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Additive Effects of Citrus Juice Flavonoid Naringenin and Statins on Human Ether-a-go-go-Related Gene Channels Expressed in *Xenopus* Oocytes

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Highlights of the Study

- Naringenin, abundantly found in citrus juice, causes QT prolongation in electrocardiograms of healthy volunteers.
- Statins, cholesterol-reducing drugs, have also been reported to inhibit human ether-a-go-go-related gene (HERG) channels and to prolong QT interval.
- Naringenin and statins inhibit HERG channels in an additive manner highlighting the increased risk of QT prolongation in patients consuming statins and citrus juice concomitantly.

Keywords
Citrus juice · Naringenin · Statins · Human ether-a-go-go-related gene channel

Abstract
Objective: Naringenin, a major flavonoid found in citrus juice, has been shown to inhibit human ether-a-go-go-related gene (HERG) channels and cause QT prolongation. Statins, the most commonly used class of cholesterol reducing drugs, have also been reported to inhibit HERG channels and prolong QT interval in patients using these drugs. However, the interaction between naringenin and statins on the function of HERG channels has not been studied. ***Materials and Methods:*** In the present study, we expressed HERG channels in *Xenopus* oocytes, tested the effects of naringenin and statins separately, and combined on HERG channels. ***Results:*** When 30 μM naringenin was added to statins (1 μM rosuvastatin or 3 μM atorvastatin), significantly greater inhibition of HERG was demonstrated, compared to the inhibition caused by statins alone. ***Conclusions:*** The results indicate that an additive interaction occurs between naringenin and statins; this could pose an increased risk of arrhythmias by decreasing repolarization reserve.

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Introduction

Prolongation of QT interval in electrocardiogram recordings is known to be caused by the inhibition of human ether-a-go-go-related gene (HERG) channels and associated with fetal ventricular arrhythmias and sudden cardiac death [1]. Flavonoids are a class of natural polyphenolics synthetized ubiquitously in plants, vegetables, fruits, and beverages of plant origin, such as tea, wine, and citrus juice. The flavonoid polyphenol naringenin, which is abundant in citrus fruits and notably in grapefruits, has been shown to be a direct blocker of the HERG channel and to cause prolongation of QT interval [2–4]. Furthermore, additive inhibitory effects of naringenin with antiarrhythmic drugs such as amiodarone, quinidine, and dofetilide on the function of HERG channels have been reported [5].

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, are one of the most commonly used drugs in treatments of hypercholesterolemia and associated cardiovascular morbidities. Recently, analysis of large numbers of patients showed that statins can prolong QT intervals and inhibit HERG channels [6, 7]. However, the interaction between citrus flavonoids such as naringenin and statins on the function of HERG channels has not been studied. In the present study, we evaluated the effect of combining naringenin with statins (rosuvastatin and atorvastatin) on the function of HERG channels expressed in *Xenopus* oocytes.

Materials and Methods

Mature female *Xenopus Laevis* frogs were purchased from Xenopus 1, Ann Arbor, MI, USA. Clusters of oocytes were removed surgically under 0.15% tricaine (Sigma, St. Louis, MO, USA) anesthesia, and individual oocytes were isolated as described previously [8]. Oocytes were placed in a recording chamber and superfused at a constant rate of 3–5 mL/min. The bathing solution contained (in mM): NaCl, 96; KCl, 2; $CaCl_2$, 1; and HEPES 5 (pH 7.4). Microelectrodes filled with 3 M KCl had tip resistances ranging from 1 to 3 MΩ. HERG currents were recorded by a GeneClamp-500B amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA) and acquired by using pCLAMP 5 software. Drugs were applied by addition to the superfusate. Naringenin, statins, and all chemicals were purchased from Sigma (St. Louis, MO, USA). Stock solutions of naringenin (100 mM) and statin (10 mM) were prepared in DMSO. The DMSO up to 0.5% v/v had no effect on the currents (online suppl. Fig. 1; for all online suppl. material, see https://

