge tubes for about 2 hours before processing. The subjects (except A and H) received oral doses of 1 or 2 tablets of chloroquine phosphate, each tablet containing 150 mg chloroquine base; subjects H and A received 1 and 2 doses, respectively, of 100 mg chloroquine syrup. Two hours after drug intake a second sample of venous blood was taken from each subject for analysis.

Test for malaria. Thin and thick smears of all blood samples collected were made on glass slides. After air-drying, the smears were treated with Giemsa

Table 1. Vital data on the *in vivo* study subjects

Subject	Age (years)	Sex	Weight (kg)	Blood genotype[a]	Erythrocyte volume fraction	Chloroquine dose (mg base)
A	4	F	12	SS	0.24	2 × 100[d]
B	24	M	75	SS	0.26	1 × 300
C	9	F	26	SS	0.27	1 × 150
D	—[b]	M	73	SS	—[c]	1 × 300
E	19	M	52	AA	0.38	1 × 300
F	17	F	53	AA	0.36	1 × 300
G	35	F	75	AA	0.35	1 × 300
H	3.5	M	18	AS	0.33	1 × 100
I	43	M	63	AS	0.44	1 × 300

[a] SS = subject with sickle-cell anaemia; AA = normal subject; AS = subject with sickle-cell trait.
[b] Age not known but subject was an adult.
[c] Not determined.
[d] Subject A was hospitalized and received the first dose of chloroquine on admission; the second dose was given 10 hours later, 2 hours before the test blood sample was collected.

stain and examined microscopically for parasitaemia.

Detection of chloroquine and desethylchloroquine. The concentrations of chloroquine and desethylchloroquine in the blood samples were determined by the Centres for Disease Control (CDC), Atlanta, GA, USA using high-performance liquid chromatography (HPLC) and a fluorescence method (*4*). Blood samples were first centrifuged at 2500 *g* for 20 minutes, and 0.1 ml of the plasma layer was carefully removed and absorbed on to a strip of thick filter-paper (*4*). The residual plasma in the tube and the blood leukocytes were then carefully removed and discarded, and 0.1 ml of the erythrocyte pellet was smeared on another filter-paper. The smears were carefully dried in air at about 27 °C for 24 hours. After drying, the samples were packed in cellophane bags and forwarded to CDC for analysis.

In vitro *test*

Samples of blood from 18 blood donors aged 17–42 years who were resident in the USA were used for the *in vitro* chloroquine accumulation test. The distribution of haemoglobin genotypes was as follows: 8 SS, 5 AA, and 5 AS. Donors of SS genotype had sickle-cell anaemia and were patients at either the Saint Louis University Medical Center or Saint Louis City Hospital. Analysis of haemoglobin in the blood samples by electrophoresis indicated that a few of the SS patients had recently received blood transfusions, and this was confirmed by examination of their hospital records. The AA and AS donors were in good health, and there were two males in each of the three genotype groups.

Heparinized venous blood from each subject was depleted of leukocytes and platelets as described previously (*5*). The erythrocytes were then washed (×4) by centrifugation and resuspension in a standard medium (containing sodium chloride (68 mmol/l), potassium chloride (4.8 mmol/l), magnesium sulfate (1.2 mmol/l), and disodium hydrogen phosphate (50 mmol/l)) that was adjusted to pH 7.4 with hydrochloric acid. A 5% suspension of the washed erythrocytes in the standard medium was then incubated with a solution of radiolabelled chloroquine (2.06 μmol/l)[a] in plastic tubes at 37 °C for 1 hour. Subsequently, the erythrocyte pellet was precipitated from the medium by centrifugation and the concentration of chloroquine in each fraction determined radiochemically (*6*).

RESULTS

Parasitology

Because malarial parasitaemia increases the uptake of chloroquine by infected erythrocytes (*7*), all blood samples were examined for malarial infection:

[a] From New England Nuclear Corporation, Boston, MA, USA.

Giemsa-stained thick and thin smears were negative in all instances.

In vivo *levels of chloroquine and desethylchloroquine in blood*

Before drug administration. Chloroquine and desethylchloroquine were not detected in the plasma and erythrocytes of samples of blood from AA and AS subjects collected before administration of chloroquine; the detection limit of the method was 5 μg/l. The plasma of SS subjects B and C contained neither chloroquine nor its metabolite; however, the erythrocytes of subject B contained chloroquine (14 μg/l) and desethylchloroquine (39 μg/l). Subject B had been taking chloroquine as a malaria prophylactic but had changed to pyrimethamine several months before the present study. In contrast, subject C had not taken chloroquine for at least 6 months prior to the study, but exhibited chloroquine in erythrocytes (20 μg/l) without any detectable metabolite. The blood of subject D had no trace of either chloroquine or desethylchloroquine prior to commencement of the study, while subject A was already receiving chloroquine when the study began.

