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APPLICATION NUMBER:NDA 20931

PHARMACOLOGY REVIEW(S)

ROEDER

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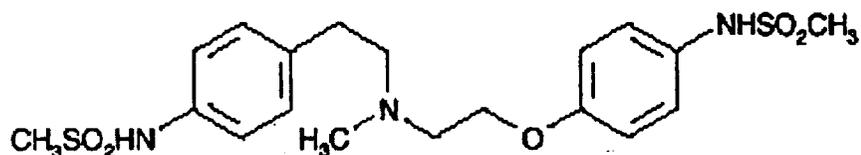
NDA # 20931

PHARMACOLOGY/TOXICOLOGY REVIEW

Reviewer: P. Gill-Kumar, M.D.
Completion dt: Nov 16, '98.

CDER receipt dt.: March 9, 1998
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Sponsor: Pfizer Pharmaceuticals Production Corp Ltd
Eastern Point Rd, Groton, CT 06340
Drug: Dofetilide
Proprietary name: Tikosyn
Molecular Formula: C₁₉H₂₇N₃O₅S₂
Molecular wt: 441.6
Formulation: Capsules
Pharmacological Class: Class III Antiarrhythmic
Proposed Therapeutic use: Treatment of supraventricular arrhythmias:
Atrial fibrillation, atrial flutter, and paroxysmal supraventricular
tachycardia (PSVT)

Structural Formula:



Maximum Proposed dose:
0.5 mg b.i.d.

Related INDs:
IND,
IND,

Submissions Reviewed:
NDA 20931
Amendment to NDA 20931, BP dt 9/9/98
Amendment to NDA 20931, BZ dt 9/28/98

Original Pharmacology/Toxicology Review

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Summary, Conclusions & Recommendations

Note: In the text below, s-s stands for statistically significant; s-ns for statistically not significant; s-rev for statistical tests done by this reviewer. s-s and s-ns mentioned without a qualifier indicate statistical tests done by the sponsor.

Pharmacodynamics

Note: In this section, APD is action potential duration; APD_n is action potential duration measured up to n% repolarization; APA is action potential amplitude; RP is resting (membrane) potential; MRD is maximum rate of depolarization; ERP is duration of effective refractory period; CT is conduction time; I_x is current carried by ion 'x'; v or V are voltages. HR is heart rate; SBP is systolic, and DBP is diastolic arterial pressure.

Cardiac effects (In-vitro studies)

- In canine cardiac cells, dofetilide increased APD and ERP of ventricular myocytes and Purkinje fibers. In the concentration range tested (5nM-1 μ M for ventricular myocytes; 1nM-500 nM for Purkinje fibers) these effects were dose related and s-s. RP, APA, and MRD were not affected at any concentration (study #1, p 11).
- In guinea pig myocytes also, dofetilide increased ERP dose relatedly, in the dose range 5nM-1 μ M. Δ ERP was greater at stimulation rate of 1 Hz than at 5 Hz. Dofetilide had no effect on ventricular conduction time (CT) at either of the stimulation rates. (study #1, p 12).
- Up to 100 nM concentration, dofetilide did not reduce peak force of contraction and dF/dt_{max} of papillary muscle preparations at 0.5-2Hz stimulation rates. (study #6, p 18).
- In voltage clamp studies on guinea pig cardiac myocytes, effect of dofetilide on delayed rectifier K current (I_{kr} the outwardly directed current that underlies the repolarization phase of cardiac action potential), was studied by measuring tail currents after 500 ms depolarizing pulses of various magnitude. 50 nM dofetilide reduced tail currents at the end of depolarizing pulses of -30 to 20 mv magnitude. 2 μ M dofetilide caused a greater reduction of I_{kr} than 50 nM (study #1, p 13).

I_{k1}, the outwardly directed time independent K current that flows at membrane potentials of <-20 mv and > equilibrium potential of K⁺ in cardiac cells, was not affected even at 2 μ M concentration of dofetilide (study #1, fig 5, p 14).

- In voltage clamp studies on guinea pig cardiac myocytes, effects of dofetilide on delayed rectifier I_{kr}, I_{ks} (the slow component of the delayed rectifier K⁺ current), and on I_{Na} and I_{Ca} were studied. Dofetilide dose relatedly reduced I_{kr} at $\geq 10^{-6}$ M concentrations; I_{ks} was only reduced at 10⁻⁴M, but τ of decay of I_{ks} seemed to be prolonged even at $\geq 10^{-6}$ M dofetilide. Peak I_{Na} and inactivation parameters of the fast Na channel were not affected by 10⁻⁴M dofetilide (study # 2, PP). This study however did not demonstrate that dofetilide has no effect on I_{Ca} in cardiac cells (discussion on p 15).
- 10 nM dofetilide increased ADP₉₀ in guinea pig papillary myocytes even when the cell membrane was depolarized by \approx 20 mv by increasing external [K⁺] to 10 mM (extra cellular [K⁺] is likely to increase in the vicinity of damaged myocardial cells, as e.g. during ischemia); s-s increase in APD₉₀ was seen even at 3 Hz pacing rate (fig 7 in study #3, p 16).
- In isolated guinea pig atria, dofetilide at concentrations ≥ 10 nM dose relatedly reduced the spontaneous beating rate. Equimolar concentration of propranolol had a smaller negative chronotropic effect (study #4, fig. 8, p 17). This study also showed that the negative chronotropic effect of dofetilide on the SA node is not due to a β blocking effect.
- In whole cell patch clamp studies on rabbit SA node cells, 100 nM and 1 μ M dofetilide dose relatedly reduced the amplitude of tail currents after 40 mv depolarizing pulses; 10 nM had no effect on the amplitude of tail currents in these cells (study #5, p 18).

The results of this study show that at least one mechanism for the negative chronotropic effect of dofetilide on spontaneous beating rates of atria is prolongation of the APD of the SA nodal cells due to inhibition of I_{Kr} in these cells.

- In study #6 (p 18), effects of dofetilide on force of contraction of paced guinea pig papillary muscle preparations were studied. 10 and 100 nM dofetilide (concentrations that prolong APD in ventricular and SA nodal cells as the studies summarized above show) did not reduce peak force of contraction; did not increase time to peak, and did not reduce dF/dt_{max} at 0.5 Hz and 2 Hz stimulation rates. This study thus showed that dofetilide, at concentration at which it increases APD of cardiac cells, does not have a negative inotropic effect.

Cardiac effects (In-vivo studies)

- In anesthetized dogs, effects of i/v dofetilide on VERP, HR, and ventricular fibrillation threshold (VFT) were studied (study #1, p 19). Dofetilide, in the dose range 3-100 $\mu\text{g}/\text{kg}$, dose relatedly increased VERP, and reduced HR (fig 11, p 19). VFT was s-s increased only at 100 $\mu\text{g}/\text{kg}$ (fig 12, p 20).
- Effect on atrial flutter (AF): In an atrial flutter model in the dog, in which sustained atrial flutter could be induced by programmed electrical stimulation or rapid atrial pacing, effects of dofetilide and quinidine (administered i/v, as loading doses and maintenance infusions) on termination and re-induction of flutter were studied (study #2, p 20-21). Experimental preparation allowed measurements of ERP at different sites in the atria, and these measurements were used to determine the effect of dofetilide on ERP dispersion. AF could be terminated in all 7 dogs of the dofetilide group, and sustained flutter could not be re-induced in any. In the quinidine group, flutter could be terminated in 3/8 and sustained flutter could be re-induced in 5. Plasma levels of dofetilide were 9.6 ± 2.7 ng/ml (mean \pm SD), and ERP dispersion in the dofetilide group was s-s reduced compared to baseline values (table, p 21).
- Effect on ventricular fibrillation (VF) during acute myocardial ischemia (MI): In anesthetized pigs, dofetilide s-s reduced the incidence of VF during myocardial ischemia (6/16 dofetilide group and 13/16 vehicle group animals developed VF (study #3, p22).

Miscellaneous studies

- Receptor binding studies: IC_{50} of dofetilide for α_2 and β adrenoceptors, and for Adenosine A1, dopamine D2, 5-HT2, muscarine, opioid, and dihydropyridine receptors was $> 10\mu\text{M}$; for α_1 -adrenoceptors, it was $4.5 \pm 1.1 \mu\text{M}$ (p 26). Since the targeted cardiac effects occur in the nM range, dofetilide at therapeutic doses is not likely to have any effects on these receptor sites.
- Effects on Acetylcholinesterase, and Na/K-activated ATPase activities: dofetilide had practically no effect on acetylcholinesterase and Na/K-activated ATPase activities at 1 μM , and 10 μM concentrations respectively (pp 26-27).

Conclusions

Studies summarized above show that dofetilide at nM concentrations terminated atrial flutter in a canine model of AF, and reduced the incidence of ischemia induced ventricular fibrillation in the pig. The mechanism of these antiarrhythmic effects is prolongation of APD (class III effect) due to inhibition of I_{Kr} ; I_{Kr} was also inhibited in SA nodal cells, and dofetilide reduced the spontaneous beating rate of isolated atria.

At 100 nM (the highest concentration tested), dofetilide had no negative inotropic effect on papillary muscle preparations. IC_{50} of dofetilide for several types of receptors, that affect myocardial function, was $>10\mu\text{M}$. At concentrations $\leq 1 \mu\text{M}$, dofetilide had no effect on acetylcholinesterase and ATPase activities.

Toxicology

Note: In all studies summarized below, mice were CrI:COBS-VAF-CD1(ICR)BR; rats, CrI:COBS-CD(SD)BR; and dogs, beagle. Extrapolations of pharmacokinetic parameters to doses at which plasma drug levels were not determined, assumes linear pharmacokinetics. Only treatment related adverse findings are described and discussed in this section. For comparisons of exposure to that of man in the clinical setting, estimated upper limits of 95% of human parameters are used; these estimates are: C_{max} , 4 ng/ml, and AUC_{0-24h} , 75 ng*h/ml (method of arriving at this estimate, 'Note' on p 29). These estimates are referred to as maximum likely human parameters (MLHP/s) in the text below. For assessing adverse effects, unless stated otherwise, values of different parameters are compared to those in control groups at the same time point.

§1: Acute (Rodents)

LD_{50} (oral), in both rat and mouse, was > 300 mg/kg (there were no deaths at 300 mg/kg, the only dose used).

§2: Subchronic & Chronic (Rodents)

Mouse (3 Month Dietary Study)

5 mg/kg/day, was the NOAED. At 20 mg/kg/day, the only treatment related adverse effects were a 7-8% reduction in testicular wt (s-ns). 80 mg/kg/day caused a s-s 17% reduction in testicular wt and testicular atrophy (histopathology) in 3/10 males (p 36). In a toxicokinetic study, 20 mg/kg/day dietary administration of dofetilide resulted in a C_{max} of 63 ± 24 ng/ml, and AUC of 724 ng*h/ml (p 15, attachment-I). These values are ≈ 15 and ≈ 10 times the respective MLHPs.

Extrapolating from the above, C_{max} and AUC at the NOAED in this study would be ≈ 4 times and 2.5 times the respective MLHPs.

Rat

One Month Gavage Study:

1 mg/kg/day was the NOAED. At this dose C_{max} and AUC were 5 times and 0.87 times the respective MLHPs (p 37). At 6 and 40 mg/kg/day doses, there were: 1) A 3-4%, s-s increase in serum Ca. 2) A dose related 8-15%, s-s reduction of testicular wts. There were no histopathological lesions in the testes. C_{max} and AUC at the high dose were ≈ 300 and ≈ 60 times the respective MLHPs (p 37).

Comments: The mechanism of the small increase in serum Ca in the mid and high dose groups is not understood. Since the increase was small, it probably does not have any toxicological significance.

Three Month Dietary Study

10 mg/kg/day was the NOAED. Mean C_{max} and AUC at this dose would be ≈ 39 and ≈ 26 times the MLHPs (p43). At 30 mg/kg/day: In males: 1) Body wt was reduced by 8% (s-s) for the last 2 weeks of the study. 2) Testicular wt was reduced by $\approx 28\%$ (s-s), and 4/10 males had testicular atrophy. Estimated C_{max} and AUC at this dose would be ≈ 120 times and ≈ 80 times the respective MLHPs (estimated by interpolation from table on p 43). At 80 mg/kg/day: In Males: 1) There was a progressive, s-s reduction in body wt; body wt was reduced by 15% at the end of the study; food consumption was 11-13% lower (s-s) at almost all time points. 2) 32% increase in alkaline phosphatase (s-s, s-rev). 3) Testicular wt was reduced by $\approx 40\%$; 2/10 animals had small, flaccid testes; and 10/10 males had testicular atrophy. Atrophy was more severe than in the mid dose group (p 37).

Six Month Gavage Study

0.5 mg/kg/day was the NOAED. At this dose, C_{max} and AUC were ≈ 9 and 1.3 times the respective MLHPs (males and females combined, p 40). At 5 mg/kg/day, there was 8-9% reduction (s-s) in testicular wt; 1/20 males had small and/or soft testes, and 2/20 males had testicular atrophy more severe than in control group. C_{max} and AUC at this dose are 70, and 10 times the respective MLHPs. At 40 mg/kg/day, at study end there were: 1) Approximately 3% increase (s-s) in serum Ca. 2) 8/40 animals vs 2/40 control group had hydronephrosis (s-s, p 39). In males: 3) There was 35% increase in triglycerides, and 11% increase in blood urea (p 39). 4) 7/20 males had small and/or soft testes, and 13/20 males had testicular atrophy of > severity than seen in control group ($p \leq 0.0001$, Fishers exact test, s-rev).

