Clinical Epidemiology

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ORIGINAL RESEARCH

Effects of chronic exposure of hydroxychloroquine/chloroquine on the risk of cancer, metastasis, and death: a population-based cohort study on patients with connective tissue diseases

This article was published in the following Dove Press journal:
Clinical Epidemiology
3 November 2017
Number of times this article has been viewed

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Background: Hydroxychloroquine and chloroquine may reduce the risk of cancer as they inhibit autophagy, in particular, in people with connective tissue diseases.

Methods: The hazard ratios of cancers, metastases, and death were assessed in adults with connective tissue diseases prescribed hydroxychloroquine/chloroquine for at least 1 year in comparison with unexposed individuals with the same underlying conditions. A competing risk survival regression analysis was performed. Data were extracted from the Health Improvement Network UK primary care database.

Results: Eight thousand nine hundred and ninety-nine individuals exposed to hydroxychloroquine (98.6%) or chloroquine (1.4%) and 24,118 unexposed individuals were included in the study (median age: 56 [45–66] years, women: 76.8%). When compared to the unexposed group, individuals exposed to hydroxychloroquine/chloroquine were not at lower risk of non-skin cancers (adjusted sub-distribution hazard ratio [sHR]: 1.04 [0.92–1.18], p=0.54), hematological malignancies (adjusted sHR: 1.00 [0.73–1.38], p=0.99), or skin cancers (adjusted sHR: 0.92 [0.78–1.07], p=0.26). The risk of metastasis was not significantly different between the two groups. However, it was significantly lower during the exposure period when compared with the unexposed (adjusted sHR: 0.64 [0.44–0.95] for the overall population and 0.61 [0.38–1.00] for those diagnosed with incident cancers). The risk of death was also significantly lower in those exposed to hydroxychloroquine/chloroquine (adjusted HR: 0.90 [0.81–1.00] in the overall population and 0.78 [0.64–0.96] in those diagnosed with incident cancer).

Conclusion: Individuals on long-term exposure to hydroxychloroquine/chloroquine are not at lower risk of cancer. However, hydroxychloroquine/chloroquine may lower the risk of metastatic cancer and death.

Keywords: antimalarial drugs, cancer, death, connective tissue diseases

Background

For decades, chloroquine and hydroxychloroquine have been used to treat malaria. Nowadays, they are mainly used to treat connective tissue diseases. More recently, their potential use as anticancer agents has led to renewed interest in these old drugs. Hydroxychloroquine and chloroquine are thought to have antitumor properties mainly because they inhibit autophagy, although they can also affect the growth and spread of

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Dovepress
http://dx.doi.org/10.2147/CLEP.S143563

tumor cells independent of this mechanism.[1–4] Autophagy is a complex cellular mechanism that is thought to play a dual role in tumor growth progress – that is, preventing tumor initiation by suppressing chronic tissue damage, inflammation, and genome instability and sustaining tumor metabolism, growth, and survival via nutrient recycling.[4–6] However, most of the physiological functions of autophagy are still unclear, even though tremendous advances have been made during the past decade. Thus, in October 2016, the Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi "for his discoveries of mechanisms for autophagy". Using autophagy as a tumor treatment target is under investigation, and there are many ongoing trials assessing the clinical benefits of adding hydroxychloroquine or chloroquine to conventional therapies in individuals with solid or hematologic cancers (unpublished data, 2017). The limited data on this topic are encouraging,[7–15] in particular, in people with connective tissue diseases.[9] Most of these studies, however, are Phase I or Phase II studies and are limited by small numbers of patients.

The aim of this cohort study was to assess the effects of hydroxychloroquine or chloroquine as prescribed in people with connective tissue diseases on 1) incident risk of cancers, 2) incident risk of metastases, and 3) all-causes mortality.

Methods

Study design

The Health Improvement Network (THIN) is a primary care database of anonymized general practice records from the early 1990s to the present day on more than 12 million individuals from nearly 700 practices in the UK. Participating general practitioners systematically and prospectively enter clinical information on individuals, including demographic data, diagnoses, and prescriptions so that the database provides a longitudinal medical record for each individual. THIN is a representative of the UK population and the clinical diagnostic and prescribing data compare favorably with external statistics and other independent studies.[16,17] Since these data are entered in routine general practice, they reflect "real-life" clinical care. We used data from January 1, 1990, to December 31, 2015 from all general practices that contributed to the database during this period. We further restricted our data to the time when there was evidence that the practices were entering most clinical information on their computer systems.[18] All diagnoses and symptoms were recorded in THIN using the Read classification system.[19] This Read classification was used to create medical code lists that enabled us to identify cases of non-skin cancers, hematological malignancies, skin cancers, or metastasis.[20]

