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Effect of Tenofovir/Emtricitabine/Efavirenz with and without Chloroquine in Patients with HIV/AIDS C3: Double Blinded Randomized Clinical Trial

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Abstract

Background: The use of antiretroviral therapy (ART) reduces both mortality and morbidity of patients with HIV/AIDS. The effects of chloroquine against HIV replication are widely known.

Objective: To demonstrate that beginning HIV treatment with ART (tenofovir/emtricitabine/efavirenz) + chloroquine increases by at least 20% the proportion of patients with viral load (VL) <50 copies/mL and >200 CD4+/mcL compared to patients receiving only ART at six months follow up.

Method: A randomized double-blind clinical trial was performed in 95 patients at the Infectious Diseases Department of the Hospital General de Mexico diagnosed with HIV/AIDS classification C3 before their first ART: 48 patients in the ART + placebo group and 47 in the ART + chloroquine group.

Results: A difference of 37% at six months of treatment in advantage of the group of ART + chloroquine ($p<0.001$), and seven times greater chance of improvement using chloroquine in the first six months of treatment. Chloroquine decreased the frequency of IRIS in 20%, compared with the group that initiated ART only at six months follow-up with statistical difference ($p=0.029$).

Conclusion: Chloroquine can improve the immune response and decrease the frequency of inflammatory syndrome by immune reconstitution at six months if added to the initial ART with tenofovir/emtricitabine/efavirenz.

Introduction

The primary goal of combination antiretroviral therapy is to increase disease free survival by supressing HIV replication and improving immunologic function [1]. Clinical disease caused by HIV appears because of a progressive decline of T CD4+ lymphocytes and immunological dysregulation associated with viral replication. When T CD4+ cells diminish to less than 200 cells/mm^3, opportunistic infections or malignancies arise. The mechanism of action of the most employed antiretroviral drugs during initial treatment is to inhibit one out of two HIV's enzymes: the reverse transcriptase or the protease [2-4]. In our country, the therapeutic guidelines are similar to the ones suggested in most international literature [5]. One of the most frequent antiviral combination recommended for initial antiretroviral therapy (ART) is Efavirenz+2 nucleoside reverse transcriptase inhibitors (NRTIs), being the first choice of these tenofovir+emtricitabine, based in the power showed in clinical essays, relatively low frequency of severe adverse events, substantial clinical experience and low frequency of dosage (once a day) [6].

Chloroquine is a 9 aminoquinoline known since 1934 which, as well as its analogue hydroxychloroquine, has been used as therapy for arthritis and malaria for decades; both have shown antiretroviral activity. These are weak alcalis which increase endosomal pH, therefore inhibiting postraductional changes of glycoprotein 120 (gp120), thus resulting in new HIV non-infectious particles [7,8]. The presence of chloroquine at non cytotoxic concentrations has antiretroviral activity in infected cells both acutely and chronically, and it is primarily stored in monocytes, an important HIV reservoir; this antiretroviral activity has been shown both in vitro and in vivo [9,10]. The efficiency and tolerance of monotherapy with hydroxychloroquine has been demonstrated in patients with HIV infection in clinical essays [11,12]. Moreover, chloroquine has immunomodulatory effects as it abates tumor necrosis factor alfa (TNF alfa) and interleukin (IL) 6 synthesis and release, both of which are mediators of inflammatory complications of several viral diseases [13,14]. Chloroquine has several effects in the life cycle of HIV, which include inhibiting HIV integrase as well as Tat mediated transactivation, therefore modifying GP120 posttranscriptional maturation, and reduction of iron deposits at infected cells, thus modifying the reverse transcription [15]. Several studies have reported that chloroquine activity is not only against HIV 1 strains class B, but also against A,C,D,E subtypes and HIV 2 strains, besides not being dependent on the presence of coreceptor CXCR4/

CCR5. Recent case reports have described hydroxychloroquine as a successful therapy for immune reconstitution inflammatory syndrome (IRIS) associated to opportunistic diseases [16].