After drug administration. The concentrations of chloroquine and desethylchloroquine, respectively, in the plasma and erythrocytes of blood samples collected 2 hours after the administration of chloroquine phosphate are shown in Tables 2 and 3. Except for subject A who received multiple doses of chloroquine, mean levels of chloroquine in plasma were consistently lower for SS (47.8 ± 14.0 μg/l) than for AA (84.5 ± 15.2 μg/l) subjects. Conversely the mean concentration of chloroquine in the erythrocytes was higher in SS subjects (1614.3 ± 709.6 μg/l) than in AA subjects (375.2 ± 115.2 μg/l).

The concentration of desethylchloroquine in erythrocytes of AA subjects was 3–4 times higher than that in plasma; however, for SS subjects desethylchloroquine was detected only in erythrocytes. The level of desethylchloroquine 2 hours after taking chloroquine was similar in samples of blood from both SS and AA subjects, suggesting that there was no genotype-dependent difference in chloroquine metabolism.

Table 2. *In vivo* distribution of chloroquine in blood of study subjects

Subject	Blood genotype[a]	Chloroquine concentration (μg/l) in: Plasma	Erythrocytes	Distribution ratio[b]
A	SS	179.5	5057.5	28.2
B	SS	40.0	2431.0	60.8
C	SS	39.5	1148.0	29.1
D	SS	64.0	1264.0	19.8
E	AA	102.0	454.5	4.5
F	AA	76.0	243.0	3.2
G	AA	75.5	428.0	5.7
H	AS	72.0	228.0	3.2
I	AS	93.0	–[c]	–

[a] SS = subject with sickle-cell anaemia; AA = normal subject; AS = subject with sickle-cell trait.

[b] Ratio of the concentration of chloroquine in erythrocytes to that in plasma.

[c] Not available.

Table 3. *In vivo* distribution of desethylchloroquine in blood of study subjects

Subject	Blood genotype[a]	Desethylchloroquine concentration (μg/l) in: Plasma	Erythrocytes	Distribution ratio
A	SS	96.5	3968.0	41.1
B	SS	<5	260.6	65.1–260.5[b]
C	SS	<5	127.5	31.9–127.5[b]
D	SS	<5	153.0	38.3–153.0[b]
E	AA	26.5	115.5	4.4
F	AA	23.5	74.0	3.1
G	AA	31.5	116.0	3.7
H	AS	40.0	103.5	2.6
I	AS	32.5	–[c]	–

[a] SS = subject with sickle-cell anaemia; AA = normal subject; AS = subject with sickle-cell trait.

[b] Detection limit is 5 μg/l. The range given refers to a maximum and minimum undetectable concentration of desethylchloroquine in plasma of 4 and 1 μg/l, respectively.

[c] Not available.

Table 4. *In vitro* accumulation of chloroquine by a 5% suspension (w/v) of erythrocytes in 2.06 μmol/l chloroquine solution

Blood genotype[a]	No. of samples	Distribution ratio[b]	
		Mean	Range
SS	8	31.0	6.0–65.6
AA	5	3.5	3.2–3.7
AS	5	2.7	2.2–3.4

[a] SS = subject with sickle-cell anaemia; AA = normal subject; AS = subject with sickle-cell trait.

[b] μmol chloroquine per kg erythrocytes : μmol chloroquine per litre medium.

In vitro *test*

Erythrocytes were treated for 1 hour with a solution containing chloroquine (2.06 μmol/l) and the distribution of the drug in the erythrocytes and the medium was determined. The results are summarized in Table 4. The mean concentration of chloroquine in the erythrocytes was 21.9 ± 9.5 μmol/kg for SS, 6.2 ± 0.5 μmol/kg for AA, and 4.7 ± 0.8 μmol/kg for AS subjects, while the corresponding mean chloroquine distribution ratios were 31.0 ± 19.9, 3.5 ± 0.3, and 2.7 ± 0.5.

DISCUSSION

Variability in the level of chloroquine in plasma is common (*8*), but the reason for this is not clearly understood; incorrect intake or malabsorption are, nevertheless, suspected when the level of plasma chloroquine is lower than normal (*9*). The findings reported here indicate that genetic factors can also influence the distribution of chloroquine in blood, e.g., erythrocytes from SS individuals accumulated a higher amount of chloroquine *in vivo* than those from AA individuals. In contrast, the level of chloroquine in the plasma was lower in SS subjects than in AA subjects. The apparently high level of chloroquine in one of the SS patients (plasma: 179.5 μg/l) arose because several large doses of the drug were administered during the illness; however, the drug distribution ratio indicates that the plasma chloroquine level was low. The erythrocytes of SS subjects accumulated a greater level of chloroquine *in vitro* than those of AA subjects. Uptake of chloroquine by AS subjects was comparable to that of AA, and this may also be the case *in vivo*.

