

Effects of Chloroquine, Mefloquine and Quinine on Natural Killer Cell Activity *in vitro*

An Analysis of the Inhibitory Mechanism

B. K. PEDERSEN,¹ I. C. BYGBJERG,² T. G. THEANDER,² and B. J. ANDERSEN³

¹Laboratory of Medical Immunology TA and ²Lymphocyte Laboratory, Dept. of Communicable and Tropical Diseases M, Rigshospitalet, University Hospital, Copenhagen and ³Dept. of Toxoplasmosis, Statens Seruminstitut, Copenhagen, Denmark

Natural killer (NK) cell activity against K 562 target cells was inhibited by pharmacological concentrations of chloroquine, mefloquine and quinine. The most potent were mefloquine and quinine. The drug-induced inhibition of the NK cell activity was abolished by addition of α -interferon (IF) or interleukin 2 (IL-2); preincubation of mononuclear cells with IF or IL-2 followed by addition of anti-malarial drugs decreased the inhibitory effects of the drugs. The drug-induced inhibition of the NK cell activity was not dependent on the presence of monocytes. Using monocyte depleted Percoll fractionated NK cell enriched populations in a single cell agarose assay, it was shown that the inhibitory effects of mefloquine, but not of chloroquine and quinine were due to an inhibition of the formation of effector/target cell conjugates.

Key words: anti-malarial drugs; natural killer cells.

Accepted for publication 2 June 1986

There is increasing evidence that natural killer (NK) cells play an important role in immune surveillance against tumors and certain infections. Therefore, regulation of NK cell activity has attracted considerable attention (22, 24). NK cells seem to be identical with large granular lymphocytes (LGL). Besides mediating NK cell activity and antibody dependent cell cytotoxicity (ADCC) LGL's release cytokines, including interferon (IF) and interleukin 2 (IL-2) (19), in response to various stimuli. The cytotoxicity capacity of NK cells is dependent upon an intact secretory system (7, 28). NK cell activity is augmented by a number of agents including IF (35) and IL-2 (16, 29) while NK cell activity is inhibited by e.g. immune complexes (18, 30) and prostaglandins (3). Many drugs used for treatment of cancer and immunological disorders influence NK cell activity. Corticosteroids, azathioprine and other cytotoxic drugs inhibit NK cell activity (25, 26, 27).

Anti-malarial drugs are widely used and chloroquine and quinine are employed not only against malaria but also against rheumatological disorders (32). The present study was undertaken in order to characterize the *in vitro* effect of chloroquine, mefloquine and quinine on the NK cell activity, with special emphasis on concentrations corresponding to those achieved in clinical practice. Chloroquine was examined at concentrations corresponding to the levels obtained in serum during malaria prophylaxis (0.25 μ g/ml) and treatment of rheumatoid arthritis (0.50 μ g/ml) (20).

MATERIAL AND METHODS

Determination of NK cell activity

The NK cell activity was determined as previously described (29). In brief, mononuclear

cells were obtained from heparinized blood isolated by Ficoll Isopaque gradient centrifugation. In some experiments mononuclear cells were incubated at 37°C on 60 mm plastic Petri dishes (Teknunc A/S Roskilde, Denmark) for 1 h (30). The nonadherent cells contained 0–2% monocytes measured by peroxidase staining (36). In other experiments NK cell-enriched suspensions were prepared on discontinuous Percoll (Pharmacia Chemicals, Uppsala, Sweden) gradients as described previously by Timonen & Saksela (34). NK cell activity against K 562 target cells was determined in a ⁵¹Cr release assay. The NK cell activity was measured at effector/target (E/T) cell ratios 80/1, 40/1 and 20/1. After incubation for 4 h the tubes were centrifuged, half of each supernatant was transferred to another tube and the radioactivity was counted in a Packard scintillation gamma counter. The experimental release was calculated by the formula:

$$\frac{{}^{51}\text{Cr release in the supernatant}}{\text{total } {}^{51}\text{Cr activity}} \times 100\%$$

The specific release (the NK cell activity) was the difference between experimental release in the presence of effector cells and experimental release when target cells were incubated with medium only.

