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Decreasing the infusion rate reduces the proarrhythmic risk of NS-7: confirming the relevance of short-term variability of repolarisation in predicting drug-induced torsades de pointes

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1 The rate of infusion has been suggested to be important for drug-induced torsades de pointes (TdP) arrhythmias. We investigated the repolarisation-prolonging effects and proarrhythmic properties of NS-7, a neuroprotective drug in development, using two different infusion rates.

2 A fast (5 min intravenously (i.v.)) escalating dosing regimen (0.3 and 3.0 mg kg^{-1}, $n=4$) of NS-7 was investigated in anaesthetised control dogs in sinus rhythm (SR). This was compared to a slow infusion (60 min i.v.) of one dose (3.0 mg kg^{-1}, $n=4$) NS-7. The similar dosing regimens were investigated in anaesthetised dogs with chronic, complete AV block (CAVB), an animal model of TdP ($n=6$).

3 No electrophysiological effects were seen after 0.3 mg kg^{-1} NS-7. Fast infusion of 3.0 mg kg^{-1} caused prolongation of repolarisation, for example, heart rate corrected QT interval (QT_c): in SR: $6\pm1\%$; in CAVB: $10\pm7\%$, which was accompanied by TdP in three of six CAVB dogs. No TdP were seen in SR dogs.

4 Slow infusion did not cause TdP in the same CAVB dogs, although NS-7 caused repolarisation to prolong with a similar magnitude (QT_c: $12\pm7\%$) as in the fast-infusion experiment.

5 Short-term variability (STV) is a novel parameter for the prediction of drug-induced TdP analysing the beat-to-beat variability of repolarisation. STV was only increased after the fast infusion in CAVB dogs (2.6 ± 0.3 *versus* 6.0 ± 1.4 ms, $P<0.05$), while there was no increase (2.1 ± 0.2 *versus* 2.5 ± 1.0 ms) after the slow infusion of NS-7.

6 Peak plasma concentrations attained were lower in slow (0.5 ± 0.1 μg ml^{-1} after 50 min) than in fast-infusion regimen (2.1 ± 0.4 μg ml^{-1} after 5 min; $P<0.05$).

7 The results support the conclusion that limiting peak plasma concentration by decreasing the rate of infusion of NS-7 reduces the proarrhythmic risk despite comparable prolongation in repolarisation parameters. The relevance of STV in predicting drug-induced TdP was confirmed.
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Abbreviations: BVR, beat-to-beat variability of repolarisation; CAVB, chronic atrioventricular block; ΔMAPD, interventricular dispersion; LV, left ventricle; MAP, monophasic action potential; MAPD, monophasic action potential duration; QT_c, heart rate corrected QT interval; RV, right ventricle; SR, sinus rhythm; STV, short-term variability; TdP, torsades de pointes arrhythmias

Introduction

A large number of cardiovascular and noncardiovascular drugs have been withdrawn from the market in the past decade due to postmarketing reports of clinical drug-induced lethal proarrhythmia (Haverkamp *et al*., 2000; Belardinelli *et al*., 2003; Redfern *et al*., 2003). Most of these drugs have in common that they delay the ventricular repolarisation seen as QT prolongation on the ECG (Haverkamp *et al*., 2000; Belardinelli *et al*., 2003). However, numerous studies have shown that there is no straightforward relation between prolongation of repolarisation and the potentially lethal arrhythmia known as torsades de pointes (TdP) (Carlsson *et al*., 1993; Hondeghem *et al*., 2001a, b; van Opstal *et al*., 2001b; Antzelevitch & Shimizu, 2002; Milberg *et al*., 2002; Thomsen *et al*., 2003, 2004). Still, the regulatory authorities require that any new potential drug is investigated to determine both its repolarisation delaying and proarrhythmic properties.

NS-7 (enecadine) is a multichannel blocker (I_{Na}, I_{Ca} and I_{K1}) reported to show promising efficacy in animal models of ischaemic stroke (Suma *et al*., 1997; Oka *et al*., 2000; 2001; Sopala *et al*., 2002; Tanaka *et al*., 2002; Katsumata *et al*., 2003). In preclinical electrophysiological studies, 100 μM NS-7

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prolonged the ventricular action potential in guinea-pigs (Satoh, 2003). In the present study, the electrophysiological and proarrhythmic characteristics of the drug were assessed in anaesthetised dogs with normal and electrically remodelled hearts. For the latter purpose, we used the animal model with chronic, complete atrioventricular block (CAVB) that shows a high susceptibility to drug-induced TdP (Weissenburger *et al*., 1991; Verduyn *et al*., 1997; 1999; Vos *et al*., 1998; Thomsen *et al*., 2003; Chiba *et al*., 2004).

Previously, the rate of drug administration has been shown to be critical for TdP induction at similar prolongation of the QT intervals in rabbits (Carlsson *et al*., 1993). Infusion rate has later been included as a risk factor for TdP observed in the clinical setting (Roden, 2004). Therefore, we employed two different infusion rates of the same total dose in this investigation, along with the analysis of beat-to-beat variability of repolarisation (BVR). This methodology was recently demonstrated to be a superior predictor for drug-induced TdP in the preclinical setting (Hondeghem *et al*., 2001a; Thomsen *et al*., 2004).

Methods

Animal handling was in accordance with the European directive for the protection of vertebrate animals used for experimental and other scientific purposes (86/609/EU). The committee for experiments on animals of Maastricht University approved all experiments.

