Biochemical Pharmacology, Vol. 57, pp. 907–911, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(98)00368-2

SHORT COMMUNICATION

Effect of Chronic Chloroquine Administration on Iron Loading in the Liver and Reticuloendothelial System and on Oxidative Responses by the Alveolar Macrophages

Rachida Legssyer, Roberta J. Ward,† Robert R. Crichton*‡ and Johan R. Boelaert,§*

*Unité de Biochimie and †Biologie du Comportement, Université Catholique de Louvain, Louvain-la-Neuve; and §Unit of Renal and Infectious Diseases, Algemeen Ziekenhuis St Jan, Brugge, Belgium

ABSTRACT. The ability of chloroquine to alter iron loading in the liver, spleen, and alveolar macrophages was investigated in iron-loaded or -depleted rats. Chloroquine significantly reduced incorporation of iron into the liver, spleen, and alveolar macrophages of animals loaded *in vivo* with iron dextran. The ability of these macrophages to respond to oxidative stress was assayed by their capacity to release reactive nitrogen intermediates after lipopolysaccharide (LPS) stimulation. A significant reduction in nitrite release was observed in primary cultures of macrophages isolated from chloroquine/iron dextran-administered rats in comparison to macrophages lavaged from rats iron-loaded alone. Macrophages isolated from iron-deficient rats showed a significant increase in nitrite after LPS stimulation, whereas nitrite release in the macrophages lavaged from the rats which had also received chloroquine during the iron depletion regime was much lower. These results indicate that the use of agents which decrease the iron content and diminish the oxidative response of the cell to altered iron status may be of therapeutic value in patients with iron loading, particularly of the reticuloendothelial system. BIOCHEM PHARMACOL **57**;8:907–911, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. iron loading, iron depletion, chloroquine, alveolar macrophages

Macrophages are phagocytic cells which accumulate at the site of infection or inflammation to eliminate pathogenic agents. The macrophage will bind to and ingest only certain particles, depending on the chemical composition and properties of the particle surface. This is accomplished by a course of metabolic events known as the respiratory burst, where a number of powerful oxidising agents, including hydrogen peroxide and hypochlorous acid together with a battery of highly reactive free radicals such as hydroxyl radicals and nitric oxide [1, 2], are released to combat the foreign agent. Various substances, such as iron and ethanol, have been shown to adversely effect this respiratory burst *in vivo* [3].

There is a moderate increase of iron in the RES‖ under certain inflammatory (e.g. rheumatoid arthritis) or infectious (e.g. HIV) conditions [4–6]. This is accompanied by the so-called "anaemia of chronic diseases". Much higher amounts of iron accumulate in the RES secondary to iron-loading diseases that principally affect the RES, such as various haemoglobinopathies (e.g. thalassaemia) and Bantu siderosis, or to the requirement for regular red cell transfusion [7]. Such an accumulation of iron may be detrimental to the normal functioning of the macrophages and may enhance the risk of certain infections [8]. Therapeutic intervention to prevent such iron accumulation might therefore have some clinical benefit.

Various agents have been demonstrated to dramatically alter intracellular trafficking, primarily by altering the intraorganelle pH of specific organelles such as lysosomes and endosomes. These acidic organelles are known to be involved in receptor-mediated endocytosis, a system known to be utilised by the transferrin–transferrin receptor pathway [9]. Chloroquine affects acidic vesicles, such as lysosomes and endosomes, and crosses membranes in its non-protonated form so that the vesicles become hyperosmotic, with water diffusing into the cytoplasm to restore the osmotic equilibrium, thereby increasing the intravesicle pH [10]. Previous *in vivo* studies which have investigated the effects of an acute chloroquine administration on ferritin trafficking showed that the release of endogenous ferritin was inhibited in normal rats, whereas the biliary ferritin concentration was decreased in iron-loaded rats [11].

Therefore, in the following studies, the effect of chronic chloroquine administration was assessed on both the accumulation of iron in specific tissues of rats of differing iron status, as well as on the alveolar macrophage function with

‡ Corresponding author: Professor R. R. Crichton, Unité de Biochimie, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium. Tel. (+32-10) 47 27 94; FAX (+32-10) 477 27 96; E-mail: crichton@bioc.ucl.ac.be.