Incubation of effector cells with IF and Il-2

Mononuclear cells were incubated with IF (6) (provided by Dr. R. Jordal) or Il-2 (Boehringer, Mannheim) (21) as previously described (29). The Il-2 activity was measured as described by Gillis et al. (14); the Il-2 preparation used in our experiments contained 300 IU/ml.

Incubation of effector cells with anti-malarial drugs

Chloroquine diphosphate, mefloquine hydrochloride and quinine hydrochloride were dissolved and diluted in RPMI medium. In most experiments the stock solutions were: chloroquine 2.5 µg/ml, 5 µg/ml and 7.5 µg/ml, mefloquine 15 µg/ml and quinine 150 µg/ml. 2×10^6 cells/ml were mixed with either of the drugs for

1 h at 37°C. In most experiments the final concentrations of the drugs were 10% of the stock solutions. In some experiments incubation of the anti-malarial drugs was followed by incubation with IF or Il-2. In other experiments the effector cells were preincubated with IF or Il-2 followed by addition of the drugs. In most experiments the drugs were present during the incubation with target cells, but in one series of experiments, the cells were either washed 3 times in RPMI + 10% FCS, 37°C, or unwashed before incubation with target cells. Possible toxic effects of chloroquine, mefloquine and quinine were tested by staining the effector cells with Nigrosin 0.02% (w/v). After 5 h, 37°C, of incubation with the drugs, cell viability was more than 95%.

Single cell agarose assay

Nonadherent mononuclear cells or NK cell-enriched suspensions and unlabelled K 562 target cells were used as effector and target cells, respectively.

The assay was performed as described by Grimm et al. (15) with minor modifications. A 0.5% agarose solution in RPMI was kept in a waterbath at 50°C. 2.5×10^5 effector cells and 2.5×10^5 target cells were mixed in a total volume of 0.25 ml of RPMI with 10% FCS. The cells were centrifuged at 500 g for 5 min and incubated for 5 min at 37°C. During this time some of the lymphocytes were bound to target cells and formed effector/target cell conjugates. Subsequently, the pellet was resuspended by shaking for 3 s on a whirlmixer. The cells were then added to 0.6 ml of the agarose solution, which had been precooled to about 40°C, resuspended with a Pasteur pipet and added dropwise from a height of 30 cm onto 90 mm plastic Petri dishes (Teknunc A/S, Roskilde, Denmark). A thin layer of agarose, which contained the cells, was formed at the bottom of the dishes. The percentage of conjugates, referred to as the percentage of target binding cells (% TBC), was determined by counting the number of lymphocytes bound to target cells and the total number of lymphocytes. At least 400 lymphocytes were counted.

RESULTS

*Effects of anti-malarial drugs
on NK cell activity*

Chloroquine, mefloquine and quinine inhibited NK cell activity. Incubation of mononuclear cells with chloroquine 0.25–0.75 µg/ml decreased NK cell activity to 83.8 and to 86.4% of controls, while mefloquine 1.5 µg/ml and quinine 15.0 µg/ml decreased the activity to 50.6% and 41.6% respectively (Table 1). When a group of experiments were assessed statistically for the inhibitory effect of chloro-

Table 1

Effect of interferon (IF) and interleukin 2 (Il-2) on the drug-induced inhibition of NK cell activity

