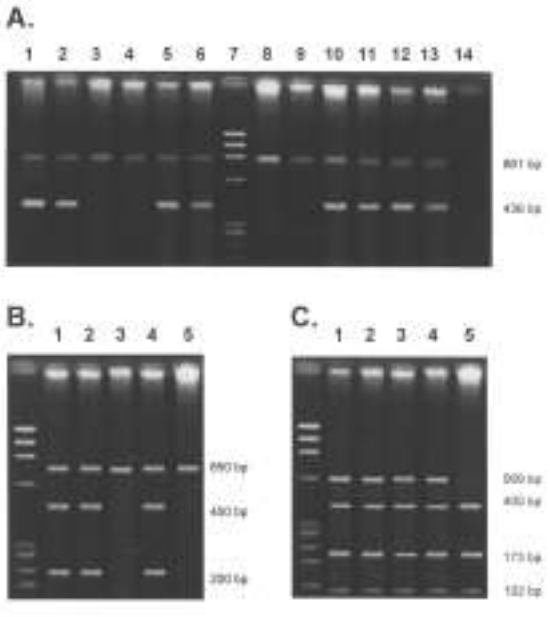

Figure 1.

A. Ethidium bromide-stained gel illustrating the products obtained after amplification using primers specific for detection by PCR-ARMS of the point mutation CD39 (C→T) (436 bp). The first six lanes correspond to the normal assay. Lane 1 and 2 are heterozygote controls, Lanes 3 and 4 represent the propositus II_1 and II_2 respectively. Lanes 5 represent the father (I_1) lane 6 the mother (I_2), respectively. Lane 7 is the DNA Marker X174 *Hae* III. Lines 8 to 13 correspond with the mutate assay; lane 8 and 9 heterozygote controls, lanes 10 and 11 represent the propositus II_1 and II_2, respectively. Lane 12 represent the father (I_1) and line 13 the mother (I_2). Lane 14 is the H_2O control. The internal control corresponds to the 861 bp fragment.

B. Ethidium bromide-stained gel illustrating the products obtained after amplification using specific primers and digestion with *Xmn* I for the polymorphic variation of C→T in the 158 bp 5' of the Cap site of the $^{G}\gamma$ gene. Lane 1 represents propositus II_1 [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 2 represents propositus II_2 [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 3 represents the father (I_1) (*Xmn* I– (–) haplotypes) with a 650 bp fragment, lane 4 the mother (I_2) [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 5 represents a negative control with a 650 bp fragment. DNA Marker X174 *Hae* III.

C. Agarose gel electrophoresis of Fnu 4HI-digested A promoter DNA amplified by PCR. Lanes 1 and 2 represent the propositus II_1 and II_2 respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lanes 3 and 4 represent the father (I_1) and mother (I_2) respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lane 5 is a normal control DNA with 400, 173 and 102 bp fragments. DNA Marker X174 *Hae* III.

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 $^{A}\gamma$ when it is associated in *trans* with haplotype I and IX β^0 39.[8] It has not been well determined whether this decrease in $^{A}\gamma$ expression can affect expression of the gene $^{G}\gamma$ in *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 $^{A}\gamma$ gene, in our two cases (II_1 and II_2), would favor the expression of $^{G}\gamma$[10] 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 $^{G}\gamma$ gene is in a region which contains sequences which are important in regulation of γ gene expression[5] and probably, in addition to being a genetic marker, is responsible for most of the $^{G}\gamma$ 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 $^{G}\gamma$ HPFH, homozygous $\beta^0$39 thalassemia, $^{A}\gamma$ gene, thalassemia intermedia

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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[4] 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 ±3.75mg/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

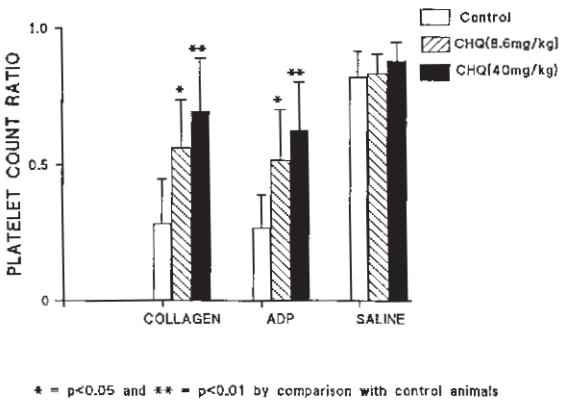

Figure 1. Platelet count ratio in rats administered collagen, adenosine diphosphate (ADP) or normal saline (iv) following ip administration of normal saline (1 mL/kg) to control rats (n=6) and chloroquine diphosphate to test rats at either 8.6 mg/kg (n=6) or 40 mg/kg (n=6). Values are represented as means±SD.

0.283±0.165, 0.560± 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 compromized 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 compromized 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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