12 Month Dietary Study

2 mg/kg/day was the NOAED. Estimated mean C_{max} and AUC at this dose would be ≈ 5 and 4 times the respective MLHPs (estimates by extrapolation from the values at 5 mg/kg/day, p 43). At 6 mg/kg/day: Spleen wt was reduced by $\approx 14\%$; testicular wt was decreased by 7% (s-s), and testes in 3/20 males were small and/or soft. Estimated C_{max} and AUC at this dose would be 15 and 12 times the respective MLHPs (estimates by extrapolation from the 2 mg/kg/day values). At 20 mg/kg/day, serum Ca was increased by $\approx 3\%$ (s-s) in females, and serum inorganic phosphorus (P_i) was increased by $\approx 12\%$ in both sexes. Males: Testes were small and/or soft in 12/20 males ($p \leq 0.001$, s-rev). 16/20 vs 7/20 control animals had testicular atrophy ($p \leq 0.01$, s-rev); 4/20 vs 1/20 control animals had atrophy of severity > grade-2; and 8/20 vs 1/20 control animals had abnormal content in epididymides ($p \leq 0.01$, s-rev). Estimated C_{max} and AUC at this dose would be ≈ 78 and 50 times the respective MLHPs.

§3: Subchronic & Chronic (Dog)

Two Week Oral Study (capsules)

10 mg/kg/day (highest dose studied) had no adverse effects other than aggressive behavior in both males on day-1 only. This dose is thus a 'no serious adverse effect dose'. C_{max} and AUC at this dose were > 900 and >250 times the respective MLHPs (p 44).

One Month Oral Study (gavage)

2 mg/kg/day (mid dose) was the NOAED. C_{max} and AUC at this dose were ≈ 240 and 58 times the respective MLHPs. At 10 mg/kg/day, cellular debris was seen in the epididymal heads of 3/3 males.

Six Month Oral Study (gavage)

2 mg/kg/day (mid dose) may be the NOAED (discussion on p 46). C_{max} and AUC at this dose were ≈ 200 and 33 times the respective MLHPs. At 10 mg/kg/day: 1) In one animal blood urea increased 57% more than the greatest increase in the control group. 2) Testicular wts in two males were 19-27% lower than the smallest wt in the control group, and these two animals had bilateral mild-moderate testicular atrophy.

12 Month Oral Study (capsules)

0.1 mg/kg/day was the 'no serious adverse effect dose' (there was 5-6% wt reduction vs control (s-ns) in males from month-4; absence of s-s for wt reduction may be due to a small group size; $n=4$). C_{max} and AUC at this dose were $\approx 6-8$ times and 1.3-1.5 times the respective MLHPs (females had somewhat higher values than males, p 48). At 1 mg/kg/day, there was 5-9%, s-ns body wt reduction in females and 6-12% body wt reduction in males (s-s at 12 months). In one male there was bilateral, multi-focal, mild testicular atrophy (vs no lesions of this severity in control group), and testes were 16% lighter than the lightest testes in the control group. (Comments: This animal died on day 168, and another animal died on day 22. These deaths most probably were due to exaggerated pharmacological response to dofetilide (discussion on p 48). C_{max} and AUC at this dose were ≈ 100 and 19 times the respective MLHPs. At 10 mg/kg/day: 1) 3 males had testicular atrophy more severe than in any other animal in this study, and two of these animals had

testes 16-21% lighter than the lightest testes in the control group (p 47). 2) In one male, triglycerides and bilirubin increased much more than the largest increases in control group (p 47).

§4: Reproduction toxicity: (drug was administered by gavage in all studies)

Fertility Study in the Rat

At 1 mg/kg/day (highest dose used in the study), there were no treatment related adverse effects on fertility, and on F0 dams. Reproduction parameters: There were no treatment related adverse effects on any reproduction parameter (p 49). Fetuses (F1): There were: 1) Increased litter incidence of visceral abnormalities (8/10 vs 3/7), and of dilated cerebral ventricles (4/10 vs 0/7). 2) Increased litter incidence of skeletal abnormalities (7/10 vs 3/7). None of the increases are s-s (s-rev). However, lack of s-s may be due to small sample sizes. F1 (pups): There were no adverse effects on any developmental or reproduction parameter (p 50). F2 (pups): There was a s-s increased mortality between day-1 and day-4; this was due to complete loss of one litter (p 51). At 0.25 mg/kg/day, treatment related adverse effects were: 1) s-s increased incidence of delayed ossification of 5th metacarpal in F1 fetuses. 2) s-s increased mortality of F2 pups between day-1 and day-4 (p 51).

NOAED for fertility and reproduction parameters for F0 dams is thus 1 mg/kg/day. Estimated C_{max} and AUC at this dose is \approx 13 and 2.7 times the respective MLHPs (p 51). NOAED for F1 fetuses and F2 pups is 0.05 mg/kg/day. Estimated C_{max} and AUC at this dose would be \approx 0.6 and 0.13 times the respective MLHPs (p 51).

Fetotoxicity Study in the Rat

At 2 mg/kg/day, the highest dose used in the study, there were: 1) Embryo lethality (reduced number of viable fetuses, and increased incidence of post-implantation loss), and reduced fetal wt. 2) Increased incidences of external (8/20 vs 0/19 litters), skeletal (some types), and visceral abnormalities (all s-s). External abnormalities were cleft palate (6/20 litters), adactyly (2/20 litters), and syndactyly, protruding tongue, tail agenesis, and deformed skull (1 litter each). Skeletal abnormalities were seen in all groups, but adactyly (3/20), syndactyly (1/20), and absent pelvic bones (1/20) were seen only in the high dose group. Incidences of deformed vertebrae, and asymmetrical sternaebrae were s-s increased. There were also increased incidences of marked delay in ossification of skull & vertebrae, and non ossification of 5th metacarpal and pubic bones (s-s). Visceral abnormalities: Incidences of dilated cerebral ventricles, levocardia, and hydronephrosis/hydronephrosis were s-s increased (p 52). At 1 mg/kg/day, incidence of non ossification of 5th metacarpals was s-s increased (p 52). 0.5 mg/kg/day was the NOAED.

Estimated C_{max} and AUC at the NOAED would be 25 ng/ml and 100 ng*h/ml (C_{max} estimated from toxicokinetics, p 53; AUC estimated from toxicokinetics, p 40). These parameters would be \approx 6 and 1.3 times the respective MLHPs.

Peri And Post Natal Development Study in the Rat

1 mg/kg/day, the highest dose used in the study, is the NOAED. Estimated C_{max} and AUC at this dose would be 50 ng/ml and 200 ng*h/ml; these parameters would be \approx 13 and 2.7 times the MLHPs.

Fetotoxicity Study in the Mouse

At 5 mg/kg/day, the highest dose used in the study, there was total litter absorption. At 2 mg/kg/day, there were: 1) s-s increase in post implantation loss (both in terms of litters that lost fetuses, and the incidence of fetal loss (p 54). 2) s-s reduced fetal wt. 3) Increased incidences of all skeletal abnormalities, unossified calcaneum, sternebral abnormalities, and vertebral abnormalities (all s-s). Vertebral abnormalities were only present in this group (p 55). 0.5 mg/kg/day was the NOAED. Estimated C_{max} and AUC at this dose (estimates from toxicokinetics on p 55) would be 24.6 ng/ml and 46.7 ng*h/ml. These parameters are \approx 6 and 0.6 times the MLHPs.

§5: Carcinogenicity Studies (attachment I)

Dofetilide was not carcinogenic in either the mouse or the rat. In the rat, at the highest dose used, AUC was ≥ 26 times the maximum likely AUC in the clinical setting (which meets the agency's criterion of $AUC \geq 25$ times the human AUC). The high dose in the rat study was, therefore adequate.

In the mouse, AUC at the high dose (males and females combined) was 724 ng*h/ml. This is ≈ 10 times the maximum likely human AUC. The CAC executive committee (at its meeting of 5/26/98) decided to consider the AUC ratios of estimates of free dofetilide. At the high dose in the mouse, free dofetilide AUC was estimated as 23 times the estimated mean AUC of free dofetilide in man in protocol 115-229, and 16 times the estimated 'mean+1.6*SD' AUC of free dofetilide in man in protocol 115-211 (attachment II, p2).

After perusal of attachment II, the executive CAC decided that the high dose in the mouse carcinogenicity study was adequate.

§6: Genotoxicity & Clastogenicity StudiesAmes test:

5 salmonella typhimurium and one E. Coli strain were used in the test. Dofetilide did not show mutagenic potential in the absence and presence of a metabolic activating system. (p 56).

Note: A mouse lymphoma test, and a clastogenicity test using human lymphocytes, were also conducted, but there are deficiencies in these tests (see discussion on p 57-58). There is also an in-vivo clastogenicity test in the submission which has not been reviewed, as a very small dose (2 mg/kg/day) was used, and therefore no conclusions can be drawn from the negative results of this test. These deficiencies were pointed out to the sponsor, who has agreed to conduct tests to make up the deficiency of the human lymphocyte test, and conduct another mouse bone marrow test at appropriately high doses. These tests will be reviewed when they are submitted.

Discussion and Conclusions:Sub-chronic & Chronic Toxicology Studies

Table below compares estimated/measured C_{max} and AUCs at: NOAEDs, lowest doses at which testicular wt decreases occurred, and lowest doses at which testicular atrophy occurred, in various studies in the 3 species. C_{max} and AUC are given as multiples/fractions of MLHPs.

Study	Mouse		Rat		Dog	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
One month; NOAED	nd	nd	5 (g)	0.87 (g)	240 (cap)	58 (cap)
↓ in testicular wt			47; ↓8%	8; ↓8%	>1000; X ⁿ¹	≈400; X ⁿ¹
Testicular atrophy			300; X	59; X	>1000; X	≈400; X
3 months; NOAED	4 (diet)	2.5 (diet)	39 (diet)	26 (diet)	nd	nd
↓ in testicular wt	15; ↓8%	10; ↓8%	120; ↓28%	80; ↓28%		
Testicular atrophy	60	40	120	80		
6 months; NOAED	nd	nd	9 (g)	1.3 (g)	200 (?) (g)	33 (?) (g)
↓ in testicular wt			70; ↓9%	10; ↓9%	≈1500; ↓23% ⁿ²	≈400; ↓23% ⁿ²
Testicular atrophy			70	10	≈1500	≈400
12 months; NOAED	nd	nd	5 (diet)	4 (diet)	6 (cap)	1.3 (cap)
↓ in testicular wt			15; ↓7%	12; ↓7%	100; ↓16% ⁿ²	19; ↓16% ⁿ²
Testicular atrophy			78	50	100	19
24 months; NOAED	4.5; (diet)	3 (diet)	5 (diet)	4 (diet)	nd	nd
↓ in testicular wt	15; ↓13%	10; ↓13%	39; ↓10%	26; ↓10%		
Testicular atrophy	15; X	10; X	39	26		

Note: (g), is gavage; (cap), is capsules; 'nd' is study not done.. 'X' is absence of an effect; numbers before X are the highest exposure levels at which the effect was not seen. ⁿ¹, cellular debris was seen in epididymal heads; ⁿ², decrease in testicular wt is vs the smallest testicular wt in control. '?', it is not certain that this is the NOAED (p 46).

In the rat and dog, testicular lesions occurred at lower exposure levels as study duration increased (3 month vs 24 month dietary studies in the rat, and one month vs 12 month studies in the dog). Since doses across different study durations in a species were not kept constant, and there are only two studies in the mouse, no conclusions regarding progression of lesions in the mouse is possible. Relative sensitivity of different species for testicular toxicity cannot be assessed from the results of these studies, since doses in different species were not increased by same orders of magnitude, e.g., in the one year dog study, doses were increased by a factor of 10, while in the one year rat study, doses were increased by a factor of ≈ 3 . Therefore, it is not possible to know whether testicular lesions in the dog would have occurred at a lower dose, had such a dose been tested.

Other adverse effects (seen mostly at high doses, e.g. small changes in serum Ca, P_i, bilirubin; and increases in triglycerides, and blood urea) which are described in individual study summaries, are not relevant to drug labeling and use because, by this time, extensive clinical chemistry information is available from human studies.

Reproduction Toxicity Studies

Dofetilide had no adverse effect on fertility in the rat. Estimated C_{max} and AUC at the highest tested dose in the fertility study were 13 and 2.7 times the respective MLHPs. Dofetilide was embryo-lethal and teratogenic in rat and mouse. Estimated C_{max} and AUC at the NOAEDs were ≈ 6 and 1.3 times the respective MLHPs in the rat, and ≈ 6 and 0.6 times the respective MLHPs in the mouse.

Safety Assessment of Inactive Ingredients

Table below shows the composition of dofetilide capsules.

Component	Grade	Function	0.125 mg Capsule (mg/ Unit)	0.25 mg Capsule (mg/ Unit)	0.5 mg Capsule (mg/ Unit)
Dofetilide	Pharm ⁽¹⁾	Active	0.125	0.250	0.500
Microcrystalline Cellulose	NF	Diluent			
Corn Starch	NF(Dried) ⁽²⁾	Diluent/ Disintegrant			
Colloidal Silicon Dioxide	NF	Glidant			
Magnesium Stearate	NF	Lubricant			
# 4 Orange Opaque ⁿ¹	Pharm ⁽¹⁾	Shell			
# 4 Peach Opaque	Pharm ⁽¹⁾	Shell			
# 2 Peach Opaque ⁿ¹	Pharm ⁽¹⁾	Shell			
Fill Weight					
Total Weight					

Note: ⁽¹⁾, Pharmaceutical grade material according to Pfizer specifications; ⁽²⁾, dried to meet a Pfizer specification which requires a water content $\leq 2.5\%$. ⁿ¹, these shells have bodies made of white opaque and caps of material shown.