Our goal was to demonstrate that antiretroviral therapy (ART) with tenofovir/emtricitabine/efavirenz plus chloroquine increases by 20% the proportion of patients with VL <50 copies/mL and T CD4+ >200/mcL compared to patients receiving ART plus placebo at six months follow-up. As a secondary goal we determined the frequency of IRIS related to each treatment regimen.

Materials and Methods

We performed a double-blinded randomized controlled clinical trial with two parallel groups design, and non-probabilistic of incident cases with randomized assignation type of sampling. The inclusion criteria were patients of both genres older than 18 years old, with HIV/AIDS confirmed by Western blot in CDC's (USA Centers for Disease Control and Prevention) classification C3, about to begin ART and agreeing to participate in the trial by means of a written consent. The exclusion criteria were pregnant women; history of hypersensitivity or previous use of chloroquine; previous ART; transaminases greater than five times the upper limit; hepatitis virus co-infection; creatinine clearance <60 ml/min; and psoriasis. The discarding criteria were patients who failed to attend to two or more follow-up appointments; who did not have two or more follow-up tests with VL or T CD4+ count (initial and final, at least); and patients who did not wish to go on with the study. Patients who met the inclusion criteria were randomized to receive either 150 mg of chloroquine every 24 hours or placebo along with ART. All patients were evaluated at baseline and six months after, with VL and T CD4+ cell counts, as well as drug toxicity probing. Chloroquine was provided by donation from Bayer pharmaceutical without getting involved in the design, analysis or reporting of results. CLOROPRON ® 150 mg chloroquine was used, SSA number 2030, with registration number 177 M83 SSA IV; a standard dose with less toxicity and most effect at 5 year follow up in rheumatologic diseases. Recruitment of patients took place from January 2010 to December 2010 at the Infectious Diseases Unit of the Hospital General de Mexico, with follow-up period of up to six months after the first assessment.

Statistical analysis

Data is presented with measures of central tendency. The evaluation of dichotomous variables was performed using X^2 test or Fisher exact test if expected frequencies were ≤ 5; for continuous variables mean difference was evaluated and Student t test or Mann-Whitney U were used in case of a distribution different from normal. We performed treatment effect analysis, descriptive statistics with simple frequencies and proportions; the OR was obtained to establish the group with best response and Hazard ratio analysis and statistical survival by Kaplan-Meier and Cox regression to establish the developmental characteristics of IRIS. For the statistical analysis of data STATA® software was used. The α 2-tailed value considered significant was ≤ 0.05. Confounding variables were randomized; evaluation of this distribution was made during the analysis of baseline characteristics of the study. In case of finding significant differences for any of these variables, the corresponding analysis was performed to determine its effect on outcome.

Ethical Considerations: The acceptance for protocol initiation by the Ethics and Research Committee was made through Of. No. CE/010/064, as well as approval by the Ethics and Research Committee on January 27, 2010 Of. No. DI/03/010/040. DIC/10/405/04/005 registry number.

Results

95 patients were included in the study and began treatment with tenofovir/emtricitabine/efavirenz: 48 received placebo in addition to ART and 47 received chloroquine (Figure 1). In the placebo group there were 40 men (83.3%) and 8 women (16.6%); and in the group receiving chloroquine were 39 men (82.9%) and 8 women (17%). The mean age for the placebo group was 39 years and 32 years for the chloroquine group. There was no statistical difference in gender, as there were 40 men in the placebo group and 39 in chloroquine group, as well as 8 women in each group.

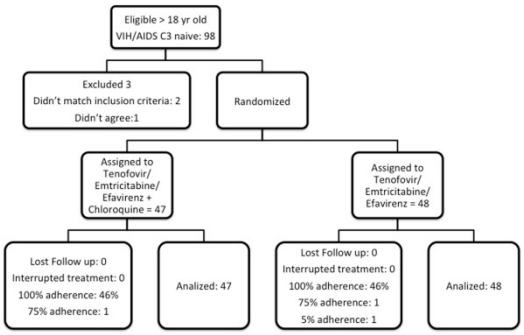

Figure 1: General distribution of patients in the study.

