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# Interaction between amiodarone and hepatitis-C virus nucleotide inhibitors in human induced pluripotent stem cell-derived cardiomyocytes and HEK-293 $Cav_{1.2}$ over-expressing cells

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ABSTRACT

Several clinical cases of severe bradyarrhythmias have been reported upon co-administration of the Hepatitis-C NS5B Nucleotide Polymerase Inhibitor (HCV-NI) direct-acting antiviral agent, sofosbuvir (SOF), and the Class-III anti-arrhythmic amiodarone (AMIO). We model the cardiac drug-drug interaction (DDI) between AMIO and SOF, and between AMIO and a closely-related SOF analog, MNI-1 (Merck Nucleotide Inhibitor #1), in functional assays of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), to provide mechanistic insights into recently reported clinical cases. AMIO co-applied with SOF or MNI-1 increased beating rate or field potential (FP) rate and decreased impedance (IMP) and $Ca^{2+}$ transient amplitudes in hiPSC-CM syncytia. This action resembled that of $Ca^{2+}$ channel blockers (CCBs) in the model, but CCBs did not substitute for AMIO in the DDI. AMIO analog dronedarone (DRON) did not substitute for, but competed with AMIO in the DDI. Ryanodine and thapsigargin, decreasing intracellular $Ca^{2+}$ stores, and SEA-0400, a $Na^{+}/Ca^{2+}$ exchanger-1 (NCX1) inhibitor, partially antagonized or suppressed DDI effects. Other agents affecting FP rate only exerted additive or subtractive effects, commensurate with their individual effects. We also describe an interaction between AMIO and MNI-1 on $Cav_{1.2}$ ion channels in an over-expressing HEK-293 cell line. MNI-1 enhanced $Cav_{1.2}$ channel inhibition by AMIO, but did not affect inhibition of $Cav_{1.2}$ by DRON, verapamil, nifedipine, or diltiazem. Our data in hiPSC-CMs indicate that HCV-NI agents such as SOF and MNI-1 interact with key intracellular $Ca^{2+}$-handling mechanisms. Additional study in a $Cav_{1.2}$ HEK-293 cell-line suggests that HCV-NIs potentiate the inhibitory action of AMIO on L-type $Ca^{2+}$ channels.



## 1. Introduction

In late 2014 and early 2015, nine post-marketing clinical reports and several successive regulatory decisions were published, regarding serious bradyarrhythmic effects when the class-III antiarrhythmic drug amiodarone (AMIO) was used with direct-acting hepatitis-C virus nucleotide inhibitor (HCV-NI) treatments including sofosbuvir (SOF) (Back and Burger, 2015a; European Medicines Agency, 2016; Gilead Sciences Inc., 2016; U.S. Food and Drug Administration, 2016a). The proposed mechanisms behind this adverse cardiac drug-drug interaction (DDI) has been a matter of speculation, generally along the lines of pharmacokinetic effects (i.e. P-glycoprotein (P-gp) inhibition, CYP450 drug transporter effect) impacting plasma concentrations of one or the other agent. DDIs of consequence involving AMIO have been widely reviewed in the literature, and include marked effects on the pharmacokinetics of several cardiovascular drugs, such as warfarin, digoxin, quinidine, and procainamide, and enhanced hemodynamic and electrophysiological effects with others, such as beta-blockers and $Ca^{2+}$ channel blockers (CCBs) (Marcus, 1983). Warnings on the AMIO label describe exacerbation of pre-existing arrhythmias in 2% to 5% of patients in various series, and significant heart block or sinus bradycardia in 2 to 5% of patients. In terms of pharmacokinetic DDIs, the AMIO label describes it as a mixed CYP3A4 and P-gp inhibitor (U.S. Food and Drug Administration, 2015). DDIs in the SOF (Sovaldi®) label, prior to the reports of bradyarrhythmia risk, were those with potent P-gp intestinal inducers, such as rifampin and St-John's Wort (U.S. Food and Drug Administration, 2016b).

*Abbreviations:* AMIO, amiodarone; CCBs, calcium channel blockers; DEA, desethylamiodarone; DDI, drug-drug interaction(s); DRON, dronedarone; FP, field potential; HCV-NI, hepatitis-C virus nucleotide inhibitor; hiPSC-CMs, human induced Pluripotent Stem Cell-derived Cardiomyocytes; IMP, impedance; MNI-1, Merck-Nucleotide Inhibitor #1; NCX1, sodium-calcium exchanger 1; P-gp, P-glycoprotein; SERCA, sarcoplasmic-endoplasmic reticulum calcium-ATPase; SOF, sofosbuvir.