In the absence of malarial parasitaemia, the distribution ratio of chloroquine in erythrocytes to that in plasma rarely exceeds 6 for AA and AS subjects. For SS subjects, on the other hand, the distribution ratio can be as high as 157 (*5*), but the reason for the high variability within the SS group is not yet known.

Previously, it was reported that, unlike AA and AS erythrocytes, SS erythrocytes have a high affinity for chloroquine, and this is probably due to the accumulation in SS erythrocytes of haemichrome, containing ferriprotoporphyrin IX (*5*, *10–12*), a compound that has a high affinity for chloroquine (*13*).

The detection of desethylchloroquine in blood 2 hours after oral administration of chloroquine phosphate is consistent with previous findings that metabolism of the drug commences shortly after its intake (*14*). The metabolite was detected both in the plasma and erythrocytes of AA and AS subjects but only in the erythrocytes of SS subjects, which bind desethylchloroquine even more avidly than chloroquine. Non-specific analytical methods that do not distinguish chloroquine metabolites from the parent drug therefore afford even higher apparent levels of chloroquine in the plasma of AA than SS individuals; for example, summation of the *in vivo* levels of chloroquine and desethylchloroquine in plasma in the present study gives levels of the drug 2 to 3 times higher in AA than in SS blood.

It would therefore be advantageous to provide some haematological data when blood chloroquine levels are reported, particularly if they refer to plasma or serum. Blood platelets and leukocytes accumulate relatively large amounts of chloroquine (*15*), and excessive thrombocytosis or leukocytosis could therefore alter the normal distribution of the drug in blood. It has also been reported that erythrocytes which are deficient in glucose-6-phosphate dehydrogenase have a high affinity for binding chloroquine (*16*).

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RÉSUMÉ

ACCUMULATION ACCRUE DE LA CHLOROQUINE ET DE LA DÉSÉTHYLCHLOROQUINE DANS LES DRÉPANOCYTES HOMOZYGOTES

Les taux plasmatiques de chloroquine varient considérablement d'un sujet à l'autre. Lors d'une étude de cette variabilité, nous avons déterminé la distribution, *in vivo* et *in vitro*, de la chloroquine dans le sang de sujets de génotypes différents.

Lors de l'étude *in vivo*, une dose de 4 à 6 mg de chloroquine base par kg de poids corporel a été administrée par voie orale à des volontaires présentant un génotype de l'hémoglobine avec anémie drépanocytaire (SS), avec trait drépanocytaire (AS) ou normal (AA). Des échantillons de sang ont été prélevés deux heures après et on a mesuré la concentration de chloroquine et de son métabolite, la déséthylchloroquine, dans le plasma et les érythrocytes par chromatograhie liquide à haute pression. Le taux moyen de chloroquine dans le plasma était plus faible chez les sujets SS (47,8 μg/l) que chez les sujets AA (84,5 μg/l). En revanche, le taux moyen de chloroquine dans les érythrocytes des sujets SS (1614 μg/l) était plus élevé que dans les érythrocytes des sujets AA (375 μg/l). Le quotient moyen de distribution (concentration de chloroquine par kg d'érythrocytes/concentration par litre de plasma) était de 36,6 pour les sujets SS et 4,5 pour les sujets AA. Le taux de déséthylchloroquine dans les érythrocytes des sujets AA était 3 à 4 fois supérieur au taux plasmatique; par contre, chez les sujets SS on ne trouvait de déséthylchloroquine que dans les érythrocytes. Le taux de déséthylchloroquine dans le sang deux heures après administration de chloroquine était analogue chez les sujets SS et AA, ce qui tend à montrer qu'il n'y a pas de différence liée au génotype en ce qui concerne le métabolisme de la chloroquine.

Lors de l'étude *in vitro*, une suspension à 5% d'érythrocytes lavés provenant de 8 sujets SS, 5 sujets AA et 5 sujets AS a été incubée avec une solution de chloroquine radiomarquée (2,06 μmol/l), et on a mesuré la concentration de médicament dans les érythrocytes et dans la solution par une méthode radiochimique. Le taux moyen de chloroquine dans les érythrocytes était de 21,9 μmol/kg pour les sujets SS, 6,2 μmol/kg pour les sujets AA et 4,7 μmol/kg pour les sujets AS. Le quotient moyen de distribution (μmol de chloroquine par kg d'érythrocytes/μmol de chloroquine par litre de milieu) était de 31,0 pour les sujets SS, 3,5 pour les sujets AA et 2,7 pour les sujets AS.

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