Abstract. Crizotinib (Xalkori®) and nilotinib (Tasigna®) are tyrosine kinase inhibitors approved for the treatment of non-small cell lung cancer and chronic myeloid leukemia, respectively. Both have been shown to result in electrocardiogram rate-corrected Q-wave T-wave interval (QTc) prolongation in humans and animals. Liposomes have been shown to ameliorate drug-induced effects on the cardiac delayed rectifier K+ current (I Kr, KV11.1), coded by the human ether-a-go-go-related gene (hERG). This study was undertaken to determine if liposomes would also decrease the effect of crizotinib and nilotinib on the I Kr channel. Crizotinib and nilotinib were tested in an in vitro I Kr assay using human embryonic kidney (HEK) 293 cells stably transfected with the hERG. Dose-responses were determined and the 50% inhibitory concentrations (IC50s) were calculated. When the HEK 293 cells were treated with crizotinib or nilotinib that were mixed with liposomes, there was a significant decrease in the I Kr channel inhibitory effects of these two drugs. When isolated, rabbit hearts were exposed to crizotinib or nilotinib, there were significant increases in QTc prolongation. Mixing either of the drugs with liposomes ameliorated the effects of the drugs. Rabbits dosed intravenously (IV) with crizotinib or nilotinib showed QTc prolongation. When liposomes were injected prior to crizotinib or nilotinib, the liposomes decreased the effects on the QTc interval. The use of liposomal encapsulated QT-prolongation agents, or giving liposomes in combination with drugs, may decrease their cardiac liability.

Crizotinib (Xalkori®) is an anaplastic lymphoma kinase (ALK) inhibitor approved for the treatment of non-small cell lung cancer in patients with ALK-positive tumors. Nilotinib (Tasigna®) is a BCR–ABL kinase inhibitor approved for Philadelphia chromosome-positive chronic myeloid leukemia. Both drugs inhibit the ion channel responsible for the delayed-rectifier K+ current in the heart (I Kr, KV11.1), encoded by the human ether-a-go-go-related gene (hERG). Inhibition of the I Kr channel can result in prolongation of the electrocardiogram (ECG) Q-wave T-wave (QT) interval, which can lead to life-threatening polymorphic ventricular tachycardia, or torsades de pointes (1). Crizotinib causes QT prolongation in humans and animals, whereas nilotinib has only been shown to cause QT prolongation in humans.

I Kr channel inhibition and cardiac toxicity can be a major liability for some classes of drugs. Detection of I Kr channel inhibition or in vivo QT prolongation during preclinical drug development can lead to the abandonment of development of promising drug classes. A number of QT-prolonging drugs have been withdrawn during development or after being on the market; examples include terfenadine, astemizole, grepafloxacin, terodilene, droperidole, lidoflazine, levomethadyl, sertindole and cisapride (2).

During development, crizotinib was shown to inhibit the I Kr channel with a 50% inhibitory concentration (IC50) of 1.1 μM (3), indicating the potential for prolongation of the QT interval. The IC50 values were below or similar to the maximum blood concentrations (C max) seen in humans at clinically-relevant doses. Dogs treated intravenously (i.v.) with crizotinib showed decreased heart rate and constrictility, increased left ventricular end diastolic pressure, and increased ECG P-wave R-wave (PR), ECG Q-wave R-wave S-wave (QRS) and QT intervals (4). These pre-clinical findings correlate with clinical findings of QTc prolongation, bradycardia and cardiac arrest observed occasionally in clinical trials (4, 5). Nilotinib was shown to inhibit the I Kr channel with a 50% inhibitory concentration (IC50) of 1.4 μM (6), which led to QTc prolongation and increased the risk of torsades de pointes (7).
channel with an IC_{50} of 0.13 μM (6). In contrast to crizotinib, dogs treated orally up to 600 mg/kg did not show QTc prolongation (7). One difference between the crizotinib and nilotinib studies in dogs was crizotinib was given i.v. and nilotinib was given orally. As with crizotinib, clinical trials showed an association of therapeutic doses of nilotinib with QTc prolongation (7,8).

A study by Doherty et al. found multiple effects of crizotinib and nilotinib on human cardiomyocytes in vitro (9). These effects included cardiac cell death, increased caspase activation, and increased superoxide generation. Cardiac cell morphology was altered, along with disruption of normal beat patterns of individual cardiac cells. For crizotinib, the cardiac ion channels IKr, NaV1.5 and CaV were inhibited, with IC_{50}s of 1.7, 3.5 and 3.1 μM, respectively. For nilotinib, IC_{50}s were 0.7, >3 and >3 μM, respectively.

