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The effect of hydroxychloroquine on cholesterol metabolism in statin treated patients after myocardial infarction

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ABSTRACT

Background and aims: To evaluate the effect of hydroxychloroquine (HCQ) on serum and lipoprotein lipids and serum biomarkers of cholesterol synthesis and absorption in myocardial infarction patients with a high-dose statin.

Methods: Myocardial infarction patients (n = 59) with a constant statin dose were randomized to receive hydroxychloroquine 300 mg (n = 31) or placebo (n = 28) daily for six months and followed up for one year.

Results: Statin reduced total-c (−26 ± 22% in hydroxychloroquine and −28 ± 19% in placebo group, P = 0.931), LDL-c (−38 ± 26% vs. −44 ± 23%, respectively, P = 0.299), and cholesterol synthesis biomarkers zymostenol, desmosterol, and lathosterol ratios from baseline to one year (e.g., serum lathosterol ratio −17 ± 45% vs. −15 ± 41%, respectively, P < 0.001 for both, P = 0.623 between groups). Compensatorily, cholesterol absorption increased during the intervention (e.g., serum campesterol ratio 125 ± 90% vs. 113 ± 72%, respectively, P < 0.001 for both, P = 0.488 between groups). Hydroxychloroquine did not affect cholesterol concentrations or cholesterol absorption. It prevented the statin-induced increase in cholesterol precursor, desmosterol ratio, from six months to one year in the hydroxychloroquine group (P = 0.007 at one year compared to placebo).

Conclusions: Combined with a high-dose statin, hydroxychloroquine had no additional effect on serum cholesterol concentration or cholesterol absorption. However, the findings suggest that hydroxychloroquine interferes with lanosterol synthesis, and thereafter, it temporarily interferes with the cholesterol synthesis pathway, best seen in halting the increase of the desmosterol ratio.

Trial Registration ClinicalTrials.gov Identifier: NCT02648464.

1. Introduction

Hydroxychloroquine (HCQ), a hydroxylated derivative of chloroquine (CQ), is an antirheumatic drug that is widely used in the treatment of autoimmune rheumatic diseases. It has several immunomodulatory and anti-inflammatory effects and has recently been the focus of increasing interest. In addition, HCQ affects lipid metabolism in subjects with autoimmune rheumatic diseases. In systematic reviews and meta-analyses, including almost 40,000 patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), HCQ significantly reduced serum total cholesterol (total-c) and low-density lipoprotein (LDL) cholesterol (LDL-c) concentrations from baseline values in comparison to HCQ non-users [1–3]. In addition, in one study HCQ has also increased high-density lipoprotein (HDL) cholesterol (HDL-c) and reduced serum triglyceride concentrations [1].

HCQ belongs to a group of cationic amphiphilic drugs like, e.g.,

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amiodarone. These drugs have a lipophilic aromatic ring and a hydrophilic side chain, and they interact with cell membranes and accumulate in acidic intracellular compartments [4–6]. Their accumulation, especially in the late endosomes/lysosomes, interferes with the cellular trafficking of cholesterol and phospholipids, and can cause a storage disorder called phospholipidosis. Furthermore, the accumulation of HCQ interferes with the cellular metabolism of lipids and other essential cellular processes such as membrane permeability, the activity of several enzymes, and autophagy. Because of the anti-inflammatory and cholesterol-lowering properties of HCQ observed in autoimmune rheumatic diseases [1–3], we initiated a randomized, placebo-controlled, double-blind OXI trial to determine whether HCQ treatment reduces the risk of recurrent cardiovascular events in patients with acute myocardial infarction (MI) [7,8]. The results showed that HCQ treatment for six months was safe, and no significant adverse effects were observed in the treatment group. A reduction of the plasma interleukin-6 levels was seen in HCQ-treated post-MI patients [8]. It is unknown whether HCQ interferes with serum and lipoprotein lipids in subjects who do not have an autoimmune rheumatic disease and whether HCQ has an additional lipid-lowering effect in patients who use statin. Therefore, we investigated the effect of HCQ combined with statins on serum lipids and cholesterol synthesis and absorption in MI patients by using assays of the serum noncholesterol sterol ratios to cholesterol, surrogate serum biomarkers of cholesterol synthesis and absorption [9–13].

2. Materials and methods

2.1. Patients

The original trial design, randomization, and patient recruitment were published previously [8]. In brief, the trial consisted of 125 myocardial infarction patients who were randomized in a median of 43 h after hospitalization to receive either HCQ (Orion Pharma, Espoo, Finland) 300 mg once daily (weight <60 kg: received 300 mg for five days per week) or placebo for six months.

Of these patients, we selected 59 patients who used a constant dose of statin and adhered to randomized study medication throughout the whole study period.

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. The trial was registered on ClinicalTrials.gov Identifier: NCT02648464. 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. Methods

Serum total-c, LDL-c, HDL-c, and triglycerides were analyzed enzymatically using the photometric method by the HUS Diagnostic Center of the Helsinki University Hospital. Serum cholesterol, cholesterol synthesis biomarkers (squalene, lanosterol, zymostenol, desmosterol, and lathosterol) (Fig. 1), cholesterol absorption biomarkers (sitosterol and campesterol, i.e., plant sterols), and cholestanol (a metabolite of cholesterol) were analyzed using gas–liquid chromatography (GLC) with a 50-m capillary column (Ultra 2, Agilent Technologies, Wilmington, DE) and flame ionization detection with 5α-cholestane as internal standard [14]. The samples from different times per subject were analyzed in the same GLC run. Serum concentrations of the noncholesterol sterols and squalene were adjusted to that of cholesterol in the same GLC run and expressed as ratios to cholesterol (10^2 μmol/mmol of cholesterol) to enable comparison between subjects with different LDL-c levels. Serum cholesterol precursors, plant sterols, and cholestanol as ratios to cholesterol are validated biomarkers of cholesterol synthesis and absorption [9–16]. Therefore, the serum noncholesterol sterol biomarkers are given as ratios to cholesterol herein.

