The Grapefruit Flavonoid Naringenin Inhibits Multiple Cardiac Ion Channels

Camille Sanson

Integrated Drug Discovery, Sanofi-Aventis R&D

Rachid Boukaiba

Integrated Drug Discovery, Sanofi-Aventis R&D

Sylvie Houtmann

Integrated Drug Discovery, Sanofi-Aventis R&D

Magali-Anne Maizières

Integrated Drug Discovery, Sanofi-Aventis R&D

Sophie Fouconnier

Integrated Drug Discovery, Sanofi-Aventis R&D

Michel Partiseti

Integrated Drug Discovery, Sanofi-Aventis R&D

Georg Andrees Bohme (✉ andrees.bohme@sanofi.com)

Integrated Drug Discovery, Sanofi-Aventis R&D

Research Article

Keywords: arrhythmogenic food, cardiac safety, Torsade de Pointe, ion channels, CiPA paradigm, patch-clamp electrophysiology, mutations

Posted Date: January 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1202805/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Drinking fresh grapefruit juice is associated with a significant prolongation of the QT segment on the electrocardiogram (ECG) in healthy volunteers. Among the prominent flavonoids contained in citrus fruits, the flavanone naringenin is known to be a blocker of the human *ether-a-go-go* related gene (hERG) potassium channel. We hypothesized that naringenin could interfere with other major ion channels shaping the cardiac ventricular action potential (AP). To this end, we examined the effects of naringenin on the seven currents comprising the Comprehensive *in vitro* Pro-Arrhythmia (CiPA) panel for early arrhythmogenic risk assessment in drug discovery and development. We used automated patch-clamp of human ion channels heterologously expressed in mammalian cell lines to evaluate half-maximal inhibitory concentrations (IC₅₀). Naringenin blocked all CiPA currents tested with IC₅₀ values in the 30 μM – 100 μM concentration-range. The rank-order of channel sensitivity was the following: hERG > K_{ir}2.1 > Na_V1.5_{late} > Na_V1.5_{peak} > K_V7.1 > K_V4.3 > Ca_V1.2. This multichannel inhibitory profile of naringenin suggests exercising caution when large amounts of grapefruit juice or other citrus juices enriched in this flavanone are drunk in conjunction with QT prolonging drugs or by carriers of congenital long QT syndromes.

Introduction

Taking certain medication or carrying inheritable mutations in genes encoding cardiac ion channels are not the sole circumstances that can lead to prolongation of the QT segment on the ECG. As recently emphasized in the concept of “arrhythmogenic foods”, components of common diet can also affect this important cardio-safety metric (Tisdale, 2019; Woosley, 2020). Grapefruit-based beverages are probably the most emblematic example in this regard. Drinking grapefruit juice was first shown to enhance the QT length brought about by terfenadine through drug-metabolism interference due to the well-known cytochrome P450 inhibition produced by grapefruit juice (Benton et al., 1996). However, it was later shown that the flavonoid naringenin, which is abundant in citrus fruits and notably in grapefruit, is actually by itself a blocker of the human *ether-a-go-go* related gene (hERG) channel (Zitron et al., 2005). Outward potassium currents flowing through hERG channels are primarily responsible for the repolarization of the cardiac ventricular action potential (AP), and drug-induced inhibition of hERG function (or trafficking to the cardiomyocyte membrane) have historically been known to be associated with QT prolongation (Rampe & Brown, 2013).

The QT prolonging effect of grapefruit intake was recently confirmed in a “thorough QT” (TQT) study. Clinical TQT studies are rigorously controlled and adequately powered studies designed to anticipate the proarrhythmic risk of new chemical entities (NCEs) during their development. Notably, the sensitivity of TQT studies is monitored by the contemporary administration of a positive control (Darpo, 2010). Regarding grapefruit juice specifically, Chorin *et al.* (Chorin et al., 2019) recently established in a well powered randomized TQT study in healthy volunteers, that the length of the QT segment on the ECG corrected for heart rate (*i.e.* QTc) increased significantly as soon as three hours after drinking 1 liter of grapefruit juice. Although the effect was relatively modest (QTc increase reached a maximum of 14 ms

after ingestion of another 0.5 L juice), it was notably greater in a subgroup of patients suffering congenital long QT syndrome. Among the latter, the QTc increase peaked at nearly 22 ms, an effect that was comparable to the QTc prolongation brought about by the positive control moxifloxacin.