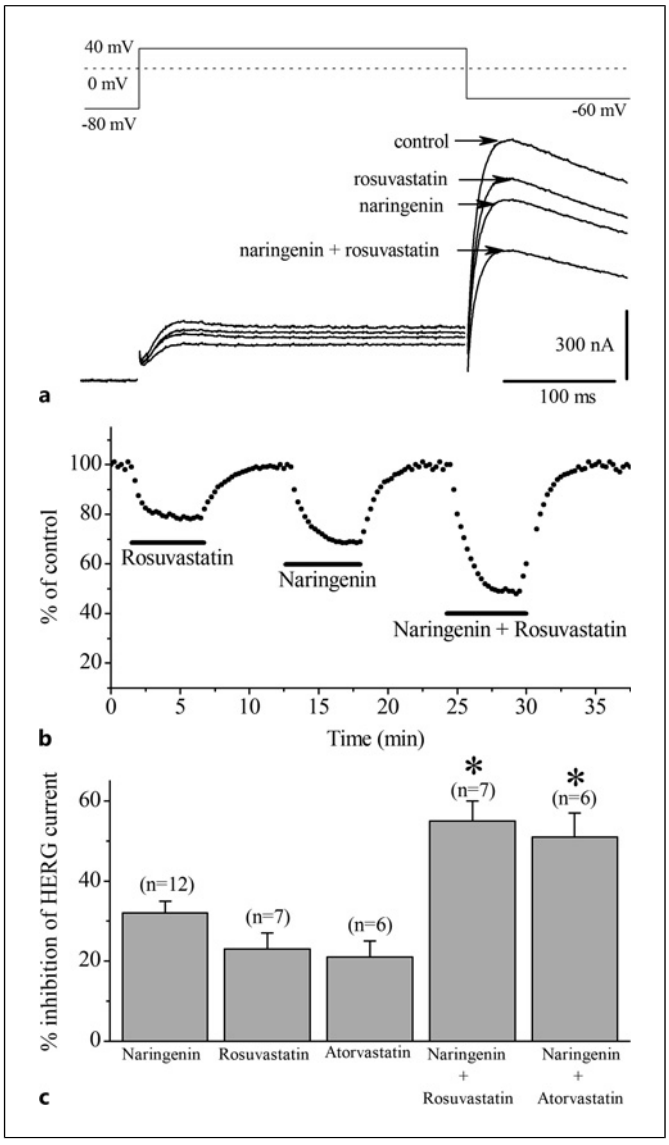

Fig. 1. Effect of naringenin and statins on HERG currents expressed in *Xenopus* oocytes. **a** Original traces of currents in control, in presence of 30 μM naringenin, 1 μM rosuvastatin, and the combination of naringenin and rosuvastatin; upper panel illustrates the single-step voltage-clamp protocol used to activate the currents. **b** The time course of the effects of naringenin and rosuvastatin on the normalized maximum amplitudes of tail currents (●) elicited with the voltage-clamp protocol. Durations of drug application were illustrated with time bars. **c** The summary of the inhibitory effects of 30 μM naringenin, statins (1 μM rosuvastatin, 3 μM atorvastatin), and combinations of naringenin and statins on the maximal amplitudes of tail currents. Bars represent the mean inhibition ± S.E.M. Number of experiments (*n*) are shown above each bar. * indicates $p < 0.05$ (unpaired *t* test, naringenin vs. naringenin + rosuvastatin or naringenin vs. naringenin + atorvastatin group).

doi.org/10.1159/000538780). Recordings were filtered at 2 kHz and acquired at 5 kHz and analyzed with Origin 8.5 (OriginLab, Northampton, MA, USA). Leak currents were not subtracted. The HERG channel cRNA from cDNA clone (GenBank accession No. hs04270) in the amount of 25 ng/50 nL was injected to each oocyte.

Results

Test pulses from a holding potential of −80 mV to +40 mV were applied to activate HERG currents every 15 s. Each pulse was followed by a constant return pulse to −60 mV to evoke tail currents (Fig. 1a inset). Figure 1a illustrates the current traces through HERG channels activated by repolarizing pulses from +40 mV to −60 mV in control, in solutions containing 30 μM naringenin, 1 μM rosuvastatin and combination of naringenin and rosuvastatin. Figure 1b shows the time course for the effects of naringenin and rosuvastatin on the normalized maximal amplitudes of tail currents. The summary of the results on the effects of naringenin and statins are shown in Figure 1c.

Discussion

Naringenin has been shown to potentiate the inhibitory effects of antiarrhythmics such as amiodarone, quinidine, and dofetilide [5]. To the best of our knowledge, this is the first report on the additive effects of naringenin and statins on HERG channels. The outward potassium current through HERG channels is primarily responsible for repolarization of the ventricular action potential, and drug-induced inhibition of HERG function has been known to be associated with QT prolongation [1] which is closely linked to fatal ventricular arrhythmias such as torsade de pointes and sudden cardiac death. Therefore, additive inhibitory effects of naringenin and statins on HERG channels reported in this study can have deleterious clinical consequences. However, naringenin has been shown to inhibit multiple ion channels including voltage-gated sodium, potassium, and calcium channels involved in the electrophysiological characteristics of the cardiac action potential [9, 10], and at least some of these effects can counteract the action potential-prolonging effect of HERG channel inhibition by naringenin.

Naringenin is a naturally occurring flavonoid that reaches concentrations of 150 μM in orange juice and more than 1,000 μM in grapefruit juice [11]. The corresponding glycoside naringin, which is metabolized to naringenin in the intestines, is found in concentrations of over 2,000 μM in grapefruit juice [2, 11]. In vivo studies detected maximum plasma naringenin levels of 6.0 ± 5.4 μM 4–6 h after single oral ingestion of 8 mL/kg of grapefruit juice [11]. Importantly, in the *Xenopus* oocyte expression system, usually higher drug concentrations are needed to achieve effects on ion channels than in native cells [2, 3, 12]. Thus, it is possible that naringenin and statins may exhibit inhibitory effects on the human cardiac potassium channels at concentrations significantly lower than those employed in the oocyte expression system.

An additional concern for the consumption of citrus juice with concomitant use of statins is the pharmacokinetic interaction between statins and flavonoids found in citrus juice. Consuming citrus juice has been known to increase the QT length brought about by various drugs due drug-metabolism interference which results from inhibition of cytochrome P450 (CYP) enzymes such as CYP3A4 and CYP219 [13]. Inhibition of CYP3A4 and CYP219, which metabolize some of statins, can increase the plasma concentrations of these drugs [13]. In addition, flavonoids have been shown to inhibit P-glycoprotein-driven efflux activity [14] which can further increase the pharmacokinetic interaction of flavonoids with other drugs including statins. In conclusion, our results suggest that consuming citrus juice in large amounts, in addition to pharmacokinetic interactions, may have pharmacodynamically adverse consequences such as QT prolongation and ventricular arrhythmias.

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Statement of Ethics

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Animal Care Committee of Bogomoletz Institute of Physiology of National Academy of Science of Ukraine (142/37BY).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Murat Oz: conceptualization, methodology, investigation, data curation, initial draft, review and editing, and resources; Dmytro Isaev: methodology, analysis, investigation, data curation, resources, and review and editing; Keun-Hang Susan Yang: conceptualization, analysis, investigation, resources, data curation, initial draft, and review and editing.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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