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Chloroquine and Hydroxychloroquine Are Novel Inhibitors of Human Organic Anion Transporting Polypeptide 1A2

Chenghao Xu [1], Ling Zhu [2], Ting Chan [1], Xiaoxi Lu [1], Weiyong Shen [2], Michele C. Madigan [2, 3], Mark C. Gillies [2], Fanfan Zhou [1, *]

[1] Faculty of Pharmacy, The University of Sydney, Sydney, Australia
[2] Save Sight Institute, The University of Sydney, Sydney, Australia
[3] School of Optometry and Vision Sciences, University of New South Wales, New South Wales 2052, Australia

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ABSTRACT

Chloroquine (CQ) and hydroxychloroquine (HCQ) are widely used to treat malaria and inflammatory diseases, long-term usage of which often causes severe side effects, especially retinopathy. Solute carrier transporters (SLCs) are important proteins responsible for the cellular uptake of endogenous and exogenous substances. Inhibitors competing with transporter substrates for SLCs often results in unfavorable toxicities and unsatisfactory therapeutic outcomes. We investigated the inhibitory effect of CQ and HCQ on substrate uptake mediated through a range of important SLC transporters in overexpressing human embryonic kidney (HEK293) cells. Our data revealed that both CQ and HCQ potently inhibit the uptake activity of organic anion transporting polypeptide 1A2 (OATP1A2). We recently reported OATP1A2 to be expressed in human retinal pigment epithelium (RPE), where it mediates cellular uptake of all-*trans*-retinol (atROL), a key step in the classical visual cycle. In this study, we demonstrate that CQ and HCQ could markedly impair atROL uptake in OATP1A2-expressing HEK293 cells and more importantly, in primary human RPE cells. Our study shows that CQ and HCQ are novel inhibitors of OATP1A2 and significantly impair OATP1A2-mediated substrate uptake, particularly transport of atROL into the RPE. This effect may compromise the function of the classic visual cycle leading to vision impairment and contribute to the retinopathy observed clinically in patients using CQ or HCQ.

Introduction

Chloroquine (CQ) and hydroxychloroquine (HCQ) are 4-aminoquinolone derivatives that have long been used in the prevention and treatment of malaria.[1,2] These two agents also have clinical applications in many inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, and Sjogren syndrome.[3-5] The chemical structure of HCQ differs from CQ with a hydroxyl group at the end of N-ethyl side chain (Fig. 1). Clinically, HCQ is used more frequently than CQ as it has similar activity to but is less toxic than CQ.[2] CQ and HCQ are often administered orally but can also be given by intravenous injection. The common adverse effects of CQ and HCQ include gastrointestinal upset, mild nausea, and occasional stomach cramps with mild diarrhea.[6] However, since the 1960s, long-term usage of CQ or HCQ has also been reported to lead to severe retinopathy and loss of retinal function.[7-11] Lack of treatment for CQ- or HCQ-induced retinopathy results in permanent visual loss for patients and as such has significantly restricted the clinical applications of these otherwise cost-effective and widely available drugs.[12]

Influx transporters, primarily the solute carrier transporters (SLCs), are the membrane proteins responsible for the cellular uptake of various substrates including endogenous and exogenous substances. SLCs are known to transport a wide range of drugs, many of which are clinically important.[13] SLCs are broadly expressed in key human organs, such as the kidney, liver, and brain, where they facilitate drug access to these tissues.[13] The transport activities of these transporters thus largely determine the absorption, distribution, and elimination of drugs, influencing drug efficacy and toxicity.[14] Furthermore, competition between therapeutic drugs and endogenous substances for a specific SLC transporter

Abbreviations used: SLCs, solute carrier transporters; CQ, chloroquine; HCQ, hydroxychloroquine; CCK-8, cholecystokinin octapeptide; E3S, estrone-3-sulfate; PAH, 4-aminohippuric acid; MPP+, methyl-4-phenylpyridinium acetate; RPE, retinal pigment epithelium; atROL, all-*trans*-retinol.

