Chloroquine inhibits proinflammatory cytokine release into human whole blood

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Chloroquine inhibits proinflammatory cytokine release into human whole blood. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1058–R1064, 1998.—Excessive synthesis and release of proinflammatory cytokines during endotoxia causes severe pathophysiological derangements and organ failure. Because the lysosomotropic agent chloroquine has been effective in the treatment of diseases associated with increased secretion of proinflammatory cytokines such as malaria or rheumatoid arthritis, this study evaluates the potential effect of chloroquine on endotoxin-induced cytokinemia using human whole blood from healthy volunteers. Chloroquine revealed a dose-dependent inhibitory effect on endotoxin-induced secretion of tumor necrosis factor-α, interleukin-1β, and interleukin-6 that was associated with reduced cytokine mRNA expression. Moreover, ammonia and methylamine, which react as weak bases like chloroquine, reduced synthesis and secretion of proinflammatory cytokines. These data indicate a potent anti-inflammatory effect of chloroquine on endotoxin-induced synthesis of proinflammatory cytokines that may be due to its weak base effect. Thus chloroquine may be of therapeutic benefit not only during chronic inflammation but also in diseases that are related to bacteria-induced inflammation.

PROINFLAMMATORY CYTOKINES, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), play an important role in the development of local and systemic inflammation during severe infection or after severe injury, escalating in organ failure or in multiple organ dysfunction syndrome (11). Despite new therapeutic approaches using monoclonal antibodies or receptor antagonists, clinical studies using these agents did not significantly decrease mortality of patients with septic shock (1).

The antimalarial drug chloroquine has recently been used in the treatment of inflammatory diseases such as rheumatoid arthritis (6) and disseminated lupus erythematosus (3), which are related to an increased synthesis of TNF-α. It has been suggested that the therapeutic effect of chloroquine in these diseases is due to its immunoregulatory potency. Previous studies demonstrated that chloroquine reduces the responsiveness of peripheral blood mononuclear cells to mitogens (18), thus inhibiting T cell proliferation and suppressing the generation of immunoglobulin-secreting cells. These effects of chloroquine were explained by a reduced production of lymphocyte-stimulating factor IL-1β, although this hypothesis was not confirmed by measuring the effect of chloroquine on IL-1β release by monocytes. Furthermore, recent investigations using a murine model of hemorrhagic shock revealed an inhibitory influence of chloroquine on enhanced synthesis of proinflammatory cytokines by Kupffer cells (5). In addition, in vitro studies using monocyte monolayers showed a dose-dependent decrease in TNF-α and IL-6 secretion after treatment with chloroquine (16, 17).

Although these in vitro and in vivo studies suggest an inhibitory effect of chloroquine on secretion of proinflammatory cytokines using purified cell cultures and experimental models of inflammation, the precise mechanisms of its anti-inflammatory potency using a clinically relevant model of infection remain to be determined.

MATERIALS AND METHODS

Collection of blood and whole blood assay. The whole blood assay was carried out as described previously (4). In brief, blood from healthy volunteers was drawn into heparinized syringes (20 U/ml; endotoxin contamination <5 pg/ml heparin according to the limulus amoebocyte lysate assay). Aliquots of 5 ml blood were placed into sterile polypropylene tubes (Falcon, Heidelberg, Germany). One sample was processed immediately as described below to serve as the 0-h time point. The other samples were stimulated with lipopolysaccharide (LPS, 1 µg/ml; from Escherichia coli 055:B5; Difco Laboratories, Detroit, MI) in the presence or absence of different concentrations of chloroquine (10, 20, 100, and 200 µM; Sigma Chemicals, Deisenhofen, Germany), ammonia (10 and 20 mM; Merck, Darmstadt, Germany), or methylamine (10 and 20 mM; Sigma). The doses of ammonia and methylamine used were comparable to previous studies and do not cause cell death (21, 23, 27, 29). Additionally, eglin (2.5 µM; Sigma), which effectively neutralizes elastase activity, was added to LPS-stimulated whole blood in the presence of chloroquine (8).

