Chloroquine use improves dengue-related symptoms

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Dengue is the most important arboviral disease in the world. As chloroquine, an antimalarial agent, has shown some antiviral effects, this study evaluated its effect in patients with dengue. A randomised, double-blind study was performed by administering chloroquine or placebo for three days to 129 patients with dengue-related symptoms. Of these patients, 37 were confirmed as having dengue and completed the study; in total, 19 dengue patients received chloroquine and 18 received placebo. There was no significant difference in the duration of the disease or the degree and days of fever. However, 12 patients (63%) with confirmed dengue reported a substantial decrease in pain intensity and a great improvement in their ability to perform daily activities (p = 0.0004) while on the medication and the symptoms returned immediately after these patients stopped taking the medication. The same effect was not observed in patients with diseases other than dengue. Therefore, this study shows that patients with dengue treated with chloroquine had an improvement in their quality of life and were able to resume their daily activities. However, as chloroquine did not alter the duration of the disease or the intensity and days of fever, further studies are necessary to confirm the clinical effects and to assess the side effects of chloroquine in dengue patients.

Key words: dengue - therapeutics - chloroquine - activities of daily living

Dengue is the most important arthropod-borne viral infection of humans and represents an important public health problem in tropical and subtropical areas. The World Health Organization estimates that approximately 2.5 billion people in 100 countries are at risk of infection. It is reported that over 100 million people are infected with dengue viruses (DENV) every year; of these individuals, 500,000 develop a severe form, leading to approximately 20,000 deaths annually (Pinheiro & Corber 1997).

Dengue results from infection by one of four DENV serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. These viruses belong to the Flaviviridae family and are transmitted to humans through the bite of female *Aedes aegypti* mosquitoes. The clinical manifestations of dengue range from a mild flu-like syndrome, occasionally associated with a rash (dengue fever) to a more severe form of the disease that is associated with plasma leakage, thrombocytopenia, haemorrhage (dengue haemorrhagic fever) and/or shock (dengue shock syndrome). No dengue vaccine or effective antiviral agent is available for the treatment of dengue infection (Valero & Levy 2008, Noble et al. 2010).

As in many other countries in Latin America, the Brazilian population has been seriously affected by dengue infections. Indeed, approximately 80% of the reported dengue cases in the Americas occur in Brazil and more than one million cases have been reported in all five Brazilian geographic Regions in the last few years (Schatzmayr 2000).

Chloroquine has been primarily used as an antimalarial drug and, due its anti-inflammatory effects, has been used as a secondary drug to treat a variety of chronic diseases, such as rheumatoid arthritis and systemic lupus erythematosus (Tutor-Ureta & Yebra-Bango 2005). Recently, several efforts have been made to identify an effective, inexpensive and universally available antiviral agent. Chloroquine has been suggested as such an agent (Savarino et al. 2006) and has been shown to exert antiviral effects by inhibiting the replication of flaviviruses (Randolph et al. 1990, Savarino et al. 2003).

Therefore, due to the need for an effective treatment for dengue and based on the anti-inflammatory and possible anti-flavivirus effects of chloroquine, we hypothesised that chloroquine could have a clinical benefit in dengue infection.

PATIENTS, MATERIALS AND METHODS

We set up a double-blind, randomised study that administered either chloroquine or placebo (starch) to patients suspected of having dengue disease. Patients were included in the study if they presented with dengue-related symptoms, such as fever and at least two other symptoms, such as headache, retro-orbital pain, muscle and bone or joint pain, nausea, vomiting and rash, for less than 72 h. Patients were excluded if they were pregnant, younger than 18 years old or had either cardiac or neurologic disease. Once entered into the study, the patients were randomised in a double-blind fashion to receive either 500 mg chloroquine (300 mg base) BID or an identical-appearing placebo BID for three days (control group). In addition to the use of either chloroquine or placebo, both groups were similarly treated with hydration and symptomatic medication, such as analgesic and antipyretic drugs, according to medical prescription.

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Three diagnostic tests were performed according to well-defined protocols [reverse transcription-polymerase chain reaction, IgM antibody capture ELISA and the non structural 1 (NS1) antigen detection] on all the samples (Innis et al. 1989, Lanciotti et al. 1992, Dussart et al. 2006, Castro-Jorge et al. 2010). Dengue was confirmed when at least two diagnostic tests were positive. The patients were re-evaluated one week later and clinical data associated with dengue symptoms, such as fever, headache, retro-orbital pain, muscle, bone or joint pain, nausea and vomiting were recorded as well as symptoms related to the side effects of chloroquine. The axillary temperature was objectively measured and considered abnormal (fever) when ≥ 37.8ºC. Other variables were obtained actively and objectively during a medical consultation using a research form.

This trial is registered with ClinicalTrials.gov (NCT00849602). The study was approved by the Ethical Committee of the Clinical Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo, process 12603/2006, and written informed consent was obtained from all participants.

