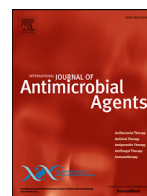




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## Review

## Cardiac effects and toxicity of chloroquine: a short update

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## ABSTRACT

There is currently increased interest in the use of the antimalarial drugs chloroquine and hydroxychloroquine for the treatment of other diseases, including cancer and viral infections such as coronavirus disease 2019 (COVID-19). However, the risk of cardiotoxic effects tends to limit their use. In this review, the effects of these drugs on the electrical and mechanical activities of the heart as well as on remodelling of cardiac tissue are presented and the underlying molecular and cellular mechanisms are discussed. The drugs can have proarrhythmic as well as antiarrhythmic actions resulting from their inhibition of ion channels, including voltage-dependent Na<sup>+</sup> and Ca<sup>2+</sup> channels, background and voltage-dependent K<sup>+</sup> channels, and pacemaker channels. The drugs also exert a vagolytic effect due at least in part to a muscarinic receptor antagonist action. They also interfere with normal autophagy flux, an effect that could aggravate ischaemia/reperfusion injury or post-infarct remodelling. Most of the toxic effects occur at high concentrations, following prolonged drug administration or in the context of drug associations.

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## 1. Introduction

Chloroquine and its related drug hydroxychloroquine, introduced more than 80 years ago and used traditionally to treat malaria and chronic inflammatory diseases, have re-emerged as possible therapeutic agents in viral diseases, including coronavirus disease 2019 (COVID-19) [1]. Renewed interest in these drugs is not new as there have also been assessments of their potential effects as anti-cancer [2,3], antiarrhythmic [4] and pulmonary vasodilator [5] agents.

Whenever considering to (re)use a drug, one should weigh the benefits against the risks (mainly the side effects) associated with use of the drug. The usefulness of chloroquine/hydroxychloroquine in the treatment of COVID-19 is currently still being debated [6,7]. Whereas on the one hand there is little doubt that chloroquine and hydroxychloroquine can display antiviral activity *in vitro* [1,8–10] and *in vivo* [11], on the other hand controversy exists on whether this effect is of use in COVID-19 patients. Clinical studies are underway to answer the question regarding an anti-COVID-19 protective action in humans [12]. While waiting for a clear answer to this issue, it is necessary to reflect on the risks of using the drugs.

The effects of chloroquine/hydroxychloroquine, especially those related to the immunomodulatory action, have been reviewed very recently [13]. Among the side effects of chloroquine/hydroxychloroquine, those involving the heart are frequently invoked as being life-threatening, and cardiotoxicity is a major concern for many other antimalarial drugs [14,15]. Cardiotoxic effects have been presented as arguments against recent trials to repurpose these drugs for the treatment of COVID-19 [16]. In the present review, we describe the major known cardiac effects of the drugs with the aim of determining which effects can be expected to occur at usual therapeutic concentrations and account for observed beneficial or side effects. We do not make a distinction between the effects of chloroquine versus those of hydroxychloroquine, the underlying assumption being that, despite potential pharmacokinetic and potency differences, their pharmacological actions are essentially identical. We suggest that (i) major side effects occur at high concentrations (e.g. following poisoning), after long-term treatment or in the context of drug associations and (ii) common effects following short-term treatment with low clinical doses are generally well tolerated. We also comment on the cellular and molecular mechanisms underlying the effects.

## 2. Cardiac effects of chloroquine/hydroxychloroquine

Chloroquine/hydroxychloroquine can be administered via parenteral or oral routes. Acute toxic effects were recorded more

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frequently when the drugs were given via the parenteral [especially the intravenous (i.v.)] route [17]. Drug absorption is practically complete even when administered via the oral route, but the concentration profile reaches lower peak levels post-enteral than post-parenteral administration [18]. The drugs accumulate in cells owing to ion trapping in more acidic environments, especially in lysosomes [19,20], where the concentration can be many orders of magnitude higher than in the blood. The biological half-life is as long as 30–60 days [21,22]. Hence, there is the possibility for progressive drug accumulation following repeated administration even of small doses, and the drug effects also develop following different time scales: from seconds or minutes, to hours or days, and to many months or years.

There are many case reports of rapidly developing cardiovascular effects following intoxication by chloroquine/hydroxychloroquine, ranging from bradycardia and hypotension to eventual cardiac arrest [23]. As mentioned, fear or real risks of cardiotoxicity has limited the use of these drugs. Cardiotoxic effects associated with chloroquine/hydroxychloroquine use in clinical conditions have recently been reviewed [14,24]. Here we summarise the major effects detected under either clinical or experimental conditions and have grouped them into (i) purely functional (electrophysiological and mechanical) effects and (ii) those associated with structural changes.

## 2.1. Electrophysiological and mechanical effects

### 2.1.1. Effects on cardiac rhythm and on conduction and duration of electrical activity

Electrocardiographic changes associated with chloroquine/hydroxychloroquine treatment have been reported as proarrhythmic in some studies and as antiarrhythmic in others.

Rhythm and electrophysiological effects of chloroquine/hydroxychloroquine include bradycardia as well as tachycardia, flattening of the T wave, prolongation of the QT interval, and various forms of conduction blocks. For example, sinus dysfunction with bradycardia [25] and atrioventricular (AV) or intraventricular conduction abnormalities [26] were noted to be frequent in rheumatoid arthritis and lupus erythematosus patients treated with chloroquine/hydroxychloroquine. In a review of case reports up to 2017, conduction disturbances were the most prevalent among all side effects [24]. However, although arrhythmias in such patients have traditionally been attributed to the drugs, a role of the underlying diseases (as a direct cause or favouring factor) cannot be excluded. Indeed, in one study ventricular conduction blocks observed in chloroquine/hydroxychloroquine-treated patients had a prevalence that did not differ from that in those not receiving these drugs [27].