Exposed and unexposed populations

All individuals in our study were ≥18 years. Individuals were included in the exposed sample if they had connective tissue diseases and had been prescribed hydroxychloroquine or chloroquine for at least 1 year at a significant daily dosage (i.e., mean daily dosage over the exposure period ≥100 mg for chloroquine and ≥200 mg for hydroxychloroquine). The medical diagnosis recorded on the date of starting hydroxychloroquine or chloroquine was used as the indication for the drug prescription. If there were no medical diagnoses recorded on this date, we searched for a diagnosis of connective tissue disease (or light eruption/photosensitivity) as entered on the records for up to 1 year prior or after this prescription. We selected only those who started the drug at least 6 months after their registration with the general practice in order to capture people with incident rather than prevalent treatment. The start of at-risk period (i.e., hereafter called "start date") was defined as the first day of the first prescription of hydroxychloroquine or chloroquine. Individuals were eligible to be included in the control, *unexposed* sample if they had never been exposed to chloroquine, hydroxychloroquine, quinine, quinacrine, or mefloquine, but were suffering from the same underlying condition as the exposed individuals. We selected up to three unexposed individuals for every exposed individual stratified within 5-year age bands, gender, and the underlying condition. For each unexposed individual, a uniformly randomly selected "start date" (i.e., start of the at-risk period) was defined as at least 6 months after their registration. Individuals had to have at least 1 year follow-up after "start date" to be included in the unexposed sample.

Study outcomes

We examined five prespecified outcomes: incident cases of 1) non-skin, non-hematological cancers (excluding in situ neoplasia), 2) hematological malignancies, 3) skin cancers (excluding in situ neoplasia), 4) metastases, and 5) all-cause mortality. The first record of each outcome was taken into account for the analyses. Cancers or metastases were defined as incident when there was no previous record of cancer or metastasis in the medical file within the previous 5 years. Lastly, we searched for any records of death and date of death for all individuals included in our study population.[21]

Covariates of interest

For each individual, we extracted data on social deprivation (i.e., Townsend deprivation index); body mass index (based on the nearest record of "start date"); smoking status (based on the nearest record of "start date"); and past history

of diabetes, hypertension, or any cancer before "start date". Further, we assessed the number of prescriptions of methotrexate, azathioprine, glucocorticoids, nonsteroidal antiinflammatory drugs (NSAIDs), vitamin D, and metformin before the record of malignancy, metastases, or death (for those with an outcome) or randomly selected dates during at-risk period (for those without these outcomes).

Statistical analyses

Baseline variables are reported as medians (interquartile range [IQR]) for continuous variables or counts (percentages) for categorical variables. Two-sided p-value ≤0.05 was considered significant.

Data on associations between hydroxychloroquine/chloroquine exposure and the risk of incident cancers or metastases were analyzed using competing risk regression using the Fine and Gray method (with death as a competing event). The sub-distribution hazard ratios (sHRs) for competing risks of malignancies/metastases and all-cause mortality were obtained. Regarding the risk of metastases, the analyses were first run in the overall population. Then they were conducted in a subgroup of individuals diagnosed with cancer (excluding basal cell carcinoma and hematological malignancies) within 3 years before "start date" as we hypothesized that cancers occurring during exposure to the drugs may be more "resistant" to the drug than those occurring outside exposure. Lastly, the analyses were run in the subgroup of individuals diagnosed with incident cancer after "start date". For these individuals, the start of at-risk period for metastasis was the date of cancer. The last step of our study was to compare the risk of death in the exposed and unexposed groups. This was performed using multivariable Cox proportional hazard models, both in the overall population and in the subgroup of individuals diagnosed with incident cancer after "start date". As for metastasis, the start of at-risk period for this subgroup analysis was the date of cancer diagnosis.

All the analyses were done by taking into account exposure both as a binary variable (exposed/unexposed) and as a time-varying variable. We did the latter as some exposed individuals may have been exposed for years before stopping the treatment.

We included age and sex as covariates in all the models, but for the other variables, we chose those associated with the outcome with a p-value ≤0.2 in univariate analyses (all covariates described in the previous paragraph were assessed). The proportional hazards assumption for Cox models was checked graphically using the Schoenfeld residuals. Linearity for continuous variables was checked by comparing two models, one with the linear term and the other with the categories using the log likelihood ratio test (when necessary, continuous variables were categorized). We examined whether the effect of exposure differed by age and gender by evaluating if there was a significant interaction between age or gender and exposure in the models. We looked for trend in the occurrence of malignancies according to the duration of exposure using the test for trend across ordered groups (Stata nptrend command).

All analyses were done using Stata, version 14.0. THIN scheme for obtaining patient data and providing them in anonymized form to researchers was approved by the National Health Service South-East Multicentre Research Ethics Committee in 2002. The present study was approved by the THIN scientific review committee.

Sensitivity analyses

Since it can be hypothesized that comparing new hydroxychloroquine/chloroquine users to individuals never exposed to the drugs can create substantial bias, in particular selection bias, we also compared individuals chronically exposed to those who received hydroxychloroquine for <1 year and stopped it, for instance, for inefficiency or occurrence of adverse events. To be eligible to be included in this shortly exposed group, individuals had to have at least 1 year of follow-up after initiation of hydroxychloroquine, reflecting at least 1-year exposure of the chronically exposed individuals. Up to three shortly exposed individuals were selected for every chronically exposed individual stratified within 5-year age bands, gender, and the underlying condition.