General

A total of 28 experiments were performed on 14 anaesthetised mongrel dogs (body weight 22–33 kg) under aseptic conditions. After overnight fasting, anaesthesia was induced by 20 mg kg^{-1} sodium pentobarbital intravenously (i.v.) and maintained by 0.5% halothane in a mixture of O_2 and N_2O (1 : 2). During artificial ventilation at a frequency of 10–12 min^{-1}, tidal volume (10–15 ml kg^{-1}) was adjusted to maintain end-expired CO_2 concentration between 3.5 and 4.0%. A thermal mattress was used to maintain body temperature. To prevent volume depletion, the dog received 0.5–1.0 l 0.9% saline i.v. Postoperative care included antibiotics (1 g ampicillin intramuscularly (i.m.)) and analgesics (15 μg kg^{-1} buprenorphine i.m.).

In all, 10 ECG leads and two monophasic action potentials (MAPs) were recorded continuously throughout the experiments. MAP catheters (EP technologies, CA, U.S.A.) were placed under fluoroscopic guidance on the endocardium of the left (LV) and right ventricle (RV). MAP signals were amplified with a customised isolated DC-coupled differential amplifier at a frequency range of 0–500 Hz with a 20-mV calibration pulse. The offset of the amplifier was variable and could be adjusted to the recorded signal. Besides minimal amplitude of 15 mV, the MAP was required to have a smooth repolarisation and a stable configuration in time.

Experimental design

Eight dogs were studied during sinus rhythm (SR) to determine (1) the dose-dependent electrophysiological effects of NS-7 in physiologically normal hearts and (2) the associated plasma concentrations of NS-7. NS-7 was administered at a fast-infusion (5 min) scheme of escalating doses (0.3, 3.0 and 10 mg kg^{-1} i.v., $n=4$) separated by 30 min. Furthermore, a slow-infusion (60 min) scheme of 3.0 mg kg^{-1} (i.v., $n=4$) was performed.

In nine dogs, complete atrioventricular block was created by radiofrequency ablation. After 4 ± 1 weeks of atrioventricular block, the dogs underwent an anaesthetised dofetilide-inducibility test (25 μg kg^{-1} i.v.) to determine their sensitivity to drug-induced TdP (van Opstal *et al*., 2001a). As NS-7 could have a low proarrhythmic potential that could escape detection in this model, we opted to exclude dogs that were not susceptible to this positive control (Thomsen *et al*., 2003). Of nine dogs with CAVB, two failed the dofetilide-inducibility test and one was lost due to drug-induced ventricular fibrillation. Thus, six dogs were used for evaluating the electrophysiological and proarrhythmic properties of NS-7 in remodelled hearts.

NS-7 was administered in a fast-infusion (5 min) scheme of escalating doses (0.3 and 3.0 mg kg^{-1} i.v.) separated by 30 min or in a slow-infusion (60 min) scheme of 3.0 mg kg^{-1} (i.v.). This was performed in a serial, random crossover design with 2 weeks between experiments. One dog was lost during a fast-infusion experiment, before the slow-infusion experiment was performed.

In two dogs, the dofetilide challenge was repeated after NS-7 to ascertain preservation of TdP inducibility. NS-7 and dofetilide were provided by PAION. Both drugs were dissolved in equal volumes of 0.9% saline.

Plasma concentrations

Blood samples were drawn from a dedicated venous access at various time points throughout the experiments. Samples were collected in citrate tubes, centrifuged for 10 min at 4000 r.p.m. at 4°C and stored at −20°C. NS-7 concentrations were determined by Scope International (Hamburg, Germany).

Data analysis

Applying a custom-made computer programme (ECGview), we measured the following parameters offline at a resolution of 4 ms: RR and QT intervals from a unipolar lead positioned on the sixth intercostal space near the edge of the sternum (Ettinger & Suter, 1970). Also, the LV and RV monophasic action potential duration (MAPD) to 50 and 100% repolarisation was determined ($MAPD_{50}$ and $MAPD_{100}$, respectively). Heart rate corrected QT intervals (QT_c) were calculated according to van de Water's formula (Van de Water *et al*., 1989). Interventricular dispersion of repolarisation (ΔMAPD) was defined as LV minus RV MAPD.

BVR was determined according to an earlier publication (Thomsen *et al*., 2004). Briefly, Poincaré plots were drawn from 30 consecutive LV MAPD and short-term variability (STV, Equation 1), representing the mean orthogonal distance to the line-of-identity, was calculated

$$\mathrm{STV} = \frac{\sum |D_{n+1} - D_n|}{30 \times \sqrt{2}} \quad (1)$$

where D_n represents LV MAPD of beat number n.

All electrophysiological parameters were measured at maximal QT prolongations, which were 10 and 60 min after the start of the fast and slow infusion, respectively.

The frequency of multiple extrasystoles was quantified in 10-min intervals after the administration of NS-7 in CAVB dogs. Extrasystoles were defined as premature ventricular complexes occurring at a coupling interval of less than 600 ms.

TdP was defined as polymorphic ventricular tachycardia of at least five beats. A dog was defined as inducible when >3 TdP occurred. If a TdP degenerated into ventricular fibrillation, electrical cardioversion was applied.

Statistical analysis

Pooled data are expressed as mean±s.d., unless otherwise stated. Comparisons of electrophysiological data were performed with repeated-measures ANOVA followed by a Bonferroni *t*-test. Statistical significance was acknowledged at $P<0.05$.

Results

Fast-infusion scheme in control SR dogs

Infusing NS-7 over 5 min resulted in a dose-dependent increase in RR interval (baseline: 460±50 ms; 0.3 mg kg^{-1}: 475±35 ms; 3 mg kg^{-1}: 530±70 ms ($P<0.05$) and 10 mg kg^{-1}: 540±25 ms ($P<0.05$)). Prolongation of electrophysiological repolarisation parameters was seen only after the higher doses of 3.0 and 10 mg kg^{-1} (Figure 1 for QT_c time and Table 1 for 3.0 mg kg^{-1}). Relatively, 3 mg kg^{-1} NS-7 caused a 5.8±1% prolongation of the QT_c interval from baseline, whereas 10 mg kg^{-1} caused a 12.6±2% prolongation. The plasma concentrations over the duration of the experiments are depicted in Figure 1, at peak reaching 0.2±0.02, 2.1±0.4 and 6.4±3.1 μg ml^{-1} after 0.3, 3.0 and 10 mg kg^{-1} NS-7, respectively.

