Short communication

Human Polymorphonuclear Leukocytes: Effect of Chloroquine on Aggregation, Arachidonic Acid Liberation and Thromboxane B_2 Generation

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Abstract. The effects of the antimalarial drug chloroquine (CQ) on arachidonic acid (AA) liberation from thromboxane B_2 (TXB_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_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 feed back control of aggregation by lack of prostaglandins

Key words: Human PMN leukocytes — Chloroquine — Aggregation — Arachidonic acid liberation — Thromboxane B_2 formation

The antimalarial and antiinflammatory drug chloroquine (CQ) was reported to inhibit phospholipid methylation, protein kinase and calmodulin-dependent kinases in human monocytes (Hurst et al 1986) and the generation of reactive oxygen species in stimulated human PMN leukocytes (Hurst et al 1987) Inhibition of blood platelet aggregation and histamine secretion from mast cells was suggested to be due to the high affinity of CQ to the plasma cell membrane of these cells (Nosal et al 1991 Jančinova et al 1994) In both platelets and mast cells, CQ dose-dependently inhibited arachidonic acid pathway at the phospholipase A_2

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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-fenyl-alanine, 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 dextrane sedimentation and centrifugation on Lymphoprep by modified Boyum's method (Boyum 1968) PMN were resuspended in phosphate buffer saline (PBS) solution (137 mmol/l NaCl, 2 7 mmol/l KCl, 8 1 mmol/l Na_2HPO_4, 1 5 mmol/l KH_2PO_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 ($7\ 10^6$/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/10^6PMN/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 Nosaľ 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

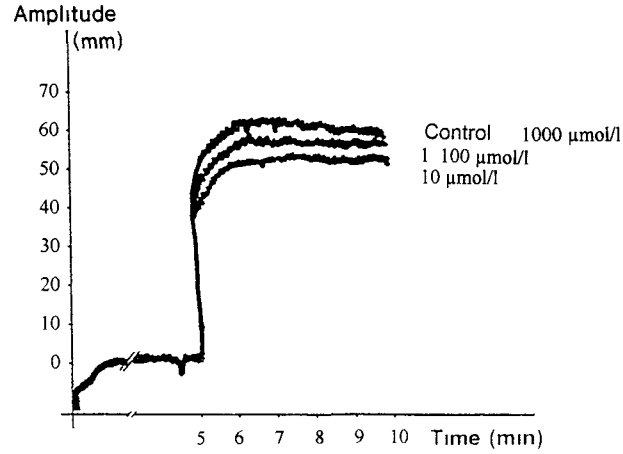

Figure 1 Representative aggregation curves of human PMN leukocytes treated with different concentrations of CQ and subsequently stimulated with FMLP

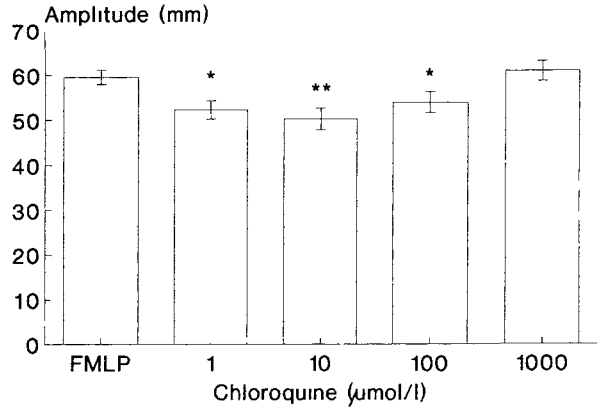

Figure 2. Effect of CQ on aggregation of FMLP-stimulated isolated human PMN leukocytes Each value represents the mean from 14 experiments ± S E M $*p < 0.05$ $**p < 0.01$

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 ^{3}H-AA liberation as percentage of FMLP stimulation CQ in the concentrations of 1 and 10 µmol/l nonsignificantly increased ^{3}H-AA liberation from stimulated PMN, to 109 48±14 1 and 116 6±13 5%, respectively Higher concentrations of CQ (100 and

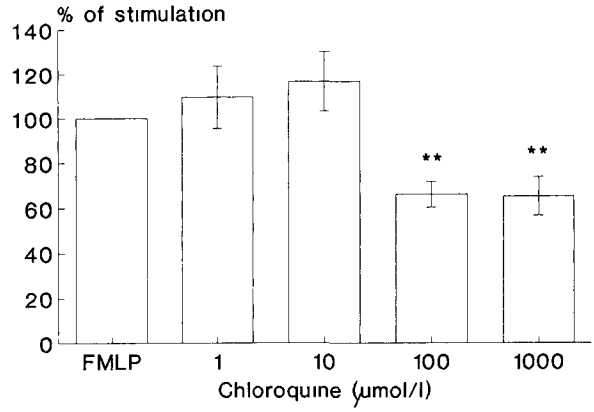

Figure 3. Effect of CQ on FMLP-stimulated ^{3}H-arachidonic acid liberation from phospholipids of isolated human PMN leukocytes Values are expressed as percentage of values obtained after stimulation with FMLP 26 014 ± 2757 dpm Each value is the mean from 6 experiments ± S E M, $^{**}p < 0\ 01$