Several other currents besides hERG-mediated I_{Kr} significantly impact the AP duration and are increasingly considered as important cardio-safety targets for NCEs. In this perspective, the Comprehensive *in vitro* Pro-Arrhythmia (CiPA) initiative has been defined and is increasingly being adopted early in the discovery process to refine the prediction of arrhythmogenic risk of NCEs heading towards the clinic (Fermini et al., 2016). The CiPA paradigm aims at better evaluating the degree of ECG surveillance that will be needed later to safely accompany the development of NCEs. Interestingly enough, the CiPA paradigm predicts that drug effects at multiple cardiac ion channels may compensate each other, a contention that has received support from the retrospect profiling of a series of marketed drugs that were revealed safer than their sole “hERGo-centric” pharmacology would have suggested (Kramer et al., 2013; Vicente et al., 2019).

We hypothesized that the primary grapefruit flavonoid naringenin may exercise inhibitory activity at other major ion channels besides hERG. Using automated patch clamp, we established for the first time the full pharmacological profile of this common alimentary component on the seven ion channels shaping the ventricular AP comprised in the upfront screening panel of the CiPA paradigm.

Materials And Methods

Studies were performed using high standard gigaseal automated patch clamp technology (Bell & Fermini, 2021) as detailed before (Le Marois et al., 2020). Briefly, CHO cells expressing hERG or $K_{V7.1}/minK$ were obtained from B'Sys GmbH (Switzerland), and $Ca_v1.2/\beta_2/\alpha_2\delta_1$, $K_{ir2.1}$ or $K_{V4.3}/KChIP2.2$ from Charles River Laboratories (USA), respectively. HEK cells expressing $Na_v1.5$ were constructed in house. All expressions were constitutive except $K_{ir2.1}$, $Ca_v1.2$ and $Na_v1.5$ which were induced overnight by exposure to 1 μ g/mL doxycycline (in the presence of 3 μ M verapamil for $Ca_v1.2$). Patch-clamp recordings were performed in whole-cell patch-clamp mode at room temperature on a Qpatch® 48X or Model II workstation (Sophion, Denmark). The voltage-protocols, holding potentials and triggering frequencies used for each current are schematized as insets in their respective panel in the figure. Extracellular buffers for potassium channels contained (in mM) NaCl, 150 ; KCl, 4 ; $CaCl_2$, 2 ; $MgCl_2$, 1 and HEPES, 10. For the hERG channels, glucose (10 mM) was added. The pH was adjusted to 7.4 with NaOH. The Intracellular buffers contained (in mM): KF, 120 ; KCl, 20; EGTA, 10 ; $MgCl_2$, 1; HEPES, 10. For $K_{V7.1}$ channels, 10 mM EDTA was added as a second chelator, EGTA was decreased to 5 mM and no $MgCl_2$ was added. The pH was adjusted to 7.2 with KOH. The extracellular buffer for $Ca_v1.2$ channels contained (in mM): NaCl, 145 ; KCl, 4 ; $BaCl_2$, 10 and HEPES, 10, and the pH was adjusted to 7.4 with NaOH. The intracellular buffer contained: CsF, 27 ; CsCl, 112 ; EGTA, 8.2 ; NaCl, 2 ; HEPES, 10 and Mg-ATP, 4. The pH was adjusted to 7.2 with CsOH. For $Na_v1.5$ channels, the extracellular buffer contained: NaCl, 137 ; KCl, 4 ; $CaCl_2$, 2 ; $MgCl_2$, 1 and HEPES, 10, and the pH was adjusted to 7.4 with NaOH. The intracellular buffer

contained: CsF, 150 ; EGTA/CsOH, 1/5 ; NaCl, 10 ; MgCl₂, 1 ; CaCl₂, 1 ; HEPES, 10, and the pH was adjusted to 7.2 with CsOH. For the recording of the late component of Na_v1.5, intracellular CsF was reduced to 130 mM and the extracellular buffer was supplemented with 10 nM of the sea anemone toxin ATX-II to slow channel inactivation (Wu et al., 2019). All channels were exposed to 6 concentrations of Naringenin applied cumulatively in an ascending order up to 300 μM. At the end of each recording, a reference inhibitor was added at a maximally active concentration to isolate leak currents if any. E-4031 (10 μM) was used for the hERG currents, lidocaine (3 mM) for the Na_v1.5 currents, CdCl₂ (0.2 mM) for Ca_v1.2, SKF-96365 (30 μM) for K_v4.3, HMR-1556 (30 μM) for K_v7.1 and BaCl₂ (3 mM) for the K_{ir}2.1 channels.