Results

Study population

In total, 8,999 individuals exposed to hydroxychloroquine or chloroquine and 24,118 unexposed individuals were included in the study. The median age was 56 (45–66) years and 25,445 (76.8%) were women (Table 1). Most exposed individuals received hydroxychloroquine (n=8,871, 98.6%). Only 128 individuals received chloroquine, with no hydroxychloroquine exposure during follow-up. The median duration of hydroxychloroquine/chloroquine exposure was 37 (22–66) months (mean duration ± SD: 50±39 months).

Incident cases of primary malignancies

Respectively, 1,329 incident cases of non-skin cancers, 198 incident cases of hematological malignancies, and 959

Table 1 Characteristics of the study population

Characteristic	Exposed, N=8,999	Unexposed, N=24,118
Age (years)	56 (46–66)	55 (45–65)
Female, n (%)	6,909 (76.8)	18,536 (76.9)
"Start date"	April 2010 (August 2006–October 2012)	Dec 2009 (May 2006–May 2012)
Duration of follow-up after "start date" (days)	1,509 (848–2,595)	1,475 (1,071–2,285)
Time between the end of exposure and the end of follow-up (days)	5 (0–202)	–
Dosage (mg/day)		
HCQ	307 (238–377)	–
CQ	169 (132–234)	–
Underlying diseases, n (%)		
Rheumatoid arthritis	6,139 (68.2)	15,995 (66.3)
Systemic lupus erythematosus	1,644 (18.3)	4,932 (20.4)
Sjogren syndrome	603 (6.7)	1,657 (6.9)
Dermatomyositis/ polymyositis	29 (0.3)	26 (0.1)
Other connective tissue diseases[a]	531 (5.9)	1,358 (5.7)
Light eruption	53 (0.6)	150 (0.6)
Number of prescriptions of[b]		
Methotrexate	0 (0–71)	0 (0–50)
Azathioprine	0 (0–2)	0 (0–0)
Glucocorticoids	2 (0–60)	0 (0–34)
NSAIDs	11 (0–108)	5 (0–85)
Metformin	0 (0–13)	0 (0–10)
Vitamin D	0 (0–43)	0 (0–25)
Smoking status, n (%)		
Nonsmokers	4,133 (45.9)	11,800 (48.9)
Ex-smokers	2,878 (32.0)	6,657 (27.6)
Smokers	1,723 (19.2)	5,090 (21.1)
Missing	265 (2.9)	571 (2.4)
Townsend deprivation index, n (%)		
0 (less deprived)	398 (4.4)	897 (3.7)
1	2,304 (25.6)	6,540 (27.1)
2	1,956 (21.8)	5,284 (21.9)
3	1,809 (20.1)	4,675 (19.4)
4	1,496 (16.6)	3,961 (16.4)
5 (more deprived)	930 (10.3)	2,483 (10.3)
Missing	106 (1.2)	278 (1.2)
BMI (kg/m^2)	26.7 (23.4–31.0)	26.6 (23.4–30.8)
Past history of, n (%)		
Non-skin cancer	431 (4.8)	1,063 (4.4)
Hematological malignancy	64 (0.7)	112 (0.5)
Skin cancer	277 (3.1)	751 (3.1)
Hypertension	2,401 (26.7)	5,852 (24.3)
Diabetes	840 (9.3)	1,943 (8.1)

Notes: [a]Systemic sclerosis, antiphospholipid syndrome, mixed and undifferentiated connective tissue diseases. [b]Before the date of cancer with those with cancer and before a randomly selected date during follow-up for those without cancer. Continuous variables are reported as medians and interquartile range except for the number of medication prescriptions that are reported as medians and 5th–95th percentile range.

Abbreviations: BMI, body mass index; CQ, chloroquine; HCQ, hydroxychloroquine; NSAIDs, nonsteroidal antiinflammatory drugs,

incident cases of skin cancers were observed in the overall population (Table 2). There were no differences in incident non-skin cancers (adjusted sHR: 1.04 [0.92–1.18], p=0.54), hematological malignancies (adjusted sHR: 1.00 [0.73–1.38], p=0.99), or skin cancers (adjusted sHR: 0.92 [0.78–1.07], p=0.26) between exposed and unexposed individuals. Taking into account hydroxychloroquine/chloroquine exposure as a time-varying covariate (i.e., exposed period time compared to unexposed period time) gave similar results (Table 3). The annual incidence rates between the exposed and unexposed period times are reported in Table 4. An increasing temporal trend over time was observed for the unexposed group, while there was no clear increase over time during hydroxychloroquine/chloroquine exposure (p for comparison of trends: 0.009).