The blood-containing tubes were placed on a rotator and incubated at 37°C in a 5% CO2 atmosphere. To detect spontaneous expression of mRNA and secretion of proinflammatory cytokines, blood samples were incubated without LPS or additional chemicals. After 4 and 8 h of incubation, blood samples were removed and processed immediately. Plasma and peripheral blood mononuclear cells (PBMC) were separated using Ficoll-Hypaque density gradient centrifugation (density = 1.077; Seromed, Berlin, Germany). The plasma samples were filtered, separated into aliquots, and stored at −80°C until assayed for cytokine levels. The PBMC were washed two times with phosphate-buffered saline containing phenylmethylsulfonyl fluoride (100 mM; Sigma) and aprotinin (1 µg/ml; Sigma) to block unspecific cytokine degradation during the preparation procedures. After determination of cell counts and viability of PBMC using the trypan blue exclusion technique, PBMC were pelleted at 800 g for 5 min at 4°C. The pellet was resuspended in lysis buffer for Northern blot.
analysis and stored at −20°C. To study kinetics of cytokine release, human whole blood was stimulated with 1 µg/ml LPS for 0, 1, 2, 4, 8, and 24 h in the presence or absence of 100 µM chloroquine. The samples were processed as described above.

Cell counts and differentiation of PBMC were determined with trypan blue exclusion and fluorescent-activated cell sorter (FACS) analysis. They were similar in all groups independent of the addition of LPS, one of the chemicals, or the incubation time. A cytotoxic effect of chloroquine on PBMC was investigated using trypan blue exclusion and measurement of lactate dehydrogenase release in plasma using a commercially available kit (Boehringer, Mannheim, Germany). Chloroquine-induced apoptosis was studied using staining of PBMC with propidium iodide and flow cytometry (Coulter) as previously described (9). Chloroquine in the highest concentration (200 µM) only slightly increased the release of lactate dehydrogenase by 17% after an incubation time of 24 h, whereas no effect was observed with low concentrations. In addition, staining of intracellular DNA with propidium iodide did not reveal chloroquine-induced apoptosis of PBMC, even in high concentrations. Therefore, chloroquine neither causes significant necrosis nor apoptosis of PBMC in whole blood.

To study the direct effect of chloroquine on monocytes and to exclude an indirect impact through granulocytes, monocyte monolayers were prepared and incubated with chloroquine (10 and 100 µM) over 4 and 8 h. TNF-α, IL-1β, and IL-6 were measured in monocyte supernatants as described below.

Cytokine assays. The biological activity of TNF-α was determined by its cytotoxic effect on the fibrosarcoma cell line WEHI 164 subclone 13 (kindly provided by S. Kunkel, Ann Arbor, MI) as previously described (5). The degree of lysis induced by TNF-α was measured by proliferation of nonlysed WEHI cells using thiazolyl blue (Sigma). To confirm that the bioactivity measured in the WEHI bioassay was due to TNF-α, a neutralizing monoclonal anti-TNF-α antibody (Genzyme, Cambridge, MA) was added to samples containing high amounts of TNF-α. This antibody completely abolished TNF-α activity. Neither chloroquine nor ammonia, methylamine, or elastase in the highest concentrations used in the whole blood assay affected the WEHI 164 cytotoxicity assay. Because chloroquine and ammonia significantly depressed the blasto-

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**Fig. 1.** Dose-dependent effect of chloroquine (10, 20, 100, and 200 µM) on tumor necrosis factor (TNF)-α (A and B), interleukin-1β (IL-1) (C and D), and interleukin-6 (IL-6) (E and F) secretion into human whole blood (n = 8) stimulated with lipopolysaccharide (LPS; 1 µg/ml) for 4 (A, C, and E) and 8 h (B, D, and F), respectively. Plasma was assayed for TNF-α, IL-1β, and IL-6 as described in MATERIALS AND METHODS. Data are presented as means ± SE. *P ≤ 0.01, plus vs. minus chloroquine.
genesis of D10.G4.1 and 7TD1 cells, which are used as target cells in bioassays measuring IL-1 and IL-6 (W. Ertel and J. Karres, unpublished data). Plasma levels of IL-1β and IL-6 were determined by specific enzyme-linked immunosorbent assay (ELISA), as described previously (4). The sensitivity of the IL-1β and IL-6 ELISA was 15 and 50 pg/ml, respectively.

