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Improvement of QT analysis for Evaluating the Proarrhythmic Risk of Drug: The Importance of Spatial and Temporal Dispersion of Repolarization

Gilles Hanton*

GH Toxconsulting, Brussels, Belgium

***Corresponding author:** Dr. Gilles Hanton, 27 avenues Everard, B-1190, Bruxelles, Tel: +32 473 351 459; E-mail: gilles.hanton@yahoo.fr

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Abstract

Cardiac arrhythmias, in particular life-threatening Torsades de Pointes (TdP) are serious adverse effects associated with a number of pharmaceuticals belonging to different classes. It is therefore critical to have reliable biomarkers for assessing this risk during pre-clinical testing of new compounds. Prolongation of cardiac action potential and consequently of the QT interval of the ECG is generally considered as indicative of a risk of arrhythmia. Evaluation of drug effects on QT in preclinical studies is therefore requested by ICH (International Conference on Harmonization) guideline (S7B). However there is now growing evidence that the prolongation of mean QT interval is not an accurate indicator of the risk of arrhythmia and that other parameters of cardiac repolarization are more predictive. They include instability of action potential duration and increase in transmural heterogeneity of myocardial repolarization (spatial variability), which can be investigated in specific *in vitro* tests. We have conducted a number of experiments in dogs for evaluating the ECG correlates of both markers in studies testing the effects of isoproterenol, cisapride, astemizole and hypokaliemia, which are known to be associated with a proarrhythmic risk. Instability of action potential duration is associated with an increase in the beat-to-beat (temporal) variability of the QT interval that is evaluated by calculating the coefficient of variation of this parameter or by plotting QT from each beat versus QT of previous beat. Spatial variability of repolarization correlates with changes in the morphology of the T wave, in particular increase in the interval between the peak and the end of the T wave and notching of this wave. In these experiments, we have therefore established a simple method for *in vivo* assessment of spatial and temporal variability of cardiac repolarization, which may help in the evaluation the pro-arrhythmic risk of drugs.

Keywords: Cardiac arrhythmia; Cardiac repolarization; QT variability; T wave morphology

Introduction

Prolongation of the QT interval of the ECG corresponding to a delayed cardiac repolarization is produced by a number of drugs, in laboratory animals and humans and is generally considered as indicative of a risk of arrhythmia. Evaluation of drug effects on QT in preclinical studies is therefore requested by the ICH (International Conference on Harmonization) guideline S7B [1].

However there is now growing evidence that the prolongation of QT interval as such is not an accurate indicator of pro-arrhythmic risk and that other parameters of cardiac repolarization are more predictive [2-5]. They include time-related and spatial variability of cardiac action potential duration (CAPD) [6,7]. Time related variability of CAPD can be evaluated *in vitro* in isolated rabbit heart or *in vivo* as changes in beat-to-beat variability of the QT interval [8,9].

Spatial variability of CAPD, can be assessed *in vitro* in cardiac wedges preparation or *in vivo* by changes in the morphology of the T wave of the ECG [10].

We have established the methodology for these *in vivo* investigations in a few studies testing the effects of astemizole, cisapride, isoproterenol, and hypokaliemia, which are known to be associated with a proarrhythmic risk. Studies were conducted in dogs, which is the most frequent non-rodent species used in preclinical toxicity and safety pharmacology studies.

In vitro evaluation of time-related variability of CAPD associated instability of cardiac action potential

One of the best ways for this investigation is to use the Screenit model developed by Pr. Hondeghem [8]. Cardiac action potentials are recorded *in situ* in isolated rabbit heart. In addition to time-related variability (instability) of CAPD, two additional parameters, triangulation and reverse use dependency are recorded and constitute together a so called *TRIad*, which give critical information on the proarrhythmic risk.