Table 2 Outcomes of interest

Outcome of interest	Exposed, N=8,999	Unexposed, N=24,118
Incident outcomes after "start date", n (%)		
Non-skin cancer	382 (4.2)	947 (3.9)
Breast	89 (1.0)	219 (0.9)
Colon	44 (0.5)	99 (0.4)
Prostate	39 (0.4)	79 (0.3)
Lung	58 (0.6)	175 (0.7)
Other or nonspecific codes[a]	152 (1.7)	375 (1.6)
Hematological malignancy	59 (0.6)	139 (0.6)
Skin cancer	270 (3.0)	689 (2.8)
Metastasis	59 (0.6)	171 (0.7)
Death	636 (7.1)	1,665 (6.9)
Median time between "start date" and record of incident outcome (days)		
Non-skin cancer	1,159 (573–2,258)	1,096 (623–1,786)
Hematological malignancy	1,220 (670–2,229)	971 (469–1,766)
Skin cancer	1,061 (489–2,099)	868 (436–1,585)
Metastasis	1,399 (679–2,789)	1,205 (811–1,815)
Death	1,611 (918–2,518)	1,461 (1,118–2,191)
Solid cancers diagnosed within 3 years before "start date", n (%)	149 (1.6)	317 (1.3)
Breast	42 (0.5)	96 (0.4)
Colon	9 (0.1)	31 (0.1)
Prostate	24 (0.3)	35 (0.1)
Lung	12 (0.1)	15 (<0.1)
Skin cancer (excluding basal cell carcinoma)	23 (0.2)	53 (0.2)
Other or nonspecific codes[a]	39 (0.4)	87 (0.4)
Median time between cancers diagnosed within 3 years before "start date" and "start date" (days)	535 (314–816)	486 (224–781)

Note: [a]For example, "adenocarcinoma".

Table 3 Risk of incident malignancies occurring after "start date" – comparison of the hydroxychloroquine/chloroquine exposed and unexposed periods

	Exposed time	Unexposed time	Crude sHR*	*p*-value	Adjusted sHR*	*p*-value
	Time at risk (year)	Time at risk (year)				
	Incidence rate (per 100 PY)	Incidence rate (per 100 PY)				
Non-skin cancers	36,320	127,188	0.97 (0.85–1.11)	0.67	1.00 (0.88–1.15)[a]	0.96
	0.76	0.83				
Hematological malignancies	36,718	129,192	0.99 (0.70–1.39)	0.95	0.89 (0.62–1.29)[b]	0.55
	0.11	0.12				
Skin cancers	36,247	127,342	1.02 (0.87–1.20)	0.79	0.94 (0.80–1.12)[b]	0.50
	0.55	0.55				

Notes: *Exposed compared to unexposed. [a]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of NSAIDs, metformin, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions. [b]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of NSAIDs, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions.
Abbreviations: NSAIDs, nonsteroidal antiinflammatory drugs; PY, person-years; sHR, sub-distribution hazard ratios.

Table 4 Annual incidence rate of incident malignancies after "start date" (excluding basal cell carcinomas)

	Person-years	Number of outcomes	Incidence rate (95% CI) per 100 person-years
First year			
Unexposed time	24,040	163	0.68 (0.58–0.79)
Exposed time	8,959	74	0.83 (0.66–1.04)
Second year			
Unexposed time	23,605	245	1.04 (0.92–1.18)
Exposed time	7,364	75	1.02 (0.81–1.28)
Third year			
Unexposed time	21,017	259	1.23 (1.09–1.39)
Exposed time	5,229	52	0.99 (0.76–1.30)
Fourth year			
Unexposed time	15,568	194	1.25 (1.08–1.43)
Exposed time	3,829	42	1.10 (0.81–1.48)
Fifth year and over			
Unexposed time	42,101	499	1.19 (1.09–1.29)
Exposed time	10,654	116	1.09 (0.91–1.31)

Incident cases of metastases

In total, 230 incident cases of metastases were recorded in the overall population (Table 2). There was no difference in the risk of metastasis in the exposed and unexposed individuals except for those diagnosed with cancer within the 3 years before "start date" (adjusted sHR: 0.27 [0.10–0.80], p=0.02), even though the number of events was low (Table 5). However, the risk of metastasis was significantly lower during the exposed period when compared with the unexposed (adjusted sHRs: 0.64 [0.44–0.95] for the overall population, 0.61 [0.38–1.00] for those diagnosed with incident cancers, and 0.26 [0.08–0.83] for those diagnosed with cancer within 3 years before "start date", respectively) (Table 5). For those with a diagnosis of metastasis recorded after the end of hydroxychloroquine/chloroquine exposure (n=27), the median time between the end of exposure and the record of metastasis was 484 (52–1791) days.

