International Journal of Cardiology 337 (2021) 21–27

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Hydroxychloroquine reduces interleukin-6 levels after myocardial infarction: The randomized, double-blind, placebo-controlled OXI pilot trial

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ARTICLE INFO

Article history:
Received 30 December 2020
Received in revised form 6 April 2021
Accepted 28 April 2021
Available online 4 May 2021

Keywords:
Myocardial infarction
ST-elevation myocardial infarction
Non-ST-elevation myocardial infarction
Hydroxychloroquine
Inflammation
Interleukins

ABSTRACT

Objectives: To determine the anti-inflammatory effect and safety of hydroxychloroquine after acute myocardial infarction.
Method: In this multicenter, double-blind, placebo-controlled OXI trial, 125 myocardial infarction patients were randomized at a median of 43 h after hospitalization to receive hydroxychloroquine 300 mg ($n = 64$) or placebo ($n = 61$) once daily for 6 months and, followed for an average of 32 months. Laboratory values were measured at baseline, 1, 6, and 12 months.
Results: The levels of interleukin-6 (IL-6) were comparable at baseline between study groups ($p = 0.18$). At six months, the IL-6 levels were lower in the hydroxychloroquine group ($p = 0.042$, between groups), and in the on-treatment analysis, the difference at this time point was even more pronounced ($p = 0.019$, respectively). The high-sensitivity C-reactive protein levels did not differ significantly between study groups at any time points. Eleven patients in the hydroxychloroquine group and four in the placebo group had adverse events leading to interruption or withdrawal of study medication, none of which was serious ($p = 0.10$, between groups).
Conclusions: In patients with myocardial infarction, hydroxychloroquine reduced IL-6 levels significantly more than did placebo without causing any clinically significant adverse events. A larger randomized clinical trial is warranted to prove the potential ability of hydroxychloroquine to reduce cardiovascular endpoints after myocardial infarction.

Trial Registration

ClinicalTrials.gov Identifier: NCT02648464.

1. Introduction

Hydroxychloroquine, an antimalarial and antirheumatic drug, has significant anti-inflammatory properties targeting the activation of the innate immune system [1]. Chloroquine, a hydroxychloroquine analog, has been shown to reduce the production of cytokines such as interleukin (IL)-1β, and IL-6 by macrophages [2]. Blocking IL-1β with the monoclonal antibody canakinumab lowered the risk of cardiovascular events, but also IL-6 levels [3]. Similarly, colchicine reduced ischemic cardiovascular events possibly by inhibition of inflammasome activation and thus

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https://doi.org/10.1016/j.ijcard.2021.04.062

IL-1β production [4]. Tocilizumab has shown to attenuate IL-6 response and troponin T release in non-ST-elevation myocardial infarction patients [5].

Hydroxychloroquine has also been shown to lower cholesterol and glucose levels, and to possess antithrombotic properties, all of which are likely to be beneficial in the treatment of coronary artery disease [6]. In a retrospective study of over 1000 rheumatoid arthritis patients, the use of hydroxychloroquine was associated with a significant decrease in the risk for the composite outcome of various cardiovascular endpoints [7].

Hydroxychloroquine is also potentially harmful, as it may prolong the QTc interval on ECG, and thereby predispose toward severe arrhythmias and sudden cardiac death [8]. Moreover, long-term use of hydroxychloroquine has been associated with cardiomyopathy, neuropathy, and retinal toxicity [9]. Therefore, before large clinical trials of hydroxychloroquine for the prevention of cardiovascular events can be conducted, the anti-inflammatory effect and safety of hydroxychloroquine should be addressed in a pilot study in patients with recent myocardial infarction.

Thus, the available retrospective clinical evidence suggests that the use of hydroxychloroquine reduces the risk of atherosclerotic cardiovascular disease [7]. However, no studies have addressed the potential benefits or risks of hydroxychloroquine in patients with a recent myocardial infarction who are at high risk for recurrent cardiovascular events. We hypothesized that hydroxychloroquine would reduce cardiovascular inflammation in this highly selected and well-defined patient population.

2. Methods

2.1. Study design

The trial design has been published previously [10]. Hydroxychloroquine for the Prevention of Cardiovascular Events in Myocardial Infarction Patients - a Safety Pilot Trial (OXI) was a randomized, double-blind, investigator-initiated pilot study in which participants received oral hydroxychloroquine (Orion Pharma, Espoo, Finland) 300 mg once daily (for patients who weigh <60 kg: 300 mg for 5 days per week) or placebo for 6 months. The trial was approved by the Ethics Committee of Helsinki University Hospital (Approval number: 148/13/03/01/2015) and conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent. The study was organized, coordinated, and executed by researchers at the Heart and Lung Center at Helsinki University Hospital who were also responsible for data management and statistical analysis.