We have reported that liposomes mitigate curcumin-induced inhibition of the IKr channel (10). The present study was conducted to characterize the effects of crizotinib and nilotinib on the IKr channel and QTc prolongation, and determine if the addition of liposomes ameliorates these effects.

Materials and Methods

Animals. New Zealand White rabbits (Elevage Cunicole Jacques Gagnon, Inc., Cheneville, QC, Canada), 3 to 4 kg, were used in the present study. All experimental protocols were approved by, and conducted in accordance with, the guidelines of the Institutional Animal Care and Use Committee of IPS Therapeutique, Inc (approval numbers 20130814-1, 20130829-1, 20130923-1, 20130924-1, 20131115-1 and 20131202-1).

Reagents. Crizotinib and nilotinib (molecular weights 450 and 530, respectively) were obtained from Reagents Direct (Encinitas, CA, USA). The positive control for the IKr assay, E-4031 (anhydrous N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbon-yl]phenyl]methylene sulfonamide dihydrochloride), and the positive control for the ex vivo heart assay (cisapride) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Empty liposomes were obtained from Polymun GmbH (Vienna, Austria). The liposomes were made up of a 9.7:1 ratio of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DMPG). For crizotinib or nilotinib plus liposomes, the crizotinib or nilotinib was mixed with liposomes at a 9:1 ratio (on a μg/ml basis) and vortexed for 10 minutes at room temperature. For example, for the high dose of crizotinib plus liposomes, 56 μM (25 μg/ml) crizotinib was vortexed with 225 μg/ml liposomes. Cell culture maintenance media: minimum essential medium complemented with 10% fetal bovine serum (Wisent Inc, St. Bruno, QC, Canada), 1% minimum essential medium sodium pyruvate, 1% nonessential amino acids, 1% L-glutamine, 1% penicillin/ streptomycin, and 400 μg/ml G-418 (Geneticin) as the selection agent (all ingredients from Gibco/Invitrogen, Burlington, ON, Canada). Internal pipette solution: 140 mM KCl, 1.0 mM MgCl2, 4.0 mM Mg-ATP, 5.0 mM EGTA, 10 mM HEPES, and 10 mM sucrose, pH 7.4±0.05. hERG external solution: 140.0 mM NaCl, 5.0 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 10.0 mM HEPES, and 10.0 mM dextrose, pH 7.3±0.05.

IKr assay. Human embryonic kidney (HEK) 293 cells stably-transfected with the hERG were maintained in cell culture maintenance media, and used between passages 12 and 16. Those cells from which a gigaohm seal could not be obtained or that did not generate currents with a distinctive tail current were eliminated during the equilibration period. The whole-cell patch-clamp technique was used. HEK 293 cells plated onto 35-mm petri dishes were washed twice with 1 ml of hERG external solution followed by the addition of 2 ml of hERG external solution. The petri-dish was mounted on the stage of an inverted phase-contrast microscope and maintained at constant temperature (35°C ± 2°C). A borosilicate glass micropipette filled with the internal pipette solution was positioned above a single cell using an Eppendorf PatchMan micromanipulator (Eppendorf Canada, Mississauga, ON, Canada). The micropipette was lowered to the cell until close contact was achieved. The gigaohm-range membrane-pipette seal was then created by applying a slight negative pressure (resistances were measured using a 5 mV square pulse). Cell capacitance was immediately measured to evaluate cell surface area, using a conversion factor of 1 pF/μm². A cell surface area that was later used to calculate net current density. All currents were recorded following analog filtering using a 4-pole Bessel filter (Frequency Devices, Haverhill, MA, USA) 1 kHz. Through the computer-controlled amplifier, the cell was depolarized to a maximum value of -40 mV (cultured cells), starting at ~10 mV, in 10 mV increments, for 1 second. The membrane potential was then returned to ~55 mV for 1 second, and finally depolarized to the resting potential value. This allowed the channels to go from activated to inactivated mode, and back to activated mode, to measure robust tail currents. All K⁺ selective currents passing through IKr channels were recorded using Axopatch-1D or Axopatch 200B amplifiers and digitized with Digidata 1322A or 1440A AD-DA interfaces (Axon instruments Inc, Foster City, CA, USA; now Molecular Devices Inc). The recording of the cell current started 500 ms before cell depolarization to -40 mV and lasted for 500 ms after the cell had been repolarized to ~80 mV.