Table 5 Risk of metastasis – comparison of exposed and unexposed individuals and exposed and unexposed periods

	Exposed group, N=8999	Unexposed group, N=24118	Crude sHR	*p*-value	Adjusted sHR	*p*-value
Overall population, n (%)	59 (0.6)	171 (0.7)	0.88 (0.65–1.18)	0.38	0.91 (0.67–1.22)[a]	0.53
In those with incident cancer diagnosed after "start date", n (%)*	40 (9.0)	112 (10.2)	0.87 (0.61–1.24)	0.44	0.87 (0.61–1.26)[b]	0.47
In those with cancer diagnosed within 3 years before "start date", n (%)*	4 (2.7)	31 (9.8)	0.28 (0.10–0.79)	0.02	0.27 (0.10–0.80)[c]	0.02
	Exposed time Time (year) Incidence rate (per 100 person-years)	**Unexposed time Time (year) Incidence rate (per 100 person-years)**	**Crude sHR**	***p*-value**	**Adjusted sHR**	***p*-value**
Overall population	36,801	129,410	0.62 (0.43–0.91)	0.01	0.64 (0.44–0.95)[a]	0.02
	0.09	0.15				
In those with newly developed cancer after "start date"*	666	2,779	0.63 (0.39–1.02)	0.06	0.61 (0.38–1.00)[b]	0.05
	2.85	4.79				
In those with cancer diagnosed within 3 years before "start date"*	530	2,132	0.27 (0.08–0.86)	0.02	0.26 (0.08–0.83)[c]	0.02
	0.57	1.50				

Notes: *Excluding hematological malignancies and basal cell carcinomas. [a]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of glucocorticoids, metformin, and vitamin D prescriptions. [b]Adjusted on age, sex, underlying condition, smoking status, deprivation index, and number of NSAIDs and metformin prescriptions. [c]Adjusted on age and sex.
Abbreviations: NSAIDs, nonsteroidal antiinflammatory drugs; sHR, sub-distribution hazard ratios.

Table 6 Risk of death according to age of the individuals (exposed compared to unexposed)

	Exposed, N=8,999	Unexposed, N=24,118	Crude HR	*p*-value	Adjusted HR	*p*-value
Death in the overall population, n (%)	636 (7.0)	1,665 (6.9)	0.93 (0.85–1.02)	0.12	0.90 (0.81–1.00)	0.05
≤70 years	306 (4.1)	923 (4.5)	0.79 (0.69–0.90)	<0.001	0.76 (0.66–0.88)	<0.001
>70 years	330 (22.3)	742 (20.6)	1.06 (0.93–1.21)	0.34	1.09 (0.93–1.26)	0.28
Death in those with newly developed cancer, n (%)[a]	151 (30.6)	464 (38.0)	0.74 (0.61–0.89)	0.001	0.78 (0.64–0.96)	0.02
≤70 years	91 (25.6)	312 (35.3)	0.64 (0.51–0.81)	<0.001	0.70 (0.55–0.91)	0.007
>70 years	60 (43.2)	152 (45.1)	1.00 (0.74–1.34)	0.98	0.98 (0.68–1.42)	0.93
Death in those without newly developed cancer, n (%)[a]	485 (5.7)	1,201 (5.2)	1.00 (0.90–1.11)	0.99	0.96 (0.86–1.08)	0.53
≤70 years	215 (3.0)	611 (3.1)	0.85 (0.72–0.99)	0.04	0.81 (0.68–0.96)	0.02
>70 years	270 (20.1)	590 (18.0)	1.09 (0.94–1.26)	0.25	1.13 (0.96–1.33)	0.14

Notes: [a]Excluding basal cell carcinoma. Models adjusted on age, sex, underlying condition, smoking status, past history of cancer, past history of diabetes, past history of hypertension, BMI, deprivation index, and number of NSAIDs, metformin, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions.
Abbreviations: BMI, body mass index; HR, hazard ratio; NSAIDs, nonsteroidal antiinflammatory drugs.

Risk of death

In total, 2,301 (6.9%) out of the 33,117 individuals died (Table 2). The risk of death was significantly lower in those exposed to hydroxychloroquine/chloroquine when compared to the unexposed (adjusted HRs: 0.90 [0.81–1.00] in the overall population and 0.78 [0.64–0.96] in those with newly developed cancer). However, as there was a significant interaction between exposure and age, separate analyses were conducted in those under 70 years and those over 70 years, and the decrease in risk was observed only in those ≤70 years (Table 6).

Sensitivity analyses

The characteristics of the 5,265 individuals shortly exposed to hydroxychloroquine are reported in Table S1. Results regarding incident malignancy and metastases were very similar to those found when comparing the chronically exposed individuals to the unexposed (Table S2). The risk of metastasis was significantly lower during exposed periods when compared with unexposed periods (sHR: 0.60 [0.38–0.94], p=0.02), while the risk of incident malignancy did not differ between the two periods. The risk of death was also lower in those ≤70 years chronically exposed when compared with the shortly exposed (adjusted HRs: 0.78 [0.64–0.94] in the overall population and 0.73 [0.52–1.01] in those with newly developed cancer; Table S3).

Discussion

In this population-based, epidemiological study, there was no difference in the risk of incident cancers in people exposed for at least 1 year to hydroxychloroquine/chloroquine for connective tissue diseases when compared to individuals with the same underlying diseases but not exposed to the drugs. On the other hand, the risk of metastasis was significantly lower during the time of exposure. Also, hydroxychloroquine and chloroquine significantly decreased the risk of death, both in the overall population and in those diagnosed with incident cancer during follow-up.