Racemic naringenin (CAS Number 67604-48-2) was obtained from Sigma-Aldrich (catalog # N5893). Concentrated stock solutions prepared in pure DMSO were diluted in extracellular buffer containing 1% Pluronic F-68 so as to obtain the following six final concentrations which were applied to the cells in ascending order : 1.2 μM, 3.7 μM, 11 μM, 33 μM, 100 μM and 300 μM. Final DMSO was kept below 1%. All current inhibitions were quantified as change in normalized peak current amplitude except for K_v4.3 currents which were quantified as change in normalized integral charge transferred calculated as the area under the current trace vs time. Half-maximal inhibitory concentrations (IC₅₀) and Hill slope values (n_H) were estimated by fitting a sigmoidal curve to the current measurements normalized with respect to pre-drug baseline. Minimal and maximal inhibition were constrained to zero and 100%, respectively. Calculations and graphs were done with Prism 8.3.0 (GraphPad Software, San Diego, CA, USA).

Results And Discussion

We observed that naringenin inhibits all seven cardiac CiPA currents tested in a concentration-dependent manner. This finding confirms our hypothesis that this prominent flavonoid of grapefruit and other citrus fruits can indeed alter the function of multiple other ion channels generating the ventricular AP besides hERG. Block potency values fall within a half-log window ranging approximately between 30 μM and 100 μM. Fig. 1 illustrates typical current traces collected before and during exposure to naringenin. Most concentration-response curves presented similar Hill slope factors around unity, suggesting that naringenin interact with single target sites on these channels (see table for IC₅₀ and n_H values).

The K_{ir}2.1-mediated outward component of the inward rectifier I_{K1} , as well as the hERG-mediated, rapidly activating component of the delayed rectifier I_K were the most sensitive currents to naringenin. The K_v7.1-mediated, slowly activating I_{Ks} component of I_K was less potently inhibited by the flavonoid. All three currents contribute to the terminal repolarization phase of the cardiac action potential and constitute the “repolarization reserve” (Roden, 2008). The potency of naringenin on hERG we determined here by automated patch-clamp in CHO cells confirms early findings by two-electrode voltage-clamp in *Xenopus* oocytes (Zitron et al., 2005). To our knowledge, the inhibitory effects of naringenin on K_{ir}2.1 and on the five other cardiac currents comprising the CiPA panel has not been documented before. Our data indicate that, among the inward cationic currents, the Ca_v1.2-mediated I_{CaL} current appeared the least sensitive to naringenin, followed by the $I_{Na,peak}$ and $I_{Na,late}$ currents transiting through Na_v1.5 channels. Overall, the

inhibitory potency of naringenin on these three depolarizing currents is roughly 2 – 3 fold weaker than its effect on hERG outward currents which are prominently associated with QT prolongation. Although it is uncertain whether the IC₅₀ differences are significant, our data suggest that the inhibitory profile of naringenin at these multiple channels incompletely compensate for each other, leaving an overall effect of prolonging the QT by a small, but significant extent.

Patients developing life threatening *Torsade de Pointe* (TdP) in response to drugs often present with additional risk factors such as hypokalemia, bradycardia or inheritable mutations in genes encoding cardiac ion channels. Regarding the latter, genotype-phenotype correlation studies have estimated that the prevalence of congenital long-QT could reach 1 : 2500 (Schwartz et al., 2009). Therefore, many clinically silent carriers of inheritable risk factors could develop proarrhythmic events when ingesting QT-prolonging substances. *De facto*, up to 1 : 3 patients presenting with drug-induced long-QT syndromes actually harbor mutations in ion channel genes related to congenital long-QT (Itoh et al., 2016). Moreover, it is noteworthy that loss-of-function mutations in *KCNQ1* or *KCNE1*, the gene encoding the pore-forming alpha-subunit K_v7.1 and its gating-modulating ancillary subunit minK, respectively, and in *KCNH2* (*i.e.* hERG), are associated with the vast majority of autosomal dominant or autosomal recessive Romano–Ward or Jervell and Lange–Nielsen long-QT syndromes (Bokil et al., 2010). We found that both channels are targeted by naringenin with half-maximal inhibitory concentrations ranging between 30 µM and 100 µM.