For quantitating the levels of intracellular and cell membrane-associated IL-1β, PBMC were isolated using Ficoll-Hypaque (density = 1.077; Sigma) density gradient centrifugation. After washing PBMC two times, cells were resuspended in 1 ml of phosphate-buffered saline. This was followed by three freeze-thaw cycles (between –20 and –70°C). IL-1β in cell lysates was determined as described above.

Northern blot analysis. After density gradient centrifugation with Ficoll-Hypaque, total mRNA from PBMC was extracted by the acid guanidinium thiocyanate-phenol-chloroform extraction procedure as previously described (4). After quantitation by spectrophotometry, equal amounts of RNA (8 µg·sample·line−1) were fractionated on 1% agarose gels containing formaldehyde and transferred to nylon filters (Hybond-N; Amersham, Braunschweig, Germany) by vacuum blotting. The filters were stained with methylene blue to visualize 18S and 28S rRNA. Prehybridization, hybridization, stringency washes, and autoradiography of blots were performed as described earlier. The blotted RNA was hybridized with fragments of human TNF-α cDNA (0.8-kb EcoRI fragment; generously provided by Genentech, South San Francisco, CA), IL-1β cDNA (1.5-kb PstI fragment; a gift from Genetics Institute, Cambridge, MA), and IL-6 cDNA (0.44 kb BamHI-TaqI fragment; kindly provided by T. Hirano, Osaka, Japan) that had been labeled with [32P]dCTP by the random priming method (Megaprime DNA labeling system; Amersham). Transfer efficiency of RNA was controlled by an additional hybridization to a murine 28S rRNA probe (obtained from I. Grummt, Heidelberg, Germany).

Statistics. Results are presented as means ± SE. Data are analyzed by means of the paired Wilcoxon test. Significance was predetermined as P < 0.05.

### RESULTS

Effect of chloroquine on synthesis of proinflammatory cytokines. A spontaneous release of TNF-α, IL-1β, or IL-6 into human whole blood obtained from healthy volunteers was not observed during the whole incubation time of 24 h, which is in line with previous studies (4). Incubation with LPS significantly increased TNF-α plasma levels at 4 h (185 ± 17 U/ml), whereas no further increase was seen at 8 h (170 ± 13 U/ml) compared with baseline levels (0 h time point). In contrast, the addition of chloroquine revealed a dose-dependent inhibitory effect on TNF-α secretion (Fig. 1, A and B). A maximum inhibition was found with 200 µM chloroquine causing a significant (P ≤ 0.05) reduction of TNF-α release at 4 h (38 ± 12 U/ml, −80%; Fig. 1A) and at 8 h (21 ± 7 U/ml, −88%; Fig. 1B) of incubation.

In parallel to TNF-α secretion, high doses of chloroquine (100 and 200 µM) caused a marked inhibition (P ≤ 0.05) of IL-1β release into LPS-stimulated whole blood by 96% (450 ± 175 pg/ml) and by 84% (2,777 ± 611 pg/ml) after 4 and 8 h of incubation, respectively, compared with whole blood without chloroquine (13,157 ± 1,155 pg/ml at 4 h; 17,301 ± 1,017 pg/ml at 8 h). Addition of low concentrations of chloroquine (10 and 20 µM) resulted only in a slight but not significant