The shells are gelatin, colored with the ingredients shown. All ingredients other than the dyes used for making capsules, are listed in the agency's list of inactive ingredients, as being used in approved marketed drugs, in quantities \geq those used in dofetilide capsules. The dyes are stated as being pharmaceutical grade by the sponsor. Therefore, the inactive ingredients are not a matter of concern from the point of view of patient safety.

Recommendations

The results of the non clinical studies reviewed and discussed in this review do not contraindicate approval of this NDA.

Labeling:

Redacted

1

pages of trade

secret and/or

confidential

commercial

information

Full Review

Pharmacodynamics

§1: In vitro studies

#1: Effects of dofetilide on Cardiac Cells (From Gwilt et al, J. Pharm & Exp Therapeutics, 256: 318-324, 1991).

Canine cells

- Dofetilide increased APD in ventricular myocytes and Purkinje fibers in a dose related manner in the dose ranges tested (5-1000 nM in the former, and 1-500 nM in the latter). Fig 1 below shows APs of a ventricular myocyte (a) and a Purkinje fiber (b).

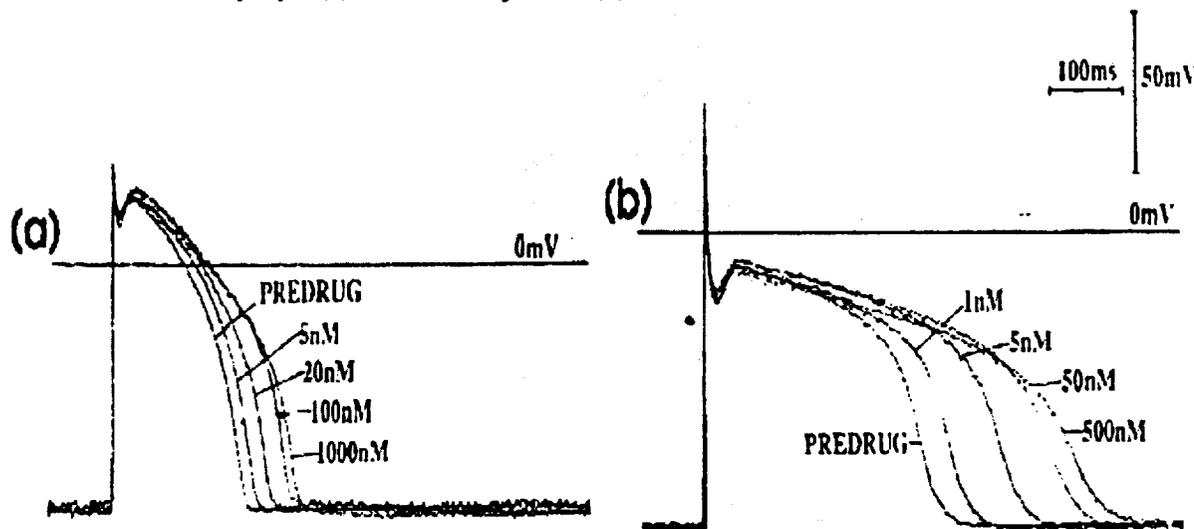


Fig 1

- Table below shows effects of dofetilide on RP, APA, MRD, APD₅₀, APD₉₀, and ERP of ventricular myocytes, and Purkinje fibers; values are mean±SE (n=5).

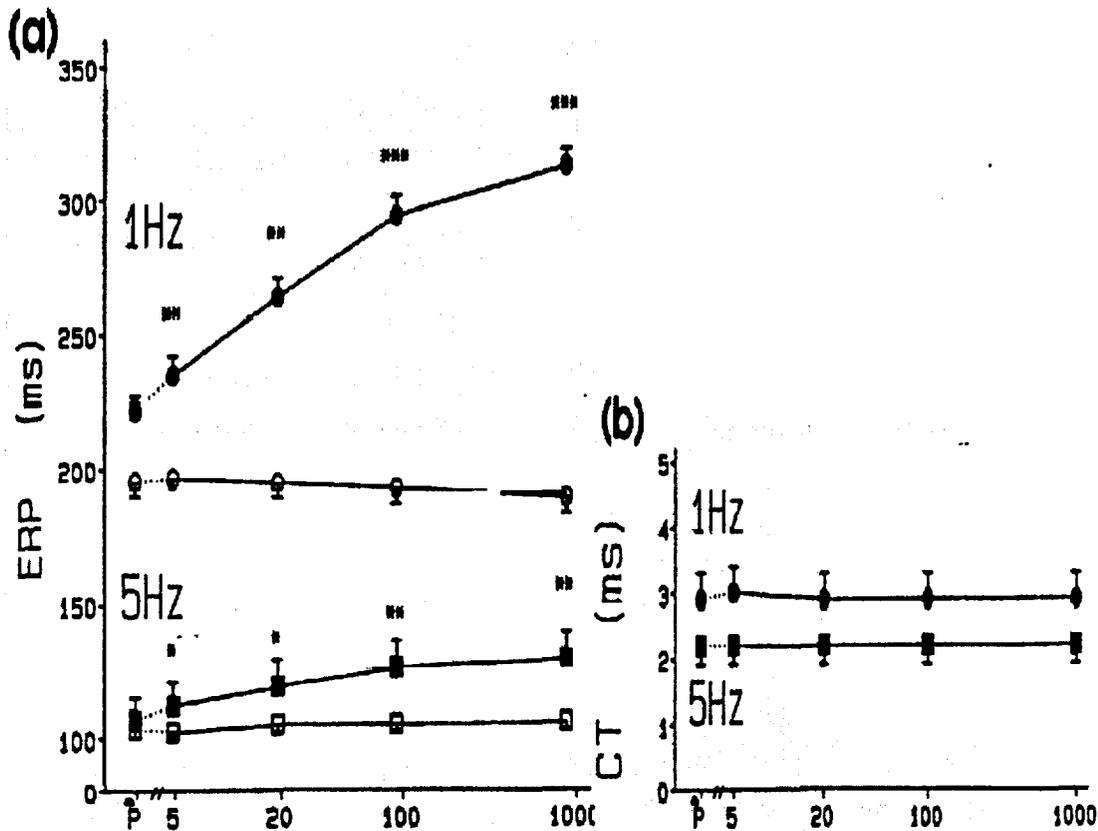
	Ventricular Myocytes					
	RP (mv)	APA (mv)	MRD (v/s)	APD ₅₀ (ms)	APD ₉₀ (ms)	ERP (ms)
Pre-drug	-83±1	106±2	304±24	140±4	183±2	200±4
Dof. 5 nM	-83±1	105±2	294±25	156±3*	207±5**	224±5*
Dof. 20 nM	-83±1	108±2	323±20	170±5*	231±11*	244±10*
Dof. 100 nM	-83±1	104±4	293±28	191±4***	261±17**	272±15**
Dof. 1000 nM	-83±2	107±1	302±20	196±6***	277±21**	286±17**
	Purkinje Fibers					
Pre-drug	-90±1	126±2	587±33	201±12	293±19	299±9
Dof. 1 nM	-90±1	125±2	579±28	215±16*	313±23*	318±11*
Dof. 5 nM	-90±2	126±2	604±39	260±10**	389±22***	394±11***
Dof. 50 nM	-89±2	124±2	608±22	296±15**	500±14***	494±8***
Dof. 500 nM	-89±1	125±1	599±23	302±11***	531±11***	535±7***

Note: * p≤0.05; ** p≤0.01; *** p≤0.001. 'Dof.' Is dofetilide.

Dofetilide had no effect on RP, APA, and MRD, and dose relatedly increased APD and ERP in the dose range tested. Lack of effect on APA and MRD indicates that the drug has no effect on Na channels.

Guinea pig myocytes

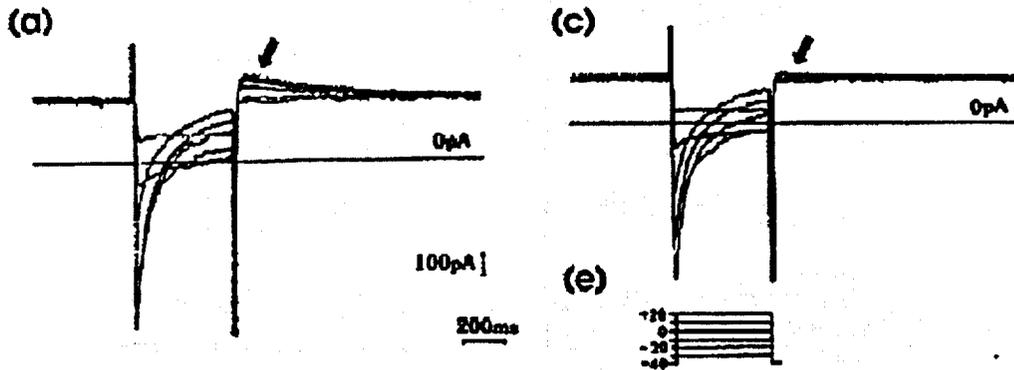
- Fig 2a shows the effect of dofetilide on the ERP of guinea pig myocytes at stimulation rates of 1 Hz and 5 Hz. ERP increased dose relatedly in the dose range tested (5nM-1 μ M). Dofetilide had no effect on ventricular CT at either frequency of stimulation (fig 2b).



Note: Abscissa is dofetilide concentration in nM; p is pre drug. Filled symbols are values with exposure to dofetilide, and hollow symbols those with vehicle. The myocytes were stimulated at 1 Hz and 5 Hz. CT (in b) is conduction time. Values are mean \pm SE from 5 cells. *, p < 0.05; **, p < 0.01

Fig 2

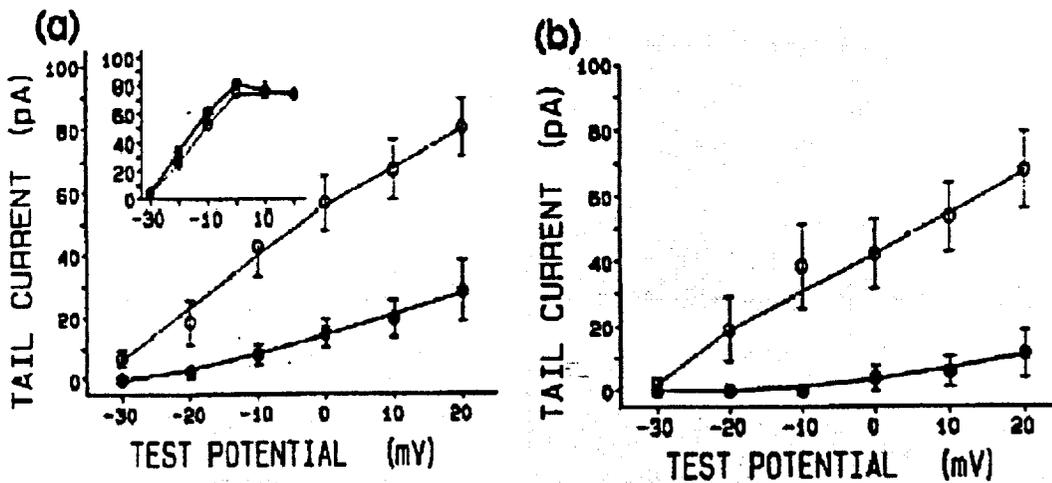
- Voltage clamp study: Holding potentials -40 mv; 500 ms depolarizing pulses. Membrane currents during depolarizing pulses were measured, and effect of dofetilide on time dependent delayed rectifier I_K (the current that underlies the repolarizing phase of the cardiac AP) was studied. Tail currents at the end of a depolarizing pulse are proportional to the magnitude of the delayed rectifier I_K current that flows at the end of the depolarizing pulse. Fig 3a shows tail currents in control condition, and fig 3c shows tail currents in the presence of 50 nM dofetilide. As can be seen, tail currents were reduced in magnitude, showing that dofetilide inhibits the delayed rectifier I_K .



Note: Membrane currents in myocytes. 'a', Pre-drug; 'c', 3-5 minutes after exposure to 50 nM dofetilide. Current and time scales shown in 'a' apply to both 'a' and 'c'; 'e' shows 500 ms depolarizing voltage pulses applied in 10 mv steps from a holding potential of -40 mv. Tail currents after the end of the depolarizing pulses (arrows) give a measure of the magnitude of the time dependent rectifier I_K .

Fig 3

- Voltage clamp study: Fig 4 shows effects of 50 nM (a), and 2 μ M (b) dofetilide on magnitudes of tail currents at the end of depolarizing pulses of various magnitude in guinea pig myocytes. Values are mean \pm SE (a, n=7; b, n=5).

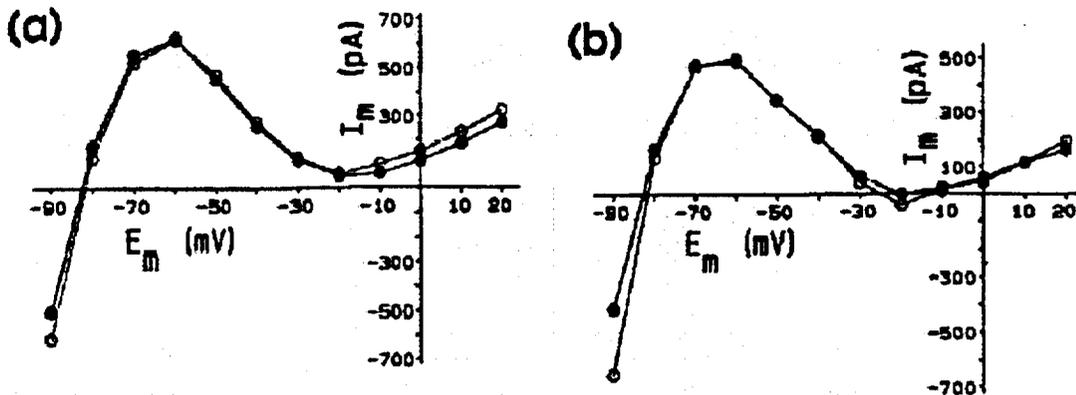


Note: Open circles, pre drug; closed circles, after 3-5 minutes exposure to dofetilide. Inset in 'a', shows mean tail current measurements repeated after a 3-5 minutes time interval (closed circles) but without application of dofetilide.