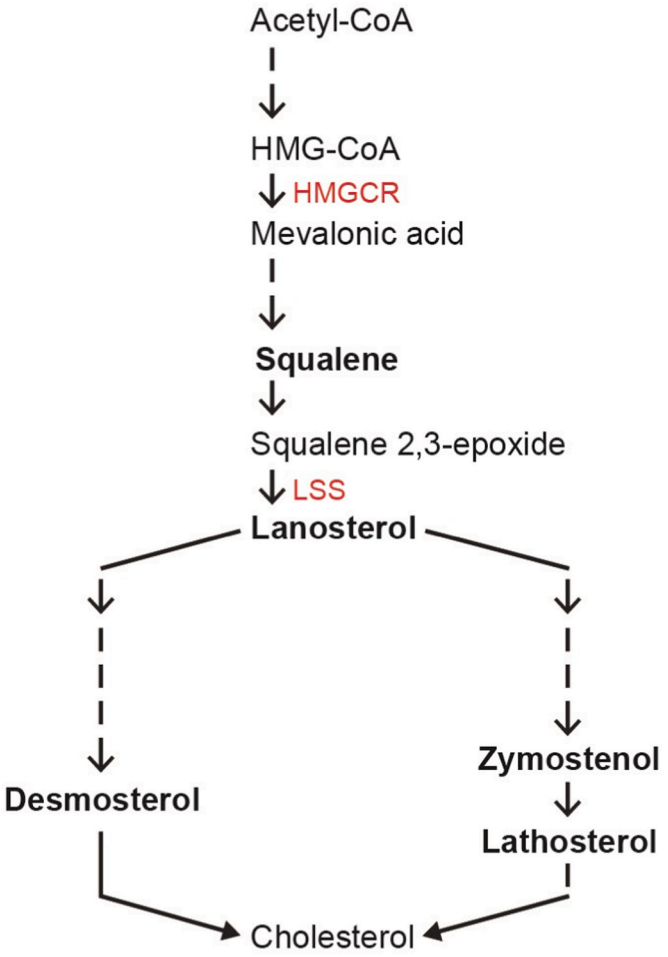

Fig. 1. The cholesterol synthesis pathway emphasizing the precursors dealt with in this study and indicated in bold font. Broken arrows denote omitted precursors. HMG-CoA = hydroxymethylglutaryl-coenzyme A; HMGCR = hydroxymethylglutaryl-coenzyme A reductase; LSS = lanosterol synthase.

2.3. Demonstration of results

Details in the supplement.

2.4. Lipid value comparison between the original study group and 59 patients analyzed in this article

Details in the supplement.

2.5. Determination of drug concentrations

Details in the supplement.

2.6. Statistics

The statistical analyses were performed with SPSS for Windows 25.0 (SPSS, Chicago, IL). Sample size calculation was based on significance levels (a = 0.05 and b = 0.20) and the essential information on HCQ cholesterol-lowering properties was obtained from the previous studies [1–3]. Using these estimates, the size of the required population was appropriate. Before analysis, the normal distribution of the data was evaluated with the Kolmogorov- Smirnov test and homogeneity of variance with Levene's test. Variables not normally distributed were

transformed logarithmically. Time-dependent continuous variables in the groups were compared by repeated measures analysis of variance (ANOVA), one-samples t-test, independent samples t-test, and paired samples t-test. We used Bonferroni correction to adjust for multiple comparisons. Noncontinuous variables were analyzed by using the chi-square test, Fisher's exact chi-square test, or Mann-Whitney U-test. Spearman's correlation coefficients were calculated. A P-value of <0.05 was considered statistically significant, whereas a P-value of >0.05 was denoted as nonsignificant (NS). The results are expressed as mean ± SD.

3. Results

3.1. Study population

The baseline characteristics of the patients are shown in Supplementary table 1. There were no significant differences between the study groups, except for sex (males > females, $p = 0.031$) and diabetic status (diabetics vs nondiabetic, $p = 0.049$). Neither sex nor diabetes type II interfered with the results. Most patients were males in their mid-fifties and had an ST-elevation myocardial infarction as an inclusion diagnosis.

The patients received study medication for six months. Five patients discontinued the study medication during the trial (three in the HCQ group; days 25, 44, and 115, and two in the placebo group; days 70 and 96) due to either suspected adverse effects (prolonged QT-time or increase of liver enzymes), medical event (pneumonia and prostate carcinoma) or due to unknown reason. The patients continued participation in the trial follow-up visits regardless of discontinuation. In addition to the study medication, all patients used the standard post-MI medication regimen (dual antithrombotic therapy, statin, angiotensin-converting enzyme (ACE) inhibitor/angiotensin receptor blocker (ARB), and β-blocker). Medication use did not differ between the study groups, except for metformin use at one year visit (Supplementary table 2).