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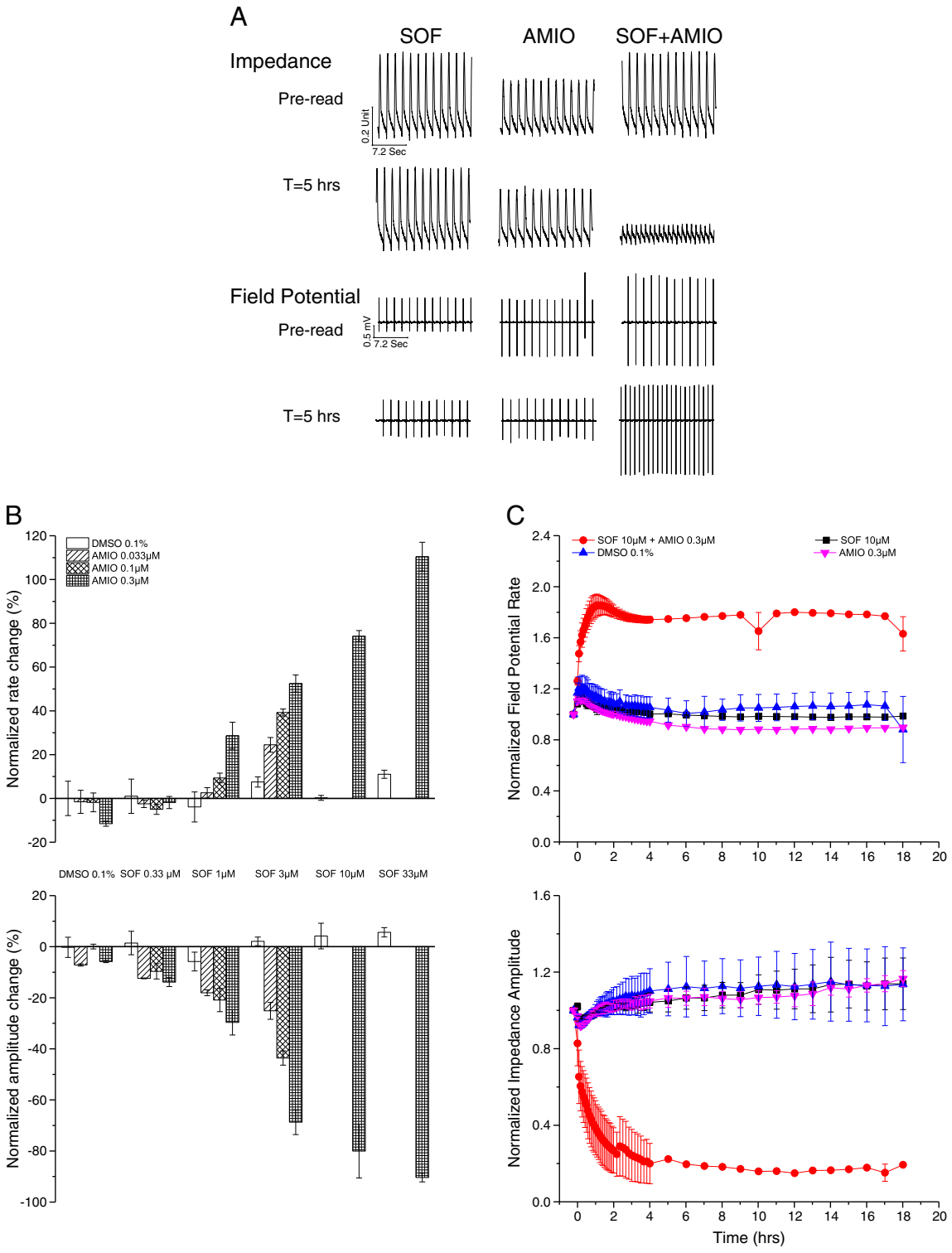


**Fig. 1.** Synergistic effect of co-application of SOF and AMIO on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) syncytia. (A) Representative impedance (IMP) and field potential (FP) traces, recorded concurrently from hIPSC-CMs syncytia, both before (Pre-read), and after application (T = 5 h) of AMIO 0.3 μM, or SOF 10 μM, or both AMIO 0.3 μM and SOF 10 μM. (B) Concentration dependence of effects on FP rate (top) and IMP amplitude (bottom) of hiPSC-CMs syncytia in response to AMIO and SOF applied alone, or in combination, 4–5 h after co-application (N = 6 for FP rate, and N = 3 for IMP amplitude). (C) 18-h time courses of the FP rate (top) and IMP amplitude (bottom) after application of AMIO 0.3 μM or SOF 10 μM alone, in combination, or vehicle alone (DMSO 0.1%) at time, t = 0 h. Values are normalized to the parameter value prior to compound application (N = 6 for FP rate, and N = 3 for IMP amplitude).

Recently, however, evidence against interactions between SOF and AMIO involving P-gp has been presented, along with data suggesting a pharmacological interaction in spontaneously beating human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) syncytia (Millard et al., 2016). Independently, and using a 2D-configuration hiPSC-CMs system and the ACEA xCELLigence® Cardio-ECR platform, we have investigated the comparative cardiac effects of SOF, applied alone or in combination with AMIO, and those of MNI-1, a closely related analog of SOF, applied alone or in combination with AMIO, on hiPSC-CMs. The goal of the present studies, more particularly those conducted on MNI-1, is to provide mechanistic insights into this reported pharmacodynamic, cardiomyocyte-delimited DDI. Relevant to our investigations