Given the role played by the duration of ventricular depolarisation (measured as QT interval on the electrocardiogram) in arrhythmogenesis, it has been of interest to examine whether chloroquine/hydroxychloroquine increase the QT duration. QT prolongation has been noted under clinical conditions with high chloroquine concentrations (25 mg base/kg/day [28]) or in experimental conditions using parenteral administration [29]. QT prolongation is dominated by the effect on the QRS duration, and marginal dose-dependent prolongation is seen on the J-T interval [30], which measures more accurately the duration of depolarisation. In most studies, no or only marginal QT prolongation is induced by clinical doses (<15 mg/kg/day) of chloroquine/hydroxychloroquine, especially by the oral route [14,15], suggesting that chloroquine/hydroxychloroquine are in this respect safer than other quinoline-based antimalarial drugs (e.g. halofantrine [31]). Clinically, low chloroquine doses (2–4 mg/kg/day) were associated with significant QT prolongation when given over

many months [32], suggesting a role of drug accumulation or myocardial remodelling.

In contrast to the proarrhythmic actions of chloroquine/hydroxychloroquine, an antiarrhythmic action, known since the middle of the past century [33,34], can be further demonstrated from experimental animal data [35] and from clinical data of patients treated for chronic inflammation [4]. Recently, it was shown that chloroquine (200–600 mg/day) can cause conversion to sinus rhythm in a patient with established atrial fibrillation [36]. Such an antiarrhythmic action is not unexpected given the drug action on voltage-dependent Na<sup>+</sup> channels (see below). It should be noted that the 'Janus-faced' (pro- and anti-) arrhythmic action is also known for classic antiarrhythmics [37].

### 2.1.2. Inotropic action

The effect of chloroquine/hydroxychloroquine on cardiac contraction remains unclear. Early studies in amphibian hearts suggested that chloroquine exerts a positive inotropic effect, attributed to an indirect action due to the activation of brain sympathetic centres [38]. Similarly, a transient positive inotropic action was obtained in guinea pig atria [39]. It was suppressed by pre-treatment with reserpine or propranolol, indicating that it was due to the release of catecholamines, which then acted on cardiomyocyte  $\beta$ -adrenergic receptors [39]. Most subsequent studies in mammalian hearts either failed to show any inotropic action or demonstrated a negative action. No change of cardiac mechanical function assessed using echocardiography was observed in rats injected with 50 mg/kg/day chloroquine for 2 weeks [40]. In contrast, negative inotropism was evident in atrial muscles, but only at concentrations >10  $\mu$ M [39,41]. Similarly, in guinea pig cardiac muscle, chloroquine decreased the contraction at 10–100  $\mu$ M in isolated atria [42]. In rat isolated left ventricular papillary muscles, chloroquine (100  $\mu$ M) was found either to have no effect [41] or to decrease the contractile force and the rates of shortening and relaxation [43]. In Langendorff-perfused guinea pig ventricles, chloroquine decreased the contraction at concentrations of >1  $\mu$ M [42], but this apparently higher potency in ventricular muscle could be an artifact due to a time-dependent rundown of the contraction in this preparation. In rat isolated perfused hearts, systolic function was decreased with chloroquine concentrations of >10  $\mu$ M and was completely suppressed at 100  $\mu$ M [44]. This negative inotropic action was associated with a decrease in heart rate. Unless heart rate is controlled, it is difficult to accurately determine the inotropic effect since part of the decrease in contraction may be related to a positive force–frequency relation prevailing in the preparation. In addition, at high chloroquine concentrations, loss of excitability and suppressed conduction (due to chloroquine effects on the upstroke of the action potential) might account for an apparent loss of contraction. Thus, a negative inotropic action of chloroquine/hydroxychloroquine is typically obtained at high drug concentrations, above those reached by usual therapeutic regimens.

The mechanism underlying the reduction of contraction could be related to the inhibition of I<sub>Ca-L</sub> discussed below. It has been noted that the effect of chloroquine (0.04 mg/mL, i.e. ~125  $\mu$ M) to reduce contractility in turtle hearts is similar to that of quinine, and that for both drugs the effect is reversed by raising the external Ca<sup>2+</sup> concentration [45]. This points to a role of decreased excitability and/or decreased Ca<sup>2+</sup> influx and makes less likely an effect on myofilament Ca<sup>2+</sup> sensitivity. However, in guinea pig hearts [42], increasing external Ca<sup>2+</sup> was not able to fully reverse the chloroquine-induced contraction decrease. Contractions obtained while inactivating Na<sup>+</sup> channels with high external K<sup>+</sup> were as sensitive to chloroquine as those recorded in normal conditions, whereas post-rest potentiation of contraction was not affected by the drug. These data point to a role of diminished Ca<sup>2+</sup> influx in the negative inotropism of chloroquine.

Cardiovascular shock is common following chloroquine poisoning [46] but it is not clear whether the condition is mainly due to vascular or cardiogenic mechanisms. Since cardiogenic mechanisms can be primarily arrhythmic and not necessarily primarily inotropic, the contribution of decreased inotropy is unclear. It is of note that besides studies of the mechanical dysfunction related to chloroquine/hydroxychloroquine cardiomyopathy, there are no studies reporting on eventual acute inotropic effects in human patients or volunteers. A dose-dependent decrease of systolic pressure can also be demonstrated in experimental animals intoxicated with i.v. chloroquine [41]. Haemodynamic failure was associated with a decrease of maximum rates of pressure development or relaxation, bradycardia and QRS widening. This suggests the participation of an important non-vascular, cardiac component.