### Table 1. Effect of chloroquine on cytosolic/membrane-bound and secreted interleukin-1β in LPS-stimulated PBMC

<table>
<thead>
<tr>
<th>Cytosolic IL-1β, ng/ml</th>
<th>Secreted IL-1β, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No additions Co LPS</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>ND ND 14.53 2.87</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>ND ND 7.69 0.95</td>
</tr>
</tbody>
</table>

Co, chloroquine; LPS, lipopolysaccharide; IL, interleukin; ND, not detectable; PBMC, peripheral blood mononuclear cells.
inhibition of IL-1β secretion (Fig. 1, C and D). Similar to secreted IL-1β, chloroquine (100 µM) decreased the amounts of cytosolic and membrane-bound IL-1β (Table 1).

In addition, low concentrations of chloroquine (10 and 20 µM) did not affect IL-6 release into whole blood after stimulation with LPS for 4 h (19,604 ± 2,928 pg/ml; Fig. 1E) and for 8 h (29,917 ± 2,790 pg/ml; Fig. 1F). However, chloroquine concentrations exceeding 20 µM significantly (P < 0.05) decreased IL-6 secretion, with a maximum inhibition of 95% (926 ± 430 pg/ml) and 90% (2,905 ± 586 pg/ml) after 4 and 8 h of incubation, respectively, in the presence of 200 µM chloroquine (Fig. 1, E and F).

Treatment of human whole blood with chloroquine even in the highest dose (200 µM) did not influence viability of PBMC or alter absolute cell numbers, as determined by their ability to exclude trypan blue, which is in line with previous studies using isolated monocyte or macrophage cultures (16, 17).

Effect of chloroquine on kinetics of proinflammatory cytokine secretion. Plasma levels of TNF-α, IL-1β, and IL-6 in human whole blood in the presence of 1 µg/ml LPS with or without 100 µM chloroquine were determined over a 24-h incubation period (Fig. 2, A–C). Kinetics of TNF-α, IL-1β, and IL-6 secretion in the presence of LPS were similar to previous results (2, 4). The addition of 100 µM chloroquine resulted in a significant (P < 0.01) inhibition of TNF-α secretion at 2, 4, 8, and 24 h of incubation (Fig. 2A). In parallel to TNF-α secretion, addition of 100 µM chloroquine significantly (P < 0.01) reduced release of IL-1β and IL-6 after 4, 8, and 24 h compared with LPS-stimulated whole blood without chloroquine (Fig. 2, B and C).

Effect of chloroquine on cytokine mRNA expression. To further elucidate a potential inhibitory effect of chloroquine on expression of cytokine mRNA, Northern blotting was carried out. In unstimulated human whole blood, mRNA for TNF-α, IL-1β, and IL-6 was not detected over an incubation period of 24 h, which is in line with previous studies (4). To investigate the effect of chloroquine on cytokine mRNA expression, an incubation time of 4 h was chosen, since TNF-α, IL-1β, and IL-6 mRNA expression provide a strong signal at this time point (4). Treatment of LPS-stimulated human whole blood with chloroquine concentrations exceeding 20 µM resulted in a dose-dependent depression of TNF-α, IL-1β, and IL-6 mRNA expression when compared with LPS-stimulated whole blood without chloroquine. The highest dose of chloroquine (200 µM) resulted in a complete inhibition of cytokine mRNA expression despite similar signals for the 28S rRNA probe (Fig. 3).

To study the effect of chloroquine on kinetics of cytokine mRNA expression, whole blood was stimulated with LPS in the absence or presence of 100 µM chloroquine for 0, 1, 2, 4, and 8 h. Chloroquine de-
Table 2. Influence of eglin on cytokine plasma levels in human whole blood

