Electrophysiological Safety of Sertindole in Dogs with Normal and Remodeled Hearts

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ABSTRACT

Inhibition of the potassium current I_{Kr} and QT prolongation are associated with drug-induced torsades de pointes arrhythmias (TdP) and sudden cardiac death. We investigated the cardiac electrophysiological effects of sertindole, an antipsychotic drug reported to prolong the QT interval in schizophrenic patients. In cell cultures, sertindole seemed to be a selective blocker of I_{HERG} over other ion currents. For I_{HERG} the IC_{50} value was 64 ± 7 nM, whereas I_{KCNQ1}, I_{Ca,T}, I_{KCNQ1/KCNE1}, and I_{Kv4.3} were blocked in the micromolar range. In canine ventricular myocytes, the IC_{50} value for I_{Kr} inhibition by sertindole was 107 ± 21 nM. Action potentials in these cells prolonged in a reverse rate- and concentration-dependent manner at 10 to 300 nM sertindole. In vivo, sertindole was administered to anesthetized dogs at clinically relevant (0.05–0.20 mg/kg) and high doses (1.0–2.0 mg/kg) i.v. At 0.05 to 0.20 mg/kg sertindole (plasma concentrations 30–157 nM), QTc was prolonged by 1 to 5% in normal dogs and by 9 to 20% in dogs with remodeled hearts due to chronic atrioventricular block (CAVB). TdP was not induced at these doses in normal dogs or in CAVB dogs with reproducible induction of TdP by dofetilide in previous experiments. At 1.0 to 2.0 mg/kg sertindole (plasma concentrations 0.5–3.1 μM), QTc prolonged by 6 to 11% in normal dogs and by 22% in dofetilide-sensitive CAVB dogs. TdP occurred in three of five animals in the latter group. Thus, at high i.v. doses sertindole can pose a serious proarrhythmic risk when electrical remodeling of the ventricles is present. At clinically relevant doses, however, sertindole does not cause TdP in anesthetized dogs with normal or remodeled hearts.

An estimated 1% of the population suffers from various degrees of schizophrenia with a significant burden on the health budget due to long-term hospitalization of these patients (WHO, 2001). The average schizophrenic patient has a 10-year shorter duration of life than the rest of the population and suicidal rates are as high as 10% (WHO, 2001). Treatment of these patients is not optimal because 30% respond poorly or not at all to available drugs and noncompliance is high, in part due to neurological side effects (Oehl et al., 2000). QTc intervals were prolonged in 4 to 5% of patients receiving sertindole (Kasper et al., 1998), and prolongation of action potential duration was confirmed in isolated feline

ABBREVIATIONS: HERG, human ether-a-go-go-related gene; TAP, transmembrane action potential; CL, cycle length; APD, action potential duration; AV, atrioventricular; CAVB, chronic atrioventricular block; TdP, torsades de pointes; MAP, monophasic action potential; LV, left ventricle; RV, right ventricle.
(Drchi et al., 1998) and rabbit hearts (Eckardt et al., 2002). After reevaluation of the existing data, and based on new preclinical, clinical, and epidemiological information, the concern about cardiac risk was outweighed by the therapeutic benefits of sertindole. This led to the reintroduction of sertindole on the European market in 2002 along with a prospective surveillance study of all patients taking the drug (Tomui, 2002).

In the present study, we investigated the cardiac electrophysiological effects of sertindole in vitro and in vivo to provide an ionic basis for repolarization prolongation by the drug in relation to possible proarrhythmic actions in intact dogs.

Materials and 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.