Chenghao Xu and Ling Zhu contributed equally to this article.

* *Correspondence to:* Fanfan Zhou (Telephone: +61-2-9351-7461; Fax: +61-29351-4391).
E-mail address: Fanfan.zhou@sydney.edu.au (F. Zhou).

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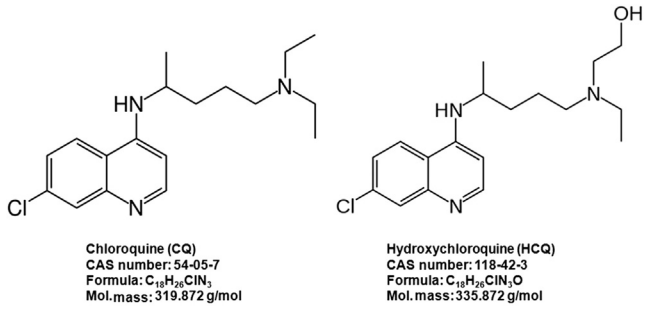

Figure 1. Chemical structures of chloroquine (CQ) and hydroxychloroquine (HCQ).

may often lead to disappointing clinical outcomes and unexpected toxicities.

The organic anion transporting polypeptides (OATPs) encoded by *SLCO* genes and the organic anion/cation transporters (OATs/OCTs/OCTNs) encoded by *SLC22A* genes represent the most important SLC members involved with drug transport.[13,15] These SLC transporters interact with a remarkably broad range of substances ranging from endogenous molecules, from hormones to various xenobiotics, particularly pharmaceutical agents such as anticancer drugs (e.g., imatinib, methotrexate) and antibiotics (e.g., penicillin).[13,15-17] SLC transporters are widely expressed in human tissues. More specifically, OATP2B1, OCT1, OCT2, OCTN1, and OCTN2 are expressed by enterocytes, whereas organic anion transporting polypeptide 1A2 (OATP1A2), OAT1, OAT2, OAT3, OAT4, OCT2, OCT3, OCTN1, and OCTN2 have been identified in the renal tubule epithelium.[13,15] OATP1B1, OATP1B3, OATP2B1, OAT2, OCT1, and OCT3 are expressed by hepatocytes, thus facilitating drug access to the liver for biotransformation, whereas OATP1A2, OATP2B1, OAT1, OAT2, OAT3, OCT1, OCT2, and OCT3 have been found in the brain, contributing to central nervous system drug transport.[13,15] Recently, we reported that OATP1A2 is expressed in the retinal pigment epithelium (RPE) within the eye and is capable of transporting all-*trans*-retinol (atROL), a retinoid essential for the classic visual cycle.[18] Overall, these transporters mediate the cellular influx of structurally diverse compounds and greatly impact on the pharmacokinetics of drugs in body. Drugs and endogenous substances competing for SLC transporters have also been widely reported,[19-21] and can significantly impact on therapeutic outcomes and toxicities.

To date, the interactions between SLC transporters and CQ or HCQ remain unclear. This study is the first to comprehensively investigate the inhibitory effect of CQ and HCQ on the substrate uptake mediated through a range of essential SLC transporters and contributes to our understanding of the observed therapeutic toxicity of these agents.

Materials and Methods

Materials

[^{3}H]-4-aminohippuric acid (PAH; 60 Ci/mmol), [^{3}H]-L-ergothioneine (1.7 Ci/mmol), and [^{14}C]-L-carnitine (56 mCi/mmol) were obtained from BioScientific Pty. Ltd. (Gymea, New South Wales, Australia). [^{3}H]-estrone-3-sulfate (E3S; 57.3 Ci/mmol), [^{3}H]-cholecystokinin octapeptide (CCK-8; 97.5 Ci/mmol), [^{3}H]-atROL (12.5 Ci/mmol), and [^{3}H]-methyl-4-phenylpyridinium acetate (MPP$^+$; 82.1 Ci/mmol) were purchased from PerkinElmer (Melbourne, Victoria, Australia). Human embryonic kidney (HEK293) cell line was obtained from the American Type Culture Collection (Manassas, VA). Culture media was purchased from Thermo Scientific (Lidcombe, New South Wales, Australia). HCQ, CQ, and all other chemicals were obtained from Sigma-Aldrich (Castle Hill, New South Wales, Australia).