In summary, although the plasma concentrations of naringenin after drinking 1 – 2 L grapefruit juice were not monitored in the available TQT study (Chorin et al., 2019), the significant QTc prolongation previously observed in the clinic combined with our present ion channel profiling data suggest that known carriers of congenital long-QT syndromes should avoid dietary sources enriched in this flavonoid. Furthermore, taking high volumes of fresh grapefruit juice with medications known to prolong the QT interval should be discouraged.

Declarations

Acknowledgements

The authors are grateful to Fiona Ducrey for language revision.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Authors Contributions

MP and GAB designed and supervised the study. CS, RB, SH, MAM and SF generated and curated the data. GAB wrote the manuscript.

All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Funding

None.

Competing Interests

All authors are current or former Sanofi employees and may hold shares and/or stock options in the company.

Data Availability

All data are included as a supplemental Prism file.

References

1. Bell, D.C. & Fermini, B. (2021) Use of automated patch clamp in cardiac safety assessment: past, present and future perspectives. *J Pharmacol Toxicol Methods*, **110**, 107072.doi: 10.1016/j.vascn.2021.107072
2. Benton, R.E., Honig, P.K., Zamani, K., Cantilena, L.R. & Woosley, R.L. (1996) Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. *Clin Pharmacol Ther*, **59**, 383-388. doi: 10.1016/S0009-9236(96)90105-8
3. Bokil, N.J., Baisden, J.M., Radford, D.J. & Summers, K.M. (2010) Molecular genetics of long QT syndrome. *Mol Genet Metab*, **101**, 1-8. doi: 10.1016/j.ymgme.2010.05.011
4. Chorin, E., Hochstadt, A., Granot, Y., Khoury, S., Schwartz, A.L., Margolis, G., Barashi, R., Viskin, D., Ghanous, E., Schnapper, M., Mekori, T., Fourey, D., Guevara-Valdivia, M.E., Marquez, M.F., Zeltzer, D., Rosso, R. & Viskin, S. (2019) Grapefruit juice prolongs the QT interval of healthy volunteers and patients with long QT syndrome. *Heart Rhythm*, **16**, 1141-1148. doi: 10.1016/j.hrthm.2019.04.039
5. Darpo, B. (2010) The thorough QT/QTc study 4 years after the implementation of the ICH E14 guidance. *Br J Pharmacol*, **159**, 49-57. doi: 10.1111/j.1476-5381.2009.00487.x
6. Fermini, B., Hancox, J.C., Abi-Gerges, N., Bridgland-Taylor, M., Chaudhary, K.W., Colatsky, T., Correll, K., Crumb, W., Damiano, B., Erdemli, G., Gintant, G., Imredy, J., Koerner, J., Kramer, J., Levesque, P., Li, Z., Lindqvist, A., Obejero-Paz, C.A., Rampe, D., Sawada, K., Strauss, D.G. & Vandenberg, J.I. (2016) A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive In Vitro Proarrhythmia Assay Paradigm. *J Biomol Screen*, **21**, 1-11. doi: 10.1177/1087057115594589