Measurements on Ion Currents in Cell Cultures. Chinese hamster ovary cells were stably transfected with human cloned HERG (I<sub>HERG</sub> GENION, Hamburg, Germany) and SCN5A (I<sub>SCN5A</sub>; obtained from Dr. R. Kallen, University of Pennsylvania, Philadelphia, PA), representing the rapidly activating delayed rectifier potassium current and fast inward sodium current, respectively. MaxiK (Pharmaceuticals, PA), representing the slowly activating delayed rectifier potassium current. Human embryonic kidney 293 cells transiently expressing Kv4.3 (ICN Biomedicals, Inc, Costa Mesa, CA) using borosilicate glass patch pipettes filled with a standard buffer solution (145 mM NaCl, 5.4 mM KCl, 1.0 mM MgCl<sub>2</sub>, 11 mM glucose, 10 mM HEPES, 1.8 mM CaCl<sub>2</sub>, 0.005 mM nifedipine; pH adjusted to 7.4 with NaOH, 37°C). Sertindole was dissolved in dimethyl sulphoxide. The rapidly activating delayed rectifier potassium current I<sub>kr</sub> was measured as the tail current fraction fully blocked by 2 μM almokalant (Carmeliet, 1993). Transmembrane action potentials (TAP) were recorded (Axoclamp 2B; Axon Instruments) using sharp glass microelectrodes filled with 3 M KCl and with a resistance between 20 and 60 MΩ. Cells were superfused with the same solution as in the whole-cell current experiments except that nifedipine was left out. Addition of 2 μM almokalant to the superfusate was used to fully block I<sub>kr</sub>. Action potentials were recorded at each cycle length (CL) of 300, 400, 500, 1000, and 2000 ms. Action potential duration at 95% of repolarization (APD<sub>95</sub>) is presented as the average of five beats >100 beats after a change in pacing CL.

In Vivo Experiments. Twenty-four anesthetized dogs (body weight 29 ± 4 kg, 11 males) were used for these experiments. In 13 animals, complete AV block was induced (van Opstal et al., 2001a). After 4 ± 1 weeks of AV block (chronic AV block; CAVB), the dogs were subjected to a TdP-susceptibility test using the I<sub>kr</sub> blocker dofetilide. Only if a dog showed reproducible TdP upon 25 μg/kg/min dofetilide, it was selected for the sertindole experiments. Thus, dofetilide was used as the positive reference compound. The average time between two experiments in a dog was 2 ± 1 weeks.

Anesthesia, perioperative care, signal processing, data recording, and off-line analysis have been described previously (van Opstal et al., 2001a). Standard and precordial ECCs were recorded. In addition, biventricular endocardial monophasic action potential (MAP) recordings were made (EP Technologies, Sunnyvale, CA).

RR and QT intervals in lead II, left and right ventricular MAP duration were measured at 260 and 340 nm, respectively. The interventricular dispersion of repolarization (MAPD<sub>100</sub>) was calculated as the difference between the LV and RV MAPD. QT intervals were corrected for heart rate (QT<sub>c</sub>) according to Van de Water’s formula (Van de Water et al., 1989). The number of ectopic beats, defined as short-coupled beats arising from a new ventricular focus before complete repolarization of the previous beat, was counted during 10 min after administration of the drug. Both single and multiple ectopic beats were counted. The latter are considered more proarrhythmic. TdP was defined as a polymorphic ventricular tachycardia consisting of five or more beats twisting around the isoelectric line of the ECG in the setting of a prolonged QT interval. Sertindole (mol. wt. 441 g/mol) was dissolved in 0.1 M HCl and diluted in 10% hydroxypropyl cyclodextrin and 0.05 M phosphate buffer (1:1). pH was adjusted to 7.4. The solution was filtered through a 22-μm pore filter before use. Sertindole was administered over 5 min through a cephalic vein and blood samples were taken from the contralateral cephalic vein to measure plasma concentrations.

Plasma Analysis. Blood samples were obtained 5, 10, and 25 min after drug administration and plasma was stored at −20°C until analysis at H. Lundbeck A/S (Copenhagen, Denmark). Sertindole plasma samples were extracted by solid phase extraction. The sample extracts were analyzed by a normal phase high-performance liquid chromatography method with a mobile phase consisting of heptane, 2% piperidine in 2-propanol, and water (100:20:0.45) and quantified by fluorescence detection with excitation/emission wavelengths at 260 and 340 nm, respectively. The method had a mean recovery of 90% with a quantification limit of 0.5 ng/ml. Total plasma concentrations (free + bound) are reported.