## 2.2. Cardiomyopathic and remodelling-associated structural changes and their functional consequences

Chronic treatment (usually over many months or years) with chloroquine/hydroxychloroquine can result in cardiomyopathy [47], characterised by wall thickening and microscopic structural changes. Cardiomyopathy appears to occur more with chloroquine than with hydroxychloroquine and involves at the microscopic level an enlargement and vacuolisation of cardiac myocytes, with deposition of myeloid and curvilinear bodies. Cardiomyopathy is accompanied by functional changes, including AV or bundle-branch conduction block [48] as well as mechanical dysfunction, with diffusive hypokinesia and decreased ejection fraction [26]. Chloroquine cardiomyopathy can develop in the absence of skeletal myopathy or retinal toxicity, although similar cellular mechanisms might be involved. In the context of short-term treatment, these effects are not expected.

Other structural changes occurring in the myocardium are not primarily due to chloroquine/hydroxychloroquine but are modified by these drugs.

Myocardial remodelling involves structural and functional changes triggered by many stress factors, including physiological conditions such as physical exercise, pregnancy or fetal body growth, as well as pathological conditions such as ischaemia/reperfusion (infarction), pressure or volume overload, inflammation, metabolic factors (e.g. hyperglycaemia and hyperlipidaemia) and reactive oxygen radicals [49]. Physiological remodelling is adaptive and leads to hypertrophy in order to increase function in athletes, pregnant women and the growing fetus. In contrast, remodelling following pathological triggers is usually maladaptive as it evolves with time towards heart failure and increased propensity to arrhythmias.

Characteristic structural changes during pathological remodelling include an increase in cardiomyocyte size (causing hypertrophy or dilatation) and alterations of the ultramicroscopic structure (e.g. loss of T-tubules [50]), modifications of the extracellular matrix, and proliferation of myofibroblasts with development of fibrosis. Following myocardial infarction, concomitantly with the development of fibrosis and formation of a scar in the infarcted zone, there are changes in the infarct border and in the remote non-infarcted myocardium. With time the heart undergoes chamber dilatation.

Functional changes in remodelled myocardium include modifications in the electrical properties (called electrical remodelling) as a result of altered expression of ion channels (e.g. Kir K<sup>+</sup> channels, L-type Ca<sup>2+</sup> channels, connexins, ryanodine receptors, etc.), transporters (e.g. Na<sup>+</sup>-Ca<sup>2+</sup> exchangers, sarcoplasmic reticulum ATPases, etc.) and other proteins, which impact on the action potential and its conduction within the cardiac tissue, on the

excitation-contraction coupling and on the contractile properties. Electrical remodelling is responsible for the increased propensity to arrhythmias. Many weeks following myocardial infarction, there is progressive deterioration of myocardial function with increased left ventricular end-diastolic volume, diastolic dysfunction and decreased systolic function.

Many processes and cell types are implicated in the remodelling process [49,51]. Inflammation, with the associated release of mediators, cytokines and chemokines by inflammatory cells, plays an important role in the remodelling process, and an experimental model of inflammation-induced remodelling resulting in dilated cardiomyopathy is obtained in mice overexpressing tumour necrosis factor (TNF) [52].

The effect of chloroquine on remodelling does not necessarily involve a direct action on cardiomyocytes and may involve primary effects on other cardiac cells such as fibroblasts. During post-infarct remodelling, cardiac fibroblasts are activated and converted to myofibroblasts, which display a higher proliferative and secretory capacity. In fibroblasts, chloroquine significantly reduced the expression of proteins such as alpha smooth muscle actinin ( $\alpha$ -SMA) and fibronectin. In myofibroblasts, chloroquine reduced cell migration and contractility [53].

Chloroquine/hydroxychloroquine may also affect electrical remodelling. As far as ion channel remodelling is concerned, reduced amplitude and slightly modified kinetics of voltage-dependent Na<sup>+</sup> current were evident in epicardial cells of the infarct border zone, and these changes were supposed to play a role in decreased conduction underlying re-entrant arrhythmias [54]. Chloroquine (100  $\mu$ M), in addition to displaying its inhibitory action on the Na<sup>+</sup> current, was shown to completely suppress the cAMP-dependent protein kinase (PKA)-mediated restoration of the ion current in cells from the infarct border zone [55].

Remodelling in infarcted hearts can be reduced by calorie restriction or pharmacological agents such as resveratrol or rapamycin, which probably act by promoting autophagy [56]. Chloroquine suppressed the anti-remodelling effects of food restriction and resveratrol. This was shown in mice that developed ventricular dilation and dysfunction a few days/weeks after induction of myocardial infarction by left coronary artery ligation. Restriction of the food intake by 20–60%, started 1 week after infarction and maintained for 2 weeks, was accompanied by an improvement of the cardiac chamber size as well as most contractile parameters [57]. In addition, the increase in cell size was also attenuated. Treatment with chloroquine (10 mg/kg/day given by continuous subcutaneous injection) for 2 weeks reversed the improvement afforded by food restriction. Like the effect of food restriction, a 2-week treatment with resveratrol significantly attenuated the ventricular dilation and improved cardiac function in mice that underwent left coronary artery ligation. This protective effect was absent in mice treated with chloroquine (10 mg/kg/day) simultaneously with resveratrol [58]. As discussed below, these effects of chloroquine are apparently due to a suppression of observed increases in autophagy by food restriction or drugs in the cells.