‖ *Abbreviations:* RES, reticuloendothelial system; LPS, lipopolysaccharide; NO_2, nitrite; RNI, reactive nitrogen intermediates; SOD, superoxide dismutase; INOS, inducible nitric oxide synthase, and NFκB, nuclear factor Kappa B.

Received 24 July 1998; accepted 10 October 1998.

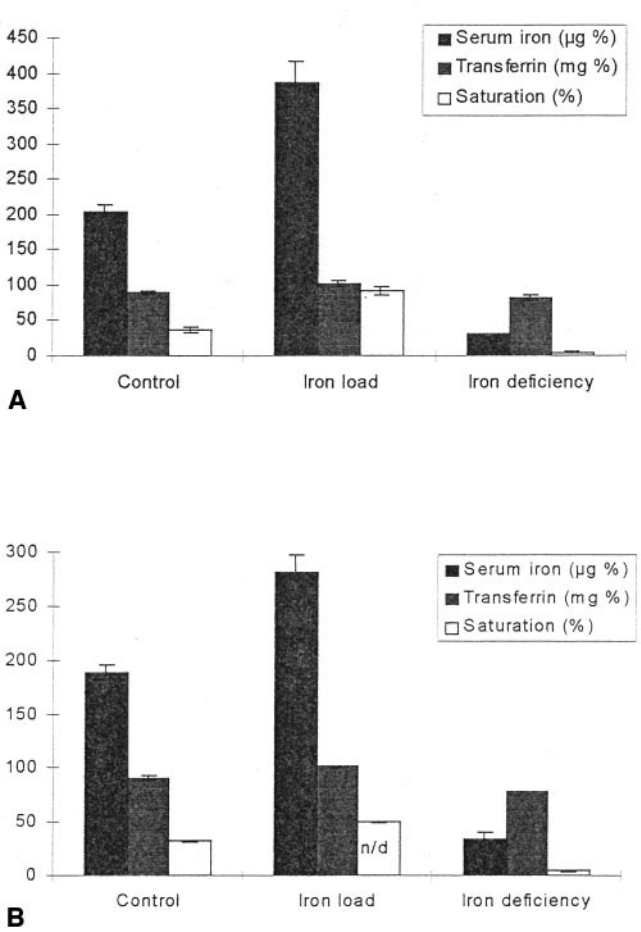

FIG. 1. Haematological parameters in rats after iron loading or depletion (A) and after supplementation with chloroquine during the iron loading and depletion period (B) with differing iron loadings supplemented or not with chloroquine.

regard to the activity of SOD and the release of RNI after stimulation with LPS.

METHODS

Groups of male Wistar rats, 70–80 g, N = 10 in each group, were either overloaded with iron dextran (Vifor Pharmaceuticals), which was administered intraperitoneally 3×/week for 4 weeks, total dose = 125 mg Fe/kg, or depleted of iron by the administration of a diet (Iffa Credo) deficient in iron (<0.001 µg Fe/kg) for a 5-week period. One week prior to the iron-loading or -depleting schedule, injections of chloroquine diphosphate (Sigma) were administered (5×/week) at a dose of 15 mg/kg. The administration of chloroquine was continued for the entire period of iron loading or depletion.

At the conclusion of the experiment, the rats were injected with a sublethal dose of Nembutal, sodium pentabarbitone, 0.6 mg/kg. The trachea of the rats was exposed and a midline incision made to expose the heart and lungs. After insertion of a tube via the trachea into the lungs, the alveolar macrophages were recovered by five lavages, each with 10 mL PBS. The lungs were observed during this procedure to ensure that there was no leakage of PBS. An aliquot of the cells was taken for the preparation of primary cultures for the *ex vivo* stimulation experiments, while the remaining cell suspensions were utilised for the assay of iron content and the activity of total SOD. The primary cultures of the macrophages were prepared as described in our previous article [3] as were the methods for estimation of SOD and the release of RNI from primary cultures of alveolar macrophages after stimulation with LPS.

Haematological parameters were assayed in the blood, collected by cardiac puncture on completion of the alveolar lavage, by standard laboratory analyses.