Instability of action potential consists of increased beat-to-beat variability of CAPD. When it reaches a critical level it can lead to chaotic behavior of the myocardium and consequent arrhythmias [5]. Instability can be evaluated by plotting each action potential duration against the preceding one (Pointcaré plot). Proarrhythmic drugs produce instability as indicated by increased degree of scattering of the successive points [11]. Instability of the cardiac action potential is considered as one of the most sensitive predictors of proarrhythmia, since it frequently precedes the arrhythmic event and occurs at a much lower drug concentration than a prolongation of the action potential duration.

Triangulation of the action potential is a more oblique repolarization phase. It is considered to be proarrhythmic because it increases the duration of the vulnerable period of repolarization during which early after-depolarizations may occur and trigger Torsade de Pointes (TdP) [12].

Reverse-use dependence is characterized by more marked effects of compounds on the action potential at lower than at higher stimulation rates and therefore reflects the likelihood of TdP.

In vivo evaluation of time-related (beat-to-beat) variablility of CAPD

The variability of CAPD over time can be evaluated from the temporal variability QT intervals measured on ECG tracings. After recording individual QT intervals over 15 sec to 1 min, mean (mean $_{QT}$) and standard deviation (SD_{QT}) are calculated and the coefficient of variation $CV_{QT}=SD_{QT}/\text{mean}_{QT}$ is established as an evaluator of beat-to-beat variability of QT, especially in dog studies. The formula of QT temporal dispersion $QT_{td}=\log_{10}(CV_{QT}/CV_{RR})^2$, which is used in clinic, is not adapted to dogs because of the marked sinus arrhythmia in this species and consequently high value of CV_{RR} [13].

Another way for evaluating the temporal variability of QT is to establish the Poincaré plot in which the QT value from each beat is plotted against the following one. The spreading of the individual points gives the degree of variability of QT interval [9].

In vitro evaluation of spatial variability of CAPD

For evaluating the variability of CAPD in the different layers of the ventricle (transmural heterogeneity of myocardial repolarization), the arterially perfused cardiac wedge is one of the best models. Action potentials are recorded on the endocardium, epicardium and mid myocardium, in dogs.

This preparation allows evaluation of differences in CAPD across the ventricle wall. Indeed the different cardiomyocytes layers repolarize at different rates, the endocardium being the first and the mid-myocardium the latest to repolarize.

An increase in this transmural heterogeneity of repolarization has been assumed to be a key trigger of arrhythmias since it may result in reentry and subsequent TdP [14]. Notably, most pro-arrhythmic I_{Kr} blockers have a more marked effect on mid-myocardial cells in dogs (M cells) than on epicardial or subendocardial cells, and thus accentuate the heterogeneity of myocardial repolarization [6].

In vivo evaluation of spatial variability of cardiac repolarization

Transmural heterogeneity of repolarization times can be evaluated in dog toxicity studies from ECG tracings by assessing the changes in the morphology of the T wave. The T wave is the result of 2 opposing voltage gradients, between mid-myocardium M cell and epicardium. The full repolarization of epicardial cell corresponds to the peak of T wave whereas the full repolarization of M cells corresponds to end of T wave. An increased heterogeneity of repolarization of these different cell layers produced an increase in the interval between the peak and the end of the T wave interval (Tp –Te), which is considered in the clinic as a marker of the risk for ventricular arrhythmias [15]. When the transmural dispersion of repolarization is still more pronounced, it may lead to a notching of the T wave [16].

Experimental Assessment

Designs of studies

Effects on astemizole: Using a cross-over design, we treated 9 dogs/group with a single intravenous injection of astemizole at doses of 0, 1 or 3 mg/kg. ECGs were recorded before treatment, then 0.5 and 1 hour after treatment (100 beats).

Another group of 3 dogs received single intravenous injections of astemizole at increasing doses (6, 9 and 15 mg/kg.) over 3 successive days. ECGs were recorded before treatment, then 15 minutes, 30 minutes, 1 hour and 3 hours after treatment (40 beats).

Effects of cisapride: Using a cross-over design, we treated 9 dogs/group with a single intravenous injection of cisapride, at doses of 0, 1.5 or 6 mg/kg. ECGs were recorded before treatment, then 0.5 and 1 hour after treatment (100 beats).