Co-infections were treated prior to ART; in the placebo group were 19 patients with pulmonary tuberculosis, 8 with pneumonia caused by *P. jiroveci*, 7 with diarrhea syndrome associated with *C. parvum*, 5 with wasting syndrome and minor infections (oropharyngeal candidiasis, molluscum contagiosum, or fever without identifying a specific pathogen by microbiological or molecular means), 4 patients with meningitis caused by *C. neoformans*, 3 patients with disseminated tuberculosis, and 2 patients with tuberculosis in central nervous system (CNS). In the group receiving chloroquine were 13 patients with pneumonia due to *P. jiroveci*, 8 with wasting syndrome and minor infections, 6 with meningitis caused by *C. neoformans*, 5 with pulmonary tuberculosis, 4 with disseminated tuberculosis, 4 with lymphatic node tuberculosis, 4 with cerebral toxoplasmosis, 2 with spread citomegalovirus and 1 with diarrhea syndrome associated with *C. parvum*. In patients who had more than one co-infection we reported the one that was the cause of hospitalization and was determined as primary infection.

At baseline the placebo group had a mean of 592,773.6 copies/mL, and the group of chloroquine 579,188 copies/mL with no statistically significant difference (p=0.47); but there was significant difference in CD4+ T cells mean in both groups: the placebo group showed the lowest levels, starting with an average of 51 CD4+ cells/mcL compared to 74.6 CD4+/mcL in the group treated with chloroquine (p=0.015).

Initial and final laboratory tests were performed in order to identify any relevant alteration caused by the therapy that was subclinical and could modify the disease outcome. There were no differences in biochemical tests values that had clinical importance. Likewise, there were no retinal changes at basal line or at the end of the six month

follow up in none of the patients. In the placebo group 33% of the patients showed side effects, most frequently nausea, cefalea, and hyporexia, and renal dysfunction less frequently. In the chloroquine group 39% of the patients showed side effects: the most frequent being epigastric pain without gastrointestinal bleeding, and the less frequent being cefalea, dysphagia and pruritus.

In accordance with our main objective, we observed that at six months of treatment with Tenofovir/Emtricitabine/Efavirenz, 25 patients (52.08%) who received placebo achieved >200 CD4+ cells/mcL and <50 copies/mL in comparison to 42 patients (89.3%) who received chloroquine. These results show a difference of 37% in favor of the treatment with chloroquine with p<0.001. On the other hand, at six months with Tenofovir/Emtricitabine/Efavirenz treatment, 29 patients (60%) with placebo achieved >200 CD4+ cells/mcL alone in comparison to 45 patients (95.7%) receiving chloroquine, showing a significative difference (p<0.001). As for VL, 39 patients (81.2%) with placebo achieved <50 copies/mcL in comparison to 43 patients (91.4%) receiving chloroquine, with no significative difference.

As we performed OR for treatment adjusted by VL copies number and CD4+ number at baseline. After adjusting for baseline means of VL and CD4+ T cells, the OR is 7.728 with p=0.001 and 95% CI (2.60-22.9).

During the six month follow-up, 19 patients in the placebo group developed IRIS (39.5%), compared with only 9 patients in the group receiving chloroquine (19.1%), with statistical difference of p=0.029. Of these patients, 16 of the placebo group (84.2%) and 5 of the chloroquine group (55.5%) received steroids to treat the IRIS (p=0.102). Moreover, 19 patients of the placebo group required hospitalization (100%), as well as 7 patients of the chloroquine group (77.7%) (p=0.095).

To calculate the risk of developing IRIS, Cox regression - Breslow method for ties was performed: there is 60% lower risk of IRIS if the patients receive chloroquine compared with the placebo group, with statistical difference p=0.025 and 95% CI (0.1830-0.8950).

Most cases of IRIS occurred within 90 days of treatment in both groups, with a tendency to lower frequency of IRIS in the group with chloroquine added to antiretroviral therapy (Figures 2 and 3).