RESULTS

Chloroquine administration to the controls and iron-loaded rats did not adversely affect the rate of growth, the weight of each group of rats at the completion of the experiment being similar (302 ± 15 g). The growth of the iron-depleted rats was slightly impeded by the iron deficiency, although the mean weight of both groups of rats at the completion of the study was 260 ± 23 g.

Figure 1a shows the mean serum iron concentration together with the mean transferrin saturation for the three groups of rats of differing iron status. The changes in each of these parameters paralleled the iron status. Iron loading for four weeks with iron dextran significantly increased the concentration of iron in the tissues investigated, namely the liver (12-fold), the spleen (45-fold), and the alveolar macrophages (4-fold). In the iron-depleted rats, a 4-fold decrease in iron content was evident in the liver, while neither the spleen nor alveolar macrophage content was significantly altered (Table 1).

Figure 1b shows the haematological parameters after chloroquine was administered during the periods of iron loading and depletion. Comparable changes in the iron status were again observed, although the values obtained

TABLE 1. Effect of chronic chloroquine administration on iron loading and depletion in the liver, spleen, and alveolar macrophages

	− Chloroquine	+ Chloroquine
Controls		
Liver	202 ± 38	119 ± 22†
Spleen	182 ± 14	126 ± 24†
Alveolar macrophages	6.1 ± 1.6	5.7 ± 0.93
Iron-loaded		
Liver	2364 ± 224	1992 ± 164*
Spleen	8210 ± 167	6468 ± 1100*
Alveolar macrophages	23.4 ± 8.9	7.02 ± 1.6*
Iron-depleted		
Liver	42 ± 4.3	35 ± 3.3*
Spleen	139 ± 17	143 ± 2
Alveolar macrophages	7.8 ± 2.7	7.6 ± 0.3

Results are presented as ug/g tissue for liver and spleen and ng/ug for macrophages.
*$P < 0.05$ and †$P < 0.01$, statistical analyses by ANOVA.

TABLE 2. Total superoxide dismutase activity in bronchoalveolar macrophages isolated from iron-loaded, iron-deficient, and control rats

	SOD activity (U/μg protein)
Rat	− Chloroquine
Control (N = 6)	0.024 ± 0.01
Iron-loaded (N = 6)	0.050 ± 0.001*
Iron-depleted (N = 5)	0.053 ± 0.04

Results are presented as means ± SE. Statistical analysis by ANOVA.
*$P < 0.05$ and refers to Fe versus control.

were diminished by comparison to those rats which did not receive this lypotrotrophic agent (Fig. 1a). In addition, the iron content of a number of these tissues decreased (Table 1). In the liver, the iron content was decreased by 40% in control rats ($P < 0.001$), and by 16% in both the iron-loaded ($P < 0.04$) and iron-deficient rats ($P < 0.02$), (Table 1). In the spleen (Table 1), chloroquine administration diminished the iron content by 31% in control rats ($P < 0.01$) and by 21% in iron-loaded rats ($P < 0.05$). However, no effect was detectable in iron-deficient rats. Furthermore, the iron content of alveolar macrophages obtained from iron-loaded rats was decreased to levels of the controls ($P = 0.04$), while no effect of this weak base on iron content was evident in macrophages from either control or iron-deficient rats (Table 1).

The oxidative tone of the macrophages of differing iron status was assessed by the measurement of SOD activity. In iron-loaded macrophages, SOD activity increased significantly compared to control rats ($P < 0.05$), which correlated with the increased iron content in these cells (Table 2). SOD activity in macrophages lavaged from iron-deficient rats, although higher than the control value, did not reach statistical significance, as the range of results was high. Attempts to assay SOD activity in the macrophages isolated from the rats treated with chloroquine was not possible, as the presence of chloroquine in the samples interfered with the superoxide-generating system of the assay.

After stimulation of the primary cultures of the alveolar macrophages with LPS, there was a small decrease in NO_2 release in the iron-loaded macrophages in comparison to controls, as previously described [3]. In the macrophages isolated from the iron-depleted rats, there was a significant increase in NO_2 release, despite the fact that their iron content was similar to controls (Fig. 2).