Statistics. Electrophysiological parameters were compared with control using (repeated-measures) ANOVA followed by Bonferroni’s test. Comparisons between controls were performed with an unpaired Student’s t test. Data are reported as mean ± s.e.m. A P < 0.05 was considered statistical significant.
Results

Sertindole Is Selective for \( I_{\text{HERG}} \). In Fig. 1A the molecular structure of sertindole is shown. Figure 1B shows concentration-response curves for the various cardiac ion channels expressed endogenously or by transfection in cell cultures. Sertindole inhibited \( I_{\text{HERG}} \) in a concentration-dependent manner over the range of 10–1000 nM, with 50% block at 64 ± 7 nM. \( I_{\text{KCNQ1/KCNE1}} \) was inhibited by 10 ± 5% at 300 nM sertindole (IC\(_{50}\) value = 6.9 ± 2 \( \mu \)M), whereas other currents (\( I_{\text{SCN5A}}, I_{\text{Ca,L}}, I_{\text{Ca,T}}, \) and \( I_{\text{Kv4.3}} \)) were only inhibited at micromolar concentrations. Representative examples of the six currents are shown in Fig. 2.

Sertindole Blocks \( I_{\text{Kr}} \) in Canine Ventricular Myocytes. Activation of \( I_{\text{Kr}} \) occurred at depolarizations to higher than \(-10 \) mV and showed saturation at conditioning voltages (\( V_{\text{cond}} \)) \( >20 \) mV (Fig. 3B). Maximal \( I_{\text{Kr}} \) density at control was 0.14 ± 0.07 pA/pF. Boltzmann fit to the data revealed a half-maximal activation at 11 ± 1 mV and a slope factor of 5.9 ± 0.9 pA/pF/mV. Half-time for \( I_{\text{Kr}} \) deactivation upon repolarization to \(-50 \) mV was 294 ± 23 ms.

An example of \( I_{\text{Kr}} \) recorded under control conditions and under the influence of 100 nM sertindole is shown in Fig. 3A. Sertindole inhibited \( I_{\text{Kr}} \) tails in a concentration-dependent and voltage-independent manner. At 300 nM, the maximal \( I_{\text{Kr}} \) tail density had decreased to 0.08 ± 0.004 pA/pF (57 ± 4%; \( P < 0.05 \); Fig. 3B). Boltzmann fit of the remaining \( I_{\text{Kr}} \) at 300 nM sertindole showed a half-maximal activation at 8 ± 2 mV (\( P = \) N.S. versus control) and a slope factor of 4.2 ± 1.4 pA/pF/mV (\( P = \) N.S. versus control). Half-time for deactivation was 273 ± 48 ms (\( P = \) N.S. versus control). Figure 3C shows an example of the effects of accumulating concentrations of sertindole to illustrate the concentration dependence of the drug on \( I_{\text{Kr}} \). Using multiple voltage protocols to analyze the properties of \( I_{\text{Kr}} \) under the influence of sertindole, a concentration-response relationship was obtained (Fig. 3D). Sertindole inhibited \( I_{\text{Kr}} \) in a concentration-dependent manner over the full range of 10 to 1000 nM, with 50% block at 107 ± 21 nM (\( n_{\text{cells}} = 10 \)).

Sertindole Prolongs the Transmembrane Action Potential. TAP in normal canine ventricular myocytes prolonged from 166 ± 5 to 278 ± 13 ms by increasing pacing CL

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Fig. 1. Selective block of \( I_{\text{HERG}} \) by sertindole. A, chemical structure of sertindole (mol. wt. 441 g/mol). B, composite data on blocking effects of sertindole on \( I_{\text{HERG}}, I_{\text{SCN5A}}, I_{\text{Ca,L}}, I_{\text{Ca,T}}, I_{\text{KCNQ1/KCNE1}}, \) and \( I_{\text{Kv4.3}} \) in heterologous and endogenous expression systems. Data points represent the means for three to eight cells. Shown are the concentration-response curves, where the remaining current is plotted relative to its control level. IC\(_{50}\) values for the different currents are given in the legend. Hill coefficients are 1.16 for \( I_{\text{HERG}} \), 1.27 for \( I_{\text{SCN5A}} \), 1.15 for \( I_{\text{Ca,L}} \), 3.98 for \( I_{\text{Ca,T}} \), 0.99 for \( I_{\text{KCNQ1/KCNE1}} \), and 2.05 for \( I_{\text{Kv4.3}} \).