<table>
<thead>
<tr>
<th>LPS</th>
<th>Chloroquine (100 µM)</th>
<th>Eglin (2.5 µM)</th>
<th>TNF-α, U/ml</th>
<th>IL-1β, pg/ml</th>
<th>IL-6, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>0.0 ± 0.0</td>
<td>16 ± 16</td>
<td>348 ± 348</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>402 ± 179</td>
<td>18,908 ± 1,980</td>
<td>13,945 ± 6,258</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>515 ± 197</td>
<td>18,284 ± 2,652</td>
<td>11,040 ± 2,919</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>284 ± 81</td>
<td>4,158 ± 284</td>
<td>3,290 ± 2,884</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>266 ± 155</td>
<td>4,772 ± 1,357</td>
<td>3,675 ± 2,020</td>
</tr>
</tbody>
</table>

Data are means ± SE. TNF, tumor necrosis factor; -, without; + with.

creased TNF-α, IL-1β, and IL-6 mRNA expression after 1, 2, and 4 h, with a maximum inhibition after 4 h (Fig. 4).

Effect of eglin on secretion of proinflammatory cytokines. The proteinase inhibitor eglin was added to LPS-stimulated whole blood in the presence of chloroquine in concentrations in which it was found to effectively neutralize the biological activity of elastase (8). Eglin did not attenuate the inhibition of proinflammatory cytokine release induced by chloroquine in LPS-stimulated whole blood (Table 2).

Effect of ammonia and methylamine on synthesis and secretion of proinflammatory cytokines. Because the observed effects of chloroquine may be due to its weak base effect, ammonia and methylamine, which also exert a weak base effect on cell functions (14, 21, 23, 24, 29), were studied. When incubating human whole blood with either ammonia or methylamine in the presence of LPS for 4 and 8 h, a significant dose-dependent decrease in TNF-α, IL-1β, and IL-6 secretion was observed in comparison with LPS-stimulated whole blood without ammonia or methylamine (Table 3). The degree of inhibition with high doses of ammonia (20 mM) and methylamine (20 mM) were similar to the effects observed with high concentrations of chloroquine (100 µM).

Using Northern blot analysis, the expression of TNF-α, IL-1β, and IL-6 mRNA was significantly reduced after treatment of human whole blood with both concentrations of ammonia compared with LPS-stimulated human whole blood without ammonia (Fig. 5).

Effect of chloroquine on cytokine release in monocyte monolayers. Incubation of isolated monocytes with different dosages of chloroquine revealed similar effects than when using whole blood (Table 4). Chloroquine significantly decreased LPS-induced release of TNF-α, IL-1β, and IL-6 in high concentrations (100 µM), whereas it was ineffective in low dosages (10 µM; Table 4).

Table 3. Dose-dependent effect of ammonia or methylamine (10 and 20 mM) on cytokine plasma levels in human whole blood

<table>
<thead>
<tr>
<th>LPS</th>
<th>Ammonia, mM</th>
<th>Methylamine, mM</th>
<th>TNF-α, U/ml</th>
<th>IL-1β, pg/ml</th>
<th>IL-6, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>108 ± 12</td>
<td>74 ± 12</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10</td>
<td>56 ± 6*</td>
<td>31 ± 6*</td>
<td>5,730 ± 840*</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>20</td>
<td>22 ± 7†</td>
<td>20 ± 5*</td>
<td>1,605 ± 288†</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>10</td>
<td>1,225 ± 311</td>
<td>1,932 ± 1,383</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>20</td>
<td>139 ± 73</td>
<td>719 ± 243</td>
</tr>
</tbody>
</table>

Data are means ± SE for 4- and 8-h incubation times. NN, not done. *P < 0.05 and †P < 0.01 plus vs. minus ammonia or methylamine.

Discussion

In the present study, we demonstrate that the antimalarial agent chloroquine markedly inhibits endotoxin-induced synthesis and release of proinflammatory cytokines in a dose-dependent manner. This downregulatory effect on proinflammatory cytokine synthesis occurs on a transcriptional level and may be caused by disturbed signaling through its weak base effect.