Whilst chloroquine appears to have deleterious effect in the remodelling induced by primary cardiac lesions, the drug may have a protective action on remodelling of the right heart associated with pulmonary arterial hypertension. Rats in which this condition was induced (by monocrotaline) and that were intraperitoneally injected with either chloroquine (20 mg/kg/day or 50 mg/kg/day) or hydroxychloroquine (50 mg/kg/day) for 3 weeks displayed less right ventricular hypertrophy and improved function owing to a decrease of pulmonary vascular resistance. In this case, the remodelling in the pulmonary vasculature was suppressed by chloroquine/hydroxychloroquine [59].

### 3. Cellular and molecular mechanisms underlying the effects

Exploring the cellular and molecular mechanisms of chloroquine/hydroxychloroquine effects allows an understanding of possible synergy or antagonism with other drugs administered simultaneously or the interaction with changes already induced by pathological conditions.

#### 3.1. Effects on cardiac ion channels

##### 3.1.1. Inactivation of voltage-dependent $\text{Na}^+$ channels: local anaesthetic effect

Chloroquine is structurally close to quinine and quinidine, which act on voltage-dependent  $\text{Na}^+$  ( $\text{Na}_V$ ) channels: they favour an inactivated state of the channels, therefore making them less available for opening upon depolarisation (excitation). Chloroquine exerts similar 'local anaesthetic' effects [60]. Given the involvement of  $\text{Na}_V$  channels in the genesis and transmission of the electrical impulse in nerves, chloroquine can suppress pain sensation as do classic local anaesthetics, but the drug is not used for this effect because of its anticoagulant effect.  $\text{Na}_V$  channels are present in other excitable cells, including cardiac atrial contracting cells and all ventricular cell types, where they are responsible for the upstroke (phase 0) of the action potential. Chloroquine decreases all indices of  $\text{Na}_V$  channel contribution to the cardiac action potential: the maximal rate of depolarisation during phase 0 and the action potential amplitude [61], both of which determine the rate of conduction within the tissue. Side effects regarding intraventricular conduction (QRS prolongation, bundle branch block) observed with the drugs [24,30] are likely explained on this basis.

A persisting, late  $\text{Na}^+$  current is among determinants of the action potential duration and favours arrhythmogenesis [62]. It contributes to intracellular  $\text{Na}^+$  overload and the subsequent  $\text{Na}-\text{Ca}^{2+}$  exchange-mediated  $\text{Ca}^{2+}$  overload during ischaemia. Suppression of this current by chloroquine [63] may counterbalance the effect of blocking  $\text{K}^+$  currents on the QT duration and partly account for the antiarrhythmic and anti-ischaemic protective actions.

##### 3.1.2. Decrease of the L-type $\text{Ca}^{2+}$ current

Chloroquine/hydroxychloroquine inhibit L-type  $\text{Ca}^{2+}$  channels since they decrease the amplitude of  $I_{\text{Ca-L}}$  in sinoatrial (SA) [64] and working myocardial cells [61]. The effect in SA cells likely contributes to the slowing of pacemaker activity. Similar effects are expected in AV nodal cells and can explain AV conduction block. Given the role of  $I_{\text{Ca-L}}$  in cardiac excitation-contraction coupling, the negative inotropic action of the drugs [42,43] might also be explained on this basis, although most studies report a lack of significant inotropic effect at clinically useful concentrations.

##### 3.1.3. Decrease of the pacemaker ( $I_f$ ) current

Cardiac automatic activity is due to the presence of a spontaneous depolarisation of nodal and conduction cells during the diastolic time. Many processes contribute to this diastolic depolarisation [65,66], among them the opening of hyperpolarisation-activated channels [67–69]. These channels are called 'funny channels' and the corresponding current 'funny current,  $I_f$ ' because of the apparently strange behaviour in comparison with other channels, which usually open upon depolarisation.  $I_f$  amplitude (measured during hyperpolarising steps under voltage clamp) was decreased by hydroxychloroquine [64]. There was no change in voltage-dependent activation, suggesting that the drug acts on the channel conductance but the underlying molecular mechanism is unknown. This inhibitory action on  $I_f$  was translated into a decrease in the rate of diastolic depolarisation and the beating rate of SA isolated cells or atrial multicellular preparations [42,64].

Based on its effect to decrease  $I_f$ , hydroxychloroquine was proposed as a relatively safe bradycardic agent [64], and bradycardia was achieved with i.v. injections in anaesthetised animals, without negative inotropic or haemodynamic action. Therefore, the bradycardic effect was proposed to be of potential value in decreasing oxygen demand, of benefit in conditions such as angina.

It should be noted that bradycardia occurred within a few (typically 5) minutes using hydroxychloroquine in anaesthetised animals, whilst tachycardia is observed for hours after a single dose of chloroquine in awake human volunteers (see below). It is likely that in humans the fast-developing rate-slowing action on pacemaker tissue is followed and opposed by a slowly developing effect due to an interaction with the autonomic nervous system (see below).

##### 3.1.4. Block of potassium channels

Chloroquine also acts on various  $\text{K}^+$  channels [61,70–75].

**3.1.4.1. Inward-rectifier  $\text{K}^+$  ( $\text{Kir}$ ) channels:  $I_{\text{K1}}$ ,  $I_{\text{K-ACh}}$  and  $I_{\text{K-ATP}}$ .** Chloroquine blocks  $\text{Kir}2.1$  inward-rectifier channels, largely responsible for the background conductance and  $I_{\text{K1}}$  current [61,75] that set the resting potential but also contribute to repolarisation and the duration of the action potential. The underlying mechanism involves an accession of chloroquine from the intracellular side and its binding near the intracellular mouth of the pore [74].