2.2. Patients

Patients were recruited from six hospitals in Finland: Heart and Lung Center in Helsinki University Hospital; Päijät-Häme Central Hospital, Lahti; Kymenlaakso Central Hospital, Kotka; South Karelia Central Hospital, Lappeenranta; North Karelia Central Hospital, Joensuu; and Seinäjoki Central Hospital, Seinäjoki.

Eligible patients were adults (18–80 years old) hospitalized due to ST- or non-ST-elevation myocardial infarction (STEMI/NSTEMI) who had undergone diagnostic angiography with or without percutaneous coronary intervention within 96 h preceding recruitment to the trial. We planned to recruit 200 patients for this safety pilot trial. All patients had 1) symptoms suggestive of myocardial infarction [11], 2) new-onset ischemic ECG changes, and 3) biochemical markers of myocardial damage. For inclusion, all patients also had to be willing to participate and able to understand and sign informed consent. Major exclusion criteria were: age > 80 years, thrombolysis <12 h earlier, cardiomyopathy, life-threatening cardiac arrhythmias, prolonged QTc interval (>470 ms), and life expectancy <1 year.

The first patients for the trial were recruited on February 15, 2016, and recruitment was completed on December 14, 2018, with the last follow-up visits at the end of 2019. Flowchart for the screening, inclusion process, and analyses are shown in online supplementary Fig. 1.

2.3. Randomization

The randomization (1:1) was computer generated and performed by an independent hospital pharmacist at Helsinki University Central Hospital, who assigned patient numbers for each center (in blocks of 10) and delivered study medication. The investigators were blinded to treatment (active drug or placebo) at all times. The randomization codes could be requested from the hospital pharmacy if considered necessary for the treatment of severe adverse events; however, unblinding was not necessary during the trial.

Hydroxychloroquine or placebo was administered in addition to patients' normal post-percutaneous coronary intervention medication, which (drugs and dosages) was at the discretion of the treating physician. Medical history was taken and physical examination, laboratory evaluation, and ECG recording were performed at randomization, and at the second (at 1 month), third (at 6 months; end of study medication), fourth (at 9 months; only telephone call from a nurse), and fifth (at 12 months; end of study) visits. Thereafter, information about the defined endpoints was obtained from hospital records and telephone inquiries. Patient information was collected until December 31, 2019.

2.4. Outcomes

The primary endpoints of the trial were combination of acute myocardial infarction, hospitalization due to recurrent unstable angina, hospitalization due to heart failure, and death within 1 year. The secondary endpoint was the composite of the primary endpoint, urgent percutaneous coronary intervention or coronary artery bypass surgery, and stroke within 1 year, and also the effect of hydroxychloroquine treatment on the level of inflammatory parameters such as hs-CRP, IL-6.

2.5. Safety and adverse events

For the safety analysis, all adverse events occurring up to 12 months were included. All known side effects of hydroxychloroquine were considered, and in particular, we attempted to monitor all cardiac adverse events such as QTc prolongation, arrhythmias, heart failure symptoms, changes in ejection fraction measured by echocardiography, and laboratory changes suggestive of heart failure (brain natriuretic propeptide [proBNP]) or cardiomyopathy (high-sensitivity troponin I).

2.6. Statistical analyses

Gaussian-distributed data were described as mean ± SD and compared by two-tailed *t*-test. Non-Gaussian data were described with medians and interquartile ranges (IQRs) and analyzed using the Mann-Whitney *U* test or t-test after logarithmic transform. Nominal data were presented using proportions and analyzed by the $\chi 2$ test or Fisher's exact test. Repeated measures ANOVA was used for studying changes of the population mean over time after logarithmic transform with non-Gaussian data, for further analysis, a Linear Mixed Model Analysis For Repeated Measures was used with classical risk factors. Event-free survival between groups was analyzed with the log-rank test. The analyses were performed by using SPSS version 25.0 (IBM) and Python language.

2.7. Patient and public involvement

Patients were not involved in the design and conduct of this research. Participants were informed of their personal results by sending letters or by personal phone calls.

3. Results

3.1. Patients

The demographic and baseline clinical characteristics of the participants were similar between the two groups (Table 1). Patients were admitted to the hospital due to acute STEMI or NSTEMI. Coronary angiogram was performed in all patients and percutaneous coronary intervention to 120 (96%) of the patients. Randomization took place on an average of 42.7 ± 26.9 h after hospitalization. The total follow-up time was 31.8 ± 10.3 months. Due to a slow recruitment rate and the fact that there were fewer endpoints than anticipated, we decided to end the study when 125 patients had been randomly allocated. As the study was underpowered for the original primary endpoint, this study aims to assess the anti-inflammatory effect and safety of hydroxychloroquine.

Table 1
Baseline characteristics.