Cell treatment. After baseline recordings were obtained, increasing concentrations of crizotinib or nilotinib, alone or mixed with liposomes, were added in 20 μl aliquots directly to the experimental chamber and were allowed to disperse through a closed-circuit perfusion system using a mini-peristaltic pump (MP-1; Harvard Instruments, Holliston, MA, USA). Exposure times for each concentration were limited to 5 minutes. Following the recording of currents in the presence of the highest concentrations of test agents, a flow-through perfusion system was used to wash out the test agent and obtain post-exposure IKr currents in the same manner as previously described. Finally, three cells were exposed to 100 nM of the positive control E-4031. The concentrations of E-4031 were added into the experimental chamber as was done with crizotinib and nilotinib. The IKr currents generated by heterologous expression systems such as HEK 293 cells are known to run down over long periods of recording. Therefore, parallel experiments were run in the absence of the test agents and in the presence of the solvent to correct for the time-dependent decrease in current density, known as current rundown.
**IKr data analysis.** The correction for the time-dependent decrease in current density involved averaging the changes in current density associated with time and solvents, and multiplying the test agent results with the resulting correction factor. All IKr results reported here have been corrected for the effect of the vehicle and for time-dependent changes in current density. IKr current amplitudes are expressed as current density [in nanoamperes/picofarad (nA/pF)] to correct for variations in cell size within the population of cells used for this study. Currents were analyzed using the Clampfit 10.0 module of the pClamp 10.0 software (Axon Instruments Inc.). The results obtained in the presence of each concentration were expressed as net current density, normalized against current density measured in baseline conditions. The amplitude of the IKr tail current was calculated as the difference between the average current recorded before the depolarizing pulse to −40 mV and the maximum transient current recorded at the beginning of the repolarizing pulse to −55 mV.

**Ex vivo heart preparation.** Two thousand units of heparin were injected intraperitoneally 20 min prior to euthanasia. The rabbit was euthanized by cervical dislocation followed by a rapid exsanguination. The heart was removed quickly and attached to a Langendorff perfusion system. A cannula connected to the system prefixed with oxygenated (95 O2 and 5% CO2) Tyrode’s solution at 35±2˚C was inserted into the aorta and sutured to secure the heart to the perfusion system. The perfusion was initiated upon injection of the heart to the Langendorff apparatus. The Tyrode’s solution perfused the heart in a retrograde manner at a pressure of approximately 80 mmHg and a flow rate of approximately 20 ml/min. The spontaneously beating heart dictates the flow rate; with each heartbeat, solution flows into the coronary arteries and perfuses the myocardial tissue, providing it with oxygen and nutrients and avoiding metabolic deficit and ischemia. A bipolar circuit of three chloride silver wire electrodes was placed on the epicardium of the heart, one on the apex, and the other on the atrium, and the third, a ground, just off the heart, providing a lead I configuration. The ECG signals were filtered at 500 Hz using an Iso-DAM8A (World Precision Instrument, Sarasota, FL, USA) and digitized at a sampling rate of 2.0 kHz using a Digidata 1322A interface (Axon Instruments Inc.). Continuous recording of the ECG was initiated 5 minutes before the start of infusion of the test agent or vehicle equivalent. The liposomes were injected 5 minutes prior to the start of infusion of each loading dose. The coronary perfusion pressure was generated by the spontaneously beating heart.

**In vivo rabbit model.** The rabbits were anesthesitized with a mixture of 2.5% isoflurane USP (Abbot Laboratories, Montreal, Canada) in 95% O2 and 5% CO2. The left jugular vein was cannulated for i.v. infusion of the test agent. ECG leads were placed on the animal, and the ECG signals were filtered at 500 Hz using an Iso-DAM8A (Word Precision Instrument) and digitized at a sampling rate of 2.0 kHz using a Digidata 1322A interface (Axon Instruments Inc.). Continuous recording of the ECG was initiated 5 minutes before beginning infusion of the first dose of the compound and was terminated at the end of infusion of the last dose. Following baseline ECG recording, the infusion of the first loading dose of the compound was started. At the end of the first loading dose, the infusion was switched to the first maintenance dose. The rabbit was exposed to each dose for 25 minutes (10 minutes of loading dose followed by 15 minutes of maintenance dose). The same procedure was applied until the rabbit was exposed to all of the selected doses of test agent or vehicle equivalent. The liposomes were injected 5 minutes prior to the start of infusion of each loading dose. The liposomes were administrated as an i.v. bolus in the left ear vein at a ratio of 9:1 (μg/ml basis). ECG parameters were analyzed and presented in the same manner as for the ex vivo heart experiment.