Statin use during the trial is shown in Supplementary table 3. The statin dose of three patients was changed during the trial (two in the HCQ group and one in the placebo group). The change did not impact the serum total-c concentration or noncholesterol sterol ratios; therefore, the patients were included in the final analysis. Apart from one patient missing the last follow-up at one year, all patients attended all follow-up visits as planned. In both study groups, most patients received a high dose of statin (atorvastatin 80 mg once a day). Only a few patients used ezetimibe (Supplementary table 3). It did not impact the measured cholesterol levels between groups (Supplementary table 4).

3.2. HCQ and statin concentration

Supplementary table 5 describes the HCQ concentrations in the intention-to-treat analysis. The measured concentrations documented adequate treatment compliance. The highest HCQ concentration was observed at one month; thereafter, the concentration declined by about 30% by six months. After discontinuing HCQ, low levels of HCQ and its metabolites could still be measured in blood at the one-year follow-up visit in 13 patients. The sum of HCQ and metabolite concentration within the therapeutic range (500–2000 mg/ml) [17] had no significant additive effect on total-c, HDL-c, LDL-c, and triglycerides, compared to patients with concentrations below <500 mg/ml or placebo users (p = NS) according to intention to treat (Supplementary table 6) or on treatment analysis (data not shown).

The concentrations of statins and their metabolites were similar in the HCQ and placebo groups (p = NS), and there were no significant differences between the groups even after normalization of the concentrations to the most common statin dose (80 mg for atorvastatin) (Supplementary table 7). The metabolic ratio of 4-OH- atorvastatin and 2-OH- atorvastatin to atorvastatin also remained unchanged by HCQ. In conclusion, HCQ did not significantly affect blood statin concentrations or their metabolism.

3.3. Serum lipids (total-c, LDL-c, HDL-c, and triglycerides)

As expected, the mean values of serum total-c, LDL-c, and serum triglycerides decreased significantly after baseline in all patients after the onset of the standard post-MI medication regimen, including a high dose of statin. Thereafter, the values remained low at all visits. On the contrary, HDL-c was low at the baseline and increased towards the six-month follow-up visit in all patients.

Serum total-c, LDL-c, HDL-c, or triglyceride values did not differ significantly between HCQ and placebo groups at any time point of the trial in intention to treat (Supplementary table 4) or on treatment analysis (data not shown, p = NS).

As shown in Supplementary table 8, evaluation of temporal change or the difference of mean serum total-c, LDL-c, HDL-c, and triglycerides between the follow-up visits showed no significant differences in patients with HCQ compared to placebo. However, the change of mean values based on repeated measures across all visits during one year follow-up period remained significant for total-c, LDL-c, HDL-c, and triglycerides ($p < 0{,}001$, data not shown). The analysis with intention to treat principles showed no lipid-lowering benefit of HCQ in comparison to the placebo group. Three patients discontinued HCQ earlier than planned, but it did not affect the lipid values.

3.4. Serum noncholesterol sterol ratios

The noncholesterol sterol ratios reflect cholesterol metabolism only if cholesterol homeostasis is intact-in other words, the absolute cholesterol synthesis and absorption, as well as their biomarkers, are inversely related [11,15,16]. Thus, the inverse correlations between the synthesis and absorption biomarkers in both groups and at different time points confirmed the intact homeostasis. E.g., the correlation coefficients between lathosterol and campesterol at six months were $r = -0.684$, $p < 0.001$ in the HCQ and $r = -0.417$, $p < 0.05$ in the placebo group (p = NS between the groups).

From the baseline to one year, the cholesterol synthesis biomarkers squalene and lanosterol were unchanged in the HCQ group (Tables 1 and 2). In the placebo group, lanosterol increased from baseline to one month. Lathosterol decreased identically in both groups up to six months. After that, it fluctuated so that the one-year lathosterol ratios were slightly but significantly higher than the six-month ratios but still lower than the baseline values in both groups.

Zymostenol and desmosterol decreased identically in both groups up to six months (Tables 1 and 2). However, from six months to one year, they fluctuated similarly to lathosterol, but only in the placebo group. The HCQ group's one-year zymostenol and desmosterol ratios were not increased from six months. The one-year desmosterol ratio was significantly lower in the HCQ than in the placebo group ($P = 0.007$).

All cholesterol absorption biomarkers increased similarly in the HCQ and placebo groups from the baseline to one year, shown for campesterol and cholestanol (Tables 1 and 2). The one-year ratio of cholestanol was significantly lower than the respective six-month ratio in the HCQ group reflecting the counteraction of cholesterol absorption to increased cholesterol synthesis.

The impact of the serum concentration of HCQ and its metabolites on serum noncholesterol sterol ratios was evaluated at one month, six months, and one year. The only significant finding was between the one-year serum desmosterol ratio and the concentrations of serum HCQ and its metabolites (Table 3). Individuals with measurable amounts of serum HCQ and its metabolites had significantly lower desmosterol ratio than individuals without detectable serum HCQ and its metabolites or individuals in the placebo group ($P = 0.007$ between the groups).

4. Discussion

HCQ has been reported to lower the mean serum total-c, LDL-c, and triglyceride concentrations [1–3,18]. The previous studies have been

Table 1
Serum noncholesterol sterol ratios in the study population during the intervention.