Chloroquine administration to the three groups of rats during the iron-loading or depletion regime induced significant and comparable changes in NO_2 release by the macrophages; the iron content of each of the alveolar macrophage preparations was now similar. However, the extent of NO_2 release before and after stimulation with LPS was markedly reduced, by approximately 10-fold (Fig. 2).

DISCUSSION

Chloroquine and its analogue hydroxychloroquine have been utilised for decades as antimalarial drugs and as anti-inflammatory drugs in diseases such as rheumatoid arthritis or lupus. In these very diverse disease indications, the exact mechanism of action of chloroquine is still debated [12, 13]. However, it is clear that the weak base chloroquine accumulates in the acidic organelles of the cell, where it blocks acidification [10]. Such an alteration in the pH of the endosomal vesicle prevents the release of iron from the transferrin–transferrin receptor complex, ultimately decreasing iron uptake via this pathway. However, other pathways exist for the entry of iron into the cell, including endocytosis [14] as well as the ferritin receptor pathway [15].

Five weeks of chloroquine administration significantly reduced the liver and splenic iron content in both the control and iron-loaded rats, while in the iron-deficient rats, a significant decrease in iron was only evident in the liver. In the control animals, chloroquine decreased the uptake of iron into the liver by 40% and into the spleen by 30%. In contrast, in the iron-overloaded animals, the chloroquine-induced reduction in iron uptake by both the liver and spleen was only 20%, possibly indicating that the major pathway for the uptake of excessive amounts of iron from iron dextran in these heterogeneous tissues was not via the transferrin receptor but by phagocytosis. An important effect of chloroquine observed in the present study was its ability to reduce the uptake of iron by the bronchoalveolar macrophages after iron dextran loading. Indeed, the iron content was substantially lower, i.e. approximately 400%, in rats treated with chloroquine. Chloroquine reputedly alters both the transferrin–transferrin receptor path-

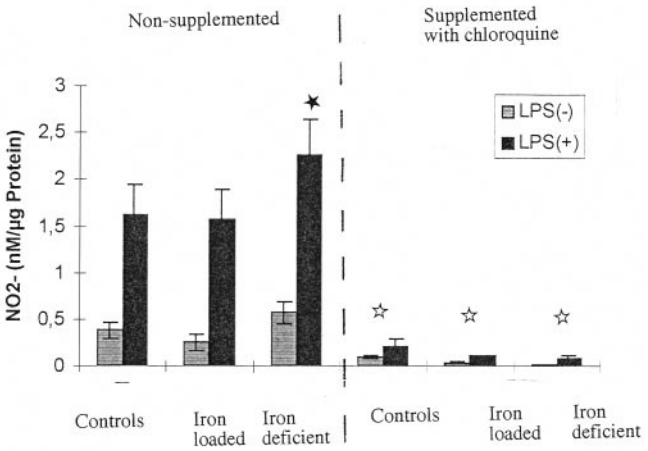

FIG. 2. NO_2 release from bronchoalveolar macrophages isolated from normal, iron-loaded, and iron-deficient rats after *ex vivo* stimulation with LPS (1 μg/mL) in the absence and presence of chloroquine supplementation. Results are presented as means ± standard deviation for 6 rats. Significance calculated (★) by comparison to controls, $P < 0.01$, and by comparison to rats not supplemented with chloroquine during the iron loading and depletion periods (☆) $P < 0.001$.

way and phagocytosis. Indeed, this antimalarial drug significantly inhibits the uptake of radioactive iron in a dose-dependent fashion in both neuronal and glial cells *in vitro* [16], this being thought to be an exclusive transferrin–iron transport system. On the other hand, macrophages obtain their iron by a variety of processes which are dependent upon the form in which it is supplied: by the transferrin–transferrin receptor pathway, by endocytosis, or by phagocytosis. However, the exact explanation as to why chloroquine decreases the degree of tissular accumulation of iron due to iron dextran remains to be elucidated.