Characteristic	Hydroxychloroquine	Placebo	p value
	n = 64	n = 61	
Demographics			
Female	15 (23.4%)	11 (18.0%)	0.46
Age (years)	59.9 ± 9.5	58.5 ± 9.9	0.41
BMI (kg/m^2)	28.4 ± 4.5	28.0 ± 5.2	0.71
Risk factors			
Current smoker	27 (42.2%)	28 (45.9%)	0.37
Diabetic	7 (10.9%)	9 (14.8%)	0.52
Hypertensive	35 (54.7%)	28 (45.9%)	0.19
Dyslipidemia	47 (73.4%)	38 (62.3%)	0.21
Family history of coronary disease	26 (40.6%)	28 (45.9%)	0.59
Type of Infarction			0.21
STEMI	44 (68.8%)	48 (78.7%)	
NSTEMI	20 (31.3%)	13 (21.3%)	
Area of ischemia			0.27
Anterior	18 (28.1%)	17 (27.9%)	
Lateral	6 (9.4%)	1 (1.6%)	
Inferior	19 (29.7%)	23 (37.7%)	
Combination of above	21 (32.8%)	20 (32.8%)	
Initial treatment			
Percutaneous coronary intervention	59 (92.2%)	61 (100%)	0.06
Number of vessels treated with PCI			0.11
0 vessel PCI	5	0	
1 vessel PCI	48	50	
2 vessel PCI	10	11	
3 vessel PCI	1	0	
LVEF %	57.2 ± 9.6	54.9 ± 9.8	0.20
LVEF normal /mild / moderate HF	52/6/1	45/15/1	0.045
Medication			
Aspirin	64 (100%)	60 (98.4%)	0.49
Other antiplatelet	64 (100%)	61 (100%)	1.0
Statin	64 (100%)	61 (100%)	1.0
β-blocker	53 (82.8%)	51 (83.6%)	1.0
ACE inhibitor/AT blocker	63 (98.4%)	60 (96.4%)	1.0
Laboratory results			
Hemoglobin (g/l)	141.0 ± 13.6	139.3 ± 14.1	0.50
GFR ml/min/1.73 m^2	88.8 ± 12.5	89.3 ± 12.3	0.82
Total cholesterol (mmol/l)	4.8 ± 1.2	4.8 ± 0.9	0.87
LDL-cholesterol (mmol/l)	3.2 ± 1.1	3.3 ± 0.9	0.43
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.3	0.53
Gluc (mmol/L)	6.3 ± 1.2	6.5 ± 1.5	0.49
HBA1c (mmol/mol)	37.6 ± 6.4	37.4 ± 7.2	0.86

Data are n (%), mean ± SD, or median (IQR). ACE = Angiotensin-converting enzyme, AT = Angiotensin,
BMI=Body mass index, GFR = Glomerular filtration rate, HBA1c = Glycated hemoglobin A1c, HDL = High-density lipoprotein cholesterol, HF=Heart failure, LDL = Low-density lipoprotein cholesterol, LVEF = Left ventricular ejection fraction, PCI=Percutaneous coronary intervention, STEMI=ST-elevation myocardial infarction, NSTEMI=Non-ST-elevation myocardial infarction.

3.2. Outcome and endpoints

3.2.1. Hs-CRP

Hs-CRP levels were elevated (>3 mg/l) in 41 patients in the hydroxychloroquine group and 46 patients in the placebo group at the onset of the study. There were no statistically significant differences between groups at any time points (Table 2). Overall hs-CRP levels declined during the study period ($p < 0.0001$, between the onset of the study and the 12-month visit, among all study patients; Fig. 1, panel A) and remained above 3 mg/l in 12 and 8 patients in the hydroxychloroquine and placebo groups, respectively. Two patients had acute respiratory infections (one in the placebo group at 1-month visit and one in the hydroxychloroquine group at three months visit). Their hs-CRP values were elevated >30 mg/l, and hs-CRP and corresponding IL-6 values were excluded from the analysis (online supplemental Fig. 1, panel A). Linear Mixed Model Analysis for Repeated Measures on hs-CRP did not change the original analysis.

3.2.2. Plasma IL-6 levels

On intention-to-treat analysis the level of IL-6 was elevated after the myocardial infarction in both groups, ($p = 0.179$, between groups, Fig. 1, panel B and Table 2), and decreased significantly thereafter up to the six-month visits in both groups ($p < 0.0001$ between the baseline and the six-month visits, among all study patients; Fig. 1, panel B). The level of IL-6 was significantly lower at the six-month visits in the hydroxychloroquine group as compared with the placebo group ($p = 0.042$, Fig. 1, Panel B and Table 2). At 12 months there were no differences between groups ($p = 0.907$). In the on-treatment analysis, at six months the decline of IL-6 levels in the hydroxychloroquine group was more pronounced ($p = 0.019$ between groups, Fig. 1, Panel D and Table 2). Linear Mixed Model Analysis for Repeated Measures on IL-6 showed that there were interactions on the intention-treat analysis between IL-6 and BMI ($p < 0.0001$), smoking ($p = 0.026$) and age ($p < 0.0001$), and that there were significant differences between study groups ($p = 0.009$). Similarly, there were interactions on on-treatment analysis between IL-6 and BMI ($p < 0.0001$), age ($p < 0.0001$), smoking ($p = 0.019$), and if the patient had STEMI/NSTEMI ($p = 0.044$), and there were significant differences between study groups ($p = 0.005$). Linear Mixed Model Analysis for Repeated Measures corroborated above more simple statistical analyses.