Incubation 1 µg/ml	Incubation 2 µg/ml	NK cell activity %
-	-	100
	IF	166.8 ± 29.2
	Il-2	179.3 ± 23.0
Chloroquine 0.25	-	86.4 ± 2.0
Chloroquine 0.50	-	83.8 ± 1.2
Chloroquine 0.75	-	88.3 ± 2.5
Mefloquine 1.5	-	50.6 ± 2.4
Quinine 15.0	-	41.6 ± 5.1
Chloroquine 0.25	IF	153.6 ± 17.8
Chloroquine 0.50	IF	167.6 ± 31.4
Chloroquine 0.75	IF	160.6 ± 27.5
Mefloquine 1.5	IF	135.4 ± 21.3
Quinine 15.0	IF	170.6 ± 31.5
IF	chloroquine 0.25	153.5 ± 8.9
IF	chloroquine 0.50	150.2 ± 10.6
IF	chloroquine 0.75	147.3 ± 15.1
IF	mefloquine 1.5	161.1 ± 11.7
IF	quinine 15.0	156.6 ± 28.3
Chloroquine 0.25	Il-2	168.5 ± 28.3
Chloroquine 0.50	Il-2	170.3 ± 25.4
Chloroquine 0.75	Il-2	117.8 ± 24.9
Mefloquine 1.5	Il-2	164.2 ± 25.4
Quinine 15.0	Il-2	128.7 ± 21.4
Il-2	chloroquine 0.25	141.3 ± 11.3
Il-2	chloroquine 0.50	133.3 ± 12.7
Il-2	chloroquine 0.75	120.3 ± 19.7
Il-2	mefloquine 1.5	104.9 ± 16.1
Il-2	quinine 15.0	49.2 ± 23.0

Mononuclear cells were incubated with or without anti-malarial drugs followed by stimulation with IF or Il-2, or preincubated with IF or Il-2 followed by incubation with the drugs. Results are given in percentages of controls (mean ± SD of five experiments).

quine, quinine and mefloquine on the NK cell activity, the differences were statistically significant at the $P < 0.05$ level for chloroquine 0.25–0.75 µg/ml and $P < 0.01$ for mefloquine 1.5 µg/ml and quinine 15 µg/ml (12 experiments).

IF and Il-2 augmented the NK cell activity markedly. The drug-induced inhibition of NK cell activity was abolished by addition of IF, and similar preincubation of mononuclear cells with IF followed by addition of anti-malarial drugs abolished the inhibitory effects of the drugs (Table 1).

When mononuclear cells were preincubated with chloroquine 0.75 µg/ml and quinine 15.0 µg/ml, addition of Il-2 only partially reverted the drug-induced inhibition of the NK cell activity, while the inhibitory effects of chloroquine 0.25–0.50 µg/ml and mefloquine 1.5 µg/ml were almost abolished by Il-2. Preincubation of mononuclear cells with Il-2 followed by anti-malarial drugs had less influence on the inhibitory effects of the drugs, in particular that of quinine, which was almost unaltered by preincubation of the cells with Il-2 (Table 1).

When mononuclear cells depleted for monocytes were used as effector cells similar results were obtained (Table 2). The inhibition of the NK cell activity was partly reversible, since washing of the effector cells decreased the drug-induced inhibition (Table 3).

Table 2

Effects of anti-malarial drugs on the NK cell activity of monocyte-depleted effector cells

µg/ml	NK cell activity (%)
Control	100
Chloroquine 0.25	86.6 ± 8.4
Chloroquine 0.50	86.6 ± 8.4
Chloroquine 0.75	61.9 ± 13.5
Mefloquine 1.5	28.0 ± 8.1
Quinine 15.0	27.2 ± 6.7

Effector cells were depleted for plastic-adherent cells before incubation with anti-malarial drugs. Results are given in percentages of controls (mean ± SD of three experiments).

Effects of anti-malarial drugs on the recognition and adhesion step

To study whether the incubation of effector cells with anti-malarial drugs inhibited cytolysis by interference with the recognition/adhesion step, or by blocking the final killing event, the adhesion of effector cells to target cells (conjugate formation) after incubation with chloroquine, mefloquine and quinine was studied in a single cell agarose assay (Table 4). Monocyte-depleted mononuclear cells, enriched for NK cells by Percoll gradient fractionation, were used as effector cells. The data show that mefloquine inhibited effector/target cell conjugate formation, while chloroquine and quinine had no effect.

DISCUSSION

The study showed that all three anti-malarial drugs decreased the NK cell activity of human blood mononuclear cells. At concentrations corresponding to the levels obtained in serum during treatment, the most potent were mefloquine and quinine.

