EVALUATION OF THE CARDIAC SAFETY OF NEW ANTIDEPRESSANT DRUGS: PREDICTIVITY OF A COMBINED *IN VITRO* AND *IN VIVO* APPROACH.

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INTRODUCTION

Cardiac toxicity of Tricyclic Antidepressant drugs (TCAs) is in part related to the blockade of cardiac ionic channels such as sodium, calcium or potassium channels (1), leading to lengthening both depolarisation and repolarisation phases of the cardiac action potential. These electrophysiological effects result in changes in the ECG, such as prolongation of the PR, QRS or QT intervals, and contribute to life threatening ventricular arrhythmia (2)(3)(4)(5).

In contrast, on the basis of animal studies (6)(7) as well as human clinical data (8), new generation of Serotonin Reuptake Inhibitor antidepressant compounds (SRIs), are considered to have fewer and more benign sided cardiovascular effects than TCAs. However, some arrhythmias associated with the use of these compounds have been reported (8). Moreover, toxicity in overdose remains an important issue (9).

The aim of this study was to develop a strategy in order to predict early the cardiotoxicity of the new antidepressant candidates using some selected preclinical models, ranging from the simplest cellular hERG or $Na_V1.5$ current assays to the more complex *in vitro* Purkinje assay and *in vivo* anaesthetised guinea pig model. Thus, cardiac profiles of some representative TCAs (imipramine, amitriptyline) as well as SRIs (paroxetine, fluoxetine, venlafaxine) antidepressant agents have been compared.

MATERIAL AND METHODS

Patch clamp hERG & $Na_V1.5$ in HEK-293 cells

Using the conventional patch-clamp technique in whole cell configuration, the effects of the compounds have been studied at room temperature (22 ± 2°C) on hERG (10) and $Na_V1.5$ (11) currents in HEK-293 cells. Cells were superfused at approximately 1 mL/min in the presence of extracellular solution (prevalue), vehicle (distilled water 0.1% or DMSO 0.1%) during 10 minutes (baseline) and increasing cumulative concentrations of paroxetine, fluoxetine, amitriptyline, imipramine or venlafaxine until the steady state was reached in 3 cells. Inhibition percentages were calculated from individual variation of tail current amplitude for each cell vs baseline.

Action potential parameters in isolated Rabbit Purkinje fibres

Groups of 3 or 4 fibres obtained from left ventricle of female rabbits anaesthetized with sodium pentobarbital were electrically stimulated at 1 Hz and superfused with Tyrode's solution (prevalue), vehicle (distilled water 0.1% or DMSO 0.1%) in Tyrode's solution (baseline) and increasing concentrations (0.1, 1 and 10 µmol/L) of paroxetine, fluoxetine, amitriptyline, imipramine or venlafaxine for 30 minutes. For each solution, after 1 Hz, fibres were stimulated at 0.2 Hz for at least 3 min followed by a 5-min recovery to 1 Hz (12). Action potential parameters were measured: maximal rate of depolarization (V_{max}), action potential durations at 40% and at 90% of repolarization (APD_{40}, APD_{90} respectively) and percentage changes from baseline were calculated.

ECG parameters in anaesthetised guinea-pigs

Using the anaesthetised guinea-pig model (13, 14), the effects of successive intravenous infusions of increasing doses of the compounds on electrocardiographic parameters were investigated and compared to those generated in the presence of vehicle. Guinea-pigs were anaesthetised with sodium pentobarbital throughout the experiment. Catheters were introduced into a carotid artery and a jugular vein for measurement of arterial blood pressure and administration of the test compound, respectively. Subcutaneous needle electrodes were placed for measurement of lead II electrocardiogram. The body temperature of the animals were maintained around 37°C using a heating pad.

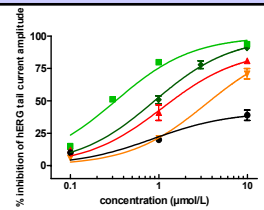
Following an equilibration period of at least 15 min, necessary to obtain stable haemodynamic conditions, guinea-pigs (n=6 per group) were sequentially administered by i.v. infusion over 15 min with increasing doses of paroxetine (1, 6 and 12.5 mg/kg), fluoxetine or venlafaxine (1, 3, 6 and 12.5 mg/kg), amitriptyline or imipramine (1, 3 and 9 mg/kg) or with 4 successive infusions of corresponding vehicle (water for injection).