IL-6 to hs-CRP correlations were as follows: Baseline, $r = 0.36$, $p < 0.0001$, Visit 2: $r = 0.39$, $p < 0.0001$, Visit 3: $r = 0.39$, $p < 0.0001$, Visit 4: $r = 0.48$, $p < 0.0001$.

3.2.3. Endpoints

Primary endpoints were observed in 1 patient in the hydroxychloroquine group (cardiac death, 2 months after the end of the study medication) and in 2 patients in the placebo group (unstable angina hospitalization and heart failure hospitalization). We observed a secondary endpoint in 1 patient in the placebo group (urgent percutaneous revascularization, a catheterization complication), and a tertiary endpoint in 1 patient in the hydroxychloroquine group (after the 1-year prespecified endpoint period; an unstable angina hospitalization); in addition, a new myocardial infarction was recorded in 1 patient in the placebo group (after the 1-year prespecified endpoint period). The difference in the number of endpoints did not reach the level of significance. The number of endpoints was clearly lower than anticipated (online supplementary Table 1).

3.2.4. Safety aspects

Of the enrolled participants, 117 (93.6%) attended the 6-month visit, and 102 (81.6%) completed 80% of the study medication (>143 of the 180 tablets). Altogether there were 226 treatment-emergent adverse events (online supplementary Table 2), and 113 patients (90.4%) had at least one event. In the hydroxychloroquine group, adverse events led to the withdrawal of the medication in 7 patients, and in 5 patients

Table 2
Selected laboratory values on intention-to-treat and on-treatment analysis.

		Hydroxychloroquine				Placebo			
Intention-to-treat	*n*	*Median*	*IQR 25*	*IQR75*	*n*	*Median*	*IQR 25*	*IQR75*	*P*
hs-CRP at baseline (mg/l)	57	7.05	1.94	22.35	59	7.58	2.63	26.96	0.791
hs-CRP at 1 mo (mg/l)	55	1.03	0.47	2.04	56	1.06	0.47	2.62	0.569
hs-CRP at 6 mo (mg/l)	53	0.70	0.41	2.14	52	1.06	0.43	1.85	0.448
hs-CRP at 1y (mg/l)	52	0.80	0.41	2.48	50	0.75	0.35	1.59	0.352
IL-6 at baseline (ng/l)	63	8.39	4.68	13.32	60	9.81	5.49	26.10	0.179
IL-6 at 1 mo (ng/l)	58	1.91	1.33	2.63	56	2.10	1.46	3.36	0.234
IL-6 at 6 mo (ng/l)	56	1.49	1.10	2.18	53	1.75	1.30	2.62	0.042
IL-6 at 1y (ng/l)	53	1.67	1.24	2.38	50	1.57	1.16	2.41	0.907
ProBNP at baseline (ng/l)	64	1024	447	1709	61	943	292	2159	0.787
ProBNP at 1 mo (ng/l)	60	338	149	781	57	290	96	749	0.780
ProBNP at 6 mo (ng/l)	58	120	68	250	54	126	54	224	0.710
ProBNP at 1y (ng/l)	56	126	57	226	50	88	34	203	0.239
TNI at baseline (ng/l)	64	6868.0	2235.0	16,328.0	61	9098.0	1998.0	19,927.0	0.422
TNI at 1 mo (ng/l)	60	10.00	6.00	18.00	57	12.00	5.00	23.00	0.645
TNI at 6 mo (ng/l)	58	6.00	4.00	10.00	54	6.50	4.75	13.00	0.588
TNI at 1y (ng/l)	56	5.00	3.25	7.00	50	6.00	4.00	11.00	0.296
QTc at baseline (ms)	64	436	418	454	61	426	415	447	0.127
QTc at 1 mo (ms)	62	428	407	445	61	416	398	435	0.087
QTc at 6 mo (ms)	61	422	403	441	56	407	392	423	0.020
QTc at 1y (ms)	60	413	397	431	55	408	397	428	0.632
On treatment	*n*	*Median*	*IQR 25*	*IQR75*	*n*	*Median*	*IQR 25*	*IQR75*	*P*
hs-CRP at baseline (mg/l)	57	7.05	1.94	22.35	59	7.58	2.63	26.96	0.926
hs-CRP at 1 mo (mg/l)	54	1.01	0.47	1.84	56	1.06	0.47	2.62	0.340
hs-CRP at 6 mo (mg/l)	45	0.70	0.37	2.14	48	1.06	0.42	1.85	0.364
hs-CRP at 1y (mg/l)	45	0.79	0.40	2.46	46	0.81	0.39	1.59	0.609
IL-6 at baseline (ng/l)	63	8.39	4.68	13.32	60	9.81	5.49	26.10	0.590
IL-6 at 1 mo (ng/l)	57	1.88	1.32	2.64	56	2.10	1.46	3.36	0.207
IL-6 at 6 mo (ng/l)	49	1.42	1.08	2.05	49	1.76	1.32	2.62	0.019
IL-6 at 1y (ng/l)	47	1.56	1.18	2.17	46	1.61	1.23	2.44	0.454
ProBNP at baseline (ng/l)	64	1024	447	1709	61	943	292	2159	0.787
ProBNP at 1 mo (ng/l)	59	328	139	736	57	290	96	749	0.710
ProBNP at 6 mo (ng/l)	50	118	68	245	50	118	54	224	0.841
ProBNP at 1y (ng/l)	49	124	58	221	46	88	34	198	0.255
TNI at baseline (ng/l)	64	6868.0	2235.0	16,328.0	61	9098.0	1998.0	19,927.0	0.422
TNI at 1 mo (ng/l)	59	10.00	6.00	18.00	57	12.00	5.00	23.00	0.710
TNI at 6 mo (ng/l)	50	6.00	3.75	9.25	50	6.50	4.75	13.00	0.689
TNI at 1y (ng/l)	49	5.00	3.00	7.00	46	6.00	4.00	11.00	0.277
QTc at baseline (ms)	64	436	418	454	61	426	415	447	0.127
QTc at 1 mo (ms)	60	427	406	446	59	416	397	431	0.118
QTc at 6 mo (ms)	51	424	410	442	50	407	390	423	0.004
QTc at 1y (ms)	50	411	397	430	48	407	398	428	0.312