The data are presented as the means ± standard deviations and number of patients (%). Differences between the two study groups were assessed using an unpaired *t* test for continuous outcomes and Fisher's exact test for proportions. All p values were two-sided and all analyses were performed with the use of GraphPad Software, version 3.05 (GraphPad Software, San Diego, CA, USA).

RESULTS

The study was conducted from mid-February to the first week of May of 2008. One hundred thirty-two patients were assessed for eligibility: three did not meet the inclusion criteria and 129 underwent randomisation. The patients enrolled in the study were adults (mean age, 31.64 ± 11.74 years), were predominantly female (51.2%) and reported a mean average duration of symptoms of 41.52 ± 20.16 h. Of the 129 patients, 63 were randomised to receive chloroquine and 66 were randomised to receive placebo. Seventy-five patients had other febrile diseases (OFDs) and 54 were confirmed to have dengue. Of the dengue patients, 17 refused to participate and 37 completed the study. Of these patients, 19 received chloroquine (mean age, 32.72 ± 11.50 years; male, 42%; duration of symptoms, 1.94 ± 0.90 day) and 18 patients received placebo (mean age, 36.74 ± 11.83 years; male, 56%; duration of symptoms, 1.56 ± 0.73 day). No patient exhibited the severe form of the disease (dengue haemorrhagic fever) and/or shock (dengue shock syndrome) (Table). There was no significant difference between group characteristics ($p > 0.05$) and the most frequent symptoms were fever (100%), body ache (99.2%), headache (96.8%), joint pain (70.4%) and retro-orbital pain (68%). There was no significant difference in the duration of the disease or the intensity and days of fever among the dengue patients treated with either chloroquine or placebo. However, 12 (63%) of the dengue patients reported a substantial reduction in the intensity of pain and a great improvement in their ability to perform daily chores after taking chloroquine, whereas no improvement was reported by the patients taking placebo ($p = 0.0004$) (Table). However, as this finding was not observed in the patients with OFDs, which included bacterial pneumonia, urinary tract infection and influenza, these diseases were not included in the statistical evaluation because the primary goal of this study was to examine the influence of chloroquine on the evolution of dengue disease. Interestingly, the symptoms returned immediately after the patients stopped taking the three-day chloroquine treatment, showing that chloroquine's effect was most likely due to the anti-inflammatory action of chloroquine rather than to its antiviral effect.

Side effects were reported by only two patients and consisted of transient blurred vision in one and a loss of consciousness in the other. The latter patient was severely dehydrated and seizure was ruled out based on clinical presentation and follow-up. Both patients were not confirmed to be dengue patients.

TABLE

Characteristics of dengue patients[a] treated with chloroquine (300 mg base) BID or identical-appearing placebo BID for three days

	Chloroquine group	Control group
Patients (n)	19	18
Age (years) (mean ± SD)	32.72 ± 11.50	36.74 ± 11.83[b]
Gender (male) [n (%)]	8 (42)	10 (56)[b]
Symptoms onset (days) (mean ± SD)	1.94 ± 0.90	1.56 ± 0.73[b]
Fever[c] [n (%)]	19 (100)	18 (100)[b]
Improvement in the intensity of pain and on ability to perform daily chores [n (%)][d]	12 (63)	0 (0)[e]

a: dengue was confirmed when at least two diagnostic tests (reverse transcription-polymerase chain reaction, IgM antibody capture ELISA and the non structural 1 antigen detection) were positive; *b*: $p > 0.05$; *c*: axillary temperature was considered abnormal (fever) when ≥ 37.8ºC; *d*: clinical variables were obtained active and objectively during a medical consultation by using a research form; *e*: $p = 0.0004$; SD: standard deviation.

DISCUSSION

Chloroquine did not alter the duration of the disease or the intensity and days of fever compared to the dengue patients taking placebo. However, the patients taking chloroquine reported a substantial reduction in the intensity of pain and a great improvement in their health status.

Dengue is a worldwide health problem that affects millions of people and causes innumerable deaths, particularly in the tropical and subtropical areas of the world. Controlling the viral vector is currently the only option for reducing dengue disease rates, as there is no a vaccine or effective antiviral agent available for the treatment of dengue infections. Accordingly, it is important to identify treatment strategies that could reduce the burden of this disease (Valero & Levy 2008).

Chloroquine is a 9-aminoquinoline known since 1934 that has a long history of use as an antimalarial agent and as an alternative drug to treat a variety of chronic diseases (Savarino et al. 2003, Tutor-Ureta & Yebra-Bango 2005). The rationales for using chloroquine in dengue patients include the drug's anti-inflammatory property, possible antiviral effect, with action on flaviviruses, low cost, universal availability and safety and global public health importance of dengue. Recently, a potential use of chloroquine has been described in some viral infections, such as influenza, human immunodeficiency virus and severe acute respiratory syndrome (coronavirus) (Lanciotti et al. 1992, Savarino et al. 2003, 2006, Vincent et al. 2005, Adachi et al. 2007, Di Trani et al. 2007, Keyaerts et al. 2009, Martinson et al. 2010).