We have previously shown that mefloquine and quinine, at therapeutic concentrations, decreased the *in vitro* proliferative responses of human blood mononuclear cells more effectively than chloroquine (5).

Quinine is a complicated quinoline-methanol, which has guided the synthesis of the

4-aminoquinoline chloroquine and the novel quinoline-methanol mefloquine.

A common anti-rheumatic and anti-malarial mechanism has been suggested (17). Both chloroquine and quinine are concentrated in the lysosomes (1) and both drugs combine strongly with DNA (2, 9, 12) while mefloquine does not (11).

Immune cytolysis mediated by NK cells is a consequence of two separate events. The first step involves recognition and adhesion of the NK cells to target cells. The second step is the lytic reaction which leads to the destruction of target cells. Mefloquine has a high affinity for membrane lipids (10) and is less water soluble than quinine and chloroquine. Interestingly, we found that mefloquine, but not quinine or chloroquine, decreased the adherence of effector cell binding to target.

On a weight base, mefloquine had the most depressing effect on NK cell activity. Others (13) have recently shown that mefloquine, but not quinine or chloroquine, when added at the initiation of the cytotoxic reaction, inhibited the NK cell activity. These authors were unable to demonstrate any effect of mefloquine on NK cell binding to target. Preincubation of the effector cells with chloroquine decreased their NK activity. Reviewing their data, it was noted that quinine also decreased NK activity, albeit non-significantly.

In the present study, the inhibitory effect of quinine and chloroquine was not related to an inhibition of effector cell binding to target cells. We also found that the inhibitory effect was not

Table 3

Effects of washing the cells after incubation with anti-malarial drugs

µg/ml	NK cell activity (%)	
	No wash	Wash
Control	100	100
Chloroquine 0.25	86.5 ± 2.0	92.4 ± 7.9
Chloroquine 0.50	82.4 ± 2.0	88.6 ± 6.8
Chloroquine 0.75	87.1 ± 2.5	90.7 ± 7.3
Mefloquine 1.5	44.1 ± 11.9	81.7 ± 2.4
Quinine 15.0	40.0 ± 12.8	60.4 ± 6.3

Mononuclear cells were incubated with anti-malarial drugs and used washed or unwashed at testing for NK cell activity. Results are given in % of the controls (mean ± SD of three experiments).

Table 4

Effect of anti-malarial drugs on effector/target cell conjugate formation

% target binding cells	
Control	11.2 ± 2.1
Chloroquine 0.75	9.4 ± 1.6
Mefloquine 1.5	5.9 ± 1.4
Quinine 15.0	10.7 ± 1.9

The percentage of target binding cells was determined by counting the number of lymphocytes bound to target cells and the total number of lymphocytes. At least 400 lymphocytes were counted. Mean ± SD of four experiments each carried out in duplicate.

dependent on the presence of monocytes. This points to an effect of the drugs on the final killing event. The drug-induced killing was abolished by addition of IF or by preincubation with IF. Addition of IL-2 after incubation with the anti-malarial drugs partially abolished the drug-induced inhibition, while IL-2 when preincubated with mononuclear cells, followed by incubation with anti-malarial drugs, had less effect. Quinine decreases the production of IL-2 by human lymphocytes, and also inhibits the mitogenesis and the number of potassium channels, which is playing an important role in T lymphocyte activation (8). It is possible that quinine inhibits the cytolytic function of NK cells through a blockage of potassium channels which play a role in the cytolytic reaction (31). Thus quinine and related drugs may prove to be useful tools on elucidating basic mechanisms of NK function.

A myriad of biological effects of chloroquine has been reported, but it remains unclear which of the numerous actions may be clinically relevant (20). Chloroquine and quinine are widely used, not only against malaria, but also for various rheumatological and skin disorders, and even for common complaints such as nocturnal muscle cramps (32). The possible adverse effect of such medication on the immune system of man, including NK cell activity, deserves clarification. As NK cells seem to be of importance in natural resistance against, e.g. virus infections (22, 24), it is possible that people who receive treatment with anti-malarials are at increased risk of developing certain infectious diseases. It has recently been shown that chloroquine, when taken for malaria prophylaxis, may decrease the response to some vaccines (4, 23, 32). While it is clear that chloroquine, mefloquine and quinine suppress NK cell activity *in vitro*, it is not clear whether this suppression may be of importance in clinical practice. Therefore further studies on NK cell activity during treatment with anti-malarials are warranted.