IL-6 = Interleukin-6, IQR = interquartile range, mo = month, ms = milliseconds, proBNP = pro brain natriuretic peptide, QTc = corrected, QT-time, TNI = Troponine I, y = year.

the withdrawal was due to a patient-related reason. In the placebo group, the corresponding numbers of patients were 4 and 7, respectively (p = ns, between groups). In the hydroxychloroquine group, 4 other patients had adverse events ($n = 2$ stomach pain, $n = 1$ diarrhea, n = 1 liver enzyme elevation) leading to interruption of medication; however, the medication could be continued later. There were 6 serious adverse events (alcohol abuse leading twice to hyponatremia in the same patient; infection in a joint prosthesis; left ventricular thrombus; repeated percutaneous coronary intervention; and an elective angiogram), none of which were considered to be related to the study medication. Arrhythmias were recorded as treatment-emergent adverse events in 6 patients in the hydroxychloroquine group (n = 1 atrial fibrillation, $n = 5$ palpitations) and 10 patients in the placebo group (n = 1 atrial fibrillation, $n = 8$ palpitations, n = 1 bradycardia leading to pacemaker implantation).

3.2.5. proBNP

ProBNP was obtained as a safety measure. It was within normal limits at the beginning of the study in 12 patients (5 and 7 in the hydroxychloroquine and placebo groups respectively). LVEF data were available for all these 12 patients, and values were normal in all cases. All other patients had elevated proBNP levels at the first visit, and there were no significant differences between study groups (Table 2). Overall proBNP levels decreased significantly during the study period ($p < 0.0001$, between the baseline and at 12 months, for all participants). ProBNP level remained above normal limits (<229 ng/l) on the last visit in 24 patients, (13 in the hydroxychloroquine group and 11 in the placebo group (p = ns)). Online supplement Fig. 1, panel C shows individual changes in ProBNP level during the study period; hydroxychloroquine did not have any negative effect on proBNP recovery.

3.2.6. Troponin I

Cardiac troponins (T- or I-type) were measured when patients arrived at the emergency department with myocardial infarction and were elevated in all patients. In addition, plasma troponin I levels were measured from blood samples collected on the day of randomization. Troponin I levels had already normalized on the day of randomization in 1 patient in the placebo group, and at the second visit for all patients except 2 patients in the hydroxychloroquine group and 6