Electrocardiographic parameters were measured before administration of the first dose (baseline, T0), and at the end of each i.v. infusion (T15, T30, T45 and T60). PR, QRS and QTcF (Fridericia correction) intervals were measured. Percentage changes from baseline were calculated.

hERG % Inhibition

Conc (µmol/L)	paroxetine	fluoxetine	venlafaxine	amitriptyline	imipramine
0.1	13	15	10	6	12
1	51	80	20	21	41
10	91	94	39	71	81

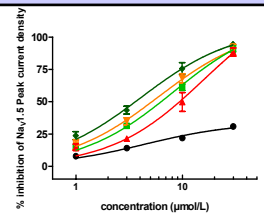
TCAs as well as SRIs dose-dependently inhibited hERG



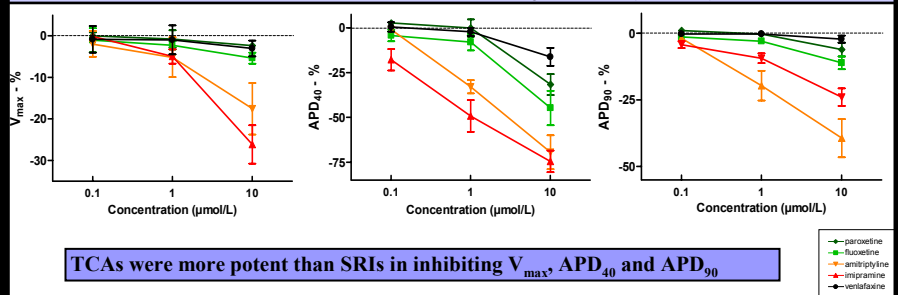
$Na_V1.5$ % Inhibition

Conc (µmol/L)	paroxetine	fluoxetine	venlafaxine	amitriptyline	imipramine
1	24	15	8	17	15
3	43	31	14	35	21
10	75	61	22	68	50

TCAs as well as SRIs dose-dependently inhibited $Na_V1.5$

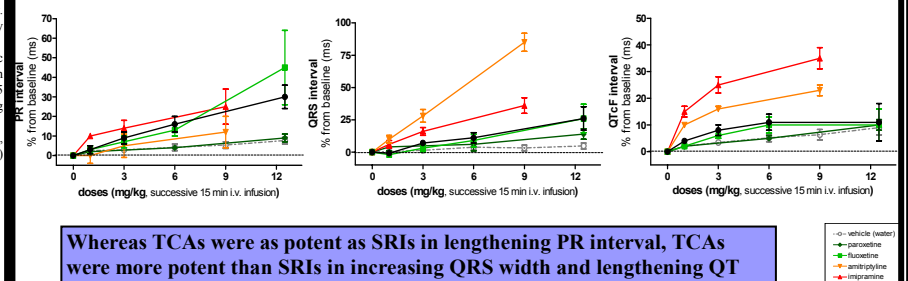


Action Potential in Rabbit Purkinje Fibres (at 1 Hz)



TCAs were more potent than SRIs in inhibiting V_{max}, APD_{40} and APD_{90}

ECG in Anaesthetised Guinea-Pig



Whereas TCAs were as potent as SRIs in lengthening PR interval, TCAs were more potent than SRIs in increasing QRS width and lengthening QT

RESULTS - CONCLUSION

→Despite dose-dependent hERG inhibition, all the antidepressant drugs tested do not lengthen APD_{90}, consistent with their multi-channel blockade.

→ Most of the compounds tested exhibited a dose-dependent inhibition of $Na_V1.5$ and, consistently, lengthened PR interval in anaesthetised guinea-pig, at relative high dosage.

→ Only TCAs reduced the V_{max} in Purkinje fibres assay and, consistently, increased QRS interval width *in vivo*.

TCAs were also more potent than SRIs in shortening the APD_{40}, related at least in part to the interaction with calcium channel (data not shown).

Finally, although TCAs appeared more potent in shortening APD_{90} as compared to SRIs, they were more potent in lengthening QTcF interval.

→Among SRIs, venlafaxine exhibited the best cardiac safety profile.

These results confirm that, for some classes of drugs such as antidepressants, hERG assay oversimplifies drug effects on the complex repolarisation process and that neither assay alone adequately predicts pro-arrhythmic risk. Moreover, PK/PD data should be considered to complete the interpretation of these data.

On the basis of these studies and clinical data, the combination of the Purkinje model data in parallel with ECG results in guinea pig appears as the most relevant assays in order to differentiate between the two classes of antidepressants and could therefore constitute a useful predictive approach for the early selection of new antidepressant drugs with improved cardiac safety.

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