ACKNOWLEDGEMENTS

We thank Dr. V. Andersen for valuable discussions. The excellent technical assistance of Kirsten Ankersø is gratefully

acknowledged. The work was supported by the Danish National Association against Rheumatic Diseases.

REFERENCES

- Allison, A. C. & Young, M. R.: Uptake of dyes and drugs by living cells in culture. *Life Sci.* 3, 1407-1414, 1964.
- Allison, J. L., O'Brien, R. L. & Hahn, F. E.: DNA: Reaction with chloroquine. *Science* 149, 1111-1113, 1965.
- Brunda, M. J., Herberman, R. B. & Holden, T.: Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* 124, 2682-2687, 1980.
- Bygbjerg, I. C. & Flachs, H.: Effect of chloroquine on human lymphocyte proliferation. *Trans. R. Soc. Trop. Med. Hyg.* (In press).
- Bygbjerg, I. C., Theander, T. G., Andersen, B. J., Flachs, H., Jepsen, S. & Larsen, P. B.: In vitro effect of chloroquine, mefloquine and quinine on human lymphocytes' response to malarial antigens. *Tropenmed. Parasitol.* (In press).
- Cantell, K. & Hirronen, S.: Large-scale production of human leucocyte interferon containing 10 units per ml. *Gen. Virol.* 39, 541-543, 1978.
- Carpen, O., Virtanen, I. & Saksela, E.: The cytotoxic activity of human natural killer cells requires an intact secretory apparatus. *Cell. Immunol.* 58, 97-106, 1981.
- Chandy, K. G., De Coursey, T. E., Calahan, M. D., McLaughlin, C. & Gupta, S.: Voltage-gated potassium channels are required for human T lymphocyte activation. *J. Exp. Med.* 160, 369-385, 1984.
- Ciak, J. & Hahn, F. E.: Chloroquine: mode of action. *Science* 151, 347, 1966.
- Chevli, R. & Fitch, C. D.: The antimicrobial drug mefloquine binds to membrane phospholipids. *Antimicrobiol. Chemother.* 21, 581-586, 1982.
- Davidson, M. W., Griggs, B. G., Boykin, D. W. & Wilson, W. D.: Molecular structural effects involved in the interaction of quinolinemethanolamines with DNA. Implications for anti-malarial action. *J. Med. Chem.* 20, 1117-1120, 1977.
- Estensen, R. D., Krey, A. K. & Hahn, F. E.: Studies on a deoxyribonucleic acid-quinine complex. *Mol. Pharmacol.* 5, 532-536, 1969.
- Ferrante, A. & Goh, D. H. B.: The effect of anti-malarial drugs on human natural killer cells *in vitro*. *Parasite Immunol.* 6, 571-580, 1984.
- Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A.: T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* 120, 2027-2032, 1978.
- Grimm, E. A., Thomas, J. A. & Bonavida, B.: Mechanism of cell-mediated cytotoxicity at the single cell level. *J. Immunol.* 123, 2870-2877, 1979.
- Henney, C. S. K., Kuribayashi, K., Kern, D. E. & Gillis, S.: Interleukin 2 augments natural killer cell activity. *Nature* 291, 335-338, 1981.
- Jones, C. J. P. & Jason, M. I. V.: Chloroquine: its effect on leucocyte auto- and heterophagocytosis. *Ann. Rheum. Dis.* 43, 205-212, 1984.