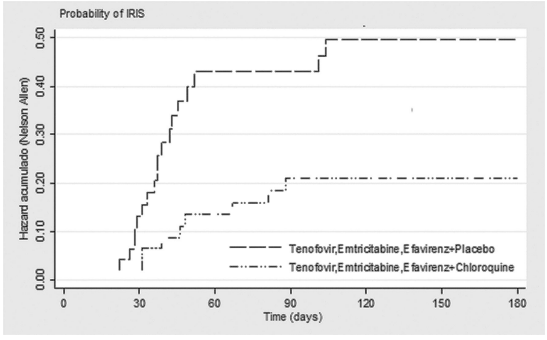

Figure 2: Hazard ratio of IRIS according to treatment.

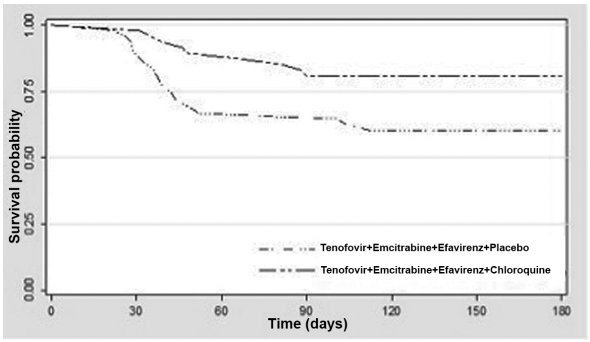

Figure 3: Time of development of IRIS according to treatment.

Regarding side effects, no retinal changes were observed at baseline or at six months follow up in any patient. It was very clear that the group using chloroquine had a higher frequency of heartburn without need of drugs that would reduce gastric acidity or reports of gastrointestinal bleeding.

Discussion

Although it's been more than 20 years since chloroquine effects on HIV have been observed, there are only a few reports comparing the efficiency of chloroquine along with ART and there are no reports with this drug in the most advanced stage of the disease. Since 1997 Sperber et al, demonstrated in a clinical essay that a chloroquine analog, hydroxychloroquine, was well tolerated and suppressed HIV replication in asymptomatic patients, describing too the anti-inflammatory effects in those patients [12]. Existing ART can decrease morbimortality associated to advanced stages of the disease; nevertheless, the time of recovery is quite long, as well as the time a patient spends within risk of opportunistic diseases. Knowing about antiviral properties of chloroquine and its immunomodulatory effects we decided to include in this research firstly the most incident and prevalent population in our care center, which is also the one with the highest mortality risk because of the opportunistic diseases like tuberculosis and the one which develops IRIS most frequently. Our goal was to prove the possibility to enhance the antiviral effect of the initial ART and at the same time to take advantage of the immunomodulatory features of chloroquine in a research solid enough to be able to consider it within the initial therapies for our patients. Although there are other reports such as Paton's in 2005, most have been open, non-comparative and in patients with low VL [17]. As chloroquine exhibits lower anti-HIV effects than present antiretrovirals, we decided to add it up to the initial standard therapeutic plan recommended by national and international guidelines: Tenofovir 300 mg/Emtricitabine 200 mg/Efavirenz 600 mg, which is a powerful plan, with easy administration because of its coformulation and need of only one dose a day, lesser secondary effects, low cost and moderate resistance barrier. Also, we used one tablet a day of chloroquine 150 mg, taken at the same time as ART to ease adherence and tolerability, and diminish secondary effects. Patients were included in the protocol after they were stabilized and treated for the opportunistic disease which caused their hospitalization, obervations that give us a glimpse about the frequency of these pathologies and infectious epidemiology in our country. Even though none of the opportunistic diseases surpassed the others in