Effects of hypokalemia: Hypokalemia was induced by oral treatment of 12 dogs with furosemide at increasing doses (5-60 mg/kg) over 12 days. ECGs were recorded before furosemide dosing, then 1.5 and 3.5 hours after each dosing (over 1 minute).

Effects of isoproterenol: A group of 3 dogs received increasing doses (2.5, 5 and 10 µg/kg) of isoproterenol by the subcutaneous route. ECGs were recorded before treatment, then 15 min, 30 min, 1 h, 3 h and 5 h after treatment (over 20 seconds).

ECG recording

Standard bipolar limb leads I, II, III, unipolar limb leads aVR, aVL, aVF and precordial leads CV6LL, CV5RL, CV6LU, V10 were recorded. T wave morphology and QT interval were assessed from CV5RL lead, since this lead gives the most accurate evaluation of end of T wave, which is monophasic and positive in untreated animals.

Evaluation of temporal variability of QT

QT values were recorded from individual beats and the coefficient of variation of QT: $CV_{QT}=SD_{QT}/\text{mean}_{QT}$ was calculated.

Evaluation of the morphology of the T wave

Modifications of the T wave were recorded from precordial lead CV5RL. In particular notching was noted. It consists in presence of 2 peaks on the wave. A grading system has been established as:

0: no notching.

1: minimal notching, mild rupture of continuity in ascending part of the T wave.

2: mild notching, plateau but single peak of the T wave.

3: moderate notching, second peak on the descending part of the wave, less than 0.1 mV between peak and trough, mild flattening

4: marked notching, second peak, with 0.1 to 0.3 mV between peak and trough, moderate flattening

5: severe notching, second peak with more than 0.3 mV between trough and peak, marked flattening and/or trough at the isoelectric line or slightly below.

Results

Detailed data have been provided in previous publications [13,17,18].

Effect of astemizole

In the cross over study, astemizole produced a dose-related increase in QT interval and in CV_{QT} at 30 and 60 min after dose (Table 1).