Fig 4

The inset in fig 4a shows that tail currents remained practically unchanged over a 3-5 minutes' time period. Therefore, reduction of tail currents in 'a' and 'b' is the effect of dofetilide. 2 μ M dofetilide reduces delayed rectifier I_K to a greater extent than 50 nM.

- Voltage clamp study: Fig 5 shows current voltage relationship of I_K s that flow at the end of 500 ms hyperpolarizing and depolarizing pulses over a range of voltage steps.



Note: Hollow symbols, pre drug; filled symbols dofetilide; Dofetilide concentration was 50 nM in '(a)', and 2 μ M in '(b)'. I_m (membrane current) values are means; in '(a)', n=7; in '(b)', n=5.

Fig 5

The current that flows when membrane potential is < -20 mV and $>$ equilibrium potential for K, is time independent K current, I_{K1} . Results depicted in fig 5 show that dofetilide even at 2 μ M concentration has no effect on this current. *Comments*: Inward K current at -90 mV membrane voltage seems to be dose relatedly reduced by dofetilide; the authors do not comment on this. The seeming lack of effect of 2 μ M dofetilide on time dependent I_K at membrane voltages > -20 mV is stated by the authors as due to an outward current shift in one of the cells; the reason for this is not known.

#2: Effects of Dofetilide on Delayed Rectifier I_K , and on I_{Na} and I_{Ca} in Guinea Pig Cardiomyocytes (From Kiehn et al (1994), J. Cardiovas. Pharm 24: 566-572).

Cells were voltage clamped using whole cell patch clamp technique; all experiments were done at room temperature. a) For studies on I_K , I_{Ca} was blocked with 1 mM $CdCl_2$ in the external solution, and Na channels were inactivated by -40 mV holding potential; depolarizing pulses of 20-80 mV magnitude and 200 and 2000 ms were used. At the end of a 200 ms pulse, I_{Kr} is the main current, and at 2000 ms pulse duration, I_{Ks} constitutes the major portion of the current (this is because I_{Ks} is a much larger current). Effect of dofetilide on time constant (τ) of decay of I_{Ks} tail currents was also studied. b) For studies on I_{Na} , holding potential was -160 mV; depolarizing pulse duration 10 ms, and amplitudes going up to 50 mV. Effect on inactivation (h_∞) parameters of Na channels was studied using 'pre-test' pulses of 1000 ms duration and -110 mV to 10 mV potentials, and constant test-pulses of 10 ms duration and 30 mV potential. Pre-test pulse potentials were then plotted against normalized peak currents elicited by the test pulses, and h_∞ parameters calculated. c) For studies on I_{Ca} , holding potential was -40 mV, and depolarizing pulse duration was 500 ms.

Results

- I_K : Table below shows the effects of different concentrations of dofetilide on tail current amplitudes of delayed rectifier I_{Kr} and I_{Ks} , and on τ of decay of I_{Ks} , during depolarizing pulses of 80 mV amplitude; values are mean \pm SD; numbers within () are no of cells studied.

Parameters (% of control)	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
I_{Kr} amplitude (pulse, 200 ms)	106 \pm 26 (6)	61 \pm 20 (4)	41 \pm 10 (6)	35 \pm 9 (8)
I_{Ks} amplitude (pulse, 2s)	104 \pm 8 (7)	100 \pm 13 (6)	113 \pm 24 (7)	71 \pm 19 (7)
τ , I_{Ks} (% τ/τ_0)	117 \pm 34 (7)	229 \pm 94 (6)	271 \pm 137 (6)	238 \pm 152 (6)

I_{Kr} was reduced dose relatedly at concentrations $\geq 1 \mu\text{M}$; I_{Ks} was only reduced at 10^{-4} M . However, τ of decay of I_{Ks} was prolonged at $\geq 1 \mu\text{M}$.

- I_{Na} : Effect of 10^{-4} M dofetilide:
 - a) Peak I_{Na} amplitude: $101 \pm 11\%$ of control (n=7)
 - b) $h-\infty$ curves in one cell are shown in fig 6 below; 'a' is control, and 'b' is after exposure to 10^{-4} M dofetilide; U_h is the membrane potential at which I is 50%; V_h is 1/slope at membrane potential= U_h

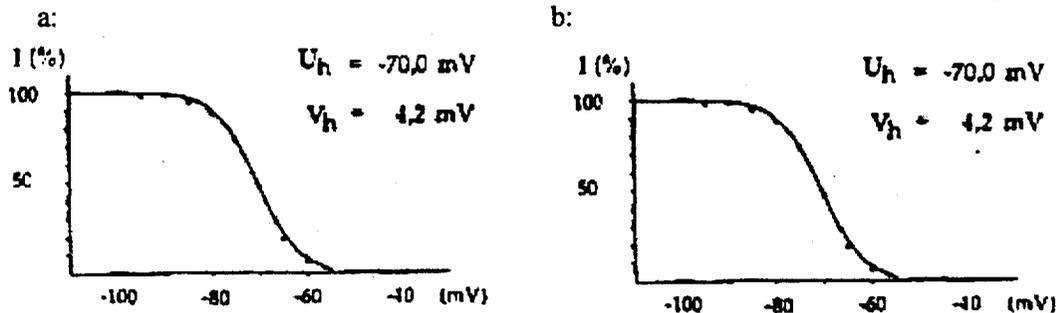


Fig 6

$h-\infty$ parameter values (mean \pm SD) in all cells tested were:

	U_h (mV)	V_h (mV)
Control (n=7)	-68.1 \pm 3.1	3.82 \pm 0.54
Dofetilide (n=7)	-71.7 \pm 3.9	4.01 \pm 0.35

- I_{Ca} : Peak I_{Ca} amplitude after exposure to 10^{-4} M dofetilide was $97 \pm 16\%$ of the control amplitude.

Discussion: 10^{-4} M dofetilide did not have any effect on peak I_{Na} , and inactivation parameters of Na channels. The current that is labeled I_{Ca} in this study would actually be the net inward current, ' I_{Ca} (inward) + I_{Kr} (outward)'. Since 10^{-4} M dofetilide would reduce I_{Kr} to a considerable extent (table, previous page), if dofetilide does not block Ca channel, theoretically the net inward current should increase compared to pre-drug value. However, this was not the case. The authors do not provide any explanation of this finding. Therefore, this study does not establish that dofetilide has no effect on I_{Ca} in cardiac myocytes.

#3: Effects of Extra Cellular K^+ Concentration on the Electrophysiological Effects of Dofetilide on Guinea Pig Papillary Myocytes (From: Yang et al, Cardiovascular Drugs and therapy 1992: 6: 429-436).

Results

- Table below shows the effects of 10 nM dofetilide on RP, APA, MRD, APD_{90} , ERP, ERP/APD_{90} , ' $ERP-APD_{90}$ ' at 4 mM and 10 mM extra cellular K^+ concentrations. Cells were paced at 1 Hz. Values are mean \pm SE (n=6).

	4 mM $[K^+]_o$		10 mM $[K^+]_o$	
	Control	10 nM Dofetilide	Control	10 nM Dofetilide
RP (mV)	-87 \pm 2	-86 \pm 2	-66 \pm 2 ^a	-65 \pm 1 ^a
APA (mV)	115 \pm 2	117 \pm 2	98 \pm 2 ^a	96 \pm 2 ^a
MRD (V/s)	168 \pm 10	170 \pm 10	114 \pm 12 ^a	109 \pm 14 ^a
APD_{90} (ms)	230 \pm 9	276 \pm ^b	215 \pm 6 ^a	252 \pm 7 ^{a,b}
ERP (ms)	242 \pm 6	294 \pm ^b	313 \pm 15 ^a	336 \pm 13 ^{a,b}
ERP/APD_{90}	1.05 \pm 0.02	1.06 \pm 0.03	1.46 \pm 0.11 ^a	1.35 \pm 0.12 ^{a,b}
$ERP-APD_{90}$ ^c	12 \pm 4	18 \pm 4	98 \pm 20 ^a	84 \pm 23 ^{a,b}

Note: ^a, $p < 0.05$ v corresponding values at 4 mM $[K^+]_o$; ^b, $p < 0.05$ compared to the control value at each $[K^+]_o$; ^c, post repolarization refractoriness.

- Effects of dofetilide on APD_{90} at different cycle lengths are shown in fig 7 below; fig 7a shows the effects at 4mM $[K^+]_o$ and fig 7b those at 10 mM $[K^+]_o$. (Fig 7 is constructed from Table 1 in the paper).

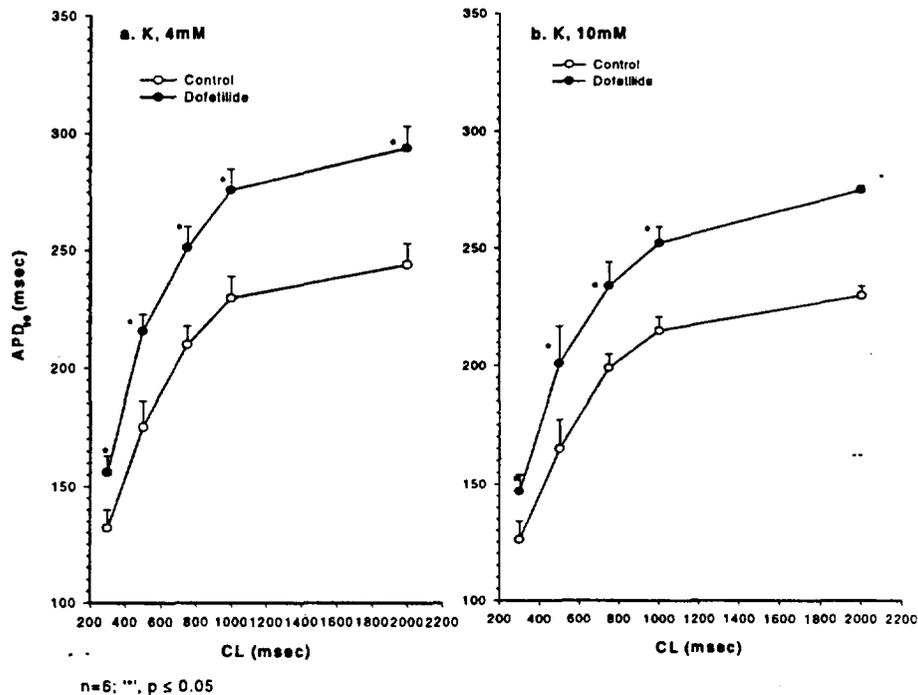


Fig. 7

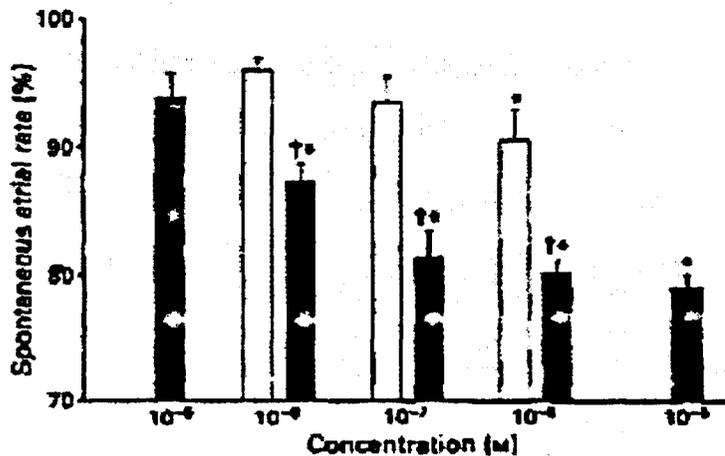
Comments: 10 nM Dofetilide increased APD_{90} s-s at all cycle lengths tested (highest pacing rate =200 bpm.) at both $[K]_o$ concentrations. ΔAPD_{90} at 10 mM $[K]_o$ seems smaller than at 4 mM $[K]_o$.

#4: Negative Chronotropic Effect of Dofetilide on Isolated Guinea Pig Atria (From Yang et al, Br J. Pharmacol (1991), 103: 1417-1420).

Effects of various concentrations of dofetilide and propranolol (a β antagonist) on spontaneous beating rates (determined from isometric contractions) of isolated guinea pig right atria and on the concentration-response (CR) curves of isoprenaline were studied.

Results

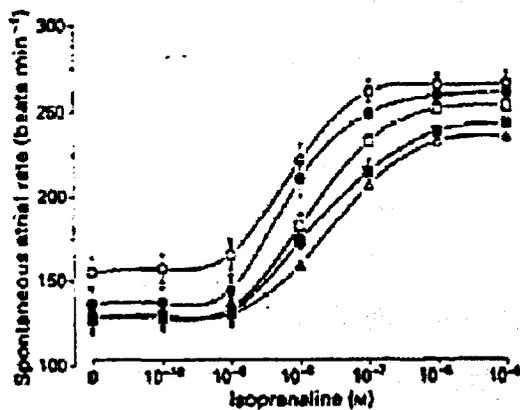
- Base line spontaneous atrial rates (SARs) were 157 ± 5 bpm (n=15). Fig 8 (next page) shows the effects of dofetilide and propranolol on SARs. Dofetilide reduced SAR dose relatedly and s-s in the dose range 10 nM-10 μ M. Propranolol reduced SAR to a much smaller extent; effect was s-s only at 1 μ M, highest concentration used.