Chloroquine represents an antimalarial agent that has been extensively used in the treatment of chronic inflammatory diseases (6). Because proinflammatory cytokines such as TNF-α have been implicated in these diseases, we hypothesized that chloroquine may also possess anti-inflammatory abilities in acute inflammation, as it occurs during severe infection. To study the effects of chloroquine in a model relevant to the clinical situation, endotoxin-stimulated human whole blood was used in this study instead of purified cultures. The use of whole blood avoids unspecific activation of monocytes by density gradient centrifugation, adherence, and further cell preparation techniques (2, 27). Although this experimental design only imitates in vivo conditions in a localized area of inflammation, the physiological environment guarantees cellular interactions between red blood cells, thrombocytes, and leukocytes and preserves the influence of complement factors, growth factors, or inhibitory peptides.

With the use of endotoxin-stimulated whole blood from healthy volunteers, chloroquine revealed a dose-dependent inhibitory effect on TNF-α, IL-1β, and IL-6 secretion, which confirms potent anti-inflammatory abilities of this agent. In addition, our data explain recent in vitro studies by Salmeron and Lipsky (18), who suggested an indirect effect of chloroquine on reduction of lymphocyte blastogenesis through blockade of IL-1β secretion by monocytes and its efficacy in the treatment of chronic inflammatory diseases such as rheumatoid arthritis (6). It could be argued that the inhibitory effect of chloroquine on proinflammatory cytokine release may be due to a cytotoxic effect on PBMC. Additional studies determining total cell counts and viability of PBMC with trypsin blue exclusion and lactate dehydrogenase release as well as FACS analysis for differentiation of leukocytes clearly excluded any toxic effect of chloroquine on absolute numbers of PBMC and on cell viability. These results are in line with previous studies by Pict et al. (16, 17) and Zhu et al. (28) using similar chloroquine concentrations without observing cell cytotoxicity.
To gain further insight into the mechanisms inducing the anti-inflammatory effect of chloroquine, its influence on mRNA expression was studied. The results demonstrate a dose-dependent inhibitory effect of chloroquine on TNF-α, IL-1β, and IL-6 mRNA expression by chloroquine. These data were validated by the fact that the total amount of mRNA per lane was similar with comparable expression of 28S rRNA. Our results are in line with previous data by Zhu et al. (28), who found a dose-dependent inhibitory influence of chloroquine on TNF-α mRNA expression in LPS-stimulated murine peritoneal macrophages.

These results do not rule out additional extracellular mechanisms of action that may contribute to the significant inhibition of proinflammatory cytokine release. In this light, chloroquine induces an enhanced release of proteases, which can cause enzymatic degradation of secreted TNF-α and IL-1β (20). However, addition of the proteinase inhibitor eglin to LPS-stimulated human whole blood failed to attenuate chloroquine-induced inhibition of proinflammatory cytokine release. Moreover, studies using isolated monocyte monolayers excluded the contribution of any mediator released from neutrophils (25) to chloroquine-induced downregulation of proinflammatory cytokine release. Thus these results exclude extracellular mechanisms underlying the inhibitory effect of chloroquine on proinflammatory cytokine synthesis.

Based on previous studies (16, 26), it could be hypothesized that the observed inhibitory effect of chloroquine on proinflammatory cytokine synthesis is due to its weak base effect. Therefore, chloroquine was compared with ammonia and methylamine, which have similar weak base effects to chloroquine (15). Both drugs have been extensively used in previous studies to emphasize the weak base effect of chloroquine as its key mechanism of action (21, 23, 24, 29). The addition of either ammonia or methylamine to LPS-stimulated human whole blood resulted in a significant reduction of TNF-α, IL-1β, and IL-6 mRNA expression and consequently of cytokine secretion. The fact that weak bases other than chloroquine are able to downregulate synthesis of proinflammatory cytokines provides further evidence that the anti-inflammatory effect of chloroquine may be due to an intracellular effect on lysosomes with an elevated lysosomal pH and a decreased intralysosomal enzyme activity.