Chloroquine had a dramatic effect upon the production of reactive nitrogen species after stimulation of the macrophages with LPS. In our previous studies, we had clearly shown that *in vivo* iron loading significantly decreases the release of RNI from macrophages stimulated with both LPS and interferon-γ [3]. The present communication confirms this, as well as showing that iron depletion of the animal significantly increases the release of RNI from alveolar macrophages, even though the total iron content of these cells is not altered. Similarly, in our previous study of ethanol-loaded rats [3], a reduction in NO_2 release was observed, despite there being no detectable change in iron content of the macrophages. The significant reduction in NO_2 release after *ex vivo* stimulation of macrophages obtained from the rats of differing iron status, which had been administered chloroquine for five weeks, was achieved despite the lack of change in macrophage iron content. Kremsner *et al.* [17] also showed that chloroquine, in a dose-dependent manner, inhibits both interferon-γ and malaria antigen-induced RNI production in murine macrophages *in vitro*. In contrast, Anstey *et al.* [18] showed that chloroquine had no effect on nitric oxide synthase activity. Very recently, chloroquine was shown to upregulate nitric oxide production in endothelial cells *in vitro* by limiting the availability of iron, but the authors did not provide results on macrophage cells [19]. Exactly how the reduction in RNI production observed in our study is achieved remains unclear, but chloroquine is known to inhibit the production of several monokines that are influential in the induction of RNI production by iNOS [20, 21].

The increased activity of SOD after iron loading alone may indicate that there is an increase in superoxide production with the formation of the reactive oxygen messenger, hydrogen peroxide. This could have a variety of actions on the iron homeostatic mechanisms of the cell and on cytokine production. Firstly, hydrogen peroxide can influence the 4Fe 4S structure of iron responsive protein 1, reducing it to the 0Fe-4S cluster in a relatively short time period, i.e. 15 min *in vitro* [22]. This would increase the uptake of iron via transferrin receptors. Secondly, hydrogen peroxide can act as a messenger for the activation of NFκB in the cell. Preliminary results from our laboratory clearly show the activation of NFκB in iron-loaded macrophages but not in iron-depleted cells.* Further studies are needed to ascertain what effects chloroquine has upon SOD activity using a method which is not adversely affected by the presence of chloroquine in the samples.

Our *ex vivo* data on the effect of iron status on RNI release therefore confirm what had previously been shown *in vitro* on the J774 murine macrophage cell line [23]. Whether there is a mechanism for the transcriptional regulation of iNOS expression by iron remains unknown. However, it was recently reported [24] that iron status modulates the binding affinities of at least two transcription factors to different sites in the iNOS promoter region, these inducible transcription factors being STAT1-a (signal transducer activators of transcription) and the LPS-inducible NFκB.

It has become increasingly clear in recent decades that iron overload predisposes to a variety of infections, particularly when iron loading principally affects the RES. This has been shown both experimentally and clinically [25, 26, 4, 8, 27]. A drug such as chloroquine which on chronic administration was shown in the present study to prevent loading of tissular macrophages with iron during experimental administration of iron dextran, might therefore play some role in preventing infection associated with iron overload. Indeed, chloroquine has been shown experimentally to inhibit the intracellular multiplication of both *Legionella pneumophila* and *Histoplasma capsulatum* by limiting the availability of iron to the microorganism [28, 29]. Chloroquine was also effective in inhibiting the growth of *Mycobacterium tuberculosis* in the human monocyte [30] and enhanced the resistance to *Cryptococcus neoformans* infection *in vivo* [31, 32]. However, in the latter type of infection, the effect of chloroquine does not seem to be mediated via restriction of iron acquisition. Finally, chloroquine or its derivative hydroxychloroquine has been reported to inhibit HIV-1 both *in vitro* and *in vivo* [33–35]. The effect of chloroquine in the latter situation is reported to result from an inhibition of posttranscriptional modifications of the virus. However, in view of the fact that iron loading of cells increases HIV-1 transcription [36], one may speculate that chloroquine might also benefit by virtue of limiting iron accumulation in macrophages due to the chronic inflammatory process. In the present study, iron loading was achieved through administration of iron dextran, and chloroquine was shown to limit iron loading in macrophages. It was clear that the release of NO_2 from the stimulated macrophages isolated from the non-chloroquine-supplemented animals inversely related to their iron content. We do not know at present whether chronic chloroquine administration will also impair chronic iron loading in macrophages, which is the hallmark of chronic inflammation.