Administration of 10 mg kg^{-1} NS-7 caused a brief fall in the QT_c interval (Figure 1) attributable to a consistent drug-induced transient shortening of the RR interval from 510±45 to 440±20 ms ($P<0.05$). No statistically significant drug-induced increase was seen in the interventricular dispersion (ΔMAPD) or BVR (STV, Table 1).

Fast-infusion scheme in proarrhythmic CAVB dogs

NS-7 (0.3 mg kg^{-1}) caused no proarrhythmia, while 3.0 mg kg^{-1} caused reproducible TdP in three of six CAVB dogs, associated with a QT_c prolongation of 1.8±1 and 10.3±7%, respectively. After the fast infusions, the peak plasma concentrations of NS-7 in CAVB dogs (0.2±0.01 and 1.6±1.9 μg ml^{-1} after 0.3 and 3.0 mg kg^{-1} NS-7, respectively) were similar to those seen in SR dogs. A representative example of TdP caused by 3.0 mg kg^{-1} NS-7 is shown in Figure 2. Representative Poincaré plots of the LV $MAPD_{100}$ at control and under the influence of 3.0 mg kg^{-1} NS-7, either at fast or slow infusion, are also shown in Figure 2. Table 2 summarises the electrophysiological changes induced by 3.0 mg kg^{-1} NS-7. During fast infusion of 3.0 mg kg^{-1} NS-7, significant increases in LV $MAPD_{100}$ and STV were observed. On average 9±20 multiple extrasystoles were encountered within the first 10 min after administration. One dog was lost after a drug-induced ventricular fibrillation, where electrical cardioversion was not feasible.

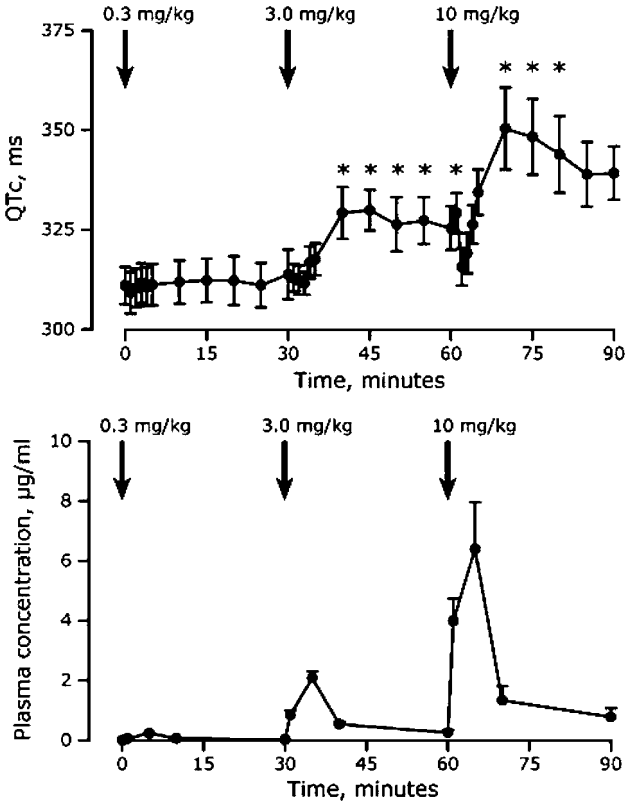

Figure 1 Dose-dependent development in QT_c interval and plasma concentration after 5-min infusions of NS-7 in anaesthetised dogs in SR. Three cumulative doses of NS-7 were administered i.v. as indicated by arrows ($n=4$ dogs). *$P<0.05$ *versus* value before last administration. Mean±s.e.m.

Table 1 Electrophysiological data from anaesthetised dogs with normally conducted SR ($n=4$)

	Control	*NS-7*
RR (ms)	460±50	530±70*
QT (ms)	265±15	290±20*
QT_c (ms)	310±10	330±15*
LV MAPD (ms)	215±5	230±15*
RV MAPD (ms)	210±5	225±5*
ΔMAPD (ms)	5±5	5±5
STV (ms)	0.8±0.1	1.0±0.4

Measurements were performed 10 min after the start of the 5-min infusion of 3.0 mg kg^{-1} NS-7 when the QT was maximally prolonged. *$P<0.05$ *versus* control.

Slow-infusion scheme in control SR dogs

Administering 3.0 mg kg^{-1} NS-7 over 60 min caused an increase in RR (550±55 *versus* 625±40 ms; $P<0.05$). The QT_c interval was prolonged to a similar extent as with the fast-infusion scheme (8.1±2 *versus* 5.8±1%; Figure 3). The maximal values were obtained 60 min after the start of the slow infusion *versus* 10–15 min in the fast-infusion experiments. The corresponding plasma concentrations of NS-7 (Figure 3) show significant differences in peak levels (0.5±0.1 *versus* 2.1±0.4 μg ml^{-1} for slow and fast infusion, respectively; $P<0.05$).