During the past years, there has been a growing interest in the potential antitumor properties of hydroxychloroquine and chloroquine through inhibition of autophagy.[1–4] Autophagy is an intracellular homeostatic pathway by which cells generate energy and metabolites by recycling their own nonessential, redundant, or damaged organelles and macromolecular components.[4,22,23] Autophagy can be activated in different cells at different stages of tumor growth and have paradoxical roles in tumor growth. Before tumorigenesis, autophagy promotes survival of normal cells and suppresses tumorigenesis by safeguarding against metabolic stress. In contrast, in tumor cells that are under metabolic stress as a result of a high proliferation rate and exposure to hypoxia from insufficient vascularization, autophagy may confer a survival advantage and may contribute to tumor growth and spreading.[4–6,22,23] Pharmacologic inhibition of autophagy may be beneficial in preventing established tumor growth and spreading. There are several ongoing trials that are assessing this effect. On the other hand, because autophagy inhibits early tumorigenesis, effects of chronic inhibition of autophagy on normal, non-tumoral cells need to be better understood.

While much is known about the beneficial effects of hydroxychloroquine or chloroquine on cancer cell proliferation and risk of metastases both in vitro and in animal studies, little is known about its effects on humans. In two studies published in 2006 and 2007 by the same team, chloroquine was used as an adjuvant therapeutic agent for the treatment of glioblastoma multiforme.[7,8] The mean survival time of patients receiving chloroquine at 150 mg/day for 12–18 months was significantly longer than that of patients

treated with conventional therapy (24 versus 11 months in the randomized, placebo-controlled trial[8] and 25±3.4 versus 11.4±1.3 months in the retrospective cohort study).[7] In a Phase II, double-blind trial, 76 patients with brain metastases were randomized to receive either chloroquine 150 mg/day for 4 weeks or placebo in association with whole brain irradiation.[10] The progression-free survival rates of brain metastases at 1 year were 83.9% (95% CI: 69.4–98.4) for the chloroquine group compared to 55.1% (95% CI: 33.6–77.6) for the control group, while the overall survival rates did not differ between the two groups. There is also a prospective, observational study that investigated the effect of hydroxychloroquine or chloroquine on the risk of developing cancer in 235 patients with systemic lupus erythematosus (median time on antimalarial: 53 [6–238] months).[9] Among patients treated with antimalarials, 2/156 (1.3%) had cancer compared with 11/79 (13%) of those not treated (adjusted HR: 0.15 [0.02–0.99]).

Our study has several strengths including the use of a large population-based sample of individuals of both sexes, across all adult age groups, and with many underlying diseases and comorbidities. This enabled us to separately compare the impact of hydroxychloroquine/chloroquine on the risk of non-skin and skin cancers, hematological malignancies, metastases, and deaths in an unselected population of individuals chronically exposed to the drugs in primary care. To our knowledge, this is the first study of this kind.

There are, however, some limitations. First, this is an observational study rather than a randomized trial, and hence, residual confounding cannot be ruled out. For instance, we compared individuals chronically exposed to hydroxychloroquine/chloroquine to individuals never exposed to the drugs during follow-up in order to examine the effects of these drugs in well-defined populations and to ease interpretation of results. However, it can be argued that this choice may have led to a selection bias as unexposed individuals may probably be different from the exposed individuals regarding general health status or severity of the underlying condition, for instance. Nevertheless, many covariates reflecting disease severity (such as concomitant prescriptions of glucocorticoids or immunosuppressive drugs) or health status (such as comorbidities) were accounted for in the analyses. Further, results comparing those chronically exposed to those exposed for a short period of time (who may be considered as less "selected" than the unexposed) showed very similar results. Second, we found that the overall incidence rate of metastasis was not different between the exposed and unexposed groups but was much lower when the individuals were exposed to the drugs compared with unexposed periods. This important result can be interpreted in two ways. First, hydroxychloroquine and chloroquine truly limit the risk of cancer spreading during exposure. Second, the drugs were stopped because of symptoms due to yet to diagnose metastases (e.g., abdominal pain and nausea), and the metastases were diagnosed (and recorded) soon after drug withdrawal. This second hypothesis could therefore lead to an interpretation bias. Noteworthy, most metastases were recorded many months or years after drug withdrawal. The third limitation is the low number of recorded metastases and hence the wide confidence interval of the effect size. This low number of events makes the analysis sensitive to misclassification of the outcome. Moreover, on account of these low numbers of events, we were unable to assess whether a subtype of primary cancer would benefit more than another to prescribed hydroxychloroquine or chloroquine. The fourth limitation is the lack of information on the chemotherapies used to treat those diagnosed with cancers since these treatments are given at the hospital and not routinely recorded in primary care. This is unfortunate as most ongoing trials are exploring the potential beneficial effects of combining hydroxychloroquine/chloroquine to conventional chemotherapies in cancer treatment. Fifth, it would have been informative to know if hydroxychloroquine/chloroquine exposure is associated with less aggressive cancers (lower stage or grade, for example), which may in turn explain the results found regarding risks of metastasis and death. Unfortunately, information regarding the stage or grade of cancer is not routinely recorded in THIN. Lastly, it is known that non-adherence to hydroxychloroquine in people with connective tissue diseases is between 10% and 50%.[24] The results of the present study may therefore be underestimations of the true effects of the drugs.