Variables	HCQ[1] group [N = 31]	Placebo group [N = 28]	*P*-value
Squalene[2]			
Baseline	11.1 (4.55)	12.3 (6.77)	0.420
1 month	11.7 (5.69)	13.6 (5.25)	0.200
6 months	11.1 (5.33)	12.7 (8.27)	0.386
1 year	11.8 (6.13)	10.9 (4.50)	0.538
Lanosterol[2]			
Baseline	11.2 (2.59)	11.4 (2.77)	0.835
1 month	11.7 (3.43)	12.2 (2.44)[a]	0.512
6 months	11.8 (2.95)	11.8 (2.15)	0.988
1 year	11.4 (2.82)	12.1 (3.12)	0.360
Zymostenol[2]			
Baseline	10.0 (4.96)	9.98 (4.48)	0.968
1 month	7.35 (1.77)[a]	7.23 (2.14)[a]	0.824
6 months	7.38 (2.19)[a]	7.78 (2.13)[a]	0.489
1 year	8.15 (2.67)[a]	8.75 (2.73)[b]	0.399
Desmosterol[2]			
Baseline	69.1 (22.7)	75.1 (23.0)	0.318
1 month	53.0 (11.2)[a]	55.3 (11.0)[a]	0.421
6 months	50.5 (15.2)[a]	54.9 (11.9)[a]	0.223
1 year	51.0 (10.9)[a]	59.3 (11.9)[a,b]	0.007
Lathosterol[2]			
Baseline	53.9 (46.7)	52.5 (36.1)	0.900
1 month	30.9 (13.2)[a]	32.4 (14.5)[a]	0.670
6 months	30.0 (12.5)[a]	33.0 (13.1)[a]	0.370
1 year	35.1 (15.0)[a,b]	39.2 (14.6)[a,b]	0.293
Campesterol[2]			
Baseline	249 (116)	240 (99)	0.753
1 month	402 (180)[a]	432 (186)[a]	0.537
6 months	537 (248)[a]	523 (224)[a]	0.820
1 year	534 (297)[a]	486 (210)[a]	0.479
Cholestanol[2]			
Baseline	155 (29.2)	161 (32.3)	0.432
1 month	198 (37.9)[a]	203 (50.1)[a]	0.681
6 months	210 (44.0)[a]	217 (50.9)[a]	0.538
1 year	203 (41.5)[a,b]	210 (53.0)[a]	0.623

Mean (SD). [1]HCQ = Hydroxychloroquine. [2]10^2 μmol/mmol of cholesterol.
[a] P < 0.05 from baseline;
[b] P < 0.05 from 6 months.

performed in patients with RA or SLE and do not report statin use in patients [1–3,18]. A few studies suggest that treatment with atorvastatin in combination with HCQ had an additive lipid-lowering effect in comparison to atorvastatin only [2,19].

The present study showed that HCQ had no significant additive effects on serum total-c, LDL-c, HDL-c, and triglyceride concentrations in post- MI patients with concomitant high-dose statin treatment. Treatment compliance was good among patients. Serum total-c, LDL-c, and triglyceride values were reduced after initiation of statin treatment in all patients without significant differences between the HCQ and placebo groups. After baseline, the lipid levels remained low throughout the trial in both groups.

Regarding cholesterol metabolism, cholesterol synthesis decreased, and compensatorily cholesterol absorption increased from baseline to one year in both groups, reflecting the strict regulation of cholesterol homeostasis. After six months, there was a slight fluctuation in cholesterol synthesis and absorption, possibly because of a time-dependent fine adjustment in cholesterol homeostasis. These changes, especially in cholesterol synthesis, were more prominent in the placebo than in the HCQ group, and their magnitudes are identical to those observed in earlier statin trials [15,20]. However, the new finding indicated that HCQ did interfere with the metabolism of desmosterol from six months to one year. On the contrary to the placebo group, desmosterol was not increased in the HCQ group and remained significantly lower at one year compared with the placebo group (P = 0.007). This association seemed to be particularly clear in patients with persisting plasma HCQ and its metabolite concentrations. HCQ might also modestly interfere with the metabolism of lanosterol and zymostenol by partly inhibiting their

Table 2
The difference in serum noncholesterol sterol ratios during the intervention.