Fig. 2. Representative tracings of \( I_{\text{HERG}}, I_{\text{SCN5A}}, I_{\text{Ca,L}}, I_{\text{Ca,T}}, I_{\text{KCNQ1/KCNE1}}, \) and \( I_{\text{Kv4.3}} \) during treatment with sertindole. Concentrations of sertindole were chosen to indicate selectivity for \( I_{\text{HERG}} \) block. Insets, voltage-clamp protocols.
from 300 to 2,000 ms ($n_{\text{cells}} = 11$). Concentration-dependent prolongation of APD$_{95}$ was observed for 10 to 300 nM sertindole, reaching statistical significance at 100 nM and higher and for CL $\geq$400 ms (Fig. 4). Under the influence of 300 nM sertindole, APD$_{95}$ was prolonged to 197 $\pm$ 15 ms (18%) and 345 $\pm$ 44 ms (24%) at 300 and 2000 ms CL, respectively ($P < 0.05$ for both CL), showing clear reverse rate dependence. Early afterdepolarizations or abnormal automaticity were not observed.

**Sertindole Causes Moderate Prolongation of Repolarization in Normal Hearts in Vivo.** Cumulative doses of 0.05, 0.10, and 0.20 mg/kg sertindole (30-min intervals) were administered to five dogs. Plasma concentrations ranged from 33 $\pm$ 1 nM after 0.05 mg/kg to 157 $\pm$ 18 nM after 0.20 mg/kg. Reported plasma concentrations after human therapeutic dosing were 22 $\pm$ 12 to 158 $\pm$ 63 nM (Wong and Graneman, 1998); hence, we considered these doses in the dogs to be clinically relevant. Representative examples of the electrophysiological effects are shown in Fig. 5. QTc interval did not prolong at 0.05 or 0.10 mg/kg sertindole. At 0.20 mg/kg QTc prolonged from 277 $\pm$ 11 to 292 $\pm$ 20 ms (5%; $P < 0.05$; Fig. 6). At this dose, the RR interval increased from 465 $\pm$ 35 to 545 $\pm$ 47 ms (17%; $P < 0.05$) and the QT interval from 231 $\pm$ 7 to 252 $\pm$ 12 ms (9%; $P < 0.05$). The LV MAPD prolonged from 191 $\pm$ 8 to 213 $\pm$ 9 ms (10%; $P < 0.05$), whereas the RV MAPD remained unchanged (181 $\pm$ 9 to 197 $\pm$ 7 ms; $P = \text{N.S.}$), leaving the interventricular dispersion of repolarization unaltered.

Cumulative doses of 0.5, 1.0, and 2.0 mg/kg sertindole (30-min interval) were administered to six other dogs. Plasma concentrations ranged from 0.5 $\pm$ 0.2 $\mu$M after 0.5 mg/kg to 3.1 $\pm$ 0.3 $\mu$M after 2.0 mg/kg. Twenty-four hours after the high dose-range experiments, the mean plasma concentration was 269 $\pm$ 31 nM. All high doses produced significant QTc increases (Fig. 6) with a maximal QTc prolongation from 294$\pm$8 to 326$\pm$19 ms (11%; $P < 0.05$) after 2.0 mg/kg sertindole. This involved a QT prolongation from 251$\pm$10 to 289$\pm$24 ms (15%; $P < 0.05$), whereas RR...
interval remained unchanged. LV MAPD increased from 214 ± 11 to 264 ± 28 ms (23%; P < 0.05) and RV MAPD from 208 ± 11 to 242 ± 22 ms (16%; P < 0.05). The interventricular dispersion of repolarization was not changed (8 ± 2 to 20 ± 12 ms; P = N.S.).