Note: Filled columns, dofetilide; hollow columns, propranolol. n=5 for 1 nM dofetilide and all concentrations of propranolol; n=7 for all other concentrations of dofetilide. '†', p<0.05 v propranolol; '*', p<0.05 v control values.

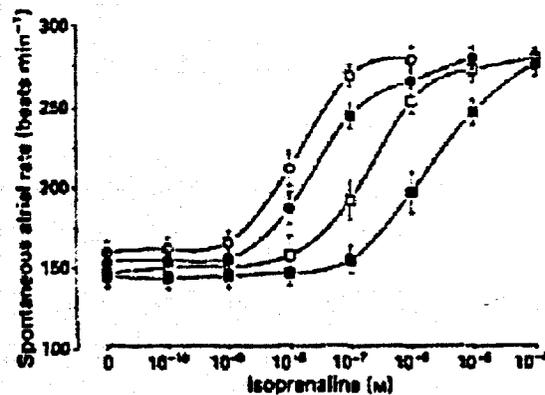
Fig 8

- Figs 9 and 10 (below) show the effects of dofetilide and propranolol respectively on CR curves of isoprenaline in the isolated right atria.



CR curves for isoprenaline in the absence and presence of increasing concentrations of dofetilide: (○) control (n = 14); (●) 10 nM, and (□) 100 nM (n = 7); (■) 1 μM, and (Δ) 10 μM (n = 7). Values are mean with SE (indicated by vertical bars).

Fig. 9



CR curves for isoprenaline in the absence and presence of increasing concentrations of propranolol: (○) control; (●) 10 nM; (□) 100 nM, and (■) 1 μM (n = 5). Values are mean with SE (indicated by vertical bars).

Fig. 10

- Table (next page) shows effects of the two drugs on pD₂ (-log EC₅₀) and E_{max} of isoprenaline (parameters calculated from the data depicted in figs 9 & 10). As seen in the table, there is a small s-n reduction in pD₂ of isoprenaline at all doses of dofetilide, but the decrease is not dose related. E_{max}, however is reduced dose relatedly, and s-s at all except 10 nM concentration.

Concentration	PD ₂		Emax (%)	
	Dofetilide	Propranolol	Dofetilide	Propranolol
Control	8.13±0.08; 8.18±0.08	8.0±0.12 (n=5)	100 (n=14)	100 (n=5)
10 nM	7.82±0.08(n=7)	7.51±0.12* (n=5)	92±3 (n=7)	99±2 (n=5)
100 nM	7.84±0.11(n=7)	6.82±0.2* (n=5)	86±4* (n=7)	98±1 (n=5)
1 μM	7.91±0.08(n=7)	5.93±0.12* (n=5)	78 ±7* (n=7)	97±2 (n=5)
10 μM	7.89±0.1 (n=7)		71 ±6* (n=7)	

Note: Effects of dofetilide were tested in two sets of 7 atria each; effects of 10 and 100 nM were tested in set-1 (control, C1); and of 1 and 10 μM in set-2 (control, C2); pD₂ control values for dofetilide are shown as C1; C2. All values are mean±SE. *, p≤0.05 v control.

Discussion: The results described above show that the effects of dofetilide on SAR are not due a β blocking action. Dofetilide thus seems to act directly on the SA node.

#5: Effects of Dofetilide on Isolated Rabbit SA Node Cells (From Tohse and Kanno (1995), 69: 303-309).

Whole cell patch clamp studies: Depolarizing pulses of 300 ms duration were applied at the rate of 0.1 Hz from a holding potential of -40 mv, and effects of 10 nM-1 μM dofetilide on tail currents (the amplitude of which is proportional to the magnitude of the delayed rectifier I_K) were studied. Using current clamp, effect of dofetilide on APs of spontaneously active SA node cells was also studied.

Results

- 100 nM and 1 μM dofetilide dose relatedly and s-s reduced the amplitude of tail currents after depolarizing pulses of 40 mv amplitude; 10 nM had no effect on the amplitude of tail currents. 1 μM dofetilide reduced tail current amplitudes by 54-59% at all test pulse amplitudes in the range 20-70 mv (n=5); decrease was 69% at 80 mv depolarization (n=3; larger SE). V-I relationship for tail currents was quite linear before as well as after exposure to dofetilide.
- In spontaneously active cells (n=3), 1 μM dofetilide reduced SA rate, and shifted the maximum diastolic potential (MDP) in the depolarizing direction by ≈ 11 mV (MDP: Control, -43.8±3.2 mv; dofetilide, -32.5±0.7 mv). *Comments:* The authors state that dofetilide also reduced the slope of the pacemaker potential, but this is not obvious from the records presented for one cell, and slope magnitudes (mean±SE) are not given.

#6: Effect of dofetilide on Guinea Pig Papillary Muscle Contraction (From Tande et al, J. Cardiovascular Pharmacology (1990), 16: 401-410).

Guinea pig papillary muscle preparations were stimulated at different rates, and force of contraction recorded; force of contraction records were differentiated to determine maximum dF/dt. Effects of 10 and 100 nM dofetilide on these two parameters were studied.

Results: Table below shows the results. (Table has been constructed from a fig in the paper by the sponsor) Values are mean±SE (n=8); 'CL' is cycle length.

Dofetilide Concentration	Peak force (mg)		Time to peak (ms)		dF/dt _{max} (gm/sec)	
	CL, 2000 ms	CL, 500 ms	CL, 2000 ms	CL, 500 ms	CL, 2000 ms	CL, 500 ms
Pre dose	102±16	211±32	118±8	103±5	17±3	39±7
10 nM	111±19	238±36*	115±4	94±3	18±4	44±8*
100 nM	109±19	235±34*	105±5*	89±3*	18±4	44±8*

s-s changes in force of contraction (increase), dF/dt_{max} (increase) and time to peak (decrease) only occurred at the 500 ms cycle length. Changes in peak force, time to peak, and dF/dt_{max}, at 1000 ms CL (not shown in the table) were similar in magnitude to those at 500 ms.

§2: In-vivo Studies

#1: Effects of Dofetilide in Anesthetized Dogs (From Gwilt et al, European Journal of Pharmacology (1992), 215: 137-144).

In groups of dogs (n=5), anesthetized with pentobarbitone and artificially ventilated, effects of dofetilide, and some other antiarrhythmic drugs on VERP, HR, dp/dt_{max}, arterial blood pressure (systolic pressure (SP) and diastolic pressure (DP)), and were studied. During measurements of VERP, hearts were paced at 200 bpm. Drugs were administered i/v cumulatively every 35 minutes, and measurements were made 10, 20, and 30 minutes post dose. Effect of time on various measurements was studied in a group of dogs that were administered saline at the same time points at which drugs were administered. Dose range of dofetilide studies was 1-100 µg/kg.

To study the effect of dofetilide on ventricular fibrillation threshold (VFT), SA node was crushed, and hearts were paced at 150 bpm. VF was initiated using 60 Hz trains of stimuli applied immediately after the R wave of the EKG; train duration was set to be 50 ms > than the duration required to elicit 3 ventricular ectopic beats at the beginning of the experiment. Trains of stimuli of increasing intensity were applied every 12th beat till VF was initiated; pacing rhythm was then reestablished; arterial pressure of each animal had recovered within 5 minutes of the fibrillation-defibrillation cycles.

Results

- In saline treated dogs, VERP remained within 1% of baseline values, and HR within 3% of baseline values, throughout the experimental period. SBP, DBP, and dp/dt_{max} decreased slightly over time in the saline group at the end of the experimental period. Except for 100 µg/kg dofetilide dose, which caused a slightly > decrease in DBP(= -15 mm Hg v ≈-10 mm Hg in saline group; s-ns), decreases in arterial pressure and dp/dt_{max} were ≤ those in the saline group. Figures 11a and 11b show the effects of dofetilide, and the other antiarrhythmic agents studied, on VERP and HR.

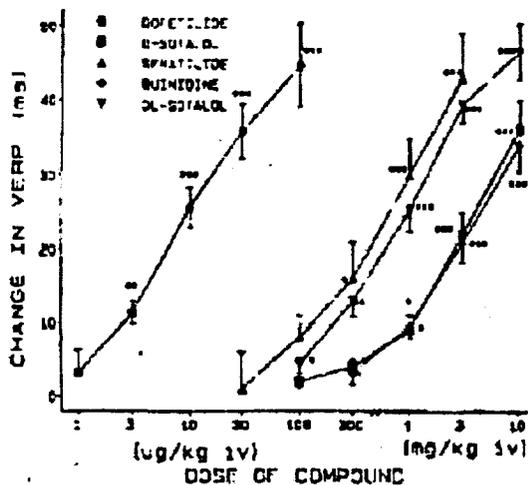


Fig 11a

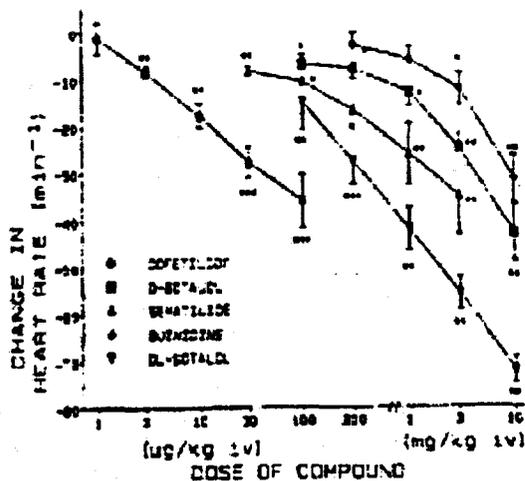


Fig 11b

Note: Changes in VERP and HR are from baseline values. Values are mean ±SE; *, p<0.05; ***, p<0.01; ****, p< 0.001; n=5 in each group. Baseline VERP was 151±4 ms, and HR, 148±8 bpm.

- Baseline VFTs were 3.5 ± 0.2 mA for the dofetilide group and 3.0 ± 0.3 mA for the saline group. During the course of the experimental period, VFT of the saline group did not change by more than 23% (so the maximum increase in mean VFT in this group would be ≈ 0.75 mA). Fig 12 shows the effects of various drugs on VFT.

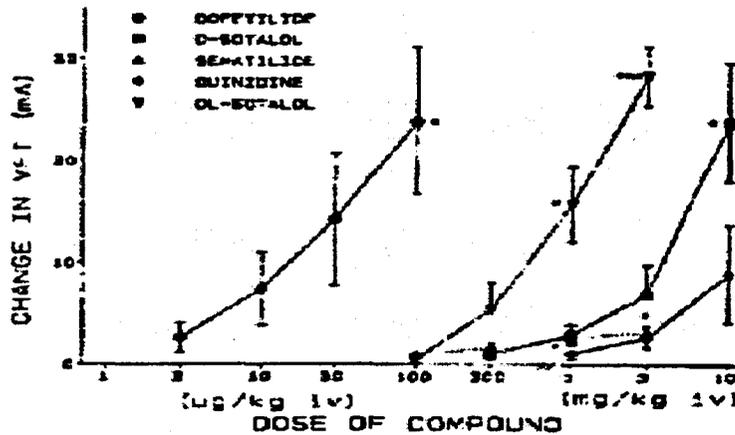


Fig 12

Dofetilide caused a dose related increase in VFT, but the increase from baseline was s-s only at 100 $\mu\text{g}/\text{kg}$ dose. *Note:* According to the authors, saline group animals always required defibrillation, whereas in all drug groups, induced tachyarrhythmia in some animals was flutter, which frequently reverted to the paced rhythm.

#2: Electrophysiological Effects of Dofetilide and quinidine in Experimental Canine Atrial Flutter (From Cha et al, J. Cardiovasc Electrophysiol (1996), 7: 809-827).

In 15 pentobarbital anesthetized, artificially ventilated dogs, a 2-3 cm long crush injury was produced on the posterior free wall of the right atrium. The crush injury was located ≈ 1.5 cm above the AV groove. A plaque containing an array of 8x8 bipolar electrodes, spaced 3 mm apart was sutured to the right atrial wall over the crush injury such that half the elements of the array were above and half below the crush injury. Atrial flutter (AF) could be induced by programmed electrical stimulation or rapid atrial pacing. Baseline ERPs were determined at each bipolar electrode of the array at pacing CLs of 200 ms (multi channel simultaneous recordings of electrical activity were done); conduction velocity of the electrical impulse in the atrial muscle could be calculated from the records. Following baseline measurements, sustained AF (defined as lasting > 10 minutes) was induced by programmed electrical stimulation or rapid atrial pacing at 150, 120, or 100 ms CLs for 10-20 beats (Sustained AF was inducible in all animals). 7 dogs were administered i/v dofetilide, and 8, i/v quinidine. Loading doses were 25 $\mu\text{g}/\text{kg}$ dofetilide, and 10 mg/kg quinidine administered over 10 minutes. Maintenance doses were 9 $\mu\text{g}/\text{kg}$ dofetilide and 3 mg/kg quinidine administered over 60 minutes. Plasma drug levels were determined approximately 30 minutes after starting maintenance infusion.

After termination of AF, multi channel ERP measurements were done, and an attempt was made to induce AF again in animals in which dofetilide terminated AF. Mean and SD of ERPs from all recording sites in the electrode plaque were calculated for each animal; SD was used as a measure of dispersion of ERP (ERP-disp). Number of pairs of electrodes for which ERPs differed by ≥ 20 ms was calculated as another measure of ERP dispersion (Elec-disp). An antiarrhythmic score was assigned to each animal in accordance with the following scheme, and mean \pm SD calculated for the group.