7. Itoh, H., Crotti, L., Aiba, T., Spazzolini, C., Denjoy, I., Fressart, V., Hayashi, K., Nakajima, T., Ohno, S., Makiyama, T., Wu, J., Hasegawa, K., Mastantuono, E., Dagradi, F., Pedrazzini, M., Yamagishi, M., Berthet, M., Murakami, Y., Shimizu, W., Guicheney, P., Schwartz, P.J. & Horie, M. (2016) The genetics underlying acquired long QT syndrome: impact for genetic screening. *Eur Heart J*, **37**, 1456-1464. doi: 10.1093/eurheartj/ehv695
8. Kramer, J., Obejero-Paz, C.A., Myatt, G., Kuryshv, Y.A., Bruening-Wright, A., Verducci, J.S. & Brown, A.M. (2013) MICE models: superior to the HERG model in predicting Torsade de Pointes. *Sci Rep*, **3**, 2100. doi: 10.1038/srep02100
9. Le Marois, M., Ballet, V., Sanson, C., Maizieres, M.A., Carriot, T., Chantoiseau, C., Partiseti, M. & Bohme, G.A. (2020) Cannabidiol inhibits multiple cardiac ion channels and shortens ventricular action potential duration in vitro. *Eur J Pharmacol*, **886**, 173542. doi: 10.1016/j.ejphar.2020.173542
10. Rampe, D. & Brown, A.M. (2013) A history of the role of the hERG channel in cardiac risk assessment. *J Pharmacol Toxicol Methods*, **68**, 13-22. doi: 10.1016/j.vascn.2013.03.005
11. Roden, D.M. (2008) Repolarization reserve: a moving target. *Circulation*, **118**, 981-982. doi: 10.1161/CIRCULATIONAHA.108.798918
12. Schwartz, P.J., Stramba-Badiale, M., Crotti, L., Pedrazzini, M., Besana, A., Bosi, G., Gabbarini, F., Goulene, K., Insolia, R., Mannarino, S., Mosca, F., Nespola, L., Rimini, A., Rosati, E., Salice, P. & Spazzolini, C. (2009) Prevalence of the congenital long-QT syndrome. *Circulation*, **120**, 1761-1767. doi: 10.1161/CIRCULATIONAHA.109.863209
13. Tisdale, J.E. (2019) Proarrhythmic food for thought. *Heart Rhythm*, **16**, 1149-1150. doi: 10.1016/j.hrthm.2019.05.010
14. Vicente, J., Zusterzeel, R., Johannesen, L., Ochoa-Jimenez, R., Mason, J.W., Sanabria, C., Kemp, S., Sager, P.T., Patel, V., Matta, M.K., Liu, J., Florian, J., Garnett, C., Stockbridge, N. & Strauss, D.G. (2019) Assessment of Multi-Ion Channel Block in a Phase I Randomized Study Design: Results of the CiPA Phase I ECG Biomarker Validation Study. *Clin Pharmacol Ther*, **105**, 943-953. doi: 10.1002/cpt.1303
15. Woosley, R.L. (2020) Arrhythmogenic foods - A growing medical problem. *Trends Cardiovasc Med*, **30**, 310-312. doi: 10.1016/j.tcm.2019.08.007
16. Wu, M., Tran, P.N., Sheng, J., Randolph, A.L. & Wu, W.W. (2019) Drug potency on inhibiting late Na(+) current is sensitive to gating modifier and current region where drug effects were measured. *J Pharmacol Toxicol Methods*, **100**, 106605. doi: 10.1016/j.vascn.2019.106605
17. Zitron, E., Scholz, E., Owen, R.W., Luck, S., Kiesecker, C., Thomas, D., Kathofer, S., Niroomand, F., Kiehn, J., Kreye, V.A., Katus, H.A., Schoels, W. & Karle, C.A. (2005) QTc prolongation by grapefruit juice and its potential pharmacological basis: HERG channel blockade by flavonoids. *Circulation*, **111**, 835-838. doi: 10.1161/01.CIR.0000155617.54749.09

Tables

Table: Half-maximal inhibitory concentrations and Hill slope estimates [with 95% confidence intervals and number of independent recordings] for Naringenin action on seven CiPA cardiac currents.

<i>Current</i>	<i>IC₅₀ [95% CI]</i>	<i>n_H [95% CI]</i>	<i>N=</i>
hERG	34 μM [31 - 37]	1.3 [1.2 - 1.5]	6
Na _V 1.5 (peak)	100 μM [91 - 111]	1.4 [1.2 - 1.6]	9
Ca _V 1.2	148 μM [138 - 158]	1.1 [1.0 - 1.2]	5
Na _V 1.5 (late)	71 μM [67 - 77)	1.5 [1.4 -1.7]	8
K _V 4.3	115 μM [108 - 122]	1.3 [1.2 - 1.5]	10
K _{ir} 2.1 (outward)	51 μM [48 - 55]	2.0 [1.7 - 2.2]	9
K _V 7.1	110 μM [92 - 133]	0.8 [0.7 - 0.9]	6

Figures

Figure 1

Effects of Narigenin on seven CiPA currents. Traces in each panel are representative population patch currents elicited by the voltage-protocol shown in inset next to them. Green traces were recorded in control buffer and superposed to red traces collected in the presence of the indicated concentration of narigenin. Panel A = $\text{Na}_V1.5_{\text{peak}}$; B = $\text{Ca}_V1.2$; C = $\text{Na}_V1.5_{\text{late}}$; D = hERG; E = $\text{K}_{\text{ir}}2.1_{\text{outward}}$; F = $\text{K}_V4.3$; G = $\text{K}_V7.1$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [NaringeninonallCiPAchannelsforpublicationNSAP.pzfx](#)