Effect of Multiple Drugs Interacting with the hERG Channel – *In Vitro* Study

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Abstract

Polypharmacy is common practice, and aims in maximizing the efficacy with lowest adverse effects. Though, when resulting in adverse effects constitute a major health problem.. The aim of the present study was to characterize possible interactions between loratadine (L), its main metabolite desloratadine (DL), and ketoconazole (K) as a loratadine enzymatic metabolism inhibitor (CYP3A4 enzyme). Ketoconazole itself can block hERG channel and is associated with QT interval prolongation, thus both PK and PD components underlie the potential clinical effect. The whole-cell configuration of the patch-clamp technique was employed on hERG channels stably expressed in human embryonic kidney 293 cells, with the use of automatic patch clamp system (CytoPatch2). All recordings were taken at room temperature (22±1° C). Up to four increasing concentrations of the same compound (K, L, DL) or mixture of two (0.25x, 0.5x and 1x IC50 value of L and DL) or three (0.25x IC50 of L and DL, with 0.25x, 0.5x, 1x, and 2x IC50 value of K) compounds were applied to each examined cell. Current inhibition was calculated by dividing the mean tail current in the presence of the drug by the mean tail current of the control phase. Concentration of a drug that is required for 50% current inhibition (IC50), and the slope parameter (Hill coefficient) for L, DL, and K were 5.17(2.24), 1.95(1.14), and 0.84(1.19) respectively. The mixture of L and DL for 0.25x, 0.5x and 1x IC50 gave 30.6, 53.8, and 76.0% of current inhibition respectively. The mixture of L, DL, and K 0.25x, 0.5x, 1x, and 2x IC50 gave 33.2, 59.3, 75.1, and 85.7% of current inhibition respectively.

Methods

hERG-transfected HEK 293 cells were routinely cultured in tissue culture flasks T-75 in Dulbecco's modified Eagle's medium with 4,5 g/l glucose (Lonza, Belgium) supplemented with 10% fetal calf serum, 1% penicillin/streptomycin, 1% nonessential amino acids, 1% sodium pyruvate and 0.5 mg/ml geneticin (all from Thermo Fisher Scientific, USA) in a 37˚C incubator with 5% CO_2. The cells were passaged every 3-4 days, using a TrypLE Express solution. For electrophysiology studies we used cells from 21st to 39th passages and the cells were seeded in tissue culture flasks T-25 at a density 1 x 10^6 for 24-48 h before patch clamping experiments. Stock solutions were prepared in DMSO for all compounds in concentrations as follows: ketoconazole (K): 50 mM, loratadine (L): 25mM and desloratadine (DL) 30mM. Aliquots of these stock solutions were stored in −20°C. For electrophysiological recordings working concentrations of the drug or mixture of two or three compound were freshly prepared by diluting to respective millimolar concentrations in DMSO and then to micromolar concentrations in EC. Obtained working solutions were used for maximum 4 hours. Final concentration of DMSO did not exceed 0.1%. For calculation of the concentration-response relationships for the inhibition of hERG peak tail current amplitudes, single drugs were studied at the following concentrations: 2.5, 3.125, 6.25 and 12.5 µM for L; 0.5, 1, 5 and 10 µM for DL and 0.5, 1, 2.5 and 5 µM for K. The number of observations ranged from 2 to 7 per test concentration.

Experiments were performed on automated patch-clamp device CytoPatch2 (CytoBioScience Inc., Tx, USA), according to a modified protocol. The sequence to whole-cell configuration (chip filling, cell capture, gigaseal formation, and patch rupture) was fully automated and executed by the device. To measure hERG outward tail currents the following pulse protocol was applied every 10 s: from a holding potential of −85 mV, a brief 100 ms prepulse to −65 mV was applied to determine the leak current, followed by a 2 s depolarizing voltage step to +40 mV and thereafter 2 s step to -65 mV to evoke outward tail currents. Amplitude of peak tail current was corrected for the leak current determined during the first short voltage step to −65 mV. A 100 ms step to -120mV ensured that all channels were fully deactivated between sweeps. The cells were then continuously perfused with EC buffer for 10 minutes to establish stable control recordings. After the control phase, drugs were continuously applied to the cell via the transport channel for 5 minutes. All recordings were taken at room temperature (22±1°C).