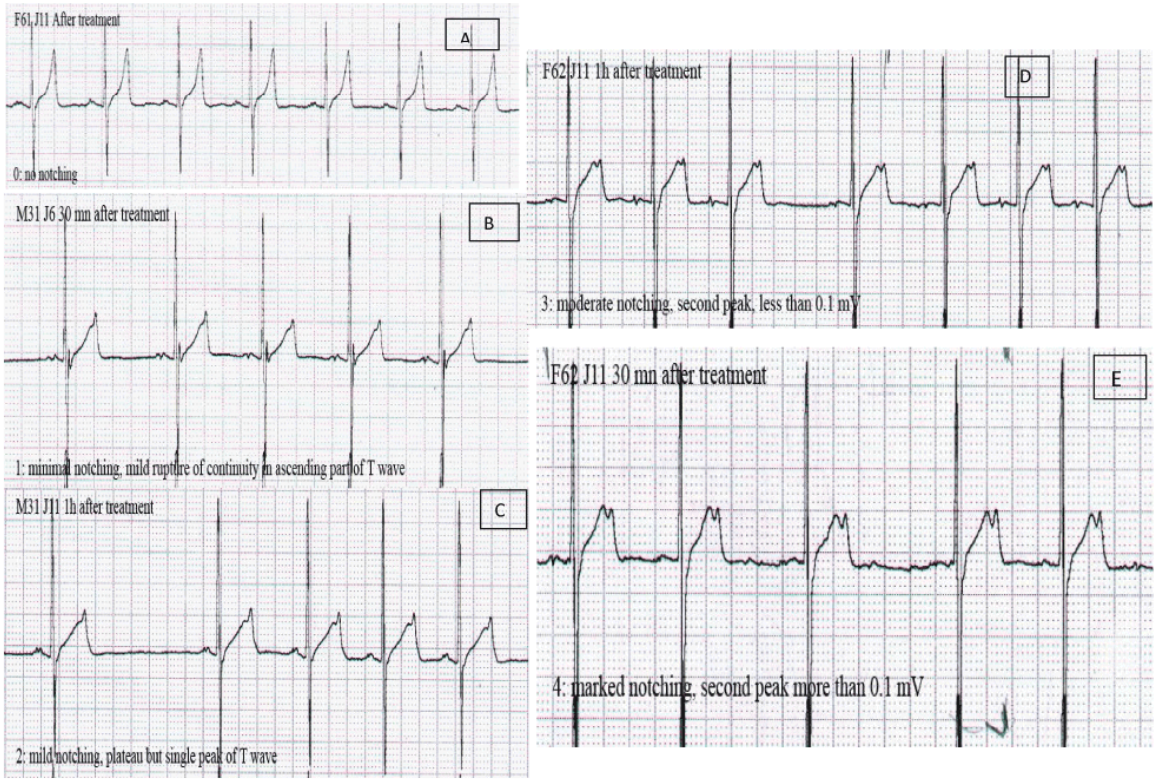

Figure 1: Changes in morphology of T wave recorded in CV5RL precordial lead, after treatment of dogs with astemizole. A: Normal T wave after vehicle treatment; B: Minimal notching after 1 mg/kg; C: Mild notching after 1 mg/kg; D: Moderate notching after 3 mg/kg; E: Marked notching after 3 mg/kg.

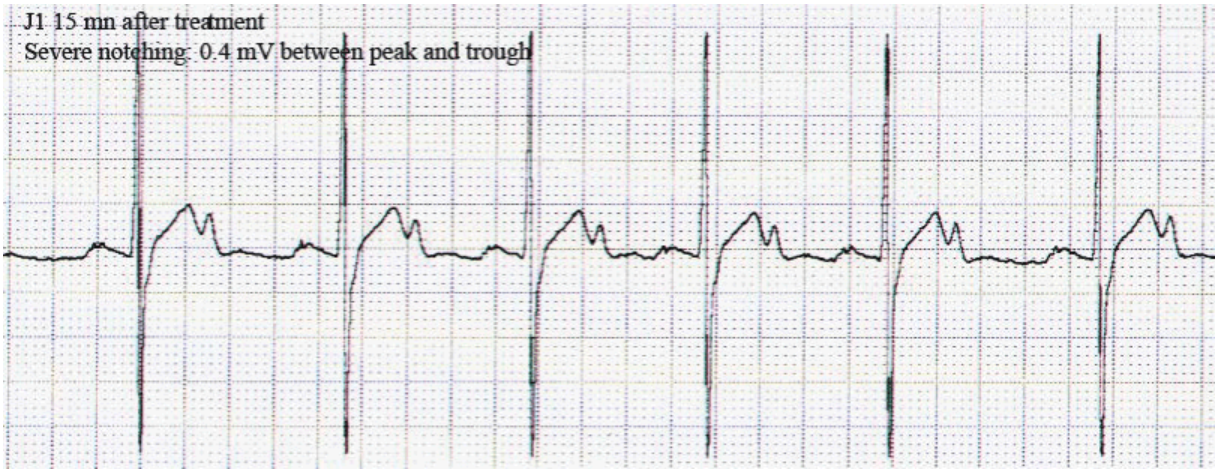

Figure 2: Severe notching of the T wave recorded in CV5RL precordial lead after treatment of dogs with 6 mg/kg of astemizole.

Notching of the T wave also occurred in both the cross over and the increased dose study and in some occasions was very pronounced and associated with a flattening of the wave (Table 2, Figure 1 and 2).

Effects of cisapride

The changes were similar to those produced by astemizole. The increase in CV_{QT} was slightly less pronounced for Cisapride than for astemizole (Table 3), but T wave notching was similar or even more marked in one animal (Table 2 and Figure 3).

Effects of hypokalemia

Hypokalemia produced an increase in QT interval and a number of changes in T wave morphology, in particular notching, flattening, inversion, biphasic of triphasic aspect (Figure 4).