Constructs containing the Open Reading Frames of human OAT1 (reference sequence: NM_004790.4), OAT2 (reference sequence: NM_006672.2), OAT3 (reference sequence: NM_004254.2), OAT4 (reference sequence: NM_018484.2), OATP1A2 (reference sequence: NM_005075.1), OATP1B1 (reference sequence: AB026257.1), OATP1B3 (reference sequence: NM_019844), OATP2B1 (reference sequence: NM_007256), OCT1 (reference sequence: NM_003057.2), OCT2 (reference sequence: NM_003058.2), OCT3 (reference sequence: NM_021977.2), OCTN1 (reference sequence: NM_003059), and OCTN2 (reference sequence: NM_003060) were purchased or cloned by us as described before.[22]

Cell Culture

Human embryonic kidney cells were grown in 75-cm^2 tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% CO_2 at 37°C. Confluent cells were routinely harvested by trypsinization and resuspended in culture medium every 3–5 days. For experiments, HEK293 cells were seeded onto 48-well plates and grown for 24 h at 37°C. Cells were then transfected with each SLC transporter plasmid using Lipofectamine 2000 (Invitrogen, Mount Waverley, Victoria, Australia) and incubated for another 24 h at 37°C.

Isolation of Human RPE Primary Cells

Three postmortem human eyes (aged between 38 and 69 year, postmortem delay <16 h) were obtained from the Lions NSW Eye Bank, with consent and ethics approval from The University of Sydney Human Research Ethics Committee in accordance with the tenets of the Declaration of Helsinki. Primary RPE cells were isolated as described previously.[23-25] Briefly, the anterior segment (cornea, iris, and lens) was removed followed by gently removing the vitreous and neurosensory retina from the underlying RPE. The eyecups were rinsed with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 1.0 mM $MgCl_2$, and 1.0 mM $CaCl_2$, pH 7.4), filled with 0.25% trypsin and 0.01% EDTA and incubated at 37°C for 45 min. The RPE was gently removed from the underlying Bruch's membrane with pipetting and collected in DMEM with 4.5 g/L glucose, 2 mM L-glutamine, and 20% fetal bovine serum. After pelleting by centrifugation (163*g* for 5 min), cells were resuspended in fresh medium and initially grown in 35 mm^2 dishes. After reaching confluence, cells were trypsinized and subsequently grown in 25 cm^2 flasks maintained at 37°C with 5% CO_2. The primary RPE cells used for experiments were between passage 2 and passage 5.

Transporter Studies

Transporter uptake was measured with cells incubated with PBS containing radiolabeled substrates with or without 10 μM CQ or HCQ for 8 min. As described previously, specific substrates were used for the SLC transporters in our uptake assays.[26] In detail, the concentration of each substrate used was: 5 μM [^{3}H]-PAH for OAT1; 300 nM [^{3}H]-E3S for OAT2, OAT3, OAT4, OATP1A2, OATP1B1, and OATP2B1; 2 nM [^{3}H]-CCK-8 for OATP1B3; 100 nM [^{3}H]-MPP$^+$ for OCT1, OCT2, and OCT3; 5 μM [^{3}H]-L-ergothioneine for OCTN1, 5 μM [^{14}C]-L-carnitine for OCTN2, and 0.1 μM [^{3}H]-atROL for OATP1A2.

After incubation, the substrate uptake was terminated by rapidly washing cells twice with ice-cold PBS. The cells were then solubilized in 0.02 N NaOH and neutralized with 0.02 N HCl. The intracellular accumulation of each radiolabeled substrate was