AF Terminated	Yes	Yes	Yes	No	No
AF Re-induced	No	Non Sustained	Sustained	Non sustained	Sustained
Score	5	4	3	2	1

Results

- Effects of the two drugs on various electrophysiological parameters are shown in the table below.

Parameter	Dofetilide (n=7)			Quinidine (n=8)		
	Baseline	Post-drug	P	Baseline	Post-drug	P
ERP (ms)	115±9	141±10 ^{***}	<0.001	112±11	128±15 ^{***}	<0.001
ERP _{max} (ms)	131±4	157±9 ^{***}	<0.001	134±5	151±11 ^{***}	<0.001
ERP-disp (ms)	8.4±1.2	6.1±0.8 ^{**}	<0.003	9.9±2.3	10.0±1.3	NS
Elec-disp	12±4.8	2.9±1.3 ^{***}	0.001	14.5±10.4	12.5±7.9	NS
AFCL (ms)	128±14	175±25 ^{**}	0.003	127±10	199±34 ^{***}	<0.001
AF terminated		7/7 ^q			3/8	
AF re-induced ^{nl}		0/7 ^q			5/8	
Antiarrhythmic score		4.86±0.38 ^{***q}			2.13±1.36	

Note: Values are mean±SD. ^{*}, p≤0.05; ^{**}, p≤0.01; ^{***}, p≤0.001; ^q, s-s v quinidine; all other significance levels are v baseline values. ^{nl}, only re-induction of sustained AF seems to have been considered as re-induction; otherwise, antiarrhythmic score in the dofetilide group would have been 5.0.

- In the dofetilide group AF was terminated at 3.5±1 minute of loading infusion; in the quinidine group, at 4.6±2.9 minutes of loading infusion. Serum levels of dofetilide were 9.6±2.7 ng/ml, and of quinidine, 2.8±0.08 µg/ml.
- The authors state that there was a s-s correlations between antiarrhythmic score and ERP-dispersion, and antiarrhythmic score and Elec-disp (p=0.01 and 0.012 respectively) but not between antiarrhythmic score and ΔERP.

#3: Effect of Dofetilide on the Incidence of VF during Acute Myocardial Ischemia in Anesthetized Pigs (From Andersen et al, Cardiovascular Research (1994), 28: 1635-1640).

In anesthetized, artificially ventilated pigs, PTCA catheters were positioned in the LAD coronary arteries, and EKGs were recorded continuously. At time t₀, EKGs were recorded during a short period of atrial pacing at 100/min and 120/min; 25 µg/kg dofetilide was then administered in 10 minutes followed by 12.5 µg/kg/hr infusion for 130 minutes (n=16). Similar volumes of vehicle were administered to another group of 16 pigs. 20 minutes after start of infusion (t₂₀), paced EKGs were repeated; LAD arteries were then completely occluded, and occlusions maintained throughout the experiment. If VF occurred, defibrillation was attempted.; experimenters were blinded to the administered agent (drug/vehicle); blood for plasma drug levels was withdrawn at 10, and 20 minutes after start of infusion and every 20 minutes thereafter.

Results:

- Table below (and continued on next page) shows EKG parameters before (t₀) and 20 minutes after start of infusion (t₂₀); QRS showed practically no change over time, and is not shown in the table.

	Dofetilide		Vehicle	
	t ₀	t ₂₀	t ₀	t ₂₀
Sinus rhythm				
RR interval (ms)	854±258	956±258 ^b	800±307	831±210
PQ interval (ms)	153±18	158±25 ^{sv}	141±19	143±14
QT _c interval (ms)	382±47	441±64 ^{sb, sv}	389±34	393±32

Table (Continued from previous page).

	Dofetilide		Vehicle	
	t ₀	t ₂₀	t ₀	t ₂₀
Atrial pacing 100/min				
PQ interval (ms)	178±27	184±25 ^{ab, *v}	171±28	175±23
QT interval (ms)	333±36	412±38 ^{ab, **v}	330±31	341±32 ^{ab}
Atrial pacing 120/min				
PQ interval (ms)	189±21 ^{*v}	195±21 ^{*v}	173±26	178±18
QT interval (ms)	317±29	381±27 ^{ab, **v}	311±24	321±22 ^{ab}

Note: ^{*}, p<0.05; ^{**}, p<0.01; ^{ab} v baseline; ^v, v vehicle at the same time point.

Except for a 10 ms (but s-s) increase in QT during atrial pacing, there were no changes in EKG parameters over time in the vehicle group. In the dofetilide group, mean HR decreased by ≈7 bpm (s-s); QT increased as expected, and PQ increased by a small (s-s) amount.

- 6/16 dofetilide v 13/16 vehicle group pigs developed VF after occlusion of LAD (p=0.014, Fishers exact test, s-rev); all VFs developed within one hour of arterial occlusion, Median time to fibrillation was 14 minutes in dofetilide group and 7 minutes in the vehicle group (s-ns). 5/6 fibrillating animals in dofetilide group and 6/13 in the vehicle group were successfully defibrillated (s-ns); median no of shocks required for defibrillation was 7 in the dofetilide and 9 in the placebo group-(s-ns).

LAD was completely occluded in all animals (angiographic evidence), and the magnitude of ST segment elevations during ischemia in the two groups was very similar. Therefore, the difference in the incidence of VF is not due to greater degree of ischemia in the vehicle group, but is a drug effect.

- Mean dofetilide plasma concentration was ≈ 83 ng/ml at 10 minutes, ≈37 ng/ml at 20 minutes, and 20-25 ng/ml for the rest of the experimental period. Dofetilide concentration during coronary ischemia was thus 20-25 ng/ml.

#4: Effects of Dofetilide and Quinidine on Pacing induced Heterogeneity of Repolarization in Ventricles of Anesthetized Dogs (From Gwilt et al, Cardiovascular Research (1992), 26: 1102-1108).

In anesthetized, artificially ventilated dogs, hearts were exposed and epicardial electrograms were recorded simultaneously from several (26-31) sites on the ventricular surfaces during cardiac pacing; electrograms were differentiated, and the differentiated signals displayed along with the electrograms. The following electrical parameters were calculated: Activation time (AT, interval between stimulus artifact and the largest negative differential during the QRS complex; AT is a measure of conduction time); repolarization time (RT, interval between stimulus artifact and the largest positive differential during the T wave).

Median values of AT and RT (from all recording sites) were averaged for all animals in a group to obtain group mean ±SE. It is stated that 'inter-quartile' ranges of AT and RT were used as measures of heterogeneity of activation and repolarization respectively, and inter-quartile ranges from individual animals in a group were averaged to determine mean±SE of dispersion for the group. Electrograms were recorded at the shortest cycle lengths at which the ventricles were paced, since it was found that heterogeneity of repolarization increased as cycle lengths decreased. After baseline measurements, cumulative doses of drugs or saline were administered i/v to different groups of animals; dofetilide group received 3, 10, 30, and 100 μg/kg dofetilide; quinidine group, 1, 3, and 10 mg/kg quinidine (the high doses of dofetilide and quinidine were chosen to increase APD, as measured by activation-repolarization interval, by approximately the same amount). Electrograms as described above were recorded after each dose of a drug/vehicle.

Results

- Conduction time (as measured by AT) in the dofetilide group remained practically unchanged during the course of the experiment; in the quinidine group it increased dose relatedly at doses of 3 and 10 mg/kg ($p < 0.01$, and < 0.001 respectively v saline group) indicating dose related reduction of conduction velocity.
- Activation-repolarization interval (measure of APD), as expected, increased dose relatedly in both drug groups (s-s v saline group, at dofetilide doses $\geq 10 \mu\text{g}/\text{kg}$, and at all quinidine doses)
- Heterogeneity of repolarization time: Fig 13 below shows the values of this parameter in different groups.

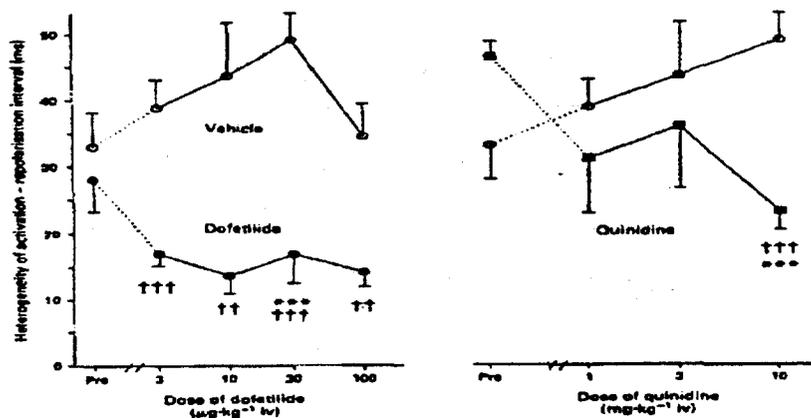


Fig 13

Comments: Values are mean \pm SE; the authors' description of the two symbols, '*' and '†' is incomprehensible.

Discussion

There are several deficiencies in this paper which make it difficult to accept that the authors have demonstrated that dofetilide reduces the heterogeneity of depolarization of ventricles in the anesthetized dog. These deficiencies are: a) It is not possible to determine what precisely the authors used as a measure of dispersion of RT. The authors state that inter-quartile range was used, but there are 4 quartiles in any data set, and the authors do not discuss which inter-quartile range was used and the reasons for using it. (It seems reasonable to assume that the distribution of RTs over the recording sites was not symmetrical around the mean RT; had it been more or less symmetrical, the authors would have used SD of RT as a measure of its dispersion.) b) Assuming the validity of the measure of dispersion used in this study, nearly maximum reduction of dispersion (from baseline value) occurred at 3 $\mu\text{g}/\text{kg}$ dofetilide dose, whereas increase in activation-repolarization interval at this dose was very small and s-ns.

#5: Electrophysiological Effects of Dofetilide in Conscious Dogs

In chronically instrumented dogs, effects of single ($n=3$) and repeated ($n=4$) oral doses of dofetilide on atrial and ventricular refractory periods and EKG were studied (refractory periods measured at sinus rhythm are called functional refractory periods, AFRP and VFRP). For the repeat dose study, the dogs were administered vehicle for 3 days, 50 $\mu\text{g}/\text{kg}/\text{day}$ dofetilide for 8 days, and 100 $\mu\text{g}/\text{kg}/\text{day}$ dofetilide for 3 days. Pre-dose measurements were done every day, and post-dose measurements on days 1, 4, 6, 8, 11, 12, and 14.

Results

- Single dose: Oral doses of 25 and 100 $\mu\text{g}/\text{kg}$ increased AFRP and VFRP dose relatedly as shown below. Values are mean \pm SE (n=3)

	AFRP (ms)		VFRP (ms)	
	Pre-dose	Maximum Post-dose	Pre-dose	Maximum Post-dose
Vehicle	131 \pm 13	135 \pm 25	183 \pm 4	183 \pm 5
Dofetilide 25 $\mu\text{g}/\text{kg}$	138 \pm 12	160 \pm 15 (Δ , 22ms)	186 \pm 2	208 \pm 2** (Δ , 22 ms)
Dofetilide 100 $\mu\text{g}/\text{kg}$	134 \pm 11	188 \pm 13* (Δ , 54ms)	187 \pm 4	226 \pm 3** (Δ , 39ms)

Note: post-dose v pre-dose, **, $p \leq 0.05$; ***, $p \leq 0.01$; s-rev. Maximum change after dofetilide occurred 2 hours post dose. s-s may actually be > than shown here, since the proper test is paired t-test, but sponsor has not provided individual animal data to do the paired test.

- Repeat dose: Pre-dose values of VFRP in the repeat dose study did not change with time. Post-dose VFRP on day-4 was 171 \pm 4 ms (mean Δ =20 ms), and was about the same on day-11. Post-dose VFRP at 100 $\mu\text{g}/\text{kg}/\text{day}$ was not > than that at 50 $\mu\text{g}/\text{kg}/\text{day}$ dose.

Comments: In the single dose study, post-dose VFRP at 100 $\mu\text{g}/\text{kg}/\text{day}$ was > than at 25 $\mu\text{g}/\text{kg}/\text{day}$ (s-s, $p=0.007$, s-rev). In this study, Δ VFRP at both doses was \approx to that at 25 $\mu\text{g}/\text{kg}$ in the single dose study. The sponsor does not offer any explanation for this difference.

- At 100 $\mu\text{g}/\text{kg}$ dose, sinus arrhythmia was accentuated. Occasionally there was A-V block during the faster HRs during inspiration.

#6: Alterations of the Effects of Dofetilide by Chronic Baseline HRs

In 12 dogs with surgically induced AV block and an implanted pacemaker, the propensity of dofetilide to induce polymorphic ventricular tachycardia (PVT) was studied. For 3 weeks, HR was maintained at 120 bpm, except during the acute experiments during which effects of different doses of dofetilide on QT intervals and occurrences of PVTs were studied under isoflurane anesthesia. During the following 3 weeks, HR was maintained at 40 bpm, and the acute experiments repeated. The first phase is called pre-bradycardia (pre-brad), and the second, chronic-bradycardia (ch-brad). The acute experiments consisted of studying the effect of different doses of dofetilide on QT interval during pacing rates of 120, 80, and 50 bpm. If PVT occurred, cardioversion was done and the experiment terminated. Comments: Details of experimental procedures regarding data handling are very poorly described, making it impossible to evaluate the results, particularly PVT incidence results. In-vitro experiments: In papillary muscle preparations from 4 normal dogs, and 6-7 dogs at the end of the chronic bradycardia phase, effects of 1-1000 nM dofetilide on APD_{50s} (measured at pacing rates of 40/min) were studied.