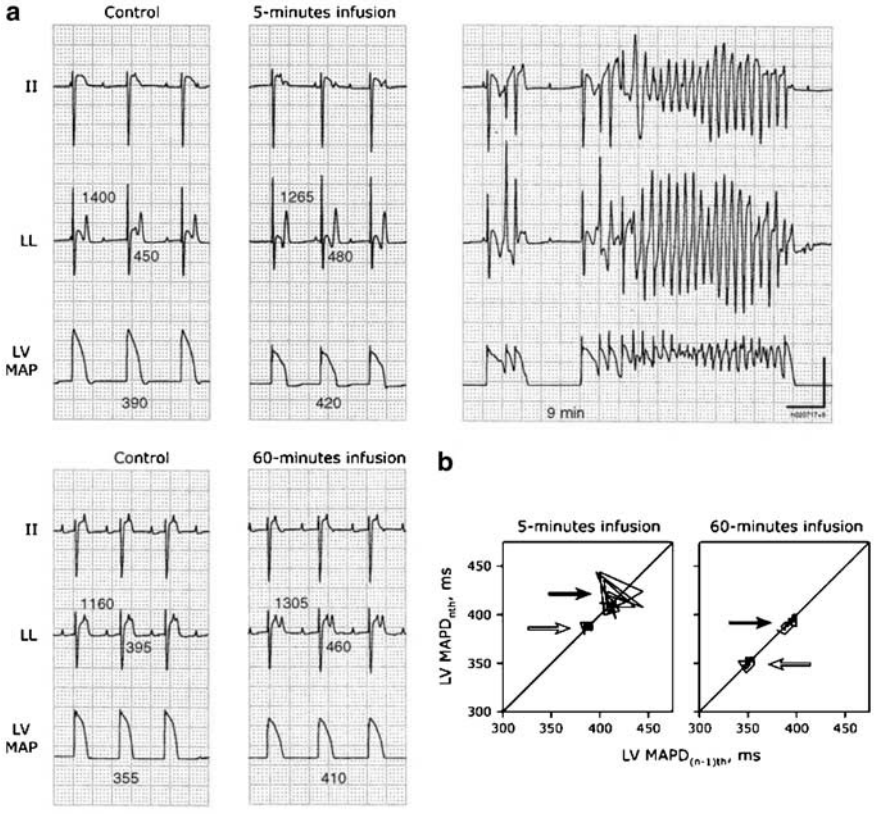

Figure 2 (a) Representative ECG traces before and after administration of 3.0 mg kg^{-1} NS-7 in an anaesthetised dog with CAVB. TdP (right-most panel) only occurred after the fast infusion of NS-7. Two ECG leads (II, lead II; LL, precordial lead placed on the left lateral side of the thorax) and LV MAP recordings are shown in each trace. RR intervals are above and QT time below lead LL. $MAPD_{100}$ is below the LV MAP trace. ECG calibrated to 1 mV cm^{-1}. Vertical scale bar, 20 mV on the MAP signal; horizontal scale bar, 1 s. (b) Poincaré plots of the LV $MAPD_{100}$ from the same dog at the two experiments. Open arrow, control; closed arrow, 3.0 mg kg^{-1} NS-7. A substantial increase in plot area is appreciable after fast administration, but not after the slow administration. STV in the fast-infusion experiment increases from 3.0 ms at control to 8.8 ms after NS-7, while STV changes from 2.3 to 2.1 ms in the slow-infusion experiment.

Table 2 Electrophysiological data from serial investigations in anaesthetised dogs with CAVB ($n = 5$)

	5-min infusion		*60-min infusion*	
	Control	*NS-7*	*Control*	*NS-7*
RR (ms)	1240±155	1300±115	1255±215	1370±275
QT (ms)	440±45	480±50	450±30	495±50
QT_c (ms)	420±40	450±45	425±20	460±30
LV $MAPD_{100}$ (ms)	365±50	420±40*	350±10	410±55*
LV $MAPD_{50}$ (ms)	285±30	325±50	275±15	295±50
RV $MAPD_{100}$ (ms)	300±35	345±35	295±30	340±55
RV $MAPD_{50}$ (ms)	245±40	280±35	240±35	280±25
$\Delta MAPD_{100}$ (ms)	65±40	75±45	55±30	70±10
$\Delta MAPD_{50}$ (ms)	40±25	45±45	35±30	15±50
STV LV $MAPD_{100}$ (ms)	2.6±0.3	6.0±1.4*	2.1±0.2	2.5±1.0†
STV LV $MAPD_{50}$ (ms)	2.1±0.7	4.9±1.6*	1.9±0.6	2.1±0.5†

NS-7 (3.0 mg kg^{-1}) was administered over 5 min or over 60 min. Measurements were performed at 10 and 60 min, respectively, when the QT was maximally prolonged. *$P<0.05$ *versus* control; $^{\dagger}P<0.05$ *versus* 5-min infusion.

Slow-infusion scheme in proarrhythmic CAVB dogs

As opposed to the fast-infusion scheme, slow infusion of 3.0 mg kg^{-1} caused no TdP, although the LV MAPD and QT_c time (11.9±9 *versus* 10.3±7%) were prolonged to similar extents (Table 2). The plasma concentration of NS-7 was similar in CAVB and SR dogs after the slow infusions (0.4±0.1 μg ml^{-1} after 3.0 mg kg^{-1} NS-7). STV was not altered by the slow infusion of NS-7. Furthermore, STV was significantly higher after a fast administration of 3.0 mg kg^{-1} NS-7 than after a slow infusion of the same dose (Table 2). No multiple extrasystoles were observed; however, this was not acknowledged as statistically significantly different from the fast infusion, partly due to