In conclusion, individuals on long-term exposure to hydroxychloroquine or chloroquine for connective tissue diseases may be at lower risk of metastatic cancer and death. However, these results are limited to observational data, and the results of the ongoing randomized controlled trials comparing hydroxychloroquine/chloroquine to placebo in individuals diagnosed with cancer are eagerly awaited.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Solomon VR, Lee H. Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *Eur J Pharmacol*. 2009;625(1–3):220–233.

2. Vlahopoulos S, Critselis E, Voutsas IF, et al. New use for old drugs? Prospective targets of chloroquines in cancer therapy. *Curr Drug Targets*. 2014;15(9):843–851.
3. Zhang Y, Liao Z, Zhang L, Xiao H. The utility of chloroquine in cancer therapy. *Curr Med Res Opin*. 2015;31(5):1009–1013.
4. Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med*. 2013;368(7):651–662.
5. Guo JY, Xia B, White E. Autophagy-mediated tumor promotion. *Cell*. 2013;155(6):1216–1219.
6. Yang X, Yu DD, Yan F, et al. The role of autophagy induced by tumor microenvironment in different cells and stages of cancer. *Cell Biosci*. 2015;5:14
7. Briceño E, Calderon A, Sotelo J. Institutional experience with chloroquine as an adjuvant to the therapy for glioblastoma multiforme. *Surg Neurol*. 2007;67(4):388–391.
8. Sotelo J, Briceño E, López-González MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2006;144(5):337–343.
9. Ruiz-Irastorza G, Ugarte A, Egurbide MV, et al. Antimalarials may influence the risk of malignancy in systemic lupus erythematosus. *Ann Rheum Dis*. 2007;66(6):815–817.
10. Rojas-Puentes LL, Gonzalez-Pinedo M, Crismatt A, et al. Phase II randomized, double-blind, placebo-controlled study of whole-brain irradiation with concomitant chloroquine for brain metastases. *Radiat Oncol Lond Engl*. 2013;8:209.
11. Rosenfeld MR, Ye X, Supko JG, et al. A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy*. 2014;10(8):1359–1368.
12. Rangwala R, Leone R, Chang YC, et al. Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. *Autophagy*. 2014;10:1369–1379.
13. Vogl DT, Stadtmauer EA, Tan KS, et al. Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy*. 2014;10:1380–1390.
14. Wolpin BM, Rubinson DA, Wang X, et al. Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. *Oncologist*. 2014;19:637–638.
15. Goldberg SB, Supko JG, Neal JW, et al. A phase I study of erlotinib and hydroxychloroquine in advanced non-small-cell lung cancer. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer*. 2012;7:1602–1608.
16. Blak BT, Thompson M, Dattani H, Bourke A. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. *Inform Prim Care*. 2011;19(4):251–255.
17. Lewis JD, Schinnar R, Bilker WB, Wang X, Strom BL. Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. *Pharmacoepidemiol Drug Saf*. 2007;16(4):393–401.
18. Horsfall L, Walters K, Petersen I. Identifying periods of acceptable computer usage in primary care research databases. *Pharmacoepidemiol Drug Saf*. 2013;22(1):64–69.
19. Chisholm J. The Read clinical classification. *BMJ*. 1990;300(6732):1092.
20. Davé S, Petersen I. Creating medical and drug code lists to identify cases in primary care databases. *Pharmacoepidemiol Drug Saf*. 2009;18:704–707.
21. Hall GC. Validation of death and suicide recording on the THIN UK primary care database. *Pharmacoepidemiol Drug Saf*. 2009;18:120–131.
22. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008;132:27–42.
23. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008;451:1069–1075.
24. Costedoat-Chalumeau N, Pouchot J, Guettrot-Imbert G, et al. Adherence to treatment in systemic lupus erythematosus patients. *Best Pract Res Clin Rheumatol*. 2013;27(3):329–340.

Supplementary materials

Table S1 Characteristics of the population shortly exposed for <1 year to hydroxychloroquine

