Human Polymorphonuclear Leukocytes:
Effect of Chloroquine on Aggregation, Arachidonic Acid Liberation and Thromboxane B\textsubscript{2} Generation

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Abstract. The effects of the antimalarial drug chloroquine (CQ) on arachidonic acid (AA) liberation from thromboxane B\textsubscript{2} (TXB\textsubscript{2}) formation in, and aggregation of isolated human polymorphonuclear (PMN) leukocytes stimulated with N-formyl methionyl leucyl phenyl alanine (FMLP) were investigated. CQ decreased aggregation of stimulated PMN leukocytes; however, in contrast to AA liberation and TXB\textsubscript{2} formation, lower concentrations were more effective than the highest one used. This effect may be associated with an increase in intracellular pH reported to be induced by higher CQ concentrations, possibly counteracting the inhibition of aggregation and/or eliminating negative feedback control of aggregation by lack of prostaglandins.

Key words: Human PMN leukocytes — Chloroquine — Aggregation — Arachidonic acid liberation — Thromboxane B\textsubscript{2} formation
and thromboxane synthase levels (Nosáľ et al. 1995, Drábiková et al. 1996). Since PMN leukocytes play a crucial role in inflammatory reactions, it was of interest to study the effects of CQ on isolated human PMN with respect to alterations of their aggregation, arachidonic acid (AA) liberation and thromboxane B$_2$ (TXB$_2$) formation.

**Materials and Methods**

FMLP (N-formyl-methionyl-leucyl-phenylalanine, Sigma) Chloroquine (CQ) (ACO, Sweden), Dextran T500 (Pharmacia Fine Chemicals), Lymphoprep (Nyegaard and Co). $^3$H-AA ($7.6 \times 10^5$ Bq/ml) and $^{125}$I-TXB$_2$ RIA kit was the kind gift from Dr. I. Mucha, Inst of Isotopes, Budapest, Hungary

**Isolation.** PMN were isolated from blood of healthy volunteers into 3.8% trisodium citrate dihydrate (9:1), after dextran sedimentation and centrifugation on Lymphoprep by modified Boyum’s method (Boyum 1968). PMN were re-suspended in phosphate buffer saline (PBS) solution (137 mmol/l NaCl, 2.7 mmol/l KCl, 8.1 mmol/l Na$_2$HPO$_4$, 1.5 mmol/l KH$_2$PO$_4$, 18 mmol/l CaCl$_2$, 10 mmol/l MgCl$_2$), pH 7.4, and washed once with PBS. For individual assays, PMN were diluted as described below. The purity of isolated PMN was > 95%.

**PMN aggregation** was measured turbidimetrically (aggregometer Chrono-log Dual Channel). PMN (7 $\times$ 10$^6$ /sample) were preincubated for 5 min with CQ (1, 10, 100, 1000 μmol/l) and subsequently stimulated with FMLP (0.1 μmol/l) for 5 min at 37°C. The results were expressed as aggregation amplitude in mm, measured 60 s after stimulation.

$^3$H-AA liberation. Labelled (3.7 $\times$ 10$^{-2}$ MBq$^3$H-AA/10$^6$ PMN/ml, 1 h/37°C) and washed cells were pretreated with CQ (1, 10, 100, 1000 μmol/l) for 5 min at 37°C and subsequently stimulated with FMLP (0.1 μmol/l) for additional 3 min. Phospholipids were extracted (Jančinova and Nosáľ 1989), and radioactivity was measured in Packard TriCarb 2500 TR.

**TXB$_2$ production**. PMN (10$^6$ cells/100 μl) were stabilised at 37°C for 3 min and incubated with CQ (1, 10, 100, 1000 μmol/l) for 5 min at 37°C. and stimulated (FMLP 0.1 μmol/l) for additional 15 min. TXB$_2$ was determined in the supernatant using radioimmunoassay (Nosáľ et al. 1993).

**Statistical evaluation.** All values are given as means ± S E M, and the results were statistically processed by Student’s t-test.

Fig 1 shows the representative aggregation curves of isolated PMN treated with CQ (1, 10, 100, 1000 μmol/l) and stimulated with FMLP (0.1 μmol/l). The lower concentration of CQ (1 μmol/l) was more effective than the higher concentration used (100 μmol/l). At 1000 μmol/l, CQ did not affect the aggregation of
Figure 1. Representative aggregation curves of human PMN leukocytes treated with different concentrations of CQ and subsequently stimulated with FMLP.