**Statistical analysis.** A paired one-way t-test was performed to determine the statistical significance of the differences in baseline values compared to each treatment. An unpaired one-way t-test, assuming unequal variances, was carried out to compare crizotinib or nilotinib alone with crizotinib or nilotinib plus liposomes.

**Results**

**In vitro IKr current.** Crizotinib, at concentrations of 11 and 56 μM, caused 57% and 89% inhibition, respectively, of the IKr tail current density at 20 mV (Figure 1A). Paired Student’s t-tests showed that the difference in normalized current density measured at baseline and in the presence of 11 and 56 μM of crizotinib reached the selected threshold for statistical significance (p<0.05). The IC50 was 8.9 μM with crizotinib-alone (Table 1). When crizotinib was mixed with liposomes at a ratio of 9:1, only the highest concentration of 56 μM crizotinib led to a statistically significant inhibition compared to baseline.
The IC₅₀ was 44 μM. Liposomes-plus-crizotinib at 11 μM did not have any effects on the Iₖr tail current density. Liposomes-plus-crizotinib at 56 μM did have a significant effect on the Iₖr tail current density when compared to baseline. However, when comparing the current density between crizotinib at 11 and 56 μM, and liposomes-plus-crizotinib, there was a significant inhibition of the effects of crizotinib when mixed with liposomes. The liposomes alone did not have any effects on the Iₖr tail current density (Figure 1A).

Figure 1. Iₖr tail current density averages obtained by measuring the Iₖr tail peak amplitude at 20 mV at baseline conditions and in the presence of crizotinib, nilotinib, liposomes alone, crizotinib plus liposomes, or nilotinib plus liposomes. The current densities were averaged, normalized against baseline current density, and corrected for time and solvent effects. A: The concentration of liposomes relative to crizotinib concentrations were 4.5, 45 and 225 μg/ml for 1.1, 11 and 56 μM crizotinib, respectively. B: Voltage dependency of the Iₖr tail current inhibition at the highest concentration of crizotinib tested (56 μM). C: The concentration of liposomes relative to nilotinib concentrations were 0.045, 0.45 and 4.5 μg/ml for 0.01, 0.1 and 1 μM nilotinib, respectively. D: Voltage dependency of the Iₖr tail current inhibition at the highest concentration of nilotinib tested (1 μM). The values plotted are the mean±standard error of the mean, for three or four cells. Paired t-test statistics comparing between baseline and post-drug exposure: *p≤0.05. Two-sample t-test statistics comparing between the liposomes-alone, and liposomes-plus-drug, at each of drug concentration: †p≤0.05.
Nilotinib, at concentrations of 0.1 and 1 μM, caused statistically significant inhibition of the I_{Kr} tail current density at 20 mV when compared to baseline of 54% and 74%, respectively (Figure 1C). The IC_{50} was 0.08 μM with nilotinib alone (Table I). When nilotinib was vortexed for 10 min at room temperature with liposomes at a ratio of 9:1, there were no effects of nilotinib on the I_{Kr} channel, even at the highest concentrations of 1 μM. The IC_{50} was >1 μM. When comparing the current density between nilotinib, and liposomes plus nilotinib, there was a significant inhibition of the effects of 0.1 and 1 μM nilotinib when mixed with liposomes. The liposomes alone did not have any effects on the I_{Kr} tail current density (Figure 1C).

The current–voltage relationships of the rectifying inward current showed that the inhibitions observed on the tail current were not voltage-dependent for both crizotinib and nilotinib (Figure 1B and 1D, respectively).

The positive control, E-4031, produced statistically significant decreases in current density at a concentration of 100 nM. E-4031 was tested twice, with 67% and 79% inhibition observed (data not shown).

Ex vivo rabbit heart QTc intervals. Crizotinib, at concentrations of 11 and 56 μM, caused a dose-dependent prolongation of the QTc interval (Figure 2A). Mixing crizotinib with liposomes at a ratio of 9:1 resulted in a significant inhibition of the crizotinib-induced QTc prolongation. Nilotinib, at concentrations of 14 and 28 μM, also caused a dose dependent prolongation of the QTc interval (Figure 2B). As with crizotinib, mixing nilotinib with liposomes, resulted in a significant inhibition of the nilotinib-induced QTc prolongation. The cisapride positive control showed the expected prolongation of the QTc interval.