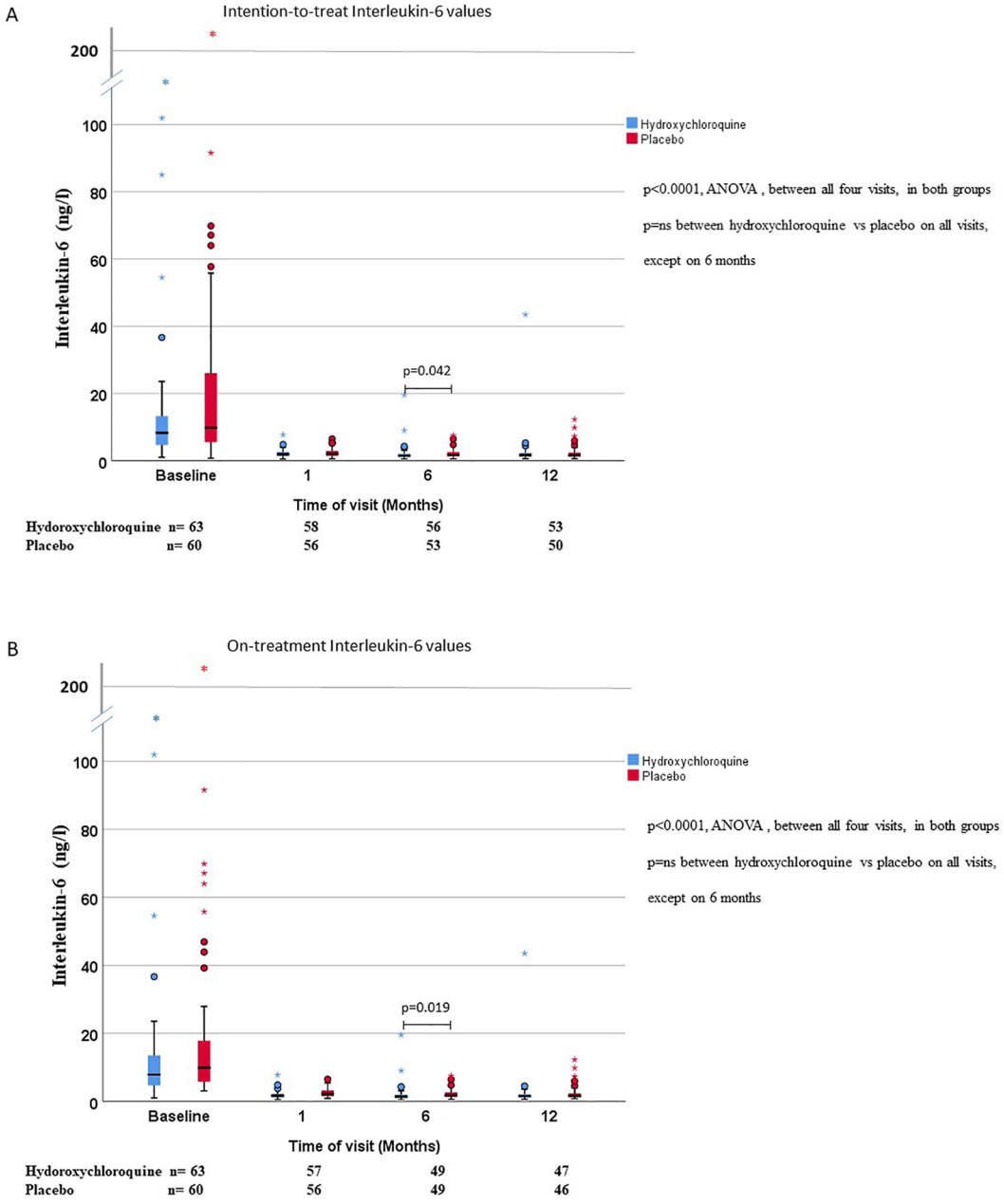

Fig. 1. IL-6 values: Intention-to-treat and on-treatment analysis. Panel A Interleukin-6 intention-to-treat analysis. Panel D–B Interleukin-6 on-treatment analysis. ANOVA for repeated measures are calculated of those cases all four measurements were available. IL-6 = Interleukin-6.

patients in the placebo group. Overall the troponin I levels declined during the study period ($p < 0.0001$, ANOVA across four study visits among all participants; Table 2).

The reason for the slightly elevated troponin I levels remains unclear. Patients were symptomless, and their creatinine levels and LVEFs were normal. In 4 patients (two in each group), proBNP was also mildly elevated (from 251 to 374 ng/l; normal range < 229 ng/l). Individual troponin I level variation is shown in online supplementary Fig. 2, panel D.

3.2.7. QTc

QTc intervals were comparable in the hydroxychloroquine and placebo groups at the beginning of the study ($p = 0.127$, Table 2) and at 1 month ($p = 0.087$). At 6 months, the QTc interval was longer in the hydroxychloroquine group than in the placebo group ($p = 0.02$; Table 2). At the end of the study medication period, the QTc intervals were again similar in the two groups ($p = 0.632$, Table 2), and lower than at study onset ($p < 0.0001$ for all patients at study onset vs. at 1 year). Individual changes during the study visits are shown in the online supplementary Fig. 2, panel E. In two cases (one in each group), QTc was prolonged during the medication period to >500 ms, leading to the withdrawal of the study medication. During the study period, 7–17% of patients received other potentially QTc-prolonging drugs, such as antidepressants or loop diuretics (online supplementary Table 3). These drugs did not have significant effect on the QTc interval (data not shown). In the on-treatment analysis, at six months visit, QTc prolongation was more pronounced ($p = 0.004$, hydroxychloroquine vs. placebo group, Table 2).