	Mean values at 3 time points (n=9) Lead CV5RL			Difference compared to pre dose values	
	Pre-dose	**30 min**	**60 min**	**30 min**	**60 min**
Control	1.51 ± 0.33	1.47 ± 0.51	1.62 ± 0.37	**-0.04**	**0.11**
1 mg/kg	1.65 ± 0.41	2.48 ± 0.57	2.25 ± 0.74	**0.84****	**0.61**
3 mg/kg	1.67 ± 0.35	2.89 ± 1.15	2.80 ± 1.22	**1.22****	**1.13****
Change compared to control at 1 mg/kg	**0.13**	**1.01*****	**0.63***		
Change compared to control at 3 mg/kg	**0.16**	**1.42*****	**1.18****		

*: p<0.05; **; p<0.01; ***: p<0.001 (data analyzed with a linear mixed model of analysis of variance with time, dose and the dosetime interaction as fixed effects and animal as a random effect).

Table 1: Effect of astemizole on coefficient of variation of QT in dogs.

	Mean score (n = 9) (range mini-max)			
		Before	**30 minutes**	**60 minutes**
Astemizole	Control	0.55 (0-2)	0.22 (0-1)	0.75 (0-2)
	1 mg/kg	0.55 (0-2)	2.11 (0-3)	2.22 (0-4)
	3 mg/kg	0.55 (0-1)	3.22 (1-4)	2.67 (0-4)
Cisapride	Control	0.4 (0-2)	0.7 (0-2)	0.6 (0-2)
	1.5 mg/kg	0.3 (0-2)	1.6 (0-3)	0.7 (0-2)
	6 mg/kg	0.2 (0-1)	2.6 (0-4)	2.3 (0-5)

Table 2: Notching of the T wave recorded in CV5RL precordial lead, after treatment of dogs with astemizole or cisapride.

	Mean values at 3 time points (n=9) Lead CV5RL			Difference compared to predose values	
	Pre-dose	**30 min**	**60 min**	**30 min**	**60 min**
Control	1.45 ± 0.42	1.41 ± 0.43	1.58 ± 0.25	-0.04	0.13
1.5 mg/kg	1.56 ± 0.41	2.06 ± 0.52	1.79 ± 0.39	0.5*	0.23
6 mg/kg	1.56 ± 0.33	2.16 ± 0.82	1.67 ± 0.58	0.6**	0.11
Change compared to control at 1 mg/kg	0.11	0.65*	0.21		
Change compared to control at 3 mg/kg	0.11	0.75*	0.09		

*: p<0.05; **; p<0.01; ***: p<0.001 (data analyzed with a linear mixed model of analysis of variance with time, dose and the dosetime interaction as fixed effects and animal as a random effect).

Table 3: Effect of cisapride on coefficient of variation of QT in dogs.