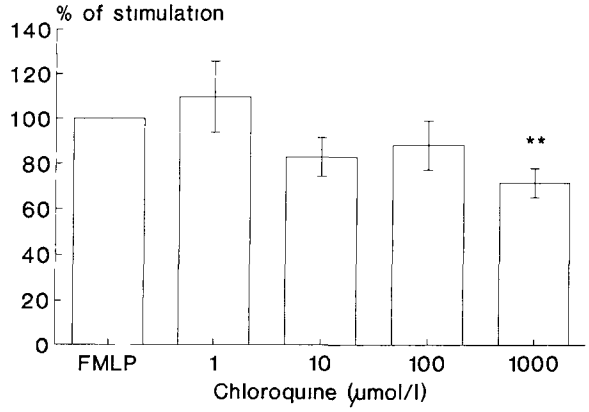

Figure 4. Effect of CQ on FMLP-stimulated TXB_2 production from isolated human PMN leukocytes Values are expressed as percentage of values obtained after stimulation with FMLP 721 ± 28 4 pg/10^6 cells Each value is the mean from 6 experiments ± S E M $^{**}p < 0\ 01$

1000 μmol/l) significantly decreased stimulated ^{3}H-AA liberation, to 66 1 ± 5 8 and 65 28 ± 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 μmol/l non-

significantly increased TXB_2 formation to 109.6 ± 15.9%. In the concentrations of 10 and 100 μmol/l CQ nonsignificanly decreased TXB_2 generation to 82.8 ± 8.5 and 88.2 ± 10.9% respectively. TXB_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_1 and PGE_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 feed back control of aggregation explaining at least partly the biphasic effect of CQ in our experiments. CQ diminished ^{3}H-AA liberation and TXB_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 this way inhibit the arachidonic acid pathway. CQ dose dependently inhibited human and rat platelet aggregation *in vitro* and *ex vivo* (Cummins et al. 1990, Jančinova et al. 1994, 1996), as well as ^{3}H-AA liberation *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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References

Boyum, M. (1968): Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. **21**, 77—90.

Cummins, D., Faint, R., Yarduman, D. A., Dawling, S., Mackie, I., Macchin, S. J. (1990): The *in vitro* and *ex vivo* effect of chloroquine sulfate on platelet function: implications

of malaria prophylaxis in patients with impaired haemostasis J Trop Med Hyg **93** 112 115

Drabikova K Pečivova J Nosal R (1996) Effect of chloroquine on arachidonic acid pathway in isolated mast cells Inflamm Res **45** S13 S14

Hurst N P French J K Bell A L Nuki G O Donnell M L Betts W H Cleland L G (1986) Differential effect of mepacrine chloroquine and hydroxychloroquine on superoxide anion generation phospholipid methylation and arachidonic acid release by human blood monocytes Biochem Pharmacol **18,** 3083—3089

Hurst N P French J K Gorjatschko L Betts W H (1987) Studies on the mechanism of inhibition of chemotactic tripeptide stimulated human neutrophil polymorphonuclear leukocyte superoxide production by chloroquine and hydroxychloroquine Ann Rheum Dis **46,** 750—756

Jancinova V Nosal R (1989) Difference among beta adrenoceptor blocking drugs in modifying platelet aggregation and arachidonic acid liberation under thrombin stimulation Thromb Res **54** 687—698

Jancinova V Nosal R Petrikova M (1991) On the inhibitory effect of chloroquine on blood platelet aggregation Thromb Res **74,** 495 501

Jančinova V Nosal R Danihelova E (1996) Inhibition of human blood platelets by chloroquine Platelets **7,** P88

Nosal R Ericsson O Sjoqvist F Ďurišova M (1988) Distribution of chloroquine in human blood fractions Meth Find Exptl Clin Pharmacol **10,** 581—587

Nosal R Drabikova K Pecivova J (1991) Effect of chloroquine on isolated mast cells Agent Action **33,** 37 40

Nosal R Petrikova M Jancinova V (1993) Alterations in lipid peroxidation of thrombin-stimulated rat platelets treated with beta-adrenoceptor blocking drugs J Lipid Med **8,** 121—132

Nosal R Jancinova V Petrikova M (1995) Chloroquine inhibits stimulated platelets at the arachidonic acid pathway Thromb Res **77,** 531 542

Ohkuma O Poole B (1978) Fluorescence probe measurement of the intralysosomal pH in leaving cells an the perturbation of pH by various agents Proc Natl Acad Sci USA **75,** 3327 3331

Poole B Ohkuma O (1981) Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages J Cell Biol **90,** 665 669

Shafi R I Molski T F P (1988) Activation of the neutrophil Progr Allergy **42,** 1—64

Titus E O (1989) Recent developments in the understanding of the pharmacokinetics and mechanism of action of chloroquine Pher Drug Monit **11,** 369 379

Wise H Jones R L (1994) Characterization of prostanoid receptors on rat neutrophils Br J Pharmacol **113,** 581 587

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