The financial support of Vifor Pharmaceuticals, St Gallen, Switzerland is gratefully acknowledged.

* Legssyer R and Piret B, unpublished observations.

References

1. Hibbs JB, Taintor RR, Vavrin Z and Rachlin EM, Nitric oxide: A cytotoxic-activated macrophage effector molecule. *Biochem Biophys Res Commun* **157:** 87–96, 1989.
2. Jorens PG, Boelaert JR, Halloy V, Zamora R, Schneider Y–J and Herman AG, Human and rat macrophages mediate fungistatic activity against *Rhizopus* species differently: *In vitro* and *ex vivo* studies. *Infect Immuni* **63:** 4489–4494, 1995.
3. Zhang Y, Crichton RR, Boelaert JR, Jorens PG, Herman AG, Ward RJ, Lallemand F and De Witte P, Decreased release of nitric oxide NO by alveolar macrophages after *in vivo* loading of rats with either iron or ethanol. *Biochem Pharmacol* **55:** 21–25, 1998.
4. Jurado RL, Iron, infections, and anaemia of inflammation. *Clin Infect Dis* **25:** 888–895, 1997.
5. Boelaert JR, Piette J, Weinberg GA, Sappey C and Weinberg ED, Iron and oxidative stress as a mechanism for the enhanced production of human immunodeficiency virus by alveolar macrophages from otherwise healthy cigarette smokers. *J Infect Dis* **173:** 1045–6, 1996.
6. Means RT Jr and Krantz SB, Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* **80:** 1639–1647, 1992.
7. Gordeuk VR, McLaren GD and Samowitz W, Etiologies, consequences, and treatment of iron overload. *Crit Rev Clin Lab Sci* **31:** 89–133, 1994.
8. Van Asbeck BS, Marx JJM, Struyvenberg A and Verhoef J, Functional defects in phagocytic cells from patients with iron overload. *J Infect Dis* **8:** 232–240, 1984.
9. Aisen P and Listowsky I, Iron transport and storage proteins. *Annu Rev Biochem* **49:** 357–393, 1980.
10. de Duve C, de Barsy T, Poole B, Trouet A, Tulkens P and van Hoof F, Lysosomotropic agents. *Biochem Pharmacol* **23:** 2495–2531, 1974.
11. Ramm GA, Powell LW and Halliday JW, Pathways of intracellular trafficking and release of ferritin by the liver *in vivo*: The effect of chloroquine and cytochalasin D. *Hepatology* **19:** 504–513, 1994.
12. Krogstad GJ and Schlesinger PH, Acid–vesicle function, intracellular pathogens, and the action of chloroquine against *Plasmodium falciparum*. *N Engl J Med* **317:** 542–549, 1987.
13. Wellems TE, Malaria. How chloroquine works. *Nature* **355:** 108–9, 1992.
14. Crichton RR and Ward RJ, Iron metabolism: Perspectives in view. *Biochemistry* **31:** 11255–11264, 1992.
15. Moss D, Fargion S, Fracanzani AL, Levi S, Cappellini MD, Arosio P, Powell LW and Halliday JW, Functional role of the ferritin receptors of human liver, hepatoma, lymphoid and erythroid cells. *J Inorg Biochem* **47:** 219–227, 1992.
16. Swainman KF and Machen VL, Chloroquine reduces neuronal and glial cell uptake. *J Neurochem* **46:** 652–654, 1986.
17. Kremsner PG, Neifer S, Rasenack T and Bienzle U, Interference by antimalerial drugs with *in vitro* production of reactive nitrogen intermediates by murine macrophages. *J Antimicrob Chemother* **31:** 385–392, 1993.
18. Anstey NN, Weinberg JB, Hassanali MY, Mwaikambo ED, Manyenga D, Misukonis MA, Arnelle DR, Hollis D, McDonald MI and Granger DL, Nitric oxide in Tanzanian children with maleria: Inverse relationship between maleria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J Exp Med* **184:** 557–567, 1996.
19. Ghigo D, Aldiere E, Todde R, Costamagna C, Garbarino G, Pescarmona G and Bosia A, Chloroquine stimulates nitric oxide synthase in murine, pocine and human endothelial cells. *J Clin Invest* **102:** 595–605, 1998.
