closely associated in cis with haplotype II in cases of β-thalassemia with the mutation of codon 39, and has been reported to cause decreased expression of γ when it is associated in trans with haplotype I and IX β 39. It has not been well determined whether this decrease in γ expression can affect expression of the γ gene cis or in trans.9,10 This way, the presence of the deletion of 4 base pairs from 225 to 222 in the promoter region of the γ gene, in our two cases (II 1 and II 2), would favor the expression of γ10 which would already be augmented by the presence of Xmn I γ (+).

Although the existence of other related genetic factors which produce an increase of HbF in the Xmn I γ (+) cannot be ruled out, the C→T substitution at position –158 to the γ gene is in a region which contains sequences which are important in regulation of γ gene expression5 and probably, in addition to being a genetic marker, is responsible for most of the γ synthesis in these Xmn I γ haplotypes. It is, therefore, important to study this factor in patients with β-thalassemia because of the prognostic implications of this disease.


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Key words
–158 C→γ HPFH, homozygous β39 thalassemia, γ, γ gene, thalassemia intermedia

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References
In vivo effect of chloroquine on platelet aggregation in anesthetized rats

Sir,

In vivo platelet aggregation was studied by a platelet count ratio (PCR) technique. Following the intravenous administration of collagen or ADP to rats the mean PCR was lower in controls than in two groups administered graded doses of chloroquine ($p < 0.05$ and 0.01 respectively). Chloroquine inhibits platelet aggregation in vivo in rats.

Previous reports on the effect of chloroquine on platelet aggregation were based on in vitro and ex vivo studies where aggregation inducers and chloroquine were added to isolated platelets, or aggregation inducers added to platelets withdrawn from chloroquine-treated human volunteers.$^1$-$^3$ Since not all the factors that affect aggregation in vivo may be available in vitro or ex vivo, the effect of chloroquine on platelet aggregation in vivo has been examined.

Rats were randomly assigned into a control or two test groups (n=6). The control group was administered 0.9% NaCl (1 mL/kg, ip). The first test group was given ADP at a dose of 8.6 mg/kg, ip$^1$ while the second test group was administered a higher dose of chloroquine (40 mg/kg, ip). After one hour, collagen (1 mg/kg, iv) was administered under urethane anesthesia (1.5 g/kg, ip) to all groups to induce platelet aggregation in vivo.

Blood (1 mL/rat) was taken by cardiac puncture for estimation of platelet aggregation. This was measured by a PCR technique $^5$ in which a lowering of the count ratio signifies an increase in platelet aggregation and vice versa. These experiments were repeated using another aggregation inducer, ADP (90 µg/kg, iv) and normal saline (1 mL/kg, iv). The doses of ADP and collagen were slightly higher than those reported for rabbits$^5$ since preliminary studies showed that lower doses were ineffective. Serum chloroquine concentration was estimated by the method of Prauty and Kuroda.$^6$

Mean serum chloroquine concentrations one hour after administration were 5.06±1.29 mg/L and 10.98±3.75 mg/L (mean±SD; $p < 0.01$) in rats administered chloroquine at doses of 8.6 mg and 40 mg/kg respectively (n=5).

In the rats given i.v. collagen, the PCR were

0.283±0.165, 0.360±0.175 and 0.694±0.193 in the control, first and second test groups respectively. The ratios for the two test groups were significantly higher ($p < 0.05$ and 0.01) than that of the control group. Results after ADP were similar. Platelet count ratios following the infusion of normal saline were 0.818±0.094; 0.830±0.073 and 0.876±0.070 for control, first and second test groups respectively. The ratios obtained with saline were not significantly different between the three groups (Figure 1).

Based on in vitro and ex vivo studies some investigators have concluded that therapeutic concentrations of chloroquine have a negligible effect on platelet aggregation and are not a significant risk to patients with compromised hemostasis.$^1$ However, in vitro and ex vivo studies may not reflect in vivo events since some endogenous aggregation inducers and inhibitors from non-platelet sources may be reduced or unavailable.

We have shown that a therapeutic dose of chloroquine inhibits platelet aggregation in vivo in rats and so, its use in patients with compromised hemostasis could be risky if the results are confirmed in humans. Conversely, chloroquine administration could be beneficial in the reduction of hyperaggregability of platelets in malaria$^7$-$^8$ and in the prevention of thrombosis.

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