Results

In-vivo tests

- Fig 14a (next page) shows mean QT values in the pre-brad and ch-brad phases of the study, during cardiac pacing at different rates; at each pacing rate, QT during the ch-brad phase was > than that in the pre-brad phase. Fig 14b shows changes in QT in response to 2-20 $\mu\text{g}/\text{kg}$ cumulative i/v doses of dofetilide. In both phases of the study, Δ QT at all doses of dofetilide was > at lower HRs, and except for the lowest pacing rate and the highest dofetilide dose, Δ QT during the ch-brad phase was > than that during the pre-brad phase. (Fig 14 is constructed from a tables of mean QT values in the submission.) Comments: Since the number of animals that contributed to each data point and the SEs of means are not known, the s-s of these findings cannot be determined.

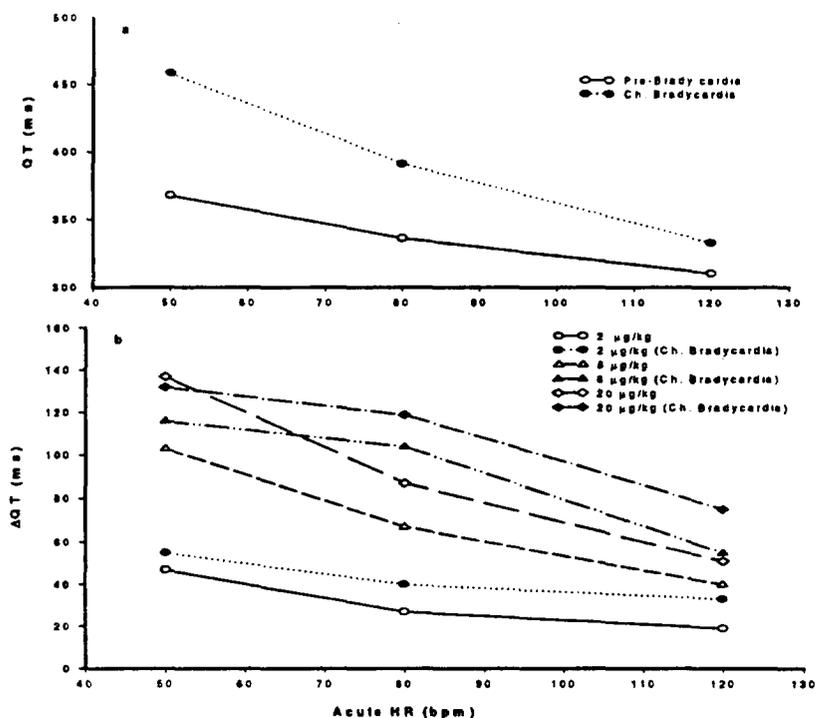


Fig 14

- The sponsor makes a statement that PVTs did not occur at any dose of dofetilide at pacing rates ≥ 80 bpm. *Comments:* It is not possible to evaluate the relationship between likelihood of occurrence of PVTs and any of the parameters because the sponsor has not provided enough information.

In-vitro tests

- Effects of dofetilide on APD_{50} in the two groups are shown in the table below.

Dofetilide Concentration nM	APD ₅₀ (ms)		Mean Δ APD ₅₀ (ms)	
	Normal	Ch. Bradycardia	Normal	Ch. Bradycardia
Pre-Drug	210 \pm 5.6 (4)	240 \pm 19.4 (6)		
1	219 \pm 5.2 (4)	257 \pm 20.7 (6)	9	17
10	226 \pm 6.8 (4)	270 \pm 23.6 (5)	16	30
100	240 \pm 7.7 (4)	305 \pm 22.3* (5)	30	65
1000	247 \pm 13.3 (4)	330 \pm 22.5* (5)	37	90

Note: Numbers within () are number of tissues; values are mean \pm SE*[†], $p \leq 0.05$ v normal.

Baseline APDs in papillary muscles from dogs paced at low heart rates for a few weeks were $>$ than in the control group, and dofetilide prolonged the APD in the chronic bradycardia group to a greater extent than in the control group. Since the values of APDs for individual animals are not provided, SEs of Δ APDs in the two groups are not known, and the s-s of differences between the two groups cannot be calculated.

- Maximum diastolic potentials and AP amplitudes in the two groups were not different, and were not affected by dofetilide.

Discussion

This study shows that prolongation of APD/QT at low HR is > during chronic bradycardia than during short term decrease in HR, and chronic bradycardia increases sensitivity of the myocardium to dofetilide.

§3: Miscellaneous**#1: Receptor Binding Studies****Results**

Table below shows IC₅₀s of standard agents and dofetilide for the receptors listed.

Receptor type/ binding site	Standard agent	IC50	
		Standard agent	Dofetilide
Alpha1- adrenoceptor	Prazosin	0.2 nM*	4.5 ± 1.1 μM
Alpha2- adrenoceptor	Clonidine	1.5 + 0.1 nM	> 10 μM
Beta- adrenoceptor	Propranolol	25 nM*	> 10 μM
Adenosine A1	Cyclohexyl adenosine	23nM*	> 10 μM
Dopamine D2	Spiroperidol	1.7 nM*	> 10 μM
5- HT2	Ketanserin	26 + 10 nM	> 10 μM
Muscarinic	Atropine	1.6 + 0.1 nM	> 10 μM
Opioid	Naloxone	3.8 + 0.9 nM	> 10 μM
Dihydropyridine	Nitrendipine	0.6 nM*	> 10 μM

Note: IC₅₀s are mean±SE of 3 experiments or average of two (indicated by '*').

Except for a weak binding affinity for Alpha-1 receptor, dofetilide has little affinity for any of the other receptors tested.

Discussion: Since the pharmacological cardiac effects are produced in the nM concentration range, dofetilide at therapeutic doses is not likely to have any effect on α1 receptors (for which dofetilide has the lowest IC₅₀).

#2: Effect on Acetylcholinesterase Activity in-vitro

Table below shows the effects of different concentrations of eserine and dofetilide on acetylcholinesterase activity. Values are mean±SE of 3 experiments

Compound	Concentration (μM)	Percentage of control activity
Eserine	0.003	74.9 +0.55
	0.005	63.3 +2.23
	0.010	46.1 +0.94
	0.030	20.6 +0.76
Dofetilide	1	93.5 +0.44
	10	58.0 +1.79

Eserine in the concentration range 3-30 nM, dose relatedly reduces the enzyme activity. 1 μM dofetilide has practically no effect on the enzyme activity; 10 μM caused approximately 40% inhibition of activity.

#3: Effect on Na/K-activated ATPase in-vitro

Table below shows the effects of different concentrations of Ouabain and dofetilide on ATPase (commercial product prepared from porcine cerebral cortex) activity. Values are mean \pm SE (n=3).

Compound	Concentration (μ M)	Percentage of control activity
Ouabain	0.01	79.6 + 3.33
	0.04	53.5 + 3.31
	0.10	31.5 + 1.07
Dofetilide	0.1	94.3 + 2.25
	1	99.6 + 1.07
	10	97.4 + 1.29

Ouabain in the dose range 10-100 nM dose relatedly inhibited ATPase activity; dofetilide even at 10 μ M concentration had practically no effect on ATPase activity.

Note: The following types of studies, which are in the submission, are not reviewed for the reasons given. a) There is a summary of a study, which consists of just statements by the sponsor about the effects of dofetilide on responses of guinea pig atria to acetylcholine. Since there is no data, the study cannot be evaluated. b) There are several studies in which effects of dofetilide on physiological functions other than cardiovascular functions were studied. These studies are not reviewed as at this time there is data from human studies as to whether dofetilide has any effects other than antiarrhythmic effects in man.

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Pharmacokinetics, Metabolism, Excretion, & Distribution

§1 Human

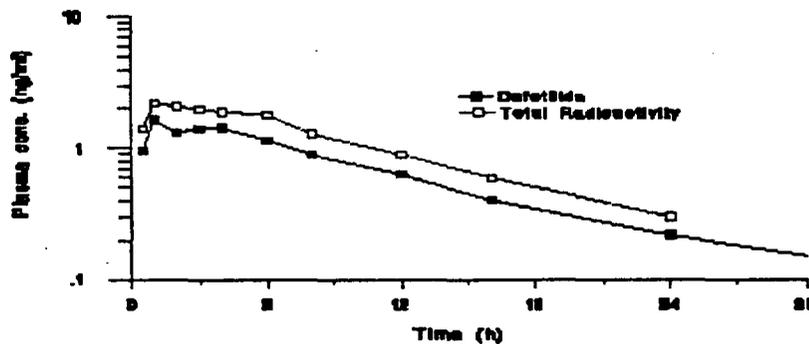
A Single Oral Dose of 0.5mg [14C]-Dofetilide

	Subject 1		Subject 2		Subject 3		Mean ± SD (n=3)	
	Dofetilide	Radioact	Dofetilide	Radioact	Dofetilide	Radioact	Dofetilide	Radioact
Elimination half life (h)	5.7	6.5	9.0	7.6	9.2	7.4	8.0 ± 2.0	7.2 ± 0.6
AUC (ng*.h/ ml)	21.0	29.2	14.8	21.0	16.6	24.4	17.5 ± 3.2	24.9 ± 4.1

Excretion of radioactivity is shown below; results are expressed as % of dose administered.

Time (h)	Subject Number			Mean ± SD
	1	2	3	
Urine				
0 - 12				45.74 ± 3.12
12 - 24				21.02 ± 1.69
24 - 48				8.59 ± 1.58
48 - 72				1.42 ± 0.09
72 - 96				0.35 ± 0.19
96 - 120				0.23 ± 0.05
120 - 144				0.12
144 - 168				0.07
Total in urine				77.53 ± 2.98
Feces				
0 - 24				-
*24 - 48				-
48 - 72				3.10 ± 2.32
72 - 96				0.40 ± 0.56
96 - 120				0.11 ± 0.18
120 - 144				0.04 ± 0.06
144 - 168				-Total
Total in feces				10.27 ± 4.01
Total recovery				87.80 ± 6.42

Fig below shows plasma profile of dofetilide and total radioactivity.



After i/v dosing, 68.6±6.61 % of the dose was excreted in the first 24 hours, and 80.04±3.59 % in 168 hours(n=3; a different set of subjects than the ones that received the oral dose). In plasma, only dofetilide which formed ≈ 75% of the radioactivity (fig. above), could be identified. Concentrations of individual metabolites must have been below detection limits.

Concentration of various moieties (as % of urinary radioactivity) are shown in the table below.

Metabolites	% radioactivity in 0- 24 hour urine							
	p. o.				i. v.			
	#1	#2	#3	Mean	#4	#6	#9	Mean
H1, Unknown				1.4 ±0.2				1.03±0.15
H2, UK- 80,725				3.3 ±1.3				3.1±0.36
H3, UK- 69,502				1.23±0.55				1.43±0.76
H4, UK- 116,856				3.17±0.35				2.23±0.32
H5, UK- 71,385				2.9 ±0.8				3.57±0.57
Dofetilide				82.9±2.45				83.57±0.49

Note: There are several pharmacokinetic studies in this submission in which C_{max} and AUC of dofetilide were determined after 0.5 mg b.i.d. repeated oral doses. In 7/17 studies, mean±SD of C_{max} is given; in 5 of these, the range of 'mean+1.6*SD' (upper limit of 95% of values) was 3.1-4.1 ng/ml. In 10/14 studies in which n=18-20, mean AUC was 41-48 ng*h/ml; in 4, 50-55 ng*h/ml; in one study (n=12), AUC was 61.5±8.033 ng*h/ml; 'mean+1.6*SD in this study is 74.4 = 75 ng*h/ml. Therefore, approximate upper limits of 95% of C_{max} and AUC values would be 4 ng/ml and 75 ng*h/ml respectively.

§2 Rat:

Excretion of Radioactivity in the Urine and Feces of Rat after Oral Administration of [¹⁴C]-Dofetilide. (SD, Charles River) Males, Report # DM-96-115-20; Females, Report # DM-96-115-20.

% of administered [¹⁴C] excreted in urine and feces

Sample and Time After Dose (h)	Males (5 mg/kg)				Females (7 mg/kg)			
	#1	#2	#3	Mean (n=3)	#1052	#1053	#1054	Mean (n=3)
Urine								
0 - 24h				43.1				52.8
24 - 48h				3.1				1.0
48 - 72h				0.6				0.2
72 - 96h				0.4				
72-120								0.4
96 - 144h				0.5				
Total in Urine (0-120/144h)				47.7				54.4
Feces								
0 - 24h				46.7				31.3
24 - 48h				1.3				9.7
48 - 72h				0.2				0.3
72-96h				0.1				0.1
96 - 144h				0.3				
Total in feces (0 -96/144h)				48.5				41.4
Carcass				0.4				
Cage residue				0.2				0.5
Total recovery (0-120/144h)				96.7				96.3

% of radioactivity excreted as various metabolites/dofetilide.

Metabolite/Dofetilide	Identity	Males		Females	
		% in urine	% in feces	% in urine	% in feces
R1	UK- 80,725	32%		28	
R2	UK- 69,502	19%	21	15	
R3	UK- 71,385	21%	18	12	14
R4					51
Dofetilide	Dofetilide	20%	56	38	20

§3: Mouse

Excretion of Radioactivity in the Urine and Feces of Male Mouse after Oral Administration of [¹⁴C]-Dofetilide. (CD-1, Charles River; Report # Dm-95-115-04). Dose, 4.4 mg/kg; 4 mice

% of administered [¹⁴C] excreted in urine and feces.

