Identifying a Lead Compound for Mitigation of Drug-Induced QTc-Interval Prolongation

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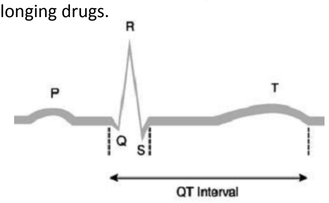
Introduction

Over 175 approved therapeutic drugs list adverse effects which include QT prolongation. Of these, 24% are oncology drugs. Arrhythmic risk is enhanced by the fact that 14-15% of cancer patients present prolonged QT intervals at screening, putting them at risk of developing Torsades the Pointes if exposed to QT-prolonging drugs.

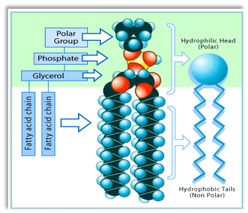
Triggers for Torsades de Pointes are generally ventricular arrhythmias, which degenerate if there is a substrate for the sustainment of the Torsades.



QT prolongation is a necessary substrate, brought about by I_{Kr} inhibition. A substrate feeds the Torsades after they are triggered. While most Torsades are self-arresting -and therefore not dangerous, and often undetected- those which are sustained are rapidly lethal.

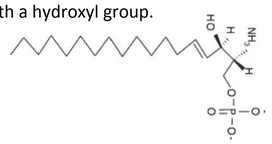
To address the risk that drugs could contribute to the genesis of a substrate for TdP, we investigated the mitigating effect of a liposome and its components administered intravenously and orally on clinically approved QT–prolonging anticancer drugs (crizotinib and nilotinib), as well as a well-characterized and often used clinical antibiotic (moxifloxacin: MF) *in vitro* and *in vivo.*

Phospholipids (PLs) and eutectic blends



Phospholipid = Polar Group + monoglyceride + fatty acid chain

A **lysophospholipid** is a phospholipid in which one of the fatty acids is replaced with a hydroxyl group.

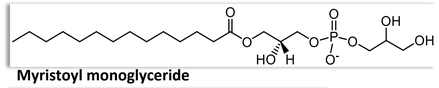


Eutectic blend: EU8120

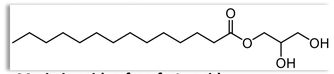
"a mixture of chemical compounds or elements that have a single chemical composition that solidifies at a lower temperature than any other composition made up of the same ingredients".

EU8120 is composed of three components, and was initially created to enhance the oral bioavailability of LysoPG.

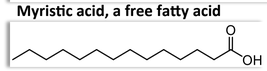
14:0 Lysophosphatidylglycerol



Myristoyl monoglyceride



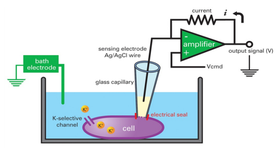
Myristic acid, a free fatty acid



In vitro candidate selection

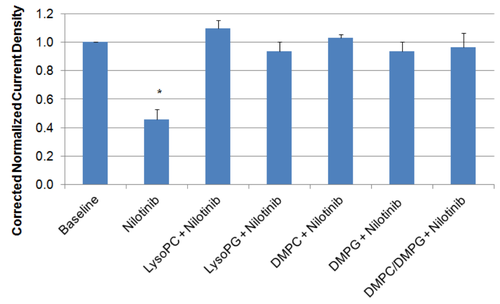
Patch-clamp current recording.

Manual, whole-cell patch-clamp experiments were conducted at physiological temperature on Human embryonic kidney (HEK) cells, line 293 (HEK 293), stably transfected with the hERG gene (HEK-hERG).



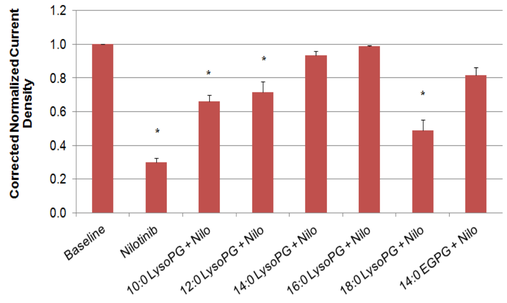
Isolated cells were plated into 2-mL experimental chambers, mounted on the platform of an inverted microscope. The cells were superfused with external solution (in mM: NaCl 140.0, KCl 5.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, HEPES 10.0, dextrose 10.0, pH 7.4 ± 0.05). A 2-10 MΩ resistance pipette was filled with the pipette solution (in mM: KCl 140.0, $MgCl_2$ 1.0, Mg-ATP 4.0, EGTA 5.0, HEPES 10.0, sucrose 10.0, pH 7.4 ± 0.05), and brought to contact the external membrane of a single cell.

Figure 1. Removal of IKr inhibition by various phospholipids (PLs)



Liposomes made with **DMPC, DMPG, DMPC/DMPG, LysoPC** and **LysoPG** did not cause any inhibition of the hERG tail current density. **Nilotinib** alone at 0.1 µM caused 54% of inhibition of the hERG current. **Nilotinib** co-formulated with DMPC, DMPG, DMPC/DMPC, LysoPC or LysoPG (Nilo/PLs ratio: 9:1) no longer inhibited the hERG tail current.

Figure 2. Fatty acid chain & Nilotinib-induced IKr inhibition



10:0, 12:0, 14:0, 16:0 and 18:0 LysoPG and **14:0 EGPG** alone did not cause any inhibition of the hERG tail current density. 1 µM **Nilotinib** caused 70% of inhibition of the hERG current.