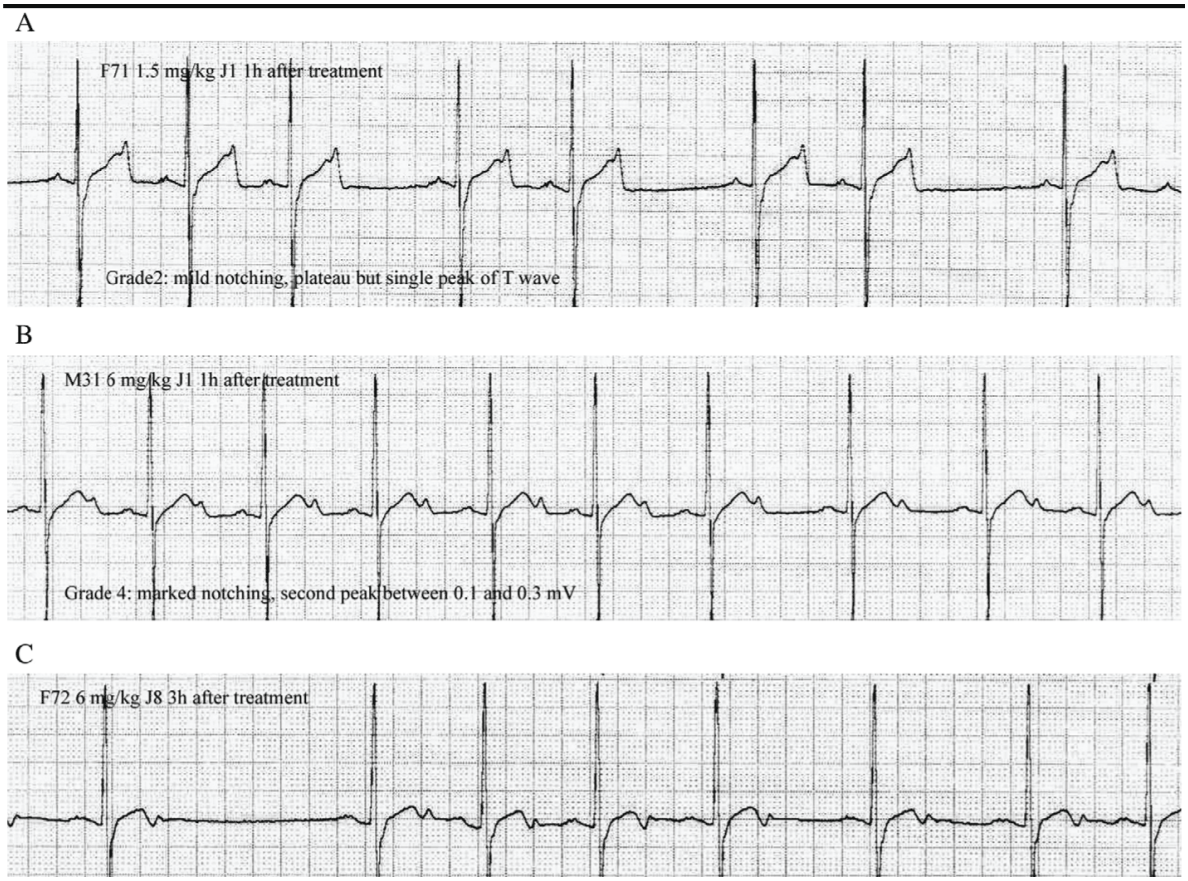

Figure 3: Changes in morphology of T wave recorded in CV5RL precordial lead, after treatment of dogs with cisapride. To be compared with normal monophasic T wave. **(A)** Mild notching 1 h after treatment with 1.5 mg/kg: (grade 2); **(B)** Marked notching with flattening of the T wave 1 h after treatment with 6 mg/kg (grade 4); **(C)** Severe notching of the T wave, 3 h after treatment with 6 mg/kg (grade 5).

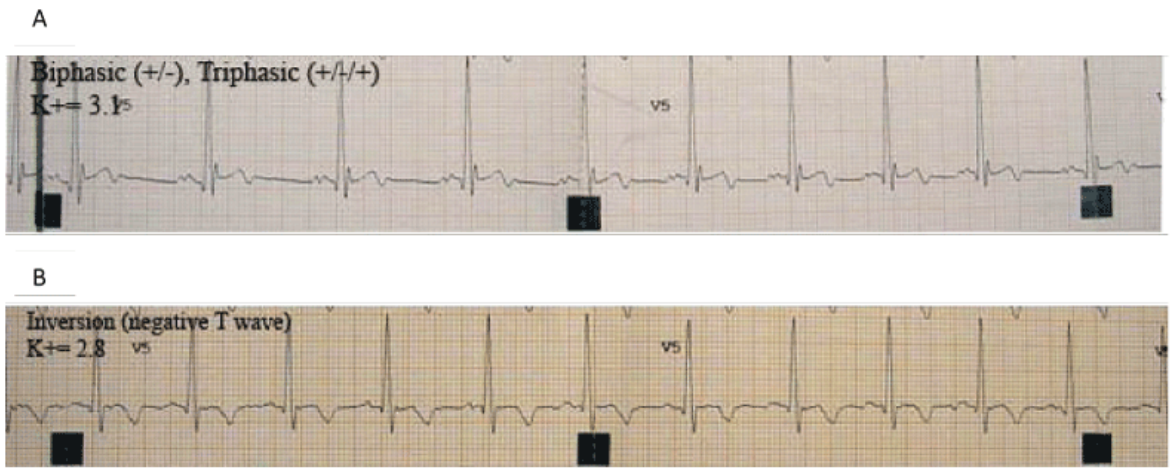

Figure 4: Severe changes inmorphology of T wave recorded in CV5RL precordial lead in hypokalemic dogs (treatment with furosemide). To be compared with normal T wave monophasic and positive. **(A)** Biphasic or triphasic T wave; **(B)** Inversion of T wave.