Characteristic	Shortly exposed, N=5,265
Age (years)	57 (46–67)
Female, n (%)	4,343 (82.5)
"Start date"	Apr 2010 (Jan 2007–Sept 2012)
Duration of follow-up after "start date" (days)	1,476 (818–2,476)
Time between the end of exposure and the end of follow-up (days)	1,369 (697–2,371)
Dosage (mg/day)	399 (267–400)
Duration of exposure (days)	105 (30–210)
Underlying diseases, n (%)	
Rheumatoid arthritis	3,938 (74.8)
Systemic lupus erythematosus	774 (14.7)
Sjogren syndrome	292 (5.5)
Dermatomyositis/polymyositis	5 (0.1)
Other connective tissue diseases[a]	251 (4.8)
Light eruption	5 (0.1)
Number of prescriptions of[b]	
Methotrexate	0 (0–63)
Azathioprine	0 (0–1)
Glucocorticoids	1 (0–56)
NSAIDs	10 (0–98)
Metformin	0 (0–6)
Vitamin D	0 (0–37)
Smoking status, n (%)	
Nonsmokers	2,551 (48.5)
Ex-smokers	1,589 (30.2)
Smokers	1,111 (21.1)
Missing	14 (0.3)
Townsend deprivation index, n (%)	
0 (less deprived)	172 (3.3)
1	1,383 (26.3)
2	1,201 (22.8)
3	1,111 (21.1)
4	818 (15.5)
5 (more deprived)	535 (10.2)
Missing	45 (0.8)
BMI (kg/m^2)	26.5 (23.2–30.9)
Past history of, n (%)	
Non-skin cancer	236 (4.5)
Hematological malignancy	34 (0.6)
Skin cancer	189 (3.6)
Hypertension	1,330 (25.3)
Diabetes	427 (8.1)
Incident outcomes after "start date", n (%)	
Non-skin cancer	
Breast	66 (1.3)
Colon	17 (0.3)
Prostate	9 (0.2)
Lung	39 (0.7)
Other or nonspecific codes	79 (1.5)
Hematological malignancy	42 (0.8)
Skin cancer	129 (2.5)
Metastasis	41 (0.8)
Death	380 (7.2)

Notes: [a]Systemic sclerosis, antiphospholipid syndrome, mixed and undifferentiated connective tissue diseases. [b]Before the date of cancer or a randomly selected date for those without cancer. Continuous variables are reported as medians and interquartile range except for the number of medication prescriptions that are reported as medians and 5th–95th percentile range.

Table S2 Risk of incident malignancies and metastases – comparison of the hydroxychloroquine/chloroquine exposed and unexposed periods

	Exposed time Time at risk (year) Incidence rate (per 100 PY)	Unexposed time Time at risk (year) Incidence rate (per 100 PY)	Crude sHR*	*p*-value	Adjusted sHR*	*p*-value
Non-skin cancers	38,128 0.76	33,340 0.91	0.93 (0.78–1.10)	0.38	0.99 (0.83–1.17)[a]	0.87
Hematological malignancies	38,527 0.12	34,157 0.16	0.79 (0.52–1.21)	0.28	0.69 (0.44–1.07)[b]	0.09
Skin cancers	38,054 0.54	33,620 0.51	1.14 (0.92–1.41)	0.24	1.18 (0.95–1.48)[b]	0.14
Metastases	38,611 0.09	34,259 0.19	0.56 (0.36–0.86)	0.008	0.60 (0.38–0.94)[c]	0.02

Notes: *Chronically exposed compared to shortly exposed. [a]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of NSAIDs, metformin, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions. [b]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of NSAIDs, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions. [c]Adjusted on age, sex, underlying condition, smoking status, past history of cancer, deprivation index, and number of metformin, vitamin D, and glucocorticoids prescriptions.
Abbreviations: NSAIDs, nonsteroidal antiinflammatory drugs; PY, person-years; sHR, sub-distribution hazard ratio.

Table S3 Risk of death according to age of the individuals (chronically exposed compared to short-term exposed)

	Chronically exposed, N=8,999	Short-term exposed, N=5,265	Crude HR	*p*-value	Adjusted HR	*p*-value
Death in the overall population, n (%)	636 (7.0)	380 (7.2)	0.91 (0.80–1.03)	0.15	0.94 (0.82–1.08)	0.37
≤70 years	306 (4.1)	197 (4.5)	0.80 (0.67–0.96)	0.01	0.78 (0.64–0.94)	0.01
>70 years	330 (22.3)	183 (20.0)	1.20 (1.00–1.43)	0.05	1.10 (0.89–1.34)	0.38
Death in those with newly developed cancer, n (%)[a]	151 (30.6)	107 (38.5)	0.79 (0.61–1.00)	0.05	0.84 (0.65–1.09)	0.20
≤70 years	91 (25.6)	62 (32.5)	0.74 (0.54–1.02)	0.07	0.73 (0.52–1.01)	0.06
>70 years	60 (43.2)	45 (51.7)	0.93 (0.63–1.38)	0.74	0.98 (0.64–1.51)	0.94
Death in those without newly developed cancer, n (%)[a]	485 (5.7)	273 (5.5)	0.97 (0.84–1.13)	0.72	0.98 (0.84–1.16)	0.85
≤70 years	215 (3.0)	135 (3.3)	0.83 (0.67–1.03)	0.09	0.81 (0.64–1.02)	0.08
>70 years	270 (20.1)	138 (16.7)	1.25 (1.01–1.53)	0.04	1.17 (0.92–1.47)	0.19

Notes: [a]Excluding basal cell carcinomas. Models adjusted on age, sex, underlying condition, smoking status, past history of cancer, past history of diabetes, past history of hypertension, BMI, deprivation index, and number of NSAIDs, metformin, vitamin D, glucocorticoids, methotrexate, and azathioprine prescriptions.
Abbreviations: BMI, body mass index; HR, hazard ratio; NSAIDs, nonsteroidal antiinflammatory drugs.