Figure 2. Effect of CQ on aggregation of FMLP-stimulated isolated human PMN leukocytes. Each value represents the mean from 14 experiments ± SEM. *p < 0.05. **p < 0.01.

PMN leukocytes induced with FMLP. Fig 2 summarises the effect of CQ (1, 10, 100, 1000 μmol/l) on FMLP-induced aggregation of isolated PMN. CQ in the concentration range from 1 μmol/l to 100 μmol/l decreased significantly the amplitude of aggregation curves. The most effective concentration was 10 μmol/l (decrease from 59.6 ± 1.59 mm to 50.2 ± 2.39 mm). Fig 3 shows the effect of CQ on 3H-AA liberation as percentage of FMLP stimulation. CQ in the concentrations of 1 and 10 μmol/l nonsignificantly increased 3H-AA liberation from stimulated PMN, to 109.48 ± 14.1 and 116.6 ± 13.5%, respectively. Higher concentrations of CQ (100 and
Figure 3. Effect of CQ on FMLP-stimulated $^3$H-arachidonic acid liberation from phospholipids of isolated human PMN leukocytes. Values are expressed as percentage of values obtained after stimulation with FMLP $26044 \pm 2757$ dpm. Each value is the mean from 6 experiments $\pm$ S.E.M. **$p < 0.01$

Figure 4. Effect of CQ on FMLP-stimulated TXB$_2$ production from isolated human PMN leukocytes. Values are expressed as percentage of values obtained after stimulation with FMLP $721 \pm 28.4$ pg/10$^6$ cells. Each value is the mean from 6 experiments $\pm$ S.E.M. **$p < 0.01$

1000 $\mu$mol/l) significantly decreased stimulated $^3$H-AA liberation, to 66.1 $\pm$ 5.8 and 65.28 $\pm$ 8.5%, respectively. The effect of CQ on TXB$_2$ formation as percentage of stimulus (FMLP) is illustrated in Fig 4. CQ in the concentration of 1 $\mu$mol/l non-
significantly increased TXB\textsubscript{2} formation to 109.6 ± 15.9\% in the concentrations of 10 and 100 µmol/l CQ nonsignificantly decreased TXB\textsubscript{2} generation to 82.8 ± 8.5 and 88.2 ± 10.9\% respectively. TXB\textsubscript{2} generation was significantly decreased by 1000 µmol/l CQ, to 71.6 ± 6.4\%.

Stimulation of PMN leukocytes with FMLP (surface membrane receptor stimulus) evokes a series of responses which includes aggregation and is accompanied also by induction of phospholipid metabolism via activation of specific phospholipases.

In our experimental settings CQ slightly but significantly decreased aggregation in stimulated human PMN leukocytes at concentrations close to therapeutic levels (1, 10 µmol/l) (Titus 1989), however lower concentrations of CQ were more effective than the highest one used (1000 µmol/l). Two possible explanations may be suggested. Firstly PMN leukocytes as well as other blood elements accumulate CQ in very high concentrations (Nosal et al 1988). With increasing concentrations of accumulated CQ intracellular pH keeps increasing (Ohkuma and Poole 1978, Poole and Ohkuma 1981), which may counteract inhibition of aggregation since a similar change of intracellular pH was found to be induced by FMLP (Sha'afi and Molski 1988). Secondly it is known that prostaglandins PGE\textsubscript{1} and PGE\textsubscript{2} partially inhibit aggregation induced by FMLP (Wise and Jones 1994). CQ at high concentrations decreased AA liberation (Fig 3) and thus lack of prostaglandins may have eliminated negative feedback control of aggregation explaining at least partly the biphasic effect of CQ in our experiments. CQ diminished \textsuperscript{3}H-AA liberation and TXB\textsubscript{2} generation in PMN leukocytes in the higher concentrations used (Figs 3 4). This suggests that CQ might affect the surface membrane receptor for FMLP or associated metabolic events and thus inhibit the arachidonic acid pathway. CQ dose dependently inhibited human and rat platelet aggregation \textit{in vitro} and \textit{ex vivo} (Cummins et al 1990, Jančinova et al 1994, 1996), as well as \textsuperscript{3}H-AA liberation \textit{in vitro} in stimulated platelets (Nosal et al 1995). As for PMN leukocytes it is not known to what extent arachidonic acid cascade participates in aggregation however the obtained results indicate a possible connection between the functions studied.

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