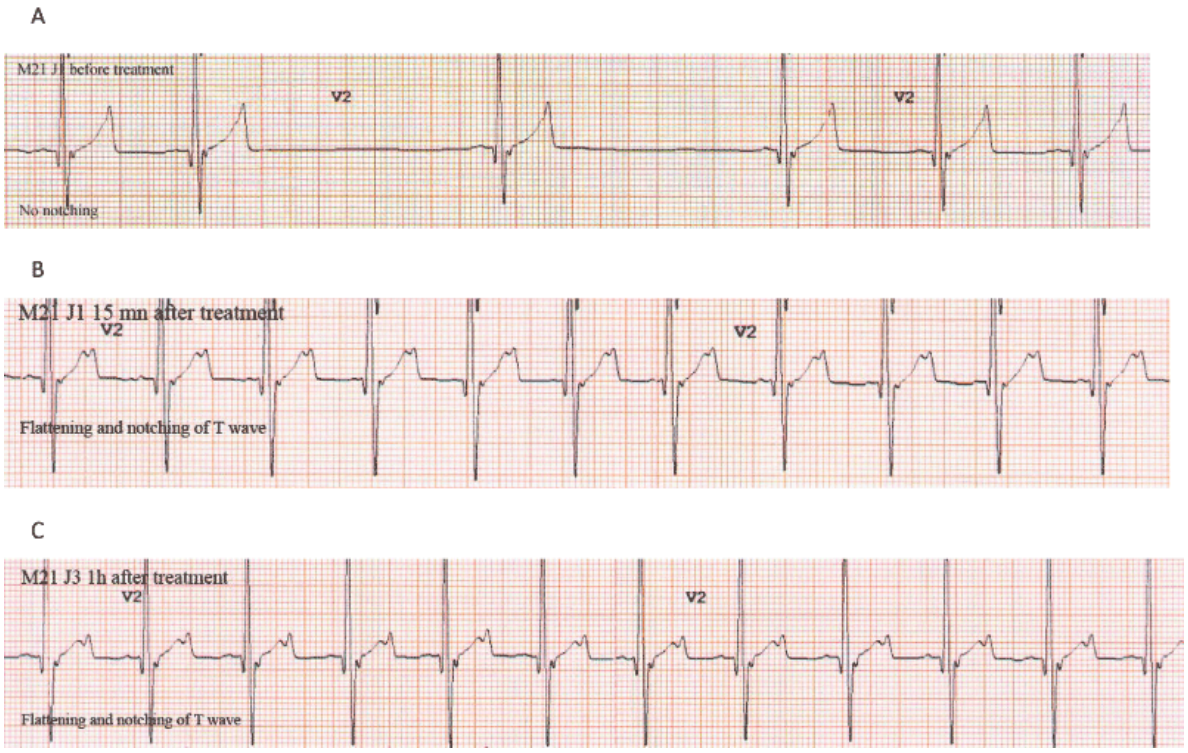

Figure 5: Changes in morphology of T wave recorded in CV5RL precordial lead, in a dog treated with 5 µg/kg of isoproterenol.

Effect of isoproterenol

Isoproterenol produced a marked increase in heart rate, a decrease in corrected QT interval (QTc) and a notching of T wave (Figure 5).