Results

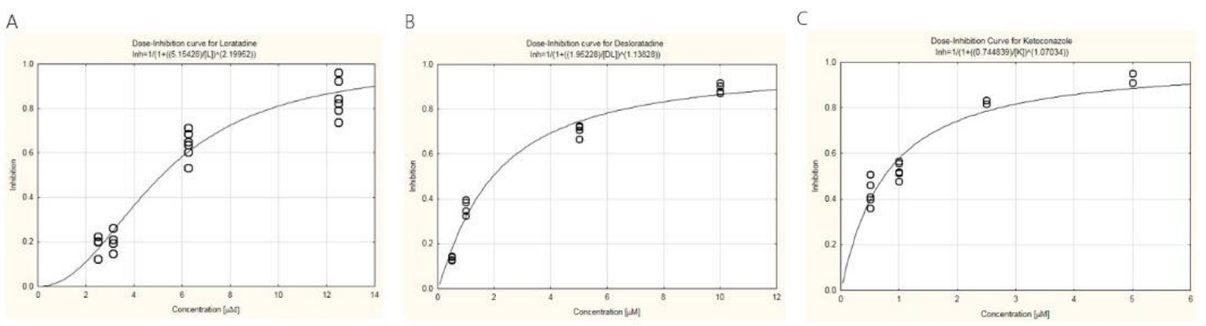
The first step of the study was to assess the IKr current inhibition potential for three single drugs, namely loratadine, its metabolite desloratadine, and ketoconazole. Table 1 presents IC50 values and Hill equation parameters.

Table 1. IC50 [µM] and Hill coefficient (nH) values for single drugs.

	Loratadine	Desloratadine	Ketoconazole
IC_{50}	5.15	1.95	0.74
nH	2.20	1.14	1.07

The individual inhibition values measured during the experiments for all three compounds are presented in Figure 1.

Figure 1. Individual hERG inhibition values and fitting curve for three tested single moieties - loratadine (A), desloratadine (B), and ketoconazole (C).



The results of experiments carried out on pairs of compounds are presented in Table 2. All three combinations were tested, and the measured IKr current inhibition ranged from 21% (L+DL for the ~25% IC50 + ~25% IC50 combination), up to almost complete block reaching 96% of the current inhibition (L+K for the ~200% IC50 + ~200% IC50 combination).

Table 2. hERG current inhibition for single drugs and drug pairs. Concentration of compound X is denoted by [X]. SEM stands for Standard Error of the Mean and n is the number of cells tested for a given concentration.

Loratadine (L) + Desloratadine (DL)				
[L] [µM]	[DL] [µM]	Mean Inhibition	n	SEM
1.35	0.65	0.216	5	0.018
2.7	1.3	0.52	5	0.034
5.4	2.6	0.769	5	0.038
10.8	5.2	0.961	3	0.008

Loratadine (L) + Ketoconazole (K)				
[L] [µM]	[K] [µM]	Mean Inhibition	n	SEM
1.35	0.163	0.296	9	0.015
2.7	0.325	0.618	11	0.016
5.4	0.65	0.866	10	0.012
10.8	1.3	0.959	10	0.008

Desloratadine (DL) + Ketoconazole (K)				
[L] [µM]	[K] [µM]	Mean Inhibition	n	SEM
0.65	0.163	0.257	5	0.01
1.3	0.325	0.575	5	0.023
2.6	0.65	0.772	5	0.018
5.2	1.3	0.9	3	0.013

Similar study was repeated for the tested triplet - combination of L, DL, and K. Results covering measured and theoretically calculated current inhibition are presented in Table 3.

Table 3. hERG current inhibition for drugs triplet. Concentration of compound X is denoted by [X]. SEM stands for Standard Error of the Mean and n is the number of cells tested for a given concentration.

Three Drugs - Mix 1					
[L] [µM]	[DL] [µM]	[K] [µM]	Mean Inhibition	n	SEM
1.35	0.65	0.16	0.343	11	0.02
1.35	0.65	0.33	0.553	12	0.036
1.35	0.65	0.65	0.735	8	0.037
1.35	0.65	1.3	0.862	8	0.024

Three Drugs - Mix 2					
[L] [µM]	[DL] [µM]	[K] [µM]	Mean Inhibition	n	SEM
2.7	1.3	0.16	0.426	4	0.023
2.7	1.3	0.33	0.718	4	0.019
2.7	1.3	0.65	0.857	4	0.024
2.7	1.3	1.3	0.916	3	0.016

Conclusions

It was demonstrated in this study that L, DL and K interact at the hERG channel level. . For the combination of drugs tested in pairs the effect was concentration dependent. In lower concentrations synergistic effect was observed, while for the highest tested concentrations the total inhibition was subadditive. For triplet the effect was subadditive regardless of concentrations.

2017 SPS Annual Meeting, September 24–27, 2017
Germany, Berlin

Poster #
0142