The effects of crizotinib and nilotinib on ECGs were associated with effects on LVP (Table II). When hearts were exposed to crizotinib or nilotinib alone, there was a decrease in LVP. When liposomes were mixed with crizotinib or nilotinib, the effects on LVP were reversed.

QTc intervals after in vivo dosing of rabbits. Rabbıts given crizotinib at 1, 2 and 3 mg/kg by i.v. infusions over 10 min, followed by a maintenance dose for 15 min, showed a dose-dependent prolongation of the QTc interval (Figure 3A). Injecting liposomes 5 min prior to treatment with crizotinib resulted in a significant inhibition of the crizotinib-induced QTc prolongation. Rabbıts given nilotinib at 2, 4 and 5.5 mg/kg by i.v. infusions over 10 min, followed by a maintenance dose for 15 min, showed a dose-dependent prolongation of the QTc interval (Figure 3B). As with crizotinib, injecting liposomes 5 min prior to treatment with nilotinib, resulted in a significant inhibition of the nilotinib-induced QTc prolongation.

### Table I. Concentrations that caused 50% inhibition of the I_{Kr} current density in HEK 293 cells stably transfected with the human ether-a-go-go-related gene calculated from the data presented in Figure 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>Crizotinib</th>
<th>Nilotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes alone</td>
<td>&gt;225 μg/ml%</td>
<td>&gt;4.5 μg/ml%</td>
<td></td>
</tr>
<tr>
<td>Drug alone</td>
<td>8.9 μM</td>
<td>0.08 μM</td>
<td></td>
</tr>
<tr>
<td>Drug plus liposomes</td>
<td>44 μM</td>
<td>&gt;1 μM</td>
<td></td>
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</tbody>
</table>

<225 and 4.5 μg/ml were the highest concentrations of liposomes-alone tested in the assay, for crizotinib and nilotinib, respectively.

### Discussion

These data demonstrate that liposomes protect against the inhibitory effect of these kinase-inhibitor drugs on the I_{Kr} channel using stably hERG-transfected HEK 293 cells, and ameliorate cardiac QTc prolongation resulting from both ex vivo and in vivo exposure. These results suggest that mixing these drugs with liposomes may prevent interactions of these inhibitory drugs with the I_{Kr} channel allowing more normal gating kinetics to occur, and thus reducing the degree and incidence of QTc prolongation that may occur in the clinic.

Other tyrosine kinase inhibitors have also been shown to have effects on the QTc interval, including lapatinib, sunitinib and vandetanib (12). The most studied in vitro is lapatinib (13). Lapatinib was shown to prolong action potential duration of isolated rabbit Purkinje fibers at 5 μM. This was associated with an inhibitory effect on the I_{Kr} channel with an IC_{50} of 0.8 μM, and a slight effect on the I_{Ks} amplitude at 5 μM. No effects were observed on the I_{Na}, I_{K1} or I_{Ca} channels.

In the clinic, crizotinib is given at doses as high as 500 mg/day (250 mg bid), which is about 4.2 mg/kg or 156 mg/m^2 bid. From the Food and Drug Administration’s review of the new drug application for crizotinib, steady state C_{max} in patients with cancer given 500 mg bid averaged 650 ng/mL, or 1.5 μM (4). Mossé et al. reported steady state C_{max} in children with cancer to be 630 ng/mL (1.4 μM) after dosing 280 mg/m^2 bid (14). This is well within the range of effects on the i.v. I_{Kr} channel with an IC_{50} of 8.9 μM reported in the present study, and 1.1 μM reported during the development of crizotinib (3). Nilotinib is dosed as high as 600 mg/day (300 mg bid), which is about 5 mg/kg or 188 mg/m^2 bid. Patients with cancer given 400 mg bid had steady state C_{max} of 1,754 ng/mL, or 3.3 μM (15). Patients given 400 mg bid had steady state C_{max} of 2161 ng/mL, or 4.1 μM (16). The present study showed an IC_{50} in the I_{Kr} assay of 0.08 μM, and 0.13 μM was reported during the development of nilotinib (6).

It has been reported that liposomes mitigate inhibitory effects of curcumin on the I_{Kr} channel (10). Curcumin-alone
inhibited the I<sub>Kr</sub> channel with an IC<sub>50</sub> of 4.9 μM, with the highest concentration tested (11.4 μM) resulting in 80% inhibition. When mixed with the same type of liposomes and at the same ratio as in the present study, the highest dose of curcumin tested (11.4 μM) only achieved 45% inhibition. Curcumin that was encapsulated in the liposomes, and not just mixed, also abrogated curcumin-induced I<sub>Kr</sub> inhibition, by 25% at 11.4 μM. In the present study, when the positive control E-4031 was tested alone, the IC<sub>50</sub> was 56 nM; when E-4031 was mixed with liposomes, the IC<sub>50</sub> increased to 210 nM.