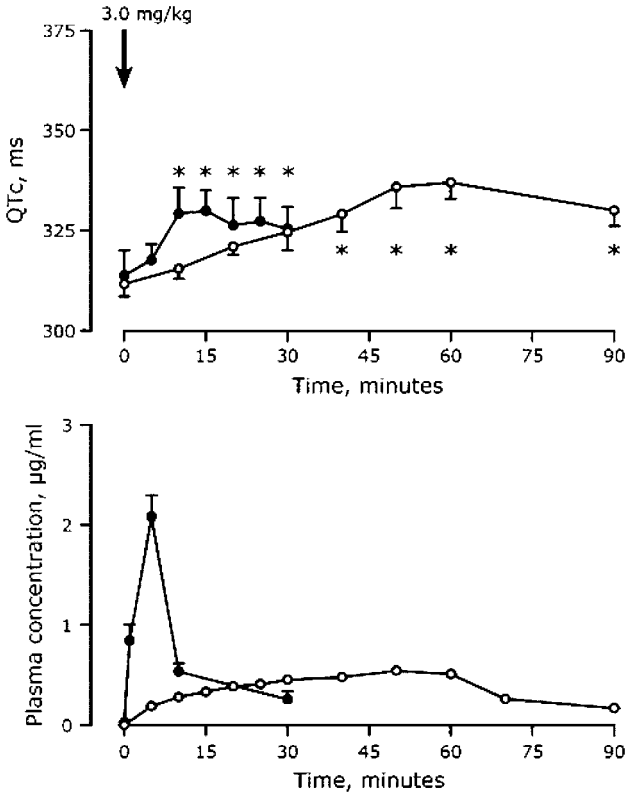

Figure 3 Comparison of the 5- and 60-min infusion of 3.0 mg kg^{-1} NS-7 in anaesthetised dogs in SR. QT_c (upper panel) and plasma concentration (lower panel) are depicted over time for the slow (open circles) and the fast (closed circles) i.v. infusion experiments ($n=4$ dogs). QT_c is similarly increased after both infusion schemes; however, peak plasma concentrations are higher after the fast infusion. *$P<0.05$ *versus* 0 min. Mean ± s.e.m.

the very large variation in the frequency in the latter experiments.

Reproducibility of TdP induction with dofetilide in time was confirmed in two experiments.

Discussion

In this study, we show that decreasing the infusion rate of NS-7 limits the peak plasma concentration and decreases or abolishes the risk of TdP. The only electrophysiological parameter that reflected the absence of TdP was BVR, quantified as STV. This confirms the inability of the prolonged QT interval to predict TdP. Furthermore, this study stresses the importance of assessing cardiac electrophysiological safety of drugs in animal models of reduced repolarisation reserve, because the same dose of NS-7 did not cause proarrhythmia in SR dogs.

Proarrhythmic assessment of NS-7

NS-7 is under development as a neuroprotective drug after ischaemic stroke. The beneficial actions of the drug are thought to be based on block of voltage-activated sodium and calcium channels in neuronal tissue (Oka *et al*., 2000; 2001; Tanaka *et al*., 2000). Block of I_{Kr} (IC_{50}: 0.4 μmol l^{-1}; PAION; data on file) may underlie the prolongation of action potential duration observed in the present study and in isolated guinea-pig ventricular myocytes (Satoh, 2003). Specifically, block of I_{Kr} has been associated with an increased risk of repolarisation-dependent proarrhythmia (Redfern *et al*., 2003).

Regulatory authorities like the US Food and Drug Administration or the European Medicines Agency encourage that all potential drugs with suspected QT-prolonging properties in humans should be tested preclinically for their ability to delay repolarisation and for their proarrhythmic characteristics. Drug testing in normal hearts is essential for the analysis of delayed repolarisation in general; however, it is not sufficient for the recognition of potential proarrhythmic effects in the diseased heart of a predisposed, vulnerable individual patient. The canine model with CAVB is known to have acquired QT prolongation and a predisposition to TdP and sudden cardiac death (Verduyn *et al*., 1997; Vos *et al*., 1998; van Opstal *et al*., 2001a, c; Thomsen *et al*., 2003). The CAVB dogs exposed to NS-7 in this investigation were selected on the basis of a positive dofetilide-inducibility test, increasing the sensitivity of the model (Thomsen *et al*., 2003). The incidence of dofetilide-induced TdP was seven of nine dogs, comparable to earlier investigations (van Opstal *et al*., 2001a; Thomsen *et al*., 2003), and was reproducible in the two dogs, which were retested after the NS-7 experiments had been performed.

To be able to compare NS-7 to other drugs in this animal model, we chose our regular infusion time of 5 min as the fast rate (Verduyn *et al*., 1997; van Opstal *et al*., 2001b; Thomsen *et al*., 2003). In an approach to the clinical setting, the slow infusion went over a period of 60 min, which is still faster than the anticipated rate in humans.

To the best of our knowledge, this is the first study to report prolonged cardiac repolarisation and proarrhythmia by NS-7 in the intact animal. A dose-dependent prolongation of the QT_c interval is appreciable after fast administration of NS-7 in both normal (Figure 1) and CAVB dogs. Peak plasma concentrations clearly exceeded the putative effective therapeutic plasma concentration of 15–29 ng ml^{-1} (Aoki *et al*., 2001). TdP was only seen after fast infusion of 3.0 mg kg^{-1} NS-7 in the remodelled hearts of the CAVB dog. The highest dose (10 mg kg^{-1}) was not administered to CAVB dogs, as TdP were already evident at 3.0 mg kg^{-1}.

Relevance of infusion time

As expected, peak plasma concentrations were markedly lower during slow than during fast infusion. Even so, prolongation of the infusion time did not reduce the effects seen on QT_c intervals, LV MAPD or interventricular dispersion, neither in control dogs (Figure 3 for QT_c) nor in CAVB dogs (Table 2). On the other hand, proarrhythmia was completely absent during the slow infusion. Thus, at two different plasma concentrations of NS-7, repolarisation was similarly prolonged, suggesting that a certain drug concentration is sufficient to produce the observed prolongation of repolarisation, but a higher concentration is necessary to destabilise repolarisation, trigger ectopic beats and sustain TdP. This vulnerability is likely restricted to areas of lowest

repolarisation reserve (Roden, 1998). In the CAVB dog, these areas seem to be located in the subendocardial regions of the LV (Thomsen *et al*., 2004).