One possible explanation for the antiviral effect of chloroquine could be related to the impairment of viral replication by raising the intracellular pH and thereby interfering with endosome-mediated viral entry. An increase in pH in acidic organelles, including endosomes, lysosomes and Golgi vesicles, leads to unwanted changes in the post-translational modifications of newly synthesised proteins, particularly the inhibition of glycosylation (Savarino et al. 2004, Rolain et al. 2007). These effects are of particular importance in DENV infection because these viruses require endosomal acidification to release their viral RNA in the cytoplasm to initiate viral replication (Lindenbach & Rice 2003). Other possible antiviral actions of chloroquine are based on either interference with the post-translational processing of viral glycoproteins, leading to imperfect glycosylation or to undefined interference with the late stages of viral replication (Savarino et al. 2003, 2004). However, the exact mechanism of action of chloroquine has not been completely elucidated and differs depending on different pathogens (Rolain et al. 2007).

Tricou et al. (2010) evaluated the effect of chloroquine in hospitalised adult patients with dengue and found no difference in viraemia or NS1 antigenaemia duration and modest anti-pyretic activity for chloroquine in the intention-to-treat population, but not in dengue laboratory-confirmed cases. Moreover, these researchers observed a trend toward a lower incidence of dengue haemorrhagic fever in dengue patients treated with chloroquine.

In our study, there was also no difference in the duration of disease or days of fever in dengue patients taking either chloroquine or placebo. However, the patients taking chloroquine in addition to routine analgesic drugs had a substantial reduction in the intensity of pain and a great improvement in health status. The pain amelioration observed in the enrolled patients taking chloroquine was not quantified by pain scales, which is a limitation of our study. However, we objectively inquired about and evaluated the dengue-related symptoms, including pain, in all patients during acute disease and one week later, at the time when the patients returned for a clinical evaluation during convalescence.

We hypothesised that the clinical benefit observed in dengue patients treated with chloroquine could be due to the drug's anti-inflammatory effects, particularly because chloroquine is a tumour necrosis factor (TNF)-α inhibitor and some of the clinical manifestations in dengue patients are due to high levels of serum TNF-α (Hober et al. 1993, Pinto et al. 1999, Gandini et al. 2011). Another possible effect of chloroquine in dengue patients could be related to its antiviral action, lowering the viral load and thus the degree of immune system activation. Other studies with different therapeutic doses, different durations and an earlier onset of treatment should be performed to evaluate the in vivo effect of chloroquine on dengue infection. Moreover, it would be interesting to compare the effect of other anti-inflammatory compounds on the treatment of dengue patients; however, non-steroidal drugs should be avoided in the care of these patients.

One limitation of this study was the small number of patients treated with chloroquine. Although only a small number of dengue patients were included in the study, approximately 63% reported an improvement in their symptoms while on the medication and the symptoms returned immediately after the patients stopped taking the medication. This finding indicates that chloroquine is a possible candidate for use in the treatment of dengue. In addition, the side effects of chloroquine observed in this study were unremarkable.

Although chloroquine has a narrow safety margin, it is usually well tolerated. Adverse effects, such as headache, nausea, malaise, dizziness, blurred vision, difficulty focusing, mild gastrointestinal upset and pruritus, may commonly occur. Severe toxicity, e.g., neuromyopathy, retinopathy and bone-marrow toxicity, is rare and may occur with long-term treatment and/or as an idiosyncratic reaction (Taylor & White 2004, Alkadi 2007). Chloroquine may worsen psoriasis, though is rarely associated with seizures and psychosis. Acute toxicity is most frequently found with therapeutic or high doses administered too rapidly by parenteral routes (AlKadi 2007). Toxic effects are related to the ingested dose and a single dose of 20 mg/kg is considered toxic (Taylor & White 2004). In this study, a safety dose of 600 mg per day for three days was used in adults and the side effects were unremarkable. Furthermore, the chloroquine dosage used in this study is very similar to the dosage used for vivax malaria treat-

ment and, in our experience, side effects are rare with this treatment. However, considering the small number of patients, more studies are necessary to evaluate the safety of chloroquine in dengue patients.

In conclusion, chloroquine promoted a reduction in the intensity of pain and an improvement in the well-being of patients with dengue infection, but did not alter the duration of the disease or the intensity and days of fever. Therefore, considering that the current options for dengue treatment are very limited, we suggest that chloroquine should be further tested in dengue disease with a larger number of patients and different chloroquine dosages and times of administration to confirm the drug's clinical effect and to assess the side effects of chloroquine in dengue patients.

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