There appears to be a dual action of chloroquine on  $\text{Kir}2.1$  channels. In contrast to the acute decrease of channel conductance, an increase in plasmalemmal expression of the channel protein has been reported in HEK cells used as an expression system [76]. This effect developed over hours and was related to inhibition of lysosomal degradation of the channels. To our knowledge, no study has tested this effect in cardiomyocytes, but such an increase in channel expression could have a corrective action against the decrease due to block, and partly contribute to limit QT prolongation.

Chloroquine/hydroxychloroquine also block the G-protein-activated inward rectifier  $\text{K}^+$  ( $\text{GIRK}$ ) channel present in cardiac supraventricular (atrial contracting and nodal) cells. The channel is also present in ventricular cells of some species but is practically absent in human ventricular contracting cells. It is activated following binding of acetylcholine ( $\text{ACh}$ ), the neurotransmitter of the parasympathetic system, on  $\text{M}_2$  muscarinic receptors, or adenosine binding on  $\text{A}_1$  receptors, hence the names  $I_{\text{K-ACh}}$  or  $I_{\text{K-Ado}}$  for the corresponding ion currents. The blocking action of chloroquine is also observed in HEK cells expressing  $\text{Kir}3.1$  and  $\text{Kir}3.4$  channels, which contribute subunits forming  $\text{GIRK}$  channels [36,77]. The underlying mechanism is similar to that of  $\text{Kir}2.1$  ( $I_{\text{K1}}$ ) channels described above: it involves binding from the intracellular side to a site within the internal mouth of the channel. The  $\text{IC}_{50}$  of chloroquine for both channels was  $1 \mu\text{M}$ , a concentration similar to the plasma concentrations following oral doses of  $\sim 10 \text{ mg/kg}$ .

The blocking effect on  $I_{\text{K1}}$ ,  $I_{\text{K-ACh}}$  or both should result in a lengthening of the action potential duration. As mentioned earlier, no or only marginal QT prolongation is induced by low doses of chloroquine/hydroxychloroquine given via the oral route [14,15]. Given that action potential shortening is associated with arrhythmogenesis, the action potential lengthening effect of chloroquine may be antiarrhythmic under pathological conditions that markedly shorten the action potential duration. Such an antiarrhythmic effect is mostly obvious in the case of short QT syndrome due to mutations of  $\text{Kir}2.1$  channels [78], but it was also shown to occur in sheep hearts with sustained atrial fibrillation induced by chronic pacing [77]. Under the latter condition,  $I_{\text{K-ACh}}$  is spontaneously activated in the absence of agonist binding on G-protein coupled receptors. Chloroquine decreased the dominant fibrillation frequency and finally induced a conversion to sinus rhythm [77], an effect that was associated with prolongation of the atrial action

potential and could be reproduced by a toxin (tertiapin) known to suppress  $I_{K-ACh}$ .

Chloroquine/hydroxychloroquine also act on ATP-sensitive  $K^+$  channels. These channels monitor cell metabolism and open following a decrease in intracellular ATP and an increase in ADP, as occur in conditions such as ischaemia. In the heart, the channels are formed by a pore unit made of Kir6.1 and a sulfonylurea-binding regulatory unit (SUR2A). Chloroquine blocks the ATP-sensitive current ( $I_{K-ATP}$ ) activated pharmacologically (by pinacidil) in ventricular cardiomyocytes [79] and the corresponding channel units expressed in HEK cells [71]. The suppression involved two mechanisms: a pore block by chloroquine entering from the intracellular side and occluding ion movement within the channel; and an inhibition, given the amphiphilic character of chloroquine, of the channel-activating interaction between Kir6.1 and the membrane phospholipid phosphatidyl-inositol bisphosphate ( $PIP_2$ ).  $I_{K-ATP}$  inhibition in cardiac myocytes occurred at micromolar concentrations ( $IC_{50}$ , 0.5  $\mu M$ ), indicating that chloroquine at therapeutic doses could also act on the heart through mechanisms involving ATP-sensitive channels, e.g. to antagonise fibrillation following action potential shortening and inhomogeneity during ischaemia.

**3.1.4.2. Rapid and delayed rectifier  $K^+$  currents:  $I_{Kr}$  and  $I_{Ks}$ .** In SA node cells, Purkinje fibres and ventricular myocytes, hydroxychloroquine was shown to decrease the amplitude of the rapid delayed rectifier  $I_{Kr}$  by 35% [61]. The drug did not affect the slow component  $I_{Ks}$ . The effect on  $I_{Kr}$  is consistent with the inhibition of hERG channels in expression systems ( $IC_{50}$ , 2.5–8.4  $\mu M$ ) and could contribute to QT prolongation, especially at high concentrations [80,81].

### 3.1.5. Channels carrying the transient outward current ( $I_{to}$ ) and other channels

A transient outward current ( $I_{to}$ ) underlies phase 1 early repolarisation in cardiac cells. It is a mixed current made of a  $Ca^{2+}$ -independent but voltage-dependent  $K^+$  component, and a  $Ca^{2+}$ -activated  $Cl^-$  component [82,83]. Chloroquine does not appear to have potent effect on  $I_{to}$  [61]. Inhibitory effects occurred only at very high concentrations ( $K_d$ , 520  $\mu M$  [84]) that are not reached under therapeutic conditions.

The effect on other (chloride-selective and non-selective sarcolemmal, sarcoplasmic reticulum and mitochondrial) cardiac channels have not, to our knowledge, been investigated.