3.2.8. Left ventricular ejection fraction (LVEF)

LVEF was measured at the beginning of the study in 119 (95.2%) patients, during the period of study medication in 33 (26.4%), and after the study medication period in 54 (43.2%) patients. LVEF was below normal

at study onset in 23 (18.4%) patients. LVEF normalized during the medication period in 4/23 (17.4%) patients, and by the end of the study (1 year) in 8/23 (34.8%). LVEF remained reduced in 3/23 (13.0%) patients. Data were missing for eight patients with initially low LVEF. There were significantly fewer patients with reduced LVEF in the hydroxychloroquine group than in the placebo group at the beginning of the study (7 vs. 16, $p = 0.045$). Among those patients for whom follow-up records are available, LVEF normalized at a similar rate in both study groups. No ICDs were implanted.

4. Discussion

Hydroxychloroquine has been shown to diminish the production of IL-6 in cell culture models [2] and to reduce cardiovascular events in patients with rheumatic diseases [12]. Here we observed, for the first time that hydroxychloroquine treatment decreased the IL-6 levels after myocardial infarction in patients who had no rheumatic disease. At the beginning of the study, acute myocardial infarction induced inflammation and elevated IL-6 levels in both study groups. Attenuation of these values was observed over time, however, the attenuation was steeper in the hydroxychloroquine group. The effect of hydroxychloroquine on cytokine IL-6 levels became apparent only at the end of the treatment period, suggesting of slow onset of hydroxychloroquine's anti-inflammatory action. A slow onset of anti-inflammatory therapeutic efficacy is typical for hydroxychloroquine as it may take up to 4 to 6 weeks for the onset, and 3 to 6 months to achieve the maximal clinical efficacy [13]. Six months after stopping the hydroxychloroquine treatment, the IL-6 level somewhat declined in placebo group, but slightly increased in hydroxychloroquine group, after which the both groups were at comparable levels. This suggests as anticipated that the effect of hydroxychloroquine on IL-6 levels lasts as long as the medication is used.

IL-6 has a causal role in myocardial infarction [14]. One dose of the IL-6 receptor antagonist tocilizumab soon after non-ST-elevation myocardial infarction reduced CRP and troponin levels, and increased myocardial salvage as measured by magnetic resonance imaging, implying that it would be beneficial for the treatment of myocardial infarction patients [5,21]. According to the CANTOS [3], the CIRT [15], and the COLCOT studies [4], the important target in the prevention of cardiovascular events would be the NLRP3–IL-1β–IL-6 axis. In the CIRT study, a low dose methotrexate did not reduce IL-6 or CRP levels, nor did it reduce cardiovascular end points [15]. In the CANTOS [3] study, blocking IL-1β with the monoclonal antibody canacinumab resulted also in lowering of IL-6 levels, but there was also an increased rate of fatal infections. Colchicine on the other hand inhibits tubulin polymerization and microtuble generation, and the action is probably attributed to NLRP3 inhibition, leading to reductions in cardiovascular end points, but not in CRP level [4]. In the present study, hydroxychloroquine lowered IL-6 levels but not those of CRP which implies that the anti-inflammatory effect of hydroxychloroquine may be limited just to IL-6. IL-6/CRP correlation coefficients were about 0.4 throughout the study, suggesting that IL-6 explains about 40% of the CRP changes [16,17]. This is corroborated by earlier literature in which the correlation coefficient varied from 0.39 to 0.79 [18,19]. The synthesis of CRP is induced most notably by IL-6 and to a lesser degree by IL-1 and tumor necrosis factor alpha. Since we do not have at this point any tumor necrosis factor alpha or IL-1 results available, we cannot speculate about their possible impacts on the CRP levels in this study.

Hydroxychloroquine's safety after myocardial infarction is not established. In our knowledge, this is the first trial were hydroxychloroquine was administered in a randomized placebo-controlled manner, starting within few days after myocardial infarction, and continued for 6 months. Patients with recent myocardial infarction are likely to be at risk for cardiac adverse events. Therefore patients were carefully selected for the absence of life-threatening arrhythmias, known previous cardiomyopathy (ejection fraction could be reduced due to recent myocardial infarction), and severely prolonged QTc interval. Study medication was started only after all above safety issues were secured. There were no differences in the rates of arrhythmia, heart failure, cardiac damage measured by troponin release, or death between groups during the medication period. Only 16% of patients stopped treatment because of adverse effects, and no serious adverse events were attributed to hydroxychloroquine.

Almost all patients received standard secondary prevention pharmacotherapies such as statins, aspirin, β-blockers, and ACE inhibitors. Indeed, it is most probable that such highly effective preventive medication contributed to the very low number of endpoints. Moreover, the use of β-blockers most likely reduced incidence of cardiac arrhythmias. Of note, 7–17% of patients received, besides hydroxychloroquine, also other QTc-prolonging drugs, such as antidepressants or loop diuretics. In contrast to previously published results, these additional drugs did not prolong the QTc interval significantly [8].