Sample & time after dose (h)	Urine	Feces
0-24h	54.5	16.4
24-48h	1.3	0.7
48-72h	0.5	0.2
72-120h	0.5	1.2
Total in (0-120h)	56.8	38.4
Cage washings	1.1	
Total recovery (0-120h)	96.3	

% of radioactivity excreted as various metabolites/dofetilide.

Metabolite/dofetilide	Identity of metabolite	% in Urine	% in Feces
M1	UK- 80,725	23	2
M2		18	25
M2a		-	16
M3	UK- 69,502	8	2
M3a		-	7
M3 (b,c, and d)		-	5
M4	UK- 71,385	27	20
M4a		-	4
Dofetilide	Dofetilide	21	17

§4: Dog (Report # DM11; beagle; female; n=4)

Body wts 13.3-16 kg; interval between i/v and oral dosing is not mentioned.

Parameter	Dog 2EA6	Dog 2EA7	Dog 4AK9	Dog 4AKO	Mean (n= 4)
Dose i/v (mg/ kg)					0.194
C _{0.1h} (ng/ml)					102.7
Elimination rate constant (h) ⁻¹					0.16
Elimination half- life (h)					4.6
Plasma clearance (ml/ min/ kg)					10.2
Volume of distribution (L/ kg)					4.0
AUC (0-∞) (ng. h/ ml)					329
Unchanged dofetilide in urine (0- 24h) (% of dose)					16(n= 2)
Dose p. o. (mg/ kg)					0.211
C _{max} (ng/ ml)					33.1
t _{max} (h)					2.9
Elimination rate constant (h) ⁻¹					0.17
Elimination half- life (h)					4.1
AUC (0- ∞) (ng. h/ ml)					249
Bioavailability (%)					72

§5: In-vitro Metabolism Using Liver Microsomes

Incubations (in triplicate) were carried out with 1M dofetilide and 0.5M cytochrome P450 in a medium containing 50mM Tris HCl (pH7.4), 5mM MgCl₂ and 5mM MnCl₂ (total volume, 12 ml). NADPH, which was regenerated in situ using anisocitrate/isocitrate dehydrogenase system, provided reducing substances required by cytochrome P450. Liver microsomes from male and female rat, mouse, dog, and human liver (gender for mouse, dog, and man is not mentioned) were studied. In these studies, the incubation was standardized to 0.5mg/ml protein, rather than to the concentration of cytochrome P450.

Results.

Disappearance half-lives of dofetilide in the presence of liver microsomes from different species are shown below ('±', are presumably SDs).

Microsome Type	Half- life (min)
Male rat	39 ± 9
Female rat	113 ± 24
Mouse	141 ± 10
Dog	297 ± 46
Human	>500

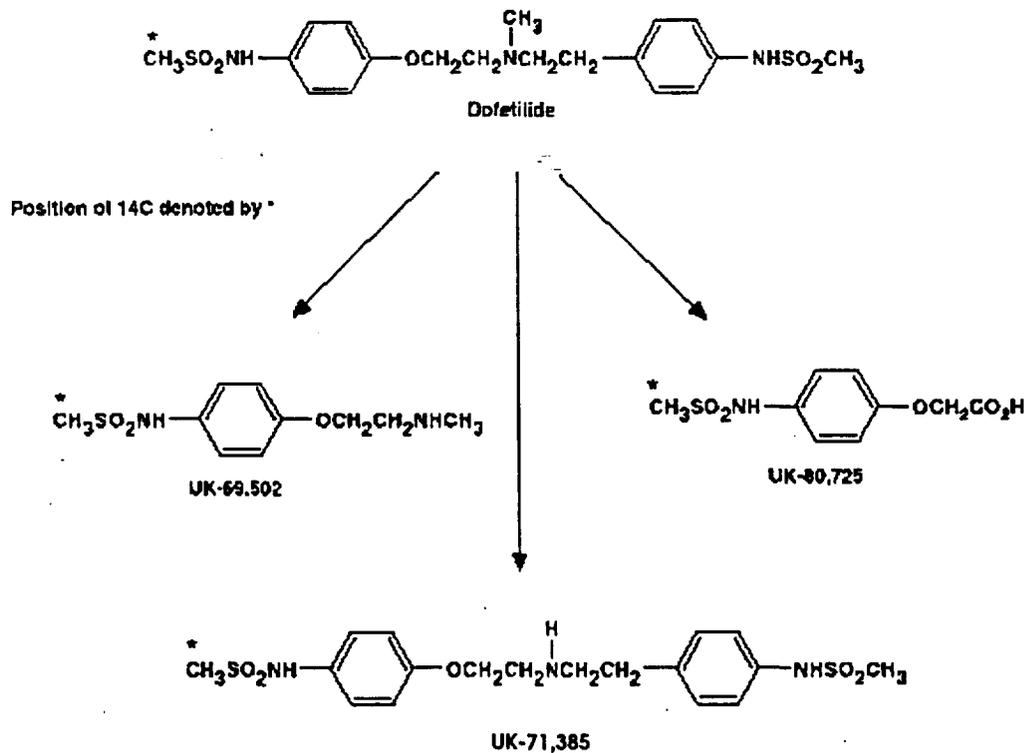
Effects of various P450 inhibitors on dofetilide metabolic rates are shown below:

Inhibitor	Species	% Change in rate	P450 Isozyme(s)
Orphenadrine	Rat	-29	2B
Cimetidine	Rat	-75	2C
Quinine/ Quinidine	Rat	No effect	2D
Chloramphenicol	Dog	-48	2B
Sulphaphenazole	Human	-20	2C

Metabolic patterns of ¹⁴C dofetilide, in rat, mouse and dog is shown below:

Species	% metabolized	% Total metabolites present as:		
		UK- 80,725	UK- 69,502	UK- 71,385
Rat (m)	64	18	25	45
Rat (f)	14	13	31	56
Mouse	22	11	13	76
Dog	7	38	19	43

Fig below shows structures of various metabolites.



Disposition Of [¹⁴C]-Dofetilide in the Isolated Perfused Rat Liver (Report # DM10)

Two male rats (250-300g) were used for this study. The animals were anaesthetized by intraperitoneal administration of sodium thiopentone and heparinized by intravenous heparin administration via the femoral vein. Cannulae were inserted into the bile duct, the portal vein, and the vena cava above the hepatic vein. The remaining systemic circulation was stopped by ligation of the vena cava above the renal vein.

Perfusion medium (150ml) consisting of 13% bovine red blood cells and 2.6% bovine serum albumin in high bicarbonate saline buffer, adjusted to pH 7.4, was oxygenated and perfused through the liver at 15ml/min via the portal vein. The outflow was allowed to flow into the reservoir from where it was recirculated around the system. 1ml vehicle (saline: 1N HCl, 195:1) containing 0.70mg [¹⁴C]-dofetilide (30 μ Ci) was added to the perfusate reservoir 15 minutes after the start of the perfusion.

Results

- After 90 minutes, concentration of [¹⁴C] in the perfusate was \approx 53% of the initial concentration, and \approx 13% of the administered [¹⁴C] was eliminated in bile; therefore, \approx 34% of administered [¹⁴C] must be in the liver. \approx 9% of [¹⁴C] in the perfusate and 7% of [¹⁴C] in bile was dofetilide at this time point.
- Peaks corresponding to UK-71,385, UK-69,502, and UK-80,725 were present in bile and the perfusate; relative amounts of these metabolites are not quantified.

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Tissue Distribution of Radioactivity in the Rat after i/v Administration Of [¹⁴C]-Dofetilide (Report # DM8). 4 mg/kg [¹⁴C]-Dofetilide was administered i/v to 4 male pigmented rats; one rat/time point was sacrificed and [¹⁴C] concentrations (as µg eq/g) in different tissues were determined.

Results

Table below shows [¹⁴C] concentration. Organs are listed in descending order of concentrations at 6 minutes post dose (except that different parts of an organ are listed together). At 1, 24 and 96 hours post dose, order of concentration is indicated by ⁽ⁿ⁾.

T (h)	0.1	1.0	24.0	96.0
Tissue concentration	µg eq/ gm			
Kidney a) Medulla	7.07	1.70 ⁽²⁾	0.04	-
b) Cortex	3.17	0.01	-	-
Liver	4.12	1.84 ⁽¹⁾	0.21 ⁽⁶⁾	0.03
Intestine Contents				
b) Large Intestine	3.44	1.05 ⁽⁶⁾	0.10 ⁽⁷⁾	0.02
a) Small Intestine	2.22	-	0.29 ⁽⁵⁾	0.02
Pituitary Gland	2.86	1.26 ⁽⁴⁾	0.09 ⁽⁸⁾	-
Salivary Gland	2.80	1.04 ⁽⁷⁾	-	-
Eye a) Retina	2.52	1.48 ⁽³⁾	0.83 ⁽¹⁾	0.57 ⁽¹⁾
b) Aqueous Humor	-	0.04	-	-
c) Vitreous Humor	-	0.04	-	-
Pancreas	2.09	0.92 ⁽⁹⁾	-	-
Preputial Gland	2.01	1.26 ⁽⁴⁾	0.51 ⁽³⁾	0.08
Harderian Gland	1.92	1.13 ⁽⁵⁾	-	-
Adrenal Gland	1.86	1.48 ⁽³⁾	0.78 ⁽²⁾	0.04
Bone Marrow	1.86	0.87 ⁽¹⁰⁾	-	-
Lung	1.83	0.82 ⁽¹¹⁾	-	-
Pineal Gland	1.81	0.55 ⁽¹⁴⁾	-	-
Spleen	1.70	0.04	-	-
Cardiac Muscle	1.62	0.47 ⁽²⁰⁾	-	-
Thyroid Gland	1.58	0.50 ⁽¹⁸⁾	-	-
Substantia Nigra	1.19	0.79 ⁽¹²⁾	0.08	-
Skeletal Muscle	1.15	0.53 ⁽¹⁶⁾	-	-
Stomach Contents	0.78	0.57 ⁽¹³⁾	-	-
Blood	0.75	0.27 ⁽²³⁾	-	-
Thymus Gland	0.75	0.02	-	-
Skin a) Sebaceous Gland	0.67	0.54 ⁽¹⁵⁾	0.42 ⁽⁴⁾	0.22 ⁽²⁾
b) Dermis	0.60	0.52 ⁽¹⁷⁾	-	-
c) Epidermis	0.60	0.52 ⁽¹⁷⁾	-	-
Male sex organs				
a) Prostate Gland	0.61	1.02 ⁽⁸⁾	-	-
b) Seminal Vesicle	0.61	0.45 ⁽²¹⁾	-	-
c) Testes	-	0.19 ⁽²⁴⁾	0.09 ⁽⁸⁾	-
Naso- Optic Sinus	0.61	0.35 ⁽²²⁾	-	-
Adipose Tissue	0.34	0.12 ⁽²⁵⁾	-	-
Bladder Wall	-	0.49 ⁽¹⁹⁾	-	-
Brain	-	0.04	-	-
Lower limit of Determination	0.14	0.016	0.008	0.008

Comments: Within 6 minutes of injection, [¹⁴C] concentrations in several organs were >> than in blood. Concentration in testes, target organ for toxicity (atrophy), was very low. Concentration in brain over all was very low, but in substantia nigra, it was comparatively high; concentration was also fairly high in retina; both these tissues are pigmented.

Discussion

Table 1 compares the % of radioactivity excreted in urine and feces in different species, and table 2 shows dofetilide and various metabolites excreted in urine in 24 hours (as % urinary radioactivity, and within () as % administered dose) in these species.

Table 1

Species	Dose (oral)	% excreted in urine		% excreted in feces		Total recovered
		24 hours	120 hours	48 hours	96 hours	
Man (n=3)	0.5 mg	66.8±4.8	77.6±3.1	6.5±6.7	10.13±4	87.7 %
Rat (m) (n=3)	5 mg/kg	43.1±3.4	47.7±2.9	48±2	48.5±2	96.2%
Rat (f) (n=3)	7 mg/kg	52.8±3.8	54.4±4.1	41±4.7	41.4	95.8%
Mouse (n=4)	4.4 mg/kg	54.5	56.8	37.1	38.3	95.1%

Table 2

Species	Dofetilide	UK- 80,725	UK- 69,502	UK- 71,385	UK- 116,856	M2	H1	Total
Man (n=3)	82.9±2.5(64.4)	3.3±1.3(2.3)	1.32±0.6(1.01)	2.9±0.8(2.3)	3.2±0.4(2.5)	-	1.4±0.2	93.6
Rat (m) (n=3)	20 (9.5)	32 (15.3)	19 (9.1)	21 (10)		-		92
Rat (f) (n=3)	38 (20.7)	28 (15.2)	15 (8.2)	12 (6.5)		-		93
Mouse (n=4)	21 (11.4)	23 (12.5)	8 (4.4)	27 (14.7)		18 (9.8)		97

In the dog, the only information regarding excretion is that ≈ 16% of the administered dose is excreted in urine as dofetilide.

As can be seen, a greater % of administered [¹⁴C] is excreted in urine of man than in urine of rat or mouse, and a much greater % of administered dose is excreted as dofetilide in urine of man than in urine of rodents. This indicates a much less extensive metabolism of dofetilide in man than in rodents. The in-vitro study with liver microsomes also showed a much slower rate of metabolism of dofetilide by human microsomes than those of other species (p). Metabolites UK-71,385, UK-69,502, and UK-69,502 were formed by the liver microsomes of dog and rodents. These 3 metabolites are present in the urine of man and rodents (excretion of dofetilide/metabolites in dog was not studied). Thus, these 3 metabolites are formed in all species studied. One metabolite, UK-116, 856, and another H1 (unknown structure) were present only in the urine of man, but only ≈ 2.5, and 1.4% respectively of administered dose was excreted as these metabolites. It is also stated