INTRODUCTION:

Recommendations of the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use concerning cardiovascular adverse effects are focused on HERG channel assay and QT prolongation. However, calcium channel (ICaL) inhibition may lead to cardiac adverse events (AV node conduction slowing, AV-block and negative inotropic effects). Thus, the aim of the present study was to present different in vitro models which allow to investigate the pharmacological ICaL inhibition by verapamil and its related electrophysiological and hemodynamic effects. Indeed, an inhibition of the ICaL current leads to a negative shift of the plateau phase of guinea pig and rabbit action potentials, an increase in the PR interval of the electrocardiogram and a negative inotropic effect.

\[ \text{IPR} = \frac{\text{PR interval}}{0.80 \pm 0.16} \]

\[ \text{k} = 3.38 \pm 0.71 \text{ E-6 mol/L} \]

\[ y = V_{\text{max}} \cdot x^n/(k^n + x^n) \]

\[ \text{IC}_{50} = 0.71 \text{ µM} \]

\[ \text{APD}_{40} = 1 \text{ Hz, APD}_{40} = 0.2 \text{ Hz (ms)} \]

\[ \text{APD}_{20} = 1 \text{ Hz, APD}_{20} = 0.2 \text{ Hz (ms)} \]

\[ \text{Max dP/dt - Min dP/dt} \text{ (mmHg/sec)} \]

\[ \text{Baseline} \]

\[ \text{Verapamil concentration-dependently decreases } \text{APD}_{40} \text{ in rabbit Purkinje fibres} \]

\[ \text{Verapamil concentration-dependently decreases } \text{APD}_{20} \text{ in guinea-pig ventricular myocytes} \]

\[ \text{Verapamil concentration-dependently decreases developed pressure, maximal rate of contraction and maximal rate of relaxation in isolated guinea-pig hearts} \]

\[ \text{Verapamil concentration-dependently increases PR interval and induced AV-blocks in isolated guinea-pig hearts} \]

Results:

The effects of verapamil (1 to 30 µM) were investigated on hCav1.2 channels, transfected in CHO cells using the patch-clamp technique in whole-cell configuration (n=4).

The repercuissions of the pharmacological block of hCav1.2 by verapamil (1 and 10 µM) were also studied on the action potential duration at 20% of total repolarization (APD20) on guinea-pig ventricular myocytes using the patch-clamp technique in whole-cell configuration. Preparations were paced at 0.5 Hz (n=3 for each concentration).

Electrophysiological effects of verapamil (0.1 to 10 µM) were also studied on the action potential duration at 40% of total repolarization (APD40) on rabbit Purkinje fibres using conventional microelectrode technique. Preparations were paced at 1 and 0.2 Hz (n=3).

Finally, the effects of verapamil (0.03 to 1 µM) on PR interval, left ventricular developed pressure and maximal rates of contraction (Max dP/dt) and relaxation (Min dP/dt) were also studied on guinea-pig Langendorff perfused hearts (n=3).

Conclusion: All the presented models may be useful for the preclinical evaluation of cardiac effects of compounds linked with ICaL inhibition.