Acute myocardial ischemia prolongs the QTc interval, which was reported to recover to the patient's normal level within 4 weeks after myocardial infarction [18]. In this OXI trial, such a QTc interval pattern was observed in the placebo group. However, the use of hydroxychloroquine delayed recovery of the QTc interval after myocardial infarction until the medication was stopped, but the QTc interval remained within normal limits, and arrhythmias were not induced. Because of concerns about QTc prolongation, careful ECG monitoring throughout the study was an essential part of the protocol. Patients with a QTc interval > 470 ms were excluded from the study, and medication was stopped in two patients (one in each group) when the QTc interval became prolonged (>500 ms) during the medication period.

Long-term use of hydroxychloroquine has been found to associate with an increased risk of cardiotoxicity, which is presumably caused by compromised lysosomal function and ensuing accumulation of metabolic products [19]. Thus, hydroxychloroquine use has been linked to ventricular hypertrophy, heart failure, and cardiomyopathy. The patients in this study received less than 60 g of hydroxychloroquine during the study, which is a low dose when compared with the wide range of cumulative doses (270–9125 g) that have been observed necessary for the induction of cardiac damage [20]. Therefore cardiomyopathy was not to be expected to occur in the present study. All of our patients had suffered a myocardial infarction with varying levels of myocardial damage, most of them were, however, relatively small. Heart failure with a reduced ejection fraction was detected only in 20%. Left ventricular ejection fraction was normalized at a similar rate after myocardial infarction in both groups, and hydroxychloroquine did not delay this improvement. Of note, a decreased ejection fraction did not provoke any arrhythmias in the patients.

4.1. Limitations

First, this was a safety pilot trial, therefore it is unclear whether hydroxychloroquine is effective for secondary prevention of myocardial infarction. Second, patient numbers were small so that rare adverse events or cardiac conditions that may appear only in a large-scale study could have been missed. Thirdly, it is possible that a treatment duration longer than 6 months could have caused other side effects such as eye-related problems, neuropathies, or cardiomyopathies. Finally, the study medication was initiated, on average, 43 h after myocardial infarction. The inflammatory process is well established by that time. Hydroxychloroquine medication could have been more effective if started immediately, but taking into consideration the relatively slow onset of effect of hydroxychloroquine the delay in onset of medication is unlikely to be significant. However, we had to balance between possible risks and the efficacy of the investigational medication, and we wanted to be on the safe side.

5. Conclusion

We conclude that hydroxychloroquine lowers IL-6 levels more than placebo, suggesting that it might be beneficial in reducing cardiovascular inflammation, Hydroxychloroquine 300 mg per day (for patients who weigh <60 kg: 300 mg on 5 days a week) can be safely administered within 96 h after myocardial infarction, provided that the patients have been carefully selected and that safety measures are carefully considered. Based on the findings of this study, a larger trial is warranted.

Contributors

The study was conceived and designed by KKE, PTK, and JS. LU, HT, OH, TTR, RP, JK, OA, TN, JY, RR, TD, SU, PK, IA, and JS collected data. All authors had full access to the data output and interpreted the data. MK and JS conducted the statistical analyses. KN, KS, KKE, and JS provided biomarker input, data analysis, and interpretation. JS, KKE, JL, and LU wrote the first draft of the manuscript. All authors critically reviewed the manuscript.

Data sharing

Qualified researchers can request access to additional data from the corresponding author.

Funding

This study was supported in part by the Aarno Koskelo Foundation, Finnish Cultural Foundation, Finnish Foundation for Cardiovascular Research, and Paavo Nurmi Foundation, and received special government funds and a non-restricted grant from the Finnish Cardiac Society/AstraZeneca. Wihuri Research Institute is maintained by the Jenny and Antti Wihuri Foundation. Orion Pharma provided the trial medication.

Declaration of Competing Interest

JS reports personal fees from Abbott, Amgen, and Bayer and a grant from AstraZeneca. PTK reports personal fees from Amgen, Novartis, Raisio Group, and Sanofi.

Acknowledgements

The authors greatly appreciate the technical assistance of Kati Oksaharju and Lauri Snellmann. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Appendix A. Appendix

The following investigators and institutions, listed in alphabetical order according to city (all located in Finland), participated in the OXI trial (number of patients enrolled in each center is shown in parentheses): Heart and Lung Center in Helsinki University Hospital: OH, HT, PK, JK, JS (99); North Karelia Central Hospital, Joensuu: TTR (11); Kymenlaakso Central Hospital, Kotka: JY, RR (2); Päijät-Häme Central Hospital, Lahti: HT, RP, OA (7); South Karelia Central Hospital, Lappeenranta: TD, SU (5); Seinäjoki Central Hospital, Seinäjoki: IA (1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.04.062.

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