frequency, as for tuberculosis, there was a difference in location: there were more patients with pulmonar tuberculosis in the placebo group and more with extrapulmonary tuberculosis in the chloroquine group. There were also more patients with *P. jiroveci* pneumonia in the chloroquine group, without statistical difference between the groups for none of the observed coinfections. Basal and final biochemical levels showed statistical differences as the values were inspected individually, although it did not require drug discontinuation or the need for further tests, as it did not suggest an associated effect in a positive or negative way with the trial. Also, such tests showed a tendency towards an improved lipid profile in the chloroquine group at six months follow up and, although time of follow up was short, there are reports about the favorable features of antimalarials towards dyslipidemia and diabetes development. We believe a research about this should be considered in patients with dyslipidemia or glycemic dysregulation associated with antiretrovirals [18,19]. The T CD4+ cells count was below than 100/mcL at baseline in both groups, which added to the opportunistic diseases, and confirmed a C3 CDC stage at the beginning of therapy. We considered a T CD4+ cells count higher than 200/mcL at the end of the six month follow up with a detectable VL could suggest an incomplete response or presence of primary resistance to antiretrovirals. Moreover, an undetectable VL at six month follow up with a T CD4+ cells count lower than 200/mcL is not enough to consider a patient without risk of developing opportunistic diseases and there wouldn't be any advantage within immune prognostic terms. When we added chloroquine to ART, we observed a statistically better response in this group with a difference of 37% in favor of chloroquine. 95% of patients in the chloroquine group achieved a T CD4+ cells count above 200/mcL at six months follow up, compared to only 60% of the patients in the placebo group. Also, 91% of the patients in the chloroquine group achieved a VL below 50 copies/mcL, compared with 81% patients in the placebo group, without statistical difference, although with a clear contrast between both groups. As we analized with OR and adjusted for basal levels of VL and T CD4+ cells the difference between both groups, we obtained an OR of 7.7, which means there's seven times more chance to achieve an undetectable VL and more than 200 T CD4+ cells if chloroquine is added to initial ART. On the other hand, we observed a higher frequency of IRIS in the placebo group without differences in the need of hospitalization or need of steroids in both groups. We performed HR in order to determine the probability to develop IRIS obtaining a figure of 0.40, which represents 60% less probability to develop IRIS during the first six months of therapy if chloroquine is added to initial ART. The outcome of survival and the risk factors for IRIS are determined by the immune status of the patient (T CD4+ count), so there's little effect when placebo or chloroquine are added or not to the coinfection therapy. These results are of considerable importance despite of such a small sample. Not all the involved mechanisms in immune recovery are clear, although a part of this could be that chloroquine shows not only antiviral properties, but also antifungal and antiparasitic and over other microorganisms which could be part of the opportunistic coinfections present at advanced stages of AIDS and are not always identified [20,21].

Another consideration is the effect of the treatment of coinfections in the decrease of the VL: altough there was a slight advantage towards the chloroquine group, close monitoring in both groups, as well as detection and oportune treatment of coinfections and side effects of the different therapies of coinfections such as tuberculosis, could have enhanced the response to the first plan initially [22]. Even though there are other manuscripts highlighting the use of hydroxychloroquine as a reducer of the immune activation in patients with HIV receiving ART and who have no immune response, Piconi S, et al, showed that immune modulation induced by hydroxychloroquine is associated with the increase in T CD4+ cells and such activity was maintained two months after discontinuing the drug, so they propose it could be a useful immunomodulator in patients with HIV infection [23].

Regarding the side effects, renal dysfunction was thought to be caused by tenofovir, so a substitution had to be made with immediate reversal of the dysfunction.

The six month follow up of the patients was so short it didn't allow us to know if there was sustained response, as well as mortality. We expect the latter to be low because of improvement of the immune response until the drug was discontinued. Likewise, there's no way to acknowledge the effects in toxicity and safety if chloroquine was used for a longer time or dispensed in a different dose. On the other hand, as we only included patients in the most advanced stage of AIDS, we don't know the response that patient in other stages and with co-infections or malignancies could show. Also, we weren't able to assess the clinical effect neither with other ARTs nor over the side effects associated to the protease inhibitors.

This research confirms again the chloroquine anti-HIV effect, establishing that adding it to the initial ART in patients with AIDS in advanced stages improve faster in their T CD4+ cells counts and reduces the IRIS associated to the beginning of the ART.

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