Variables	HCQ[1] group [N = 31]	P_1- value HCQ group	Placebo group [N = 28]	P_2-value, Placebo group	P_3- value, between the groups
Squalene[2]					
Baseline - 1 month	0.66 (5.66)	0.753	1.30 (6.71)	0.314	0.690
Baseline - 6 months	0.02 (4.95)	0.980	0.38 (10.1)	0.843	0.860
Baseline - 1 year	0.68 (5.78)	0.817	−1.40 (7.16)	0.311	0.222
6 months - 1 year	0.66 (5.72)	0.552	−1.78 (8.09)	0.255	0.172
Lanosterol[2]					
Baseline - 1 month	0.41 (3.36)	0.640	0.78 (2.05)	0.050	0.617
Baseline - 6 months	0.59 (3.32)	0.366	0.44 (3.04)	0.452	0.851
Baseline - 1 year	0.15 (2.63)	0.901	0.72 (3.64)	0.306	0.492
6 months - 1 year	−0.44 (3.35)	0.466	0.28 (2.48)	0.557	0.351
Zymostenol[2]					
Baseline - 1 month	−2.68 (4.93)	0.003	−2.75 (3.55)	0.001	0.955
Baseline - 6 months	−2.65 (5.06)	0.004	−2.20 (3.96)	0.007	0.712
Baseline - 1 year	−1.88 (5.51)	0.086	−1.23 (4.08)	0.121	0.613
6 months - 1 year	0.76 (3.03)	0.110	0.97 (2.18)	0.026	0.907
Desmosterol[2]					
Baseline - 1 month	−16.1 (19.5)	<0.001	−19.8 (16.8)	<0.001	0.446
Baseline - 6 months	−18.6 (20.4)	<0.001	−20.2 (20.3)	<0.001	0.763
Baseline - 1 year	−18.1 (22.9)	<0.001	−15.8 (22.6)	<0.001	0.686
6 months - 1 year	0.42 (9.47)	0.806	4.39 (10.6)	0.037	0.131
Lathosterol[2]					
Baseline - 1 month	−23.1 (45.4)	<0.001	−20.1 (30.3)	0.002	0.774
Baseline - 6 months	−23.9 (47.4)	<0.001	−19.5 (33.4)	0.005	0.685
Baseline - 1 year	−18.8 (49.3)	0.031	−13.3 (33.7)	0.046	0.623
6 months - 1 year	5.11 (13.8)	0.008	6.21 (8.93)	0.001	0.736
Campesterol[2]					
Baseline - 1 month	153 (129)	<0.001	191 (114)	<0.001	0.232
Baseline - 6 months	288 (189)	<0.001	282 (163)	<0.001	0.908
Baseline - 1 year	285 (245)	<0.001	245 (180)	<0.001	0.488
6 months - 1 year	−2.79 (138)	0.567	−37.0 (137)	0.166	0.907
Cholestanol[2]					
Baseline - 1 month	43.3 (32.0)	<0.001	41.7 (33.4)	<0.001	0.854
Baseline - 6 months	54.5 (40.3)	<0.001	55.8 (36.0)	<0.001	0.896
Baseline - 1 year	48.4 (39.5)	0.034	48.1 (41.1)	<0.001	0.982
6 months - 1 year	−6.10 (15.3)	0.172	−7.65 (21.9)	0.075	0.752

Mean (SD). [1]HCQ = Hydroxychloroquine. [2]10^2 μmol/mmol of cholesterol.

Table 3
Serum desmosterol ratios in the HCQ group divided into patients with low (<500 ng/ml) and high (≥500 ng/ml) sum concentrations of HCQ[1] and its metabolites[2] during the intervention and compared to the placebo group.

Variables	HCQ and placebo groups	N	Mean (SD)	*P*-value between the three groups[3]
Desmosterol, 10^2 µmol/mmol of cholesterol				
1 month	HCQ and metabolites, <500 ng/ml	12	53.7 (8.38)	0.712
	HCQ and metabolites, ≥500 ng/ml	19	52.5 (12.9)	
	Placebo	28	55.1 (10.6)	
6 months	No HCQ or metabolites	1	64.1	0.062
	HCQ and metabolites, <500 ng/ml	22	47.1 (9.06)	
	HCQ and metabolites, ≥500 ng/ml	8	58.4 (24.6)	
	Placebo	28	54.4 (11.7)	
1 year	No HCQ or metabolites	18	54.2 (11.2)	0.007
	HCQ and metabolites, <500 ng/ml	13	46.5 (9.15)[a]	
	Placebo	28	58.6 (12.0)	

[1]HCQ = Hydroxychloroquine. [2]Desethyl hydroxychloroquine, desethyl chloroquine, and didesethyl chloroquine. [3]One-way analysis of variance between the three groups at each time point.
[a] $P < 0.05$ from the placebo group.

increase observed in the placebo group. However, these effects did not impact the serum total-c level or lathosterol ratio, the late cholesterol precursor, which is not located in the same cholesterol synthesis pathway as desmosterol (Fig. 1). HCQ did not affect cholesterol absorption.

Transcription factors liver X receptors (LXRα and LXRβ) and sterol regulatory element-binding protein transcription factor 2 (SREBP-2) are responsible for the regulation of cholesterol and lipid metabolism. For example, desmosterol and oxysterols are activators of the LXR system. They regulate the metabolism of cholesterol, fatty acids, and phospholipids, as well as the immune and inflammatory responses [21–23]. LXR system works closely with the SREBP-2 pathway responsible for cholesterol biosynthesis. LXRs facilitate the elimination of excess cholesterol when the cellular cholesterol levels are high, whereas SREBP2 promotes cholesterol biosynthesis and uptake in response to low cellular cholesterol levels [21]. Desmosterol activates the LXR system resulting in the target gene *ABCA1* transcription and increases the efflux of cholesterol from the body. It also suppresses the SREBP-2 pathway and its target genes *hydroxymethylglutaryl-coenzyme A reductase* (*HMGCR)* and *LDL receptor (LDL-R)* and further prevents cholesterol synthesis. Based on mouse models it is suggested that the anti-atherogenic effects of desmosterol are based on the above-mentioned activities and suppression of the inflammatory gene expression [21,22,24]. It is unclear whether the decrease in desmosterol in HCQ users would further affect the atherosclerotic plaque formation. The decrease in desmosterol was slight, though significant after six months towards one year, and it was overshadowed by the effects of potent statins. Thus, its connection to the worsening of plaque formation can only be speculated.