Sertindole induced no changes in the PQ interval or QRS duration. Apart from the QT prolongation, no major changes were seen in the T-wave morphology at low or high doses of administration (Fig. 5).

**Sertindole Carries a Proarrhythmic Risk in Electrically Remodeled Hearts.** Ten dofetilide-susceptible CAVB dogs received sertindole. In five animals, 0.10 mg/kg was administered, followed by another 0.20 mg/kg after 30 min. The QTc interval prolonged more than in normal dogs (e.g., 20% after 0.20 mg/kg in CAVB dogs versus 5% in normal dogs). Electrophysiological data from these experiments are summarized in Table 1. The five other dogs were tested with 1.0 mg/kg sertindole (Table 1). Sertindole prolonged repolarization in a dose-dependent manner, whereas the CL of the idioventricular rhythm only increased at the high dose (Table 1). The high dose of sertindole caused reproducible TdP in three of five dogs (Fig. 7). In these three animals, the first TdP was seen on average 7 ± 2 min after start of the 1.0 mg/kg sertindole infusion (range 6–9 min). The two dogs not responding with TdP received another 1.0 mg/kg, which caused TdP in one dog. During the 1-h observation period after 1.0 mg/kg sertindole, a total of 19 TdP (6 ± 2; n_dogs = 3) were seen of which four TdP had to be cardioverted electrically. Single ectopic beats (16 ± 9) occurred in all dogs at high dosing, whereas multiple ectopic beats (5 ± 2) were seen in four dogs. Interventricular dispersion of repolarization tended to increase, e.g., from 45 ± 6 to 79 ± 19 ms at 1.0 mg/kg (P = 0.09).

**Electrophysiological Data on the Positive Reference Compound Dofetilide.** Concentration-response studies of dofetilide on I_Kr in native ventricular myocytes revealed an IC_{50} value of 46 ± 9 nM (Fig. 8A). Prolongation of TAP in the myocytes was reverse rate-dependent (Fig. 8B). In normal anesthetized dogs, i.v. doses of 12.5, 25, and 50 μg/kg dofetilide (van Opstal et al., 2001a) caused significant QTc prolongation (19–25%; P < 0.05 versus control; Fig. 8C). RR also increased, e.g., by 13% after 12.5 μg/kg (P < 0.05 versus control). Plasma concentrations of dofetilide are given in Fig. 8C. Dofetilide (25 μg/kg) induced TdP in 10/13 anesthetized CAVB dogs (Fig. 8D for n_dogs = 10 used for sertindole testing in vivo).

**Discussion**

The present study investigates the electrophysiological properties of sertindole from cloned cardiac ion channels to anesthetized dogs with normal and remodeled hearts. The results can be summarized as follows: 1) Sertindole is a selective blocker of I_{H/Kr} over other ion currents expressed in cell cultures. 2) Sertindole causes concentration-dependent block of native I_Kr, and this translates into reverse rate-dependent lengthening of myocyte action potentials. 3) In anesthetized dogs, dose-dependent prolongation of in vivo repolarization by sertindole is observed. 4) Clinically relevant doses of sertindole do not cause TdP in anesthetized normal dogs or in CAVB animals with reproducible induction of TdP by dofetilide in previous experiments. 5) High doses of sertindole induce multiple ectopic beats and TdP in the majority of these CAVB dogs.