## 3.2. Effects on membrane receptors

### 3.2.1. Atropine-like muscarinic receptor antagonist action

In contrast to slowing of the heart rate by chloroquine observed under experimental conditions as expected from the suppression of  $I_f$  and  $I_{Ca-L}$  [64], the drug increased heart rate during more than 4 h after a single per os dose of 3.75–10 mg/kg in healthy human volunteers [85]. This latter effect may be attributed to a dual effect on the autonomic nervous system. On the one hand there might be an activation of heart-accelerating mechanisms resulting from stimulation of the sympathetic system [38], but such a mechanism has not been demonstrated in humans. On the other hand there is inactivation of heart-decelerating mechanisms resulting from inhibition of the parasympathetic (or vagal) system. This latter effect has been more thoroughly investigated by the author. The heart rate increase was associated with a decrease of cardiovascular reflexes used as indices of the vagal influence on the heart. One of the most readily assessable parameters of the autonomic nervous control of the heart is the beat-to-beat variation of the heart cycle. Chloroquine caused a narrowing of the average dispersion be-

tween heart cycle durations. Other reflexes, such as the cardiac cycle increase during a Valsalva manoeuvre or post deep inspiration, were also decreased, indicating that chloroquine exerts a vagolytic action on the heart. This effect is dose-dependent and its underlying mechanism appears to involve, at least in part, a direct atropine-like antagonist action of chloroquine on muscarinic receptors. Chloroquine antagonised the negative chronotropic effect of muscarinic receptor activation under experimental conditions using animal isolated hearts [86]. Chloroquine is known to bind to muscarinic receptors ( $K_d$ , 6–15  $\mu M$  [87]). Its binding on cardiac muscarinic receptors could be demonstrated by its displacement of saturation binding curves of a radioactive label of these receptors [86]. However, this interaction with receptors occurred at rather higher concentrations than those interfering with the negative chronotropic effect of muscarinic agonists. Thus, despite the demonstrated interaction of chloroquine with muscarinic receptors, the mechanism of chloroquine effect on the heart rate appears to be complex. As discussed above, effects on channels, including a block of  $I_{K1}$  and  $I_{K-ACh}$ , which contribute to regulation of the heart rate, could play a role and partly account for the increase in heart rate caused by chloroquine.

### 3.3. Inhibition of lysosomal function and autophagy

Besides the effects described above, which basically occur at the surface membrane, chloroquine also targets intracellular processes, mainly (but not exclusively) those involving the intracellular processing of proteins by autophagy.

Autophagy is an important homeostatic mechanism that is responsible for the clearance of damaged intracellular protein complexes and organelles [88,89], including in the heart. It plays an important role in the response to stress and in disease conditions such as ischaemia/reperfusion [90,91], hypertrophy [92], heart failure [93,94], etc. Drug-induced changes in autophagy may also underlie therapeutic or toxic effects.

Macroautophagy involves the fusion of autophagosomes with lysosomes to constitute autolysosomes, where cargo brought by phagosomes is digested. Chloroquine, which accumulates in lysosomes by ion trapping [19,20] and causes alkalinisation of the intralysosomal pH, inhibits the fusion between autophagosomes and lysosomes and the basic macroautophagy flux [95]. Hearts pretreated with chloroquine (10 mg/kg/day, a dose within clinical range) for days show a decreased autophagy flux, with accumulation of autophagosomes. Another type of autophagy that involves chaperone-mediated transport of abnormal proteins into lysosomes also appears to be inhibited by chloroquine [40].

Given the importance of ischaemic diseases in cardiac pathology, it is of interest that ischaemia/reperfusion is reported to modify autophagy and that chloroquine has a potential to modulate the changes occurring under these conditions. An excessive decrease or increase of autophagy beyond its basal level may be expected to have harmful consequences. Autophagy is upregulated during myocardial ischaemia and reperfusion [89]. Whereas during the ischaemia phase activation is adenosine monophosphate-activated kinase (AMPK)-dependent, adaptive and protective, during reperfusion it is AMPK-independent, excessive and enhances myocardial death. Protection during ischaemia is likely due to the ability of autophagy to deliver metabolites and to clear damaged organelles such as mitochondria (mitophagy). Excessive autophagy and cell death during reperfusion are attributed to the formation of reactive oxygen radicals. Autophagy inhibition by chloroquine could limit the adaptive increase during ischaemia and aggravate the condition. This is the case in isolated rats submitted to episodes of intermittent hypoxia for many weeks. In this experimental model set to mimic the condition of sleep apnoea, inhibition of autophagy with chloroquine was associated with the development of my-

cardiac dysfunction, whilst no dysfunction was present with intermittent hypoxia alone [96], suggesting that autophagy provided cardiac adaptive mechanisms to hypoxia. In contrast, attenuation of the excessive increase of autophagy during reperfusion could a priori be expected to confer protection. However, reperfusion by itself, despite upregulating autophagy, is associated with decreased clearance of autophagosomes [97]. Further suppression of autophagosome clearance by chloroquine can therefore aggravate this process. Increased cell death caused by chloroquine during ischaemia/reperfusion has been linked to a decrease of the oxygen radical buffering action of mitochondria [40].

Chloroquine could be expected to be protective in other conditions that enhance autophagy flux, such as cardiac hypertrophy. The drug reversed the myocardial wall thickening caused by pressure overload in rats that underwent ascending aorta banding. It also appears to confer protection against ischaemia/reperfusion in diabetic cardiomyopathy [98,99].