Statins inhibit the conversion of hydroxymethylglutaryl-coenzyme A (HMG-CoA) into mevalonic acid by interfering with the activity of the enzyme HMGCR in the early cholesterol synthesis pathway (Fig. 1.). HMGCR is one of the important rate-limiting enzymes in the pathway. Reduced cellular cholesterol content upregulates the LDL-R expression and increases LDL-c uptake into the cell [25]. Consequently, the circulating LDL-c concentration is lowered. In the present study, the mean LDL-c reduction caused by statin in HCQ and placebo groups corresponds to the literature. The efficacy of high-dose statin is also compatible [20,26]. Atorvastatin and simvastatin are metabolized by the CYP3A4 and CYP3A5 enzymes in the small intestinal wall and the liver, which makes them sensitive to interactions with CYP3A-inhibiting drugs [25,27,28]. In our recent *in vitro* studies [29], HCQ metabolites were identified as time-dependent CYP3A4 inhibitors. From this perspective, it is reassuring that HCQ use did not affect statin concentrations, statin metabolite concentrations, or the metabolic ratio of 2-OH- or 4-OH- atorvastatin to atorvastatin. Thus, it appears that HCQ does not significantly influence the function of the CYP3A4 enzyme and is unlikely to increase the concentrations of statins or other drugs metabolized by CYP3A4. Importantly, the findings also demonstrate that any differences between the groups in cholesterol metabolism are not explained by differences in statin concentrations.

HCQ is a 4-aminoquinoline compound and a hydroxylated analogue of CQ. Both HCQ and CQ accumulate primarily in the acidic late endosomes/lysosomes [4–6,17]. Being weak bases, they increase lysosomal pH and disturb lysosomal metabolic functions [17]. Regarding cholesterol metabolism, *in vitro* studies CQ did not interfere with the important regulators of cholesterol metabolism, such as the activities of LDL-R [30,31] and HMGCR [30,32]. Thus, the possible lipid-lowering mechanism of CQ and HCQ differs entirely from that of statins. In an *in vivo* kinetic study in patients with SLE, CQ surprisingly increased the uptake of LDL-like particles compared with patients without CQ or controls [33]. This result contradicts those observed in the *in vitro* studies [30,31]. One explanation might be that the labeled particles used were LDL-like particles instead of native autologous LDL-apoprotein B100 particles, generally used in human kinetic studies. The LDL-like particles may be taken up into the cell not only via the LDL-R as the autologous LDL-apoprotein B100 particles but also via alternative routes, which might result in their increased cellular uptake.

On the other hand, CQ inhibited cholesterol synthesis in rat isolated hepatocytes, but the specific step of the inhibition was not assessed [34]. In *in vitro* studies using both mouse L cells and mouse liver homogenates, CQ inhibited the activity of the enzyme lanosterol synthase (LSS) (EC 5.4.99.7), which cyclizes squalene 2,3-epoxide to lanosterol [32] (Fig. 1.). Consequently, the concentration of squalene 2,3-epoxide was accumulated and the concentrations of squalene and lanosterol were reduced [32]. Since lanosterol is located at the intersection of the two cholesterol synthesis pathways and is a precursor for both pathways, its reduction can probably be reflected in the sterols of either pathway. In fact, in the *in vitro* studies, LSS inhibition decreased markedly the concentration of desmosterol [32].

How do the CQ *in vitro* findings explain our results on HCQ beyond the statin effects? Apart from their similar molecular structures, CQ and HCQ have almost identical pharmacokinetic properties; therefore, it is plausible that the *in vitro* CQ results can also be considered applicable to HCQ [35]. Furthermore, our findings are consistent with the earlier *in vitro* CQ findings [32]. The plausible inhibition of LSS by HCQ could explain the lack of statin-induced increase in serum desmosterol and the more modest effects on lanosterol in the HCQ group of the present study, a finding similar to the *in vitro* experiments [32]. Zymostenol was not involved in the earlier *in vitro* studies, but the decrease in its precursor, lanosterol, can explain its reduction in this study. HCQ has a long half-life (approximately 40 days) and is slowly released from the tissues after discontinuation of the drug use [17]. The long half-life could explain why HCQ inhibited the increase of the desmosterol ratio beyond its discontinuation after six months of use in our study. The HCQ-induced blockages on cholesterol metabolism were temporary and not present at each time point. This could explain the lack of LDL-c lowering effect of HCQ in the present and the earlier studies [1,2] and the reason why HCQ was entirely overpowered by statin in the present study. HCQ neither affected cholesterol absorption nor the metabolism of lathosterol, which is a powerful surrogate of the absolute whole-body cholesterol synthesis even during drug interventions [11,15].

Our study has the following limitations. The lipid-lowering effect of HCQ can be speculated based on the marginal findings in this trial on

acute post-MI patients. In general, this patient material is delicate due to its acute nature. Due to ethical aspects, it is impossible to set up a control group with post-MI patients without statin treatment to enable a more detailed and sensitive evaluation of HCQ effects. Statin treatment also has limitations: dosage changes due to adverse effects are common in statin users, leading to changes in the plasma statin concentration and interference with the lipid analysis results.