Tartar emetic is a trivalent antimonial drug that causes QT interval elongation in rats and humans. When tartar emetic was encapsulated in liposomes, the QT effects were abolished (17). One important difference between the tartar emetic study and the present study is the composition of the liposomes that were used. The liposomes used in the tartar emetic study were composed of L-α-distearoyl-phosphatidylcholine, cholesterol and polyethylene glycol 2000 distearoylphosphatidylethanolamine. Another difference is the present study showed that simply mixing the drugs with the liposomes, or injecting them prior to treatment with QT-prolonging drugs, and not encapsulating them, resulted in the inhibitory effects.

One clinical trial in healthy volunteers has shown that encapsulation with liposomes abolished QT-prolongation effects. When bupivacaine, which increases QT interval in humans and laboratory animals, was encapsulated in liposomes (Exparel<sup>®</sup>), it did not cause QT prolongation at doses as high as 750 mg given subcutaneously (18). As with the tartar emetic

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**Figure 2.** Electrocardiogram rate-corrected Q-wave T-wave interval (QTc) prolongation of isolated rabbit hearts after treatment with crizotinib, nilotinib, liposomes alone, crizotinib plus liposomes, or nilotinib plus liposomes. Hearts were exposed sequentially to the different concentrations of test agents for 10 minutes, and ECG recordings taken during the last 1 minute. Concentration of liposomes were 45 and 225 μg/ml for 11 and 56 μM crizotinib, respectively (A) and 68 and 135 μg/ml for 14 and 28 μM nilotinib, respectively (B). The values plotted are the mean±standard error of the mean, for QTc prolongation above baseline, for three hearts. Paired t-test statistics comparing between baseline and post-drug exposure, using the actual interval data: *p<0.05. Two-sample t-test statistics comparing QTc prolongation between the liposomes alone, and liposomes plus drug, at each drug concentration. The p-values for the two-sample t-test statistics are presented on the graph, †p<0.05. ND: The hearts in the crizotinib and liposomes + crizotinib groups were not treated with cisapride. WO: Washout.

**Table II.** Effects of crizotinib and nilotinib, alone and with liposomes on left ventricular pressure (LVP) in ex vivo rabbit hearts. Values are the mean (SEM), of three hearts per group.

<table>
<thead>
<tr>
<th>Concentration of drug (μM)</th>
<th>Liposomes-alone</th>
<th>Drug-alone</th>
<th>Liposomes-plus-drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crizotinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.25 (2.10)</td>
<td>-7.34 (6.19)</td>
<td>-1.03 (0.62)</td>
</tr>
<tr>
<td>56</td>
<td>0.67 (1.87)</td>
<td>-8.22 (6.23)</td>
<td>-1.15 (0.37)</td>
</tr>
<tr>
<td>Nilotinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-0.98 (1.56)</td>
<td>-0.45 (1.51)</td>
<td>-0.32 (0.93)</td>
</tr>
<tr>
<td>28</td>
<td>0.47 (2.01)</td>
<td>-9.80 (0.19)</td>
<td>-3.30 (0.34)</td>
</tr>
</tbody>
</table>
study, here the drug was encapsulated and the components of the liposome were different from those of the present study: cholesterol, 1, 2-dipalmitoyl-sn-glycero-3 phospho-rac-(1-glycerol), tricaprylin, and 1, 2-dierucoylphosphatidylcholine.

The in vitro assay assessing the effects of drugs on the I\textsubscript{Kr} (hERG) current is extensively used to help predict potential effects of a drug on QTc interval in the clinic (19). This is a useful assay, but sometime results in false-positives. The present study demonstrates an example where this in vitro assay was very predictive of in vivo QTc prolongation in both animals and humans.

Based upon the data in the present study, and the data with curcumin (10), it does not appear to be necessary to encapsulate a drug in DMPC/DMPG liposome to mitigate IKr suppression by crizotinib and nilotinib, and possibly other QTc-prolonging agents. A simple mixing of the compound with the liposomes may be sufficient. For orally-administered QT-prolonging agents, concurrent subcutaneous administration of an extended-release formulation of liposomes may suffice. This will need to be tested with QT-prolonging drugs in the in vivo animal model of QTc prolongation.

Acknowledgements

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