Modification of autophagy is at least in part responsible for the effect of chloroquine/hydroxychloroquine on the cardiac remodelling induced by stress conditions such as myocardial ischaemia/reperfusion. Autophagy is decreased during post-infarction remodelling in rabbit heart [100]. In the days following myocardial infarction in rats, there was maintained cell death by controlled necrosis (necroptosis), including in the non-infarcted border zone. Autophagy flux was increased during the first day but despite a maintained increase in autophagosome formation, with time autophagy flux was decreased at the level of autophagosome processing and there was an associated increase in heart dysfunction [101]. The impaired autophagy appeared to be responsible for the necroptosis since the latter could be attenuated by genetic overexpression of beclin, an initiator of autophagy. In this model, chloroquine caused an aggravation of the structural and functional deterioration.

As already mentioned, because of reduced autophagy during myocardial remodelling, various strategies, including food restriction and pharmacological agents, can be used to stimulate autophagy. A similar protection can also be demonstrated by targeting autophagy in fibroblasts using micro-RNA [102]. The cellular signalling involved in some of these strategies may be complex. For example, the effect of food restriction and resveratrol may implicate an increased AMPK activity and a downregulation of mTOR signalling [58]. Chloroquine appears to suppress the protective effect of these strategies by modifying the effects of these signalling pathways on autophagy. It is not known whether there are additional inhibitory effects at more proximal steps.

The scope of effects due a modulation of autophagy may be larger than heretofore known. It is plausible that inhibition of autophagy affects the expression of many proteins (e.g. Kir2.1 channels mentioned earlier) and impacts on function. Chloroquine has been reported to enhance the loss of T-tubules observed in cultured ventricular cardiomyocytes from failing human and healthy adult rat hearts cultured in the absence of glucocorticoids [103]. Chloroquine, by inhibiting lysosome function in cultured mouse neonatal cardiomyocytes, was shown to cause an accumulation of phospholamban [104], a negative regulator of the sarcoendoplasmic reticulum  $\text{Ca}^{2+}$  ATPase (SERCA), responsible for storing  $\text{Ca}^{2+}$  needed for contraction in the lumen of the sarcoplasmic reticulum. It is not known to what extent this effect occurs in adult cardiomyocytes and impacts on contractility.

## 4. Non-cardiac actions possibly relevant for cardiac effects

### 4.1. Acute neurotoxicity and psychotoxicity

Reports of neurotoxic effects of chloroquine include the development of extrapyramidal symptoms (rolling of the eyeballs, invol-

untary movements, trismus) following a few hours to days of treatment with low doses in children [105] as well as in adults. These effects were reversible within 8–48 h after stopping the treatment. Behavioural disturbances have also been described [106–108]. Whether they can be related to actions on ion channels and receptors or to rapid consequences of the interference with autophagy is not clear.

### 4.2. Retinotoxicity and myotoxicity

Retinotoxicity, a noted frequent side effect of treatment with chloroquine/hydroxychloroquine [109], and skeletal muscle myopathy, which is less frequent [110–113], are largely the result of changes in autophagy.

Administration of chloroquine can cause a degeneration of skeletal muscle cells resulting in myopathy. Chloroquine myopathy can be reproduced experimentally by high doses (50 mg/kg/day) of the drug over  $\geq 3$  weeks. The degenerated muscle fibres contain numerous autophagic vacuoles [111] in which various proteins accumulate [112,113]. As mentioned earlier, a similar accumulation can occur in cardiomyocytes and is responsible for cardiotoxicity following long-term treatment [112,113]. Such effects are unlikely to complicate treatment limited to a few days and involving low to moderate doses ( $\leq 10$  mg/kg/day).

### 4.3. Hypokalaemia

Chloroquine poisoning is known to induce hypokalaemia [114]. The degree of hypokalaemia is larger the higher the dose ingested and the blood concentration. Hypokalaemia in patients is systematic at chloroquine concentrations of  $\geq 10 \mu\text{M}$ , but in a few cases it could be observed at much lower concentrations [114]. The condition can develop within 2 h or less following large doses and its underlying mechanism is not very clear, but increased renal loss has been excluded. It has been proposed to involve reduced cellular  $\text{K}^+$  efflux due to channel block by chloroquine (described above). However, in the absence of an increased  $\text{K}^+$  influx pathway, suppression of outflux is not a satisfactory explanation for the cellular uptake of  $\text{K}^+$  that causes hypokalaemia. Since the cellular membrane potential of resting or active cells is positive to the  $\text{K}^+$  equilibrium (Nernst) potential, only outward  $\text{K}^+$  movements can occur via channels. One should therefore explore the role of a non-channel influx pathway to account for the increased transport of  $\text{K}^+$  into cells. The effect of chloroquine on the  $\text{Na}^+-\text{K}^+$  pump is unclear. Chloroquine has been shown to inhibit the  $\text{Na}^+-\text{K}^+$  ATPase in vitro [115], but a transient stimulation could be obtained in vivo at a low concentration [116]. It is conceivable that hypokalaemia is triggered by conditions such as stress and exercise that stimulate the  $\text{Na}^+-\text{K}^+$  ATPase by enhancing the sympathetic tone, as is the case in hypokalaemic periodic paralysis [117], or by an eventual increase of insulin secretion. In addition, the vagolytic action of chloroquine (described above) could remove the known 'accentuated antagonism' exerted by the parasympathetic system towards the sympathetic system. Increased  $\text{Na}^+-\text{K}^+$  pump activity could then result from increased  $\beta$ -adrenergic receptor activation [118,119]. This or other possible mechanisms have not been examined, and the main basis for hypokalaemia thus remains unclear.