20. Picot S, Peyron F, Donadille A, Vuillez JP, Barbe G and Ambroise-Thomas P, Chloroquine-induced inhibition of the production of TNF, but not IL-6, is affected by disruption of iron metabolism. *Immunology* **80:** 127–133, 1993.
21. Sperber K, Quraishi H, Kalb TH, Panja A, Stecher V and Mayer L, Selective regulation of cytokine secretion by hydroxychloroquine: Inhibition of interleukin-1 alpha and IL-6 in human monocytes and T cells. *J Rheumatol* **20:** 803–808, 1993.
22. Pantopoulos K and Hentz M, Rapid responses to oxidative stress by iron regulatory protein. *EMBO J* **14:** 2917–2924, 1995.
23. Weiss G, Werner-Felmayer G, Werner ER, Grunewald K, Wachter H and Hentze MW, Iron regulates nitric oxide synthase activity by controlling nuclear transcription. *J Exp Med* **180:** 969–976, 1994.
24. Weiss G and Dlaska M, Mechanism of iron-mediated regulation of iNOS expression. *J Allergy Clin Immunol* **S226**, Abstract No. 939, 1998.
25. Boelaert JR, Daneels RF, Schurgers ML, Matthys EG, Gordts BZ and Van-Landuyt HW, Iron overload in haemodialysis patients increases the risk of bacteraemia: A prospective study. *Nephrol Dial Transplant* **5:** 130–134, 1990.
26. Boelaert JR, Iron and infection. *Acta Clin Belg* **51:** 213–221, 1996.
27. Weinberg ED, Iron and Infection, *Microbiol Rev* **42:** 45, 1984.
28. Byrd TF and Horwitz MA, Chloroquine inhibits the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. A potential new mechanism for the therapeutic effect of chloroquine against intracellular pathogens. *J Clin Invest* **88:** 351–357, 1991.
29. Newman SL, Gootee L, Brunner G and Deepe GS Jr, Chloroquine induces human macrophage killing of *Histoplasma capsulatum* by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. *J Clin Invest* **93:** 1422–1429, 1994.
30. Crowle AJ and May MH, Inhibition of tubercle bacilli in cultured human macrophages by chloroquine used alone and in combination with streptomycin, isoniazid, pyrazinamide, and two metabolites of vitamin D3. *Antimicrob Agents Chemother* **34:** 2217–2222, 1990.
31. Mazzolla R, Barluzzi R, Brozzetti R, Boelaert JR, Luna T, Saleppico S, Bistoni F and Blasi E, Enhanced resistance to *Cryptococcus neoformans* infection induced by chloroquine in a murine model of meningoencephalitis. *Antimicrob Agents Chemother* **41:** 802–807, 1997.
32. Levitz SM, Harrison TS, Tabuni A and Liu X, Chloroquine reduces human mononuclear phagocytes to inhibit and kill *Cryptococcus neoformans* by a mechanism independent of iron deprivation. *J Clin Invest* **100:** 1640–1646, 1997.
33. Tsai WP, Nara PL, Kung HF and Oroszlan S, Inhibition of human immunodeficiency virus infectivity by chloroquine. *Aids Res Hum Retrov* **6:** 481–489, 1990.
34. Sperber K, Kalb TH, Stecher VJ, Banerjee R and Mayer L, Inhibition of human immunodeficiency virus type 1 replication by hydroxychloroquine in T cells and monocytes. *Aids Res Hum Retrov* **9:** 91–98, 1993.
35. Chiang G, Sassaroli M, Louie M, Chen H, Stecher VJ and Sperber K, Inhibition of HIV-1 replication by hydroxychloroquine: Mechanism of action and comparison with zidovudine. *Clin Ther* **18:** 1080–1092, 1996.
36. Boelaert JR, Weinberg GA and Weinberg ED, Altered iron metabolism in HIV infection: Mechanisms, possible consequences, and proposals for management. *Infect Agents Dis* **5:** 36–46, 1996.