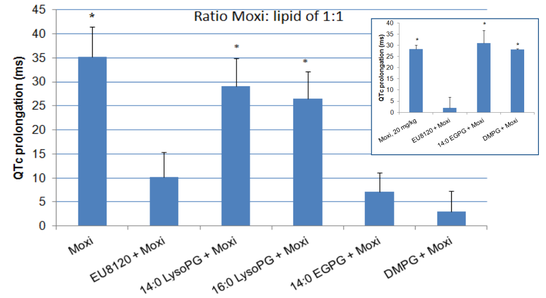
Nilotinib when formulated with 10:0, 12:0, 14:0, 16:0 and 18:0 LysoPG and 14:0 EGPG (PLs/Nilo ratio: 9:1) prevented the inhibition of the hERG current. 14:0 and 16:0 LysoPG were the most potent PLs against the inhibition of hERG currents by Nilotinib.

In vivo candidate selection

Male Hartley guinea pigs (350 - 400; Charles River) were used in these studies. The animals were anaesthetized with a mixture of 1.0 to 1.5% isoflurane USP in 95% O_2 and 5% CO_2. The jugular vein was cannulated for i.v infusion of 20 mg/kg moxifloxacin (MF). ECG leads were placed on the animals in a 3-lead configuration.

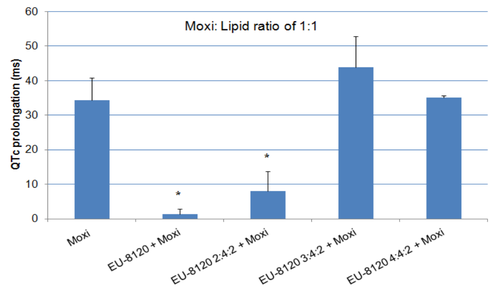
EU8120, 14:0 LysoPG, 16:0 LysoPG, 14:0 EGPG and DMPG (Avanti Polar Lipids, Inc.) were administrated as an oral gavage 2 hours prior to the infusion of MF. Three animals were exposed to each PL+ MF combination at PLs/MF ratios of 3:1, 1:1 or 0.3:1 (n=3).

Figure 3. Mitigation of QT prolongation by various PLs liposomes



20 mg/kg i.v. MF caused a 35-ms QTc prolongation in guinea pigs. EU8120, 14:0 EGPG, and DMPG prevented the MF-induced QTs prolongation.
Inset: Dropping the PLs: MF ratio to 0.3:1 revealed the greater potency of EU8120. EU8120 maintains its efficacy down to a ratio of 0.3:1

Figure 4. QTc-based optimization of EU8120

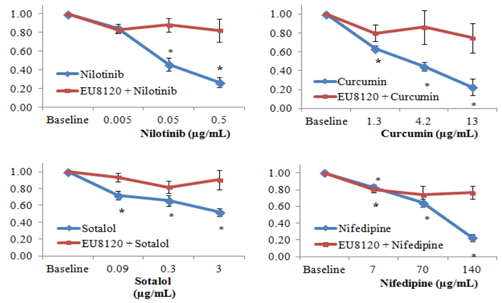


EU8120 is constituted of a 1:4:2 ratio of 14:0 LysoPG/Myristoyl monoglyceride/myristic fatty acid chain. Changing the constituent ratio to 2:4:2, 3:4:2, 4:4:2 (i.e. increasing the LysoPG content of EU8120) resulted in a loss of QTc mitigation potency. It is hypothesized that Myristoyl monoglyceride and myristic acid are necessary for oral bioavailability.

Experiments are ongoing to determine the effect of substituting the monoglyceride and fatty acid constituents on QTc mitigation potency.

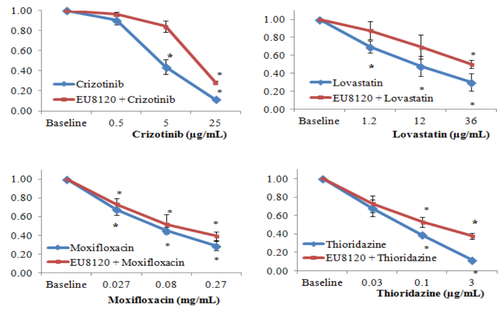
Potential mechanisms of action

Figure 5. EU8120 prevents IKr inhibition via lipid-receptor interactions



Flat concentration-response curves for some drugs suggest receptor-lipid interactions. The receptor is likely the hERG channel, with EU8120 binding a site within the pore of the channel, or a site within the cytoplasmic membrane.

Figure 6. Eu8120 could prevent IKr inhibition via PL-drug interactions



Concentration-response curves suggest a PL-drug interaction for some drugs. Inhibition is proportional to the amount of EU8120 and appears independent of a membrane-based receptor.

Conclusion

Formulation of 14:0 LPG in a eutectic mixture with a myristoyl monoglyceride and myristic acid (EU8120) given orally to guinea pigs prior to i.v. infusion of nilotinib, crizotinib and Moxifloxacin resulted in significantly reduced QTc prolongation.
Four ratios of PLs/MF were tested for mitigation of conduction delays: 3:1, 1:1, 0.3:1, and 0.1:1. Down to 0.3:1 ratio, all the compounds tested mitigated the drug-induced prolongation of QTc intervals. While EGPG induced the most protection, it caused bradycardia and was de-prioritized.

Special thanks