The drug intake of the study population was executed optimally, which was essential to keep the steady state of cholesterol metabolism constant throughout the study. Because of the overpowering effect of high-dose statin, the serum total-c and LDL-c changes by HCQ were subtle and did not reveal the possible lipid-lowering effect of HCQ without concomitant statin treatment. However, the temporal statin-induced increase in cholesterol precursors lanosterol, zymostenol, and desmosterol observed in the placebo group were inhibited by HCQ. These findings were subtle, though significant. Especially the one-year desmosterol ratio was significantly lower in the HCQ group compared with the placebo group. The available mechanistic *in vitro* studies of the effects of CQ or HCQ on cholesterol synthesis were performed only with CQ, and in these studies, CQ inhibited the synthesis of lanosterol followed by a decrease in the concentrations of squalene, lanosterol, and desmosterol [32]. However, it is plausible that HCQ has similar effects as CQ on cholesterol synthesis. The findings point to the direction that HCQ inhibits lanosterol synthesis and further cholesterol metabolism, transitorily corroborating earlier *in vitro* works.

In conclusion, HCQ had no significant additive effects on serum total-c, LDL-c, HDL-c, and triglyceride concentrations in post-myocardial infarction patients with concomitant high-dose statin treatment. Throughout the intervention, the changes in cholesterol metabolism were largely explained by statin treatment. However, HCQ interfered temporarily with the metabolism of lanosterol, zymostenol, and especially with the metabolism of desmosterol, suggesting that HCQ is able to interfere with cholesterol synthesis.

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Author contributions

The original OXI trial was designed and executed by KKE and JS. LU, HT, OH, TTR, and JS were responsible for collecting the data. Statistical analysis was performed by LU, MK, PS, HG, and JS. All authors had full access to the data. PS and HG were responsible for analyzing cholesterol synthesis and absorption biomarkers. MK, MNE, MNI, and JTB were responsible for drug quantification and pharmacokinetic interpretations. The first draft of the manuscript was written by LU, PS, HG, and JS. All authors contributed to the review of the manuscript.

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Data availability

Additional data supporting the findings of this study can be requested from the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2023.06.003.

References

[1] Rempenault C, Combe B, Barnetche T, Gaujoux-Viala C, Lukas C, Morel J, Hua C. Metabolic and cardiovascular benefits of hydroxychloroquine in patients with rheumatoid arthritis: a systematic review and meta-analysis. Ann Rheum Dis 2017;77:98–103.
[2] Guevara M, Ng B. Positive effect of hydroxychloroquine on lipid profiles of patients with rheumatoid arthritis: a veterans affair cohort. Eur J Rheumatol 2020;8:62–6.
[3] Babary H, Liu X, Ayatollahi Y, Chen XP, Doo L, Uppaluru LK, Kwak MK, Kulaga C, Modjinou D, Olech E, Yoo JW. Favorable effects of hydroxychloroquine on serum low density lipid in patients with systemic lupus erythematosus: a systematic review and meta-analysis. Int J Rheum Dis 2018;21:84–92.
[4] Nirk EL, Reggiori F, Mauthe M. Hydroxychloroquine in rheumatic autoimmune disorders and beyond. EMBO Mol Med 2020;12:e12476.
[5] Tummino TA, Rezelj Vv, Fischer B, Fischer A, O'Meara M, Monel BVT, White K, Zhang Z, Alon A, Schadt H, O'Donnell H, Lyu J, Rosales R, McGovern B, Rathnasinghe R, Jangra S, Schotsaert M, Galarneau J, Krogan N, Urban L, Shokat K, Kruse A, García-Sastre A, Schwartz O, Moretti F, Vignuzzi M, Pognan F, Shoichet B. Drug-induced phospholipidosis confounds drug repurposing for SARS-CoV-2. Science 2021;373(1979):541–7.
[6] Salata C, Calistri A, Parolin C, Baritussio A, Palù G. Antiviral activity of cationic amphiphilic drugs. Expert Rev Anti Infect Ther 2017;15:483–92.
[7] Hartman O, Kovanen PT, Lehtonen J, Eklund KK, Sinisalo J. Hydroxychloroquine for the prevention of recurrent cardiovascular events in myocardial infarction patients: rationale and design of the OXI trial. Eur Heart J Cardiovasc Pharmacother 2016;3:92–7.
[8] Ulander L, Tolppanen H, Hartman O, Rissanen TT, Paakkanen R, Kuusisto J, Anttonen O, Nieminen T, Yrjölä J, Ryysy R, Drews T, Utriainen S, Karjalainen P, Anttila I, Nurmi K, Silventoinen K, Koskinen M, Kovanen PT, Lehtonen J, Eklund KK, Sinisalo J. Hydroxychloroquine reduces interleukin-6 levels after myocardial infarction: the randomized, double-blind, placebo-controlled OXI pilot trial. Int J Cardiol 2021;337:21–7.
[9] Miettinen TA. Serum squalene and methyl sterols as indicators of cholesterol synthesis in vivo. Life Sci 1969;8:713–21.
[10] Miettinen TA. Cholesterol precursors and their diurnal rhythm in lipoproteins of patients with jejuno-ileal bypass and ileal dysfunction. Metabolism 1985;34:425–30.
[11] Miettinen TA, Tilvis RS, Kesäniemi YA. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am J Epidemiol 1990;131:20–31.
[12] Cohen JC, Pertsemlidis A, Fahmi S, Esmail S, Vega GL, Grundy SM, Hobbs HH. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. Proc Natl Acad Sci U S A 2006;103:1810.
[13] Simonen P, Gylling H, Miettinen TA. The validity of serum squalene and non-cholesterol sterols as surrogate markers of cholesterol synthesis and absorption in type 2 diabetes. Atherosclerosis 2008;197:883–8.
[14] Miettinen TA. Cholesterol metabolism during ketoconazole treatment in man. J Lipid Res 1988;29:43–51.
[15] Nissinen MJ, Miettinen TE, Gylling H, Miettinen TA. Applicability of non-cholesterol sterols in predicting response in cholesterol metabolism to simvastatin and fluvastatin treatment among hypercholesterolemic men. Nutr Metabol Cardiovasc Dis 2010;20:308–16.
[16] Grundy SM, Ahrens Jr EH, Davignon J. The interaction of cholesterol absorption and cholesterol synthesis in man. J Lipid Res 1969;10:304–15.
[17] Browning DJ. Pharmacology of chloroquine and hydroxychloroquine. In: Browning DJ, editor. Hydroxychloroquine and Chloroquine Retinopathy. New York, NY: Springer New York; 2014. p35–63.