Low extracellular  $\text{K}^+$  concentration, largely because of the decrease in conductance of inward rectifier channels and of the corresponding outward currents, is associated with a lengthening of cardiomyocyte action potential duration and the QT interval, and is arrhythmogenic. Indeed, ventricular tachyarrhythmias were the immediate cause of death in approximately one-third of patients admitted for chloroquine poisoning [114]. One should be careful when attributing any clinically observed QT lengthening to a direct

effect of chloroquine, without excluding the eventual contribution of concurrent hypokalaemia.

## 5. Discussion

The possible efficacy of chloroquine/hydroxychloroquine in the treatment of various diseases, including viral infections such as COVID-19 [1,8,10,11] but also cancer [2,3], has received increasing interest. Besides the drug efficacy in patients, issues regarding safety are also important. Evidently, if the side effects are so marked that they put the life of treated patients in danger, then, as long as beneficial effects have been confirmed, the drug use could be restricted to only very severe cases when there is no other choice than to try everything that can potentially save the patient. Such use can only be done with close monitoring of the patient. In contrast, if side effects are mild and tolerable, such that they do not jeopardise the patient's life, then one can or should consider using the drug for its proven or potential benefit.

The side effects of chloroquine/hydroxychloroquine on the heart are mainly of two types: those involving changes in electrical conduction, heart rate and rhythm [14,24,27,31,80,120,121]; and those involving structural changes associated with cardiomyopathy [47,48] or myocardial remodelling. Conduction and rhythm disturbances can be seen under acute conditions before any structural changes, but they can also be favoured by cardiomyopathy [48]. On a time frame, the rhythm changes are acute and reversible upon drug withdrawal, whereas cardiomyopathy is induced after treatment for months and is largely irreversible. All effects are also dose-dependent, and cardiomyopathy [111] and interference with remodelling can be experimentally induced over shorter periods (weeks) with high doses.

The mechanisms underlying the conduction and rhythm changes mainly involve inhibitory actions on various ion channels. Block of Na<sup>+</sup> channels (local anaesthetic action) is likely responsible for observed bundle branch and intraventricular block [24,27,48]. Block of K<sup>+</sup> channels will tend to lengthen the action potential duration, whereas inhibition of Na<sup>+</sup> and Ca<sup>2+</sup> channels will have opposite effects. That the drugs given alone at low or moderate concentrations and for a short duration produce only limited prolongation of the QT interval is likely due to this balanced contribution of opposite effects [30]. Heart rate changes also result from a balance between a bradycardic action of I<sub>f</sub> and I<sub>Ca-L</sub> inhibition [61,64] and a tachycardic effect of I<sub>K1</sub> and I<sub>K-ACh</sub> inhibition [61,74,75], in addition to suppression of the vagal tone on the heart [85,86]. The resultant action of chloroquine can be proarrhythmic or antiarrhythmic depending on the underlying condition [4,14,33,34,36,72,80].

The mechanism underlying chloroquine cardiomyopathy involves inhibition of autophagy [40,95,122]. Even in the absence of cardiomyopathic changes, such inhibition can aggravate cell damage under ischaemia/reperfusion, which by itself causes a change in the autophagic flux and recruits additional cell death mechanisms [88–92,97]. The trend to favour myocardial death may be opposed by protective mechanisms due to an inhibition of intracellular Na<sup>+</sup> and Ca<sup>2+</sup> load and of arrhythmias resulting from the effects on ion channels or due to inhibition of apoptosis [123].

It should be underlined that all cardiac effects of chloroquine/hydroxychloroquine could not be covered in this brief review. For example, metabolic effects due to phospholipase A<sub>2</sub> inhibition have been proposed to mediate an anti-ischaemic action of chloroquine [124]. In addition, other effects may not yet be known, and the consequences of autophagy inhibition on the expression of various proteins could be more complex. Finally, the effect of the drugs and their mechanisms may depend on coexisting non-cardiac (e.g. electrolyte) changes or cardiac diseases (e.g. the presence of myocarditis). The side effects of

chloroquine/hydroxychloroquine are generally mild, but they can be exacerbated by drug associations. Many macrolide antibiotics can cause QT prolongation when used in isolation [120]. If co-administered with chloroquine/hydroxychloroquine, the effects on the QT interval might add and become marked, even if the effect of each drug taken separately is mild. Use of high doses and association with other potential QT-prolonging drugs are likely the cause of the marked QT prolongation and occurrence of arrhythmias that have recently led to interrupt clinical trials testing the benefits of chloroquine in the treatment of COVID-19 [16]. Even when apparently moderate mean doses are reported to have been used in large registries of patient groups, there will be large variations among different patient groups and a significant number of them might have been exposed to high drug doses. In any case, drug associations and the role of complicating conditions such as hypokalaemia that are themselves drug dose-dependent should also be analysed before concluding on the direct cardiotoxic effect of chloroquine/hydroxychloroquine.

## 6. Conclusion

The antimalarial drugs chloroquine and hydroxychloroquine have been proposed for antiviral therapy, including for COVID-19. The drugs are relatively safe when used at low or moderate doses, with blood concentrations  $\leq 1 \mu\text{M}$ . At such doses they can display beneficial (e.g. antiarrhythmic) actions, and despite the many effects on K<sup>+</sup> channels they do not appear to be associated with prominent QT prolongation. Acute life-threatening cardiac effects, including ventricular tachyarrhythmias, are observed with high doses. Given the long half-life of the drug, other side effects are usually associated with long-term treatment. The mechanisms underlying chloroquine effects include direct actions on ion channels and receptors, whilst others (especially cardiomyopathy developing following long-term treatment) involve inhibition of autophagy. Care should therefore be taken to limit the dose and duration of treatment with the drugs as well as to avoid association with other drugs with known toxic effects on the myocardium.

## Declaration of Competing Interest

None declared.

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## Ethical approval

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