It can be speculated that chloroquine exerts its anti-inflammatory effects through alterations of certain signal transduction pathways. In particular, sphingomyelinase-regulated signaling may be altered, because the sphingomyelin-catalyzing enzyme sphingomyelinase acts in a pH-dependent manner, and chloroquine has been found to decrease its activity (22). Moreover, lysosomes, the predominant targets of chloroquine actions, play a central role in the turnover of membrane or lipoprotein-associated sphingomyelin. Although the sphingomyelinase pathway appears to be most effective for nuclear factor-κB activation, which is strongly required for induction of proinflammatory cytokine gene transcription (19), the sphingomyelinase signal transduction pathway does not represent the classical pathway activated through endotoxin after binding to its cell surface receptor. Therefore, further conclusions about the effect of chloroquine on endotoxin-induced activation of various signal transduction pathways need substantial investigations.

Table 4. Dose-dependent effect of chloroquine on proinflammatory cytokine release by LPS-stimulated monocytes

<table>
<thead>
<tr>
<th>LPS</th>
<th>Chloroquine, µM</th>
<th>TNF-α, U/ml</th>
<th>4h</th>
<th>8h</th>
<th>IL-1β, pg/ml</th>
<th>4h</th>
<th>8h</th>
<th>IL-6, pg/ml</th>
<th>4h</th>
<th>8h</th>
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<tr>
<td>+</td>
<td>-</td>
<td>162 ± 38</td>
<td>193 ± 15</td>
<td>278 ± 102</td>
<td>604 ± 251</td>
<td>1,058 ± 113</td>
<td>4,244 ± 2,299</td>
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<td></td>
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<tr>
<td>+</td>
<td>10</td>
<td>156 ± 61</td>
<td>154 ± 49</td>
<td>112 ± 24</td>
<td>269 ± 81</td>
<td>981 ± 246</td>
<td>1,728 ± 946</td>
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<td></td>
<td></td>
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<tr>
<td>+</td>
<td>100</td>
<td>50 ± 11</td>
<td>29 ± 9</td>
<td>66 ± 8</td>
<td>104 ± 27</td>
<td>49 ± 49</td>
<td>1,014 ± 870</td>
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Data are means ± SE for 4- and 8-h incubation times.
Finally, from these results, we can not rule out that decreased synthesis and release of proinflammatory cytokines into whole blood is due to a diminished responsiveness of PMBC to endotoxin as a consequence of intracellularly trapped endotoxin binding receptors, since previous studies (7, 10, 13, 24) revealed a trapping of phospholipids and cell receptors inside the cells by chloroquine.

In summary, our studies suggest a potent anti-inflammatory effect of chloroquine on endotoxin-induced synthesis of proinflammatory cytokines that is related to a significant inhibition of mRNA expression. These anti-inflammatory properties seem to be based on its weak base effect rather than on increased release of elastase or other extracellular mechanisms.

**Perspectives**

Chloroquine has previously been used as an anti-malarial agent and in the treatment of chronic inflammation related to rheumatoid arthritis or lupus erythematosus. The results from this study clearly demonstrate that, in line with previous animal studies (5, 28), chloroquine also counterregulates proinflammatory cytokine release by leukocytes induced through bacterial stimuli. Thus chloroquine as potent inhibitor of proinflammatory cytokine synthesis may gain an important role not only in the treatment of chronic inflammatory diseases of nonbacterial origin but also of acute or chronic infection. Because concentrations of chloroquine similar to those being effective in this study have been detected in the tissue of chloroquine-treated patients rather than in the circulation, chloroquine may predominantly be used for control of localized inflammatory processes due to septic stimuli.

This work was supported in part by grant ER 165/1–1 from the Deutsche Forschungsgemeinschaft (Gerhard-Hess Programm), Bonn, Germany, and in part by a grant from the SBF, Zurich, Switzerland. Address for reprint requests: W. Ertel, Div. of Trauma Surgery, Dept. of Surgery, Univ. Hospital of Zurich, Raemistrasse 100, CH-8091 Zurich, Switzerland.

Received 7 February 1997; accepted in final form 11 December 1997.

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