[18] Simental-Mendía LE, Simental-Mendía M, Sánchez-García A, Linden-Torres E. Effect of hydroxychloroquine on lipid levels: a systematic review and metaanalysis. Curr Pharmaceut Des 2021;27:4133–9.
[19] Pareek A, Chandurkar N, Thulaseedharan NK, Legha R, Agarwal M, Mathur SL, Salkar HR, Pednekar S, Pai V, Sriram U, Khyalappa R, Parmar M, Agrawal N, Dhruv U, Saxena S. Efficacy and safety of fixed dose combination of atorvastatin and hydroxychloroquine: a randomized, double-blind comparison with atorvastatin alone among Indian patients with dyslipidemia. Curr Med Res Opin 2015;31:2105–17.
[20] Miettinen TA, Gylling H. Synthesis and absorption markers of cholesterol in serum and lipoproteins during a large dose of statin treatment. Eur J Clin Invest 2003;33:976–82.
[21] Wang B, Tontonoz P. Liver X receptors in lipid signalling and membrane homeostasis. Nat Rev Endocrinol 2018;14:452–63.
[22] Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, Reichart D, Fox JN, Shaked I, Heudobler D, Raetz CRH, Wang EW, Kelly SL, Sullards MC, Murphy RC, Merrill AH, Brown HA, Dennis EA, Li AC, Ley K, Tsimikas S, Fahy E, Subramaniam S, Quehenberger O, Russell DW, Glass CK. Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. Cell 2012;151:138–52.
[23] Yang C, McDonald JG, Patel A, Zhang Y, Umetani M, Xu F, Westover EJ, Covey DF, Mangelsdorf DJ, Cohen JC, Hobbs HH. Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. J Biol Chem 2006;281:27816–26.
[24] Zhang X, McDonald JG, Aryal B, Canfrán-Duque A, Goldberg EL, Araldi E, Ding W, Fan Y, Thompson BM, Singh AK, Li Q, Tellides G, Ordovás-Montanes J, García Milian R, Dixit VD, Ikonen E, Suárez Y, Fernández-Hernando C. Desmosterol suppresses macrophage inflammasome activation and protects against vascular inflammation and atherosclerosis. Proc Natl Acad Sci USA 2021:118.
[25] Ward NC, Watts GF, Eckel RH. Statin toxicity. Circ Res 2019;124:328–50.
[26] LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart J-C, Gotto AM, Greten H, Kastelein JJP, Shepherd J, Wenger NK. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 2005;352:1425–35.
[27] Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. Clin Pharmacol Ther 2006;80.
[28] Filppula AM, Hirvensalo P, Parviainen H, Ivaska VE, Lönnberg KI, Deng F, Viinamäki J, Kurkela M, Neuvonen M, Niemi M. Comparative hepatic and intestinal metabolism and pharmacodynamics of statinsxs. Drug Metabol Dispos 2021:49.
[29] Paludetto M-N, Kurkela M, Kahma H, Backman JT, Niemi M, Filppula AM. Hydroxychloroquine is metabolized by cytochrome P450 2D6, 3A4, and 2C8, and inhibits cytochrome P450 2D6, while its metabolites also inhibit cytochrome P450 3A *in vitro*. Drug Metabol Dispos 2023;51:293–305.
[30] Goldstein JL, Brunschede GY, Brown MS. Inhibition of proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. J Biol Chem 1975;250:7854–62.
[31] Dashti N, Wolfbauer G, Koren E, Knowles B, Alaupovic P. Catabolism of human low density lipoproteins by human hepatoma cell line HepG2. Biochim Biophys Acta Lipids Lipid Metabol 1984;794:373–84.
[32] Chen HW, Leonard DA. Chloroquine inhibits cyclization of squalene oxide to lanosterol in mammalian cells. J Biol Chem 1984;259:8156–62.
[33] Sachet JC, Borba EF, Bonfá E, Vinagre CGC, Silva VM, Maranhão RC. Chloroquine increases low-density lipoprotein removal from plasma in systemic lupus patients. Lupus 2007;16:273–8.
[34] Beynen AC, Molen AJ van der, Geelen MJH. Inhibition of hepatic cholesterol biosynthesis by chloroquine. Lipids 1981;16:472–4.
[35] Reis Neto ET dos, Kakehasi AM, Medeiros Pinheiro M de, Ferreira GA, Marques CDL, Mota LMH da, Santos Paiva E dos, Pileggi GCS, Sato EI, Reis APMG, Xavier RM, Provenza JR. Revisiting hydroxychloroquine and chloroquine for patients with chronic immunity-mediated inflammatory rheumatic diseases. Adv Rheumatol 2020;60:32.