Discussion

The temporal variability of the QT interval of the ECG is the *in vivo* correlate of cardiac action potential instability, which has been shown to be markedly increased by arrhythmogenic drugs, in particular I_{Kr} blockers and is considered as a reliable and sensitive predictor for the risk of arrhythmia [12]. In the astemizole experiment, the CV of QT was markedly increased, indicating an effect on the temporal variability of cardiac repolarization. The CV of QT was also increased by cisapride but to a lesser extent as compared to astemizole. Results from both studies indicate that the CV of QT can assess the temporal variability of QT in dogs treated with pro-arrhythmic I_{Kr} blockers. These findings were consistent with those of previous authors [9].

Clinical investigations have shown that increased beat-to-beat QT variability is an indicator of temporal myocardial repolarization liability and predicts ventricular tachyarrhythmias, sudden cardiac death and cardiovascular mortality [19-21].

The second key observation after treatment with astemizole and cisapride was a clear notching of the T wave, consisting of presence of 2 peaks of the T wave, with the intermediate trough sometimes reaching the isoelectric line, giving an impression of U wave. Notching of the T wave indicates an increase in the heterogeneity of repolarization of the different layers of cardiomyocytes across the ventricular wall and consequent modification of the transmural voltage gradient [22-24]. The change is considered to be due to differences in the action of the compounds on different cardiac cells. I_{Kr} blockers like astemizole or cisapride act predominantly on the M cells, which are more sensitive to I_{Kr} blocking than epicardial or endocardial cells [25,26]. Notching of the T wave has been previously observed in dogs and humans after treatment with I_{Kr} blockers [26-28].

Notching of the T wave in dogs was therefore found to be a predictive biomarker for the evaluation of potential proarrhythmic risk of I_{Kr} blockers and the aim of further studies was to verify this finding in situations potentially producing arrhythmia by other ways than I_{Kr} blocking. These experiments indicated that diuresis-induced hypokalemia and isoproterenol, an adrenergic β-agonist both produce T wave abnormalities.

Notching and/or flattening of the T wave in CV5RL in dogs treated with isoproterenol is also probably related to an increase in heterogeneity of the repolarisation of the different cardiomyocytes layers across the ventricular wall. *In vitro* studies on canine cardiac tissues have shown that isoproterenol produced a greater shortening of the action potential in epicardial than in endocardial cardiomyocytes and prolonged the action potential of M cells [29,30]. These changes resulted from a large augmentation in I_{Ks} current in epicardial and endocardial cells but not in M cells in which I_{Ks} is weak.

The changes in T wave morphology in hypokalemic dogs is consistent with *in vivo* and *in vitro* data and find a similar explanation as the changes produced by astemizole, cisapride and isoproterenol. In isolated cardiac tissues, a decrease in extracellular potassium prolongs the duration of cardiac action potential to a greater extent in the epicardium than in other myocardial layers, which is attributed to a

predominant I_{to} current (responsible for transient early outflow of potassium) in the epicardium [14]. Electrophysiological studies in isolated cardiac cells also showed that when extracellular potassium decreases, the slope of phase 2 of the action potential becomes steeper and phase 3 slower, resulting in an increased duration of the action potential. The period of incomplete repolarization tends to be longer in Purkinje fibres than in ventricular cells, resulting in an increased dispersion of repolarization [31]. In humans, hypokalemia is known to produce typical changes in the ECG, in particular a decrease in T wave amplitude and the appearance of a U wave [32]. Similar changes have been observed in hypokalemic dogs [33].

Notching of the T wave thus occurred in different conditions known to be associated with arrhythmic events. Notably this indication of proarrhythmic risk was found in association with QT prolongation (astemizole, cisapride and hypokaliemia) but also with QT shortening (isoproterenol). The findings of the current experiments are therefore consistent with clinical investigations showing that changes in the morphology of the T wave, in particular T wave notching and increase in Tp-Te, are reliable markers of the proarrhythmic risk [34-36].

In conclusion, we have established a simple method for *in vivo* assessment of spatial and temporal variability of cardiac repolarization, which may help in the evaluation the pro-arrhythmic risk of drugs. The precordial lead CV5RL was found to be the best lead for these investigations. This methodology could help in the interpretation of findings in pre-clinical studies.

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