Our data confirm the finding of Carlsson *et al*. (1993), who showed that a fast infusion of the I_{Kr} blocker almokalant ($25\,nmol\,kg^{-1}\,min^{-1}$) produced TdP in nine of 10 methoxamine-treated anaesthetised rabbits, while a slow infusion ($5\,nmol\,kg^{-1}\,min^{-1}$) gave an inducibility of one of eight. This was associated with a 42% increase in QT_c after the slow infusion, but only 30% prolongation after the fast infusion, once more confirming that QT prolongation is not directly convertible into risk of TdP, a conclusion reached by numerous groups (Hondeghem *et al*., 2001a; van Opstal *et al*., 2001b; Antzelevitch & Shimizu, 2002; Milberg *et al*., 2002; Thomsen *et al*., 2004). We have compared amiodarone and its noniodinated successor, dronedarone, in chronically dosed CAVB dogs and demonstrated equal QT prolongations, contrary to a different proarrhythmic outcome (van Opstal *et al*., 2001b). Similar conclusions were drawn from dose-dependent investigations employing the antipsychotic drug, sertindole, or the antiarrhythmic drug, D-sotalol (Thomsen *et al*., 2003; 2004). In isolated rabbit hearts, Hondeghem *et al*. (2001a, b) showed that parameters like instability and triangulation of the action potential were proarrhythmic, while action potential prolongation was antiarrhythmic. In a comparison between the macrolide antibiotics erythromycin, clarithromycin and azithromycin, it was shown that they all caused similar increases in repolarisation duration; however, only the latter was devoid of proarrhythmia (Milberg *et al*., 2002). Other antibiotics like gatifloxacin and moxifloxacin have developed TdP in conscious CAVB dogs despite the absence of drug-induced QT prolongation (Chiba *et al*., 2004). Moreover, the experimental calmodulin inhibitor W-7 was able to suppress drug-induced TdP without shortening QT intervals (Mazur *et al*., 1999; Gbadebo *et al*., 2002).

Beat-to-beat variability of repolarisation

Repolarisation reserve has been introduced as a concept to explain susceptibility to arrhythmia (Roden, 1998; Biliczki *et al*., 2002). A reduction in repolarisation reserve generates an action potential that is more vulnerable to additional challenges upon repolarisation such as drugs with I_{Kr} blocking properties. Often I_{Kr} blockers are the final challenge on repolarisation, which unmasks an unidentified predisposition precipitating a lethal arrhythmia. This predisposition can be a congenital (long QT syndrome) or an acquired (metabolic or electrolyte disturbances, heart disease, etc.) ion channelopathy. Quantification of the repolarisation reserve and identification of the vulnerable patient are therefore important questions for many investigators.

BVR is a concept that provides data to identify unsafe drugs. With the use of Poincaré plots, STV is one way of quantifying BVR. A dose-dependent TdP occurrence after D-sotalol was tightly associated with the elevation of STV, while the absence of TdP after chronic, oral amiodarone was reflected in an unchanged STV (Thomsen *et al*., 2004). The global nature of the QT interval *versus* the relatively local recording of an MAP signal possibly explains the low predictive power of beat-to-beat variability of the QT interval. Furthermore, the predominant remodelling of the LV may underlie the absence of drug-induced increases in the BVR of the RV MAPD (Thomsen *et al*., 2004). With the assumption that it is a population of cells located in the left ventricular subendocardium that has the lowest repolarisation reserve, we would not expect to find physiologically significant drug-induced increases of the STV of the epicardial repolarisation (Sicouri & Antzelevitch, 1991). This is the first study to show that within the STV of the LV MAPD, variability is present at both 50 and 100% repolarisation levels, supporting the hypothesis that BVR arises at the plateau of the action potential rather than at the fast repolarisation.

In the present study, we have applied STV and confirmed our earlier observation that an increase in BVR predicts TdP. The only NS-7 induced increase in STV was observed in the fast-infusion experiments in the remodelled CAVB dogs. In normal hearts as well as with the slow infusion of NS-7 in CAVB dogs, STV was not altered and TdP was absent. Thus, BVR is an attractive addition to assess proarrhythmic actions of drugs. Additionally, STV is present prior to drug-induced early after-depolarisation and extrasystoles, which allows time for preventive antiarrhythmic actions.

Novel parameters in drug screening

In vivo, repolarisation-dependent arrhythmias have been associated with a QT variability index in humans (Bilchick *et al*., 2004) and with triangulation, spatial dispersion and instability of the MAPD and reverse-use dependency of the drug in isolated rabbit hearts (Hondeghem *et al*., 2001a). Transmural dispersion of repolarisation in arterially perfused canine left ventricular wedge preparations also has predictive value in proarrhythmic drug testing (Di Diego *et al*., 2003; Antzelevitch *et al*., 2004). Short-term variability of repolarisation differs from other measures of temporal lability of repolarisation in its consecutiveness. The former evaluates the direct difference between two consecutive beats, while QT variability index and instability rearranges the order of beats.

Conclusions

Limiting peak plasma concentrations of NS-7 by decreasing the infusion rate reduces the proarrhythmic risk in dogs with remodelled hearts considerably. This occurs despite similar prolongations of the repolarisation parameters, like QT intervals. Conversely, an increase in short-term variability of MAPD predicted the proarrhythmic outcome.

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References

ANTZELEVITCH, C., BELARDINELLI, L., ZYGMUNT, A.C., BURASHNIKOV, A., DI DIEGO, J.M., FISH, J.M., CORDEIRO, J.M. & THOMAS, G. (2004). Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. *Circulation,* **10,** 904–910.

ANTZELEVITCH, C. & SHIMIZU, W. (2002). Cellular mechanisms underlying the long QT syndrome. *Curr. Opin. Cardiol.,* **17,** 43–51.

AOKI, Y., TAMURA, M., ITOH, Y., SETO, T., NONAKA, K., MUKAI, H. & UKAI, Y. (2001). Effective plasma concentration of a novel Na^+/Ca^{2+} channel blocker NS-7 for its cerebroprotective actions in rats with a transient middle cerebral artery occlusion. *J. Pharmacol. Exp. Ther.,* **296,** 306–311.

BELARDINELLI, L., ANTZELEVITCH, C. & VOS, M.A. (2003). Assessing predictors of drug-induced torsade de pointes. *Trends Pharmacol. Sci.,* **24,** 619–625.

BILCHICK, K., VIITASALO, M., OIKARINEN, L., FETICS, B., TOMASELLI, G., SWAN, H., LAITINEN, P.A., VAANANEN, H., KONTULA, K. & BERGER, R.D. (2004). Temporal repolarization lability differences among genotyped patients with the long QT syndrome. *Am. J. Cardiol.,* **94,** 1312–1316.

BILICZKI, P., VIRAG, L., IOST, N., PAPP, J.G. & VARRO, A. (2002). Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of repolarization reserve. *Br. J. Pharmacol.,* **137,** 361–368.

CARLSSON, L., ABRAHAMSSON, C., ANDERSSON, B., DUKER, G. & SCHILLER-LINHARDT, G. (1993). Proarrhythmic effects of the class III agent almokalant: importance of infusion rate, QT dispersion, and early afterdepolarisations. *Cardiovasc. Res.,* **27,** 2186–2193.

CHIBA, K., SUGIYAMA, A., HAGIWARA, T., TAKAHASHI, S.-I., TAKASUNA, K. & HASHIMOTO, K. (2004). *In vivo* experimental approach for the risk assessment of fluoroquinolone antibacterial agents-induced long QT syndrome. *Eur . J. Pharmacol.,* **486,** 189–200.

DI DIEGO, J.M., BELARDINELLI, L. & ANTZELEVITCH, C. (2003). Cisapride-induced transmural dispersion of repolarization and torsade de pointes in the canine left ventricular wedge preparation during epicardial stimulation. *Circulation,* **108,** 1027–1033.

ETTINGER, S.J. & SUTER, P.F. (1970). *Canine Cardiology*. London: W.B. Saunders Company.

GBADEBO, T.D., TRIMBLE, R.W., KHOO, M.S., TEMPLE, J., RODEN, D.M. & ANDERSON, M.E. (2002). Calmodulin inhibitor W-7 unmasks a novel electrocardiographic parameter that predicts initiation of torsade de pointes. *Circulation,* **105,** 770–774.

HAVERKAMP, W., BREITHARDT, G., CAMM, A.J., JANSE, M.J., ROSEN, M.R., ANTZELEVITCH, C., ESCANDE, D., FRANZ, M., MALIK, M., MOSS, A. & SHAH, R. (2000). The potential for QT prolongation and pro-arrhythmia by non-anti- arrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology. *Cardiovasc. Res.,* **47,** 219–233.

HONDEGHEM, L.M., CARLSSON, L. & DUKER, G. (2001a). Instability and triangulation of the action potential predict serious pro-arrhythmia, but action potential duration prolongation is anti-arrhythmic. *Circulation,* **103,** 2004–2013.

HONDEGHEM, L.M., DUJARDIN, K. & DE CLERCK, F. (2001b). Phase 2 prolongation, in the absence of instability and triangulation, antagonizes class III proarrhythmia. *Cardiovasc. Res.,* **50,** 345–353.

KATSUMATA, T., MURAMATSU, H., NAKAMURA, H., NISHIYAMA, Y., AOKI, Y. & KATAYAMA, Y. (2003). Neuroprotective effect of NS-7, a novel Na^+ and Ca^{2+} channel blocker, in a focal ischemic model in the rat. *Brain Res.,* **969,** 168–174.

MAZUR, A., RODEN, D.M. & ANDERSON, M.E. (1999). Systemic administration of calmodulin antagonist W-7 or protein kinase A inhibitor H-8 prevents torsade de pointes in rabbits. *Circulation,* **100,** 2437–2442.

MILBERG, P., ECKARDT, L., BRUNS, H.J., BIERTZ, J., RAMTIN, S., REINSCH, N., FLEISCHER, D., KIRCHHOF, P., FABRITZ, L., BREITHARDT, G. & HAVERKAMP, W. (2002). Divergent proarrhythmic potential of macrolide antibiotics despite similar QT prolongation: fast phase 3 repolarization prevents early afterdepolarizations and torsade de pointes. *J. Pharmacol. Exp. Ther.,* **303,** 218–225.

OKA, M., HAYASHI, S. & ITOH, Y. (2001). Electrophysiological and neurochemical evidence for voltage-dependent Ca(2+) channel blockade by a novel neuroprotective agent NS-7. *Pharmacol. Toxicol.,* **89,** 123–128.

OKA, M., ITOH, Y. & UKAI, Y. (2000). Preferential inhibition by a novel Na(+)/Ca(2+) channel blocker NS-7 of severe to mild hypoxic injury in rat cerebrocortical slices: a possible involvement of a highly voltage-dependent blockade of Ca(2+) channel. *J. Pharmacol. Exp. Ther.,* **293,** 522–529.

REDFERN, W.S., CARLSSON, L., DAVIS, A.S., LYNCH, W.G., MACKENZIE, I., PALETHORPE, S., SIEGL, P.K., STRANG, I., SULLIVAN, A.T., WALLIS, R., CAMM, A.J. & HAMMOND, T.G. (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.,* **58,** 32–45.

RODEN, D.M. (1998). Taking the 'idio' out of 'idiosyncratic': predicting torsades de pointes. *Pacing Clin. Electrophysiol.,* **21,** 1029–1034.

RODEN, D.M. (2004). Drug-induced prolongation of the QT interval. *N. Engl. J. Med.,* **350,** 1013–1022.

SATOH, H. (2003). Cardiac electrophysiological modulation by NS-7, a novel neuroprotective drug, of guinea pig ventricular muscles. *Life Sci.,* **72,** 1039–1048.

SICOURI, S. & ANTZELEVITCH, C. (1991). A subpopulation of cells with unique electrophysiological properties in the deep subepicardium of the canine ventricle. *Mol. Cell. Circ. Res.,* **68,** 1729–1741.

SOPALA, M., DANYSZ, W. & QUACK, G. (2002). Neuroprotective effects of NS-7, voltage-gated Na^+/Ca^{2+} channel blocker in a rodent model of transient focal ischaemia. *Neurotox. Res.,* **4,** 655–661.

SUMA, C., HAYASHI, S., UKAI, Y., YOSHIKUNI, Y. & KIMURA, K. (1997). Na^+ and high-voltage-activated Ca^{2+} channel blocking actions of NS-7, a novel neuroprotective agent, in NG108-15 cells. *Eur. J. Pharmacol.,* **336,** 283–290.

TANAKA, K., ITO, D., SUZUKI, S., DEMBO, T., KOSAKAI, A. & FUKUUCHI, Y. (2002). A novel voltage-sensitive Na(+) and Ca(2+) channel blocker, NS-7, prevents suppression of cyclic AMP-dependent protein kinase and reduces infarct area in the acute phase of cerebral ischemia in rat. *Brain Res.,* **924,** 98–108.

TANAKA, K., NOGAWA, S., NAGATA, E., SUZUKI, S., DEMBO, T., KOSAKAI, A. & FUKUUCHI, Y. (2000). Effects of blockade of voltage-sensitive Ca(2+)/Na(+) channels by a novel phenylpyrimidine derivative, NS-7, on CREB phosphorylation in focal cerebral ischemia in the rat. *Brain Res.,* **873,** 83–93.

THOMSEN, M.B., VERDUYN, S.C., STENGL, M., BEEKMAN, J.D., DE PATER, G., VAN OPSTAL, J.M., VOLDERS, P.G. & VOS, M.A. (2004). Increased short-term variability of repolarization predicts D-sotalol-induced torsades de pointes in dogs. *Circulation,* **110,** 2453–2459.

THOMSEN, M.B., VOLDERS, P.G., STENGL, M., SPÄTJENS, R.L., BEEKMAN, J.D., BISCHOFF, U., KALL, M.A., FREDERIKSEN, K., MATZ, J. & VOS, M.A. (2003). Electrophysiological safety of sertindole in dogs with normal and remodeled hearts. *J. Pharmacol. Exp. Ther.,* **307,** 776–784.

VAN DE WATER, A., VERHEYEN, J., XHONNEUX, R. & RENEMAN, R.S. (1989). An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. *J. Pharmacol. Methods,* **22,** 207–217.

VAN OPSTAL, J.M., LEUNISSEN, J.D., WELLENS, H.J. & VOS, M.A. (2001a). Azimilide and dofetilide produce similar electrophysiological and proarrhythmic effects in a canine model of torsade de pointes arrhythmias. *Eur. J. Pharmacol.,* **412,** 67–76.

VAN OPSTAL, J.M., SCHOENMAKERS, M., VERDUYN, S.C., DE GROOT, S.H., LEUNISSEN, J.D., DER HULST, F.F., MOLENSCHOT, M.M., WELLENS, H.J. & VOS, M.A. (2001b). Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome. *Circulation,* **104,** 2722–2727.

VAN OPSTAL, J.M., VERDUYN, S.C., LEUNISSEN, H.D.M., DE GROOT, S.H.M., WELLENS, H.J.J. & VOS, M.A. (2001c). Electrophysiological parameters indicative of sudden cardiac death in the dog with chronic complete AV-block. *Cardiovasc. Res.,* **50,** 354–361.

VERDUYN, S.C., VOS, M.A., LEUNISSEN, H.D., VAN OPSTAL, J.M. & WELLENS, H.J. (1999). Evaluation of the acute electrophysiologic effects of intravenous dronedarone, an amiodarone-like agent, with special emphasis on ventricular repolarization and acquired torsade de pointes arrhythmias. *J. Cardiovasc. Pharmacol.,* **33,** 212–222.

VERDUYN, S.C., VOS, M.A., VAN DER ZANDE, J., KULCSAR, A. & WELLENS, H.J. (1997). Further observations to elucidate the role of interventricular dispersion of repolarization and early afterdepolarizations in the genesis of acquired torsade de pointes arrhythmias: a comparison between almokalant and D-sotalol using the dog as its own control. *J. Am. Coll. Cardiol.,* **30,** 1575–1584.

VOS, M.A., DE GROOT, S.H., VERDUYN, S.C., VAN DER ZANDE, J., LEUNISSEN, H.D., CLEUTJENS, J.P., VAN BILSEN, M., DAEMEN, M.J., SCHREUDER, J.J., ALLESSIE, M.A. & WELLENS, H.J. (1998). Enhanced susceptibility for acquired torsade de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. *Circulation,* **98,** 1125–1135.

WEISSENBURGER, J., DAVY, J.M., CHEZALVIEL, F., ERTZBISCHOFF, O., POIRIER, J.M., ENGEL, F., LAINEE, P., PENIN, E., MOTTE, G. & CHEYMOL, G. (1991). Arrhythmogenic activities of antiarrhythmic drugs in conscious hypokalemic dogs with atrioventricular block: comparison between quinidine, lidocaine, flecainide, propranolol and sotalol. *J. Pharmacol. Exp. Ther.,* **259,** 871–883.

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