18. Karsch, J., Dorval, G. & Osterland, C. K.: Natural cytotoxicity in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **19**, 437-446, 1981.
19. Kasahara, T., Djeu, J. Y., Dougherty, S. F. & Oppenheim, J. J.: Capacity of human large granular lymphocytes (LGL) to produce multiple lymphokines: Interleukin 2, interferon, and colony stimulating factor. *J. Immunol.* **131**, 2379-2385, 1983.
20. Mackenzie, A. H. & Scherbel, A. L.: Chloroquine and hydroxychloroquine in rheumatologic therapy. *Clin. Rheum. Dis.* **6**, 545-566, 1980.
21. Mier, J. W. & Gallo, R. C.: Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte conditioned media. *Proc. Natl. Acad. Sci.* **77**, 6134-6144, 1980.
22. Ortaldo, J. R. & Herberman, R. B.: Heterogeneity of natural killer cells. *Ann. Rev. Immunol.* **2**, 359-394, 1984.
23. Pappaioanou, M., Fishbein, D. B., Dreesen, D. W., Schwartz, I. K. & Campbell, G. H.: Reduced antibody response to human diploid cell rabies vaccine administered concurrently with 300 milligrams chloroquine base per week. *Proceedings of Joint Meeting of the Royal and American Societies of Tropical Medicine and Hygiene*, Baltimore, 305, 1984.
24. Pedersen, B. K.: Natural killer cells in relation to disease and treatment. A review. *Allergy*, **40**, 547-557, 1985.
25. Pedersen, B. K., Beyer, J. M., Klarlund, K. & Clemmensen, I. H.: Baseline and interferon enhanced natural killer cell activity in rheumatoid arthritis. *Acta Pathol. Microbiol. Immunol. Scand. [C]* **93**, 79-84, 1985.
26. Pedersen, B. K., Beyer, J. M., Rasmussen, A., Klarlund, K., Pedersen, B. N. & Helin, P.: Methylprednisolone pulse therapy induced fall in natural killer cell activity in rheumatoid arthritis. *Acta Pathol. Microbiol. Immunol. Scand. [C]* **92**, 319-323, 1984.
27. Pedersen, B. K., Beyer, J. M., Rasmussen, A., et al. Azathioprine as single drug in the treatment of rheumatoid arthritis induced complete suppression of natural killer cell activity. *Acta Pathol. Microbiol. Immunol. Scand. [C]* **92**, 221-225, 1984.
28. Pedersen, B. K., Norrild, B. & Krebs, H. J.: The effect of monensin on the antibody dependent cell-mediated killing. *Cell. Immunol.* **72**, 208-214, 1982.
29. Pedersen, B. K., Oxholm, P., Manthorpe, R. & Andersen, V.: Interleukin 2 augmentation of the defective natural killer cell activity in patients with primary Sjogren's syndrome. *Clin. Exp. Immunol.* **63**, 1-7, 1986.
30. Pedersen, B. K., Thomsen, B. S. & Nielsen, H.: Inhibition of natural killer cell activity by antigen-antibody complexes. *Allergy*, accepted for publication, 1986.
31. Podack, E. P.: The molecular mechanism of lymphocyte-mediated tumor cell lysis. *Immunol. Today* **6**, 21-27, 1985.
32. Rollo, I. M.: Drugs used in the chemotherapy of malaria. In Goodman, L. S., Gilman, A. G., (eds.): *Pharmacological basis of therapeutics*, p. 1043, 6th ed. Mac-Millan, New York, 1980.
33. Taylor, D. N., Wasi, C. & Bernard, K.: Chloroquine prophylaxis associated with a poor antibody response to human diploid cell rabies vaccine. *Lancet* **1**, 1405, 1984.
34. Timonen, T. & Saksela, E.: Isolation of human NK cells by density gradient centrifugation. *J. Immunol. Methods* **36**, 285-291, 1980.
35. Ullberg, M. & Jondahl, M.: Recycling and target binding capacity of human natural killer cells. *J. Exp. Med.* **153**, 615-628, 1981.
36. Yam, L. T., Li, C. Y. & Crosby, W. H.: Cytochemical identification of monocytes and granulocytes. *Am. J. Pathol.* **55**, 283-290, 1971.

Address

Bente K. Pedersen, M.D.
 Laboratory of Medical Immunology, 7544
 Tagensvej 20, Rigshospitalet
 DK-2200 Copenhagen N
 Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.