**Normal and Remodeled Hearts.** To elucidate whether a drug is devoid of proarrhythmic properties, a reproducible animal model is essential. Testing drugs in normal hearts is necessary but is not sufficient for the recognition of proarrhythmic effects in the diseased heart. We used the canine model with CAVB, known to have acquired QT prolongation. Creation of CAVB results in a bradycardia-induced volume overload. Hypertrophy is observed in ventricular myocytes (Volders et al., 1998) as well as in the whole heart (Vos et al., 1998; Verduyn et al., 2001a). Contractile remodeling in vivo restores initially depressed cardiac output (compensated function), which is associated with an increased cytosolic Ca^{2+} transient in vitro (de Groot et al., 2000; Sipido et al., 2000). Down-regulation of I_{Ks} and I_{Kr} (Volders et al., 1999; Ramakers et al., 2003) and up-regulation of the sodium-calcium exchanger (Sipido et al., 2000) contribute to the electrical alterations in remodeled CAVB hearts. This ventricular remodeling predisposes to TdP and sudden cardiac death (van Opstal et al., 2001c).

Whereas most class III antiarrhythmic drugs cause TdP in 2 to 5% of patients (Haverkamp et al., 2000), an incidence in the order of 56 to 67% is encountered in anesthetized CAVB dogs, making the model very sensitive (Verduyn et al., 1997; van Opstal et al., 2001a). In the present study, the noncardiovascular drug sertindole was tested in a number of different ways and using a broad dose regimen. Based on earlier clinical reports of low proarrhythmia of sertindole in patients (0.3% cardiac mortality rate or <10% of antiarrhythmic drugs; Kasper, 2002), we anticipated a low TdP incidence in the CAVB dog. Serial testing in this model has shown reproducible induction of TdP (Verduyn et al., 2001b). Therefore, we chose to increase the sensitivity of the model and to evaluate the proarrhythmia of sertindole only in dogs that showed reproducible TdP after administration of 25 μg/kg sertindole.

**Cardiac Safety of Sertindole.** This is the first report on sertindole in which both in vitro and in vivo investigations...
are combined. Sertindole caused prolongation of repolarization in both normal and CAVB dogs, although at variable degree, e.g., at 0.20 mg/kg, QTc interval increased by up to 5% in normal hearts and by up to 20% in CAVB dogs. The plasma concentrations measured in dogs in this study at the low doses were comparable with plasma concentrations from human volunteers (4–20 mg/day sertindole p.o., range from 22 ± 12 to 158 ± 63 nM; Wong and Granneman, 1998). These doses did not cause TdP in dofetilide-sensitive CAVB dogs. Administration of 25 μg/kg dofetilide led to a plasma concentration of 79 ± 11 nM. Reported plasma concentrations from human volunteers ranging from 5 to 23 nM (Pfizer, 1999).

Eckardt et al. (2002) reported a low torsadogenic potential of sertindole in isolated rabbit hearts. They showed a 15 to 17% prolongation of the QT interval at a perfusion concentration of 1.5 μM sertindole without induction of TdP. In the present investigation in anesthetized dogs with normal hearts, 9% prolongation of the QTc interval was observed at 1.3 ± 0.1 μM. No TdP was observed, confirming the results from Eckardt et al. (2002). Plasma protein binding in vivo and unknown levels of accumulation in cardiac tissue complicate comparisons between these models.

Relating plasma concentrations to concentrations used in the in vitro setting can only be done with great caution. Among the factors to be taken into account are plasma protein binding, tissue accumulation and the distance between the plasma protein and the receptor on the cardiomyocyte in situ. Plasma protein binding of sertindole in humans is high (>99%; Ereshefsky, 1996), indicating a free plasma concentration of maximally 1 to 2 nM after therapeutic administration, based on the plasma concentrations in human volunteers (Wong and Granneman, 1998). The level of accumulation in cardiac tissue is unknown, but a rather large volume of distribution is reported (20–40 l/kg; Ereshefsky, 1996), indicating accumulation of sertindole in various tissues. In our dogs, maximal QT prolongation was already seen 5 to 10 min after the start of infusion of sertindole, suggesting a rapid inhibition of $I_{Kr}$ once the drug is present in the circulation. The relative $I_{Kr}$ block induced by sertindole in vivo or in the clinic could be underestimated when plasma concentrations are compared with in vitro concentrations.

Apart from an inhibition of $I_{Kr}$, sertindole has also been reported to block the human dopamine D2 and the 5-hydroxytryptamine2A receptors (Arnt, 1998). It does also show α1A-blocking properties in rat mesenteric arteries (Ip-sen et al., 1997). New studies are required to test possible additional electrophysiological properties of sertindole in the heart under conditions when physiological levels of these agonists are present.

**Pharmacological Implications.** Previous studies using chronic amiodarone administration have shown that TdP can be absent in CAVB dogs despite prolongation of the QTc interval by 21% (van Opstal et al., 2001b). The present study indicates again a poor association between the degree of QTc prolongation and the incidence of TdP (Table 1); at a comparable QTc after 0.2 and 1.0 mg/kg sertindole, TdP were only
induced after the higher dose. This stresses not only the importance of testing several doses when assessing the proarrhythmic potential of a drug but also the relevance of addressing other proarrhythmic factors such as ectopic beats and dispersion.

If our in vitro data from cell cultures and isolated canine myocytes would have determined the future for sertindole, the drug would have likely been abandoned from further development (e.g., based on the recommendations of the Policy Conference of the European Society of Cardiology; Haverkamp et al., 2000). The expansion of our study to in vivo testing showed a discrepancy between the in vitro finding of $I_{Kr}$ inhibition and prolonged cellular repolarization and the absence of arrhythmias in normal anesthetized dogs. Our data strongly advocate the use of pathological animal models when testing for proarrhythmic properties of cardiovascular and noncardiovascular drugs.

A recent risk-benefit analysis of the preclinical and clinical data on sertindole by the European Committee for Proprietary Medicinal Products led to the reintroduction of sertindole to the European market in 2002.

Limitations. Steady-state plasma concentrations were not obtained in this study, as opposed to previous clinical studies, and the pharmacokinetic difference between acute i.v. and repeated oral dosing should be considered when extrapolating our data to humans. Differences in accumulated tissue concentrations after acute i.v. versus chronic oral administration will likely exist. Furthermore, species differences between dogs and patients should be taken into account.

In conclusion, in vitro studies clearly show sertindole’s selective inhibition of $I_{HERG}$ over other ion currents. Block of native $I_{Kr}$ forms the ionic basis for action potential prolongation in canine ventricular myocytes and QT prolongation in vivo. At high i.v. doses, sertindole can pose a serious proarrhythmic risk when electrical remodeling of the ventricles is present, as in dogs with CAVB. At clinically relevant doses, sertindole does not cause TdP in anesthetized dogs with normal or remodeled hearts.

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**Fig. 8.** Electrophysiological data on the positive reference compound dofetilide. A, concentration-response curve of the inhibition of 
$\text{IC}_{50}$ tails by dofetilide in normal canine ventricular myocytes (IC$_{50}$ value, 46 ± 9; Hill coefficient, 0.76; mean, $C_{\text{m}}$ = 180 ± 11.1 pF; $n_{\text{cells}}$ = 9). Almokalan (Almo; 2 µM) was used for full block of $I_{\text{Kr}}$. B, transmembrane APD$_{95}$ upon increasing concentrations of dofetilide. *, $P < 0.05$, 100, and 300 nM versus control, $n_{\text{cells}}$ = 5. Vertical axes are identical. Inset shows two representative action potentials at control and during 300 nM dofetilide, CL = 2,000 ms (scale bar, 100 ms at -80 mV, 0 mV level indicated). C, QTc prolongation by increasing doses of dofetilide i.v. to six anesthetized normal dogs and corresponding plasma concentrations. *, $P < 0.05$ versus control QTc. For comparison, plasma concentrations after oral administration to humans are 5 to 23 nM (plasma protein binding, 60–70%; volume of distribution, 3 l/kg; Pfizer, 1999). D, electrophysiological effects of 25 µg/kg dofetilide i.v. to the 10 anesthetized dogs with CAVB that were TdP-inducible and used for sertindole testing. Values in milliseconds and percentage increases in brackets. *, $P < 0.05$ versus control. Singles and multiples, numbers of single and multiple ventricular ectopic beats. TdP and shocks, number of TdP and electrical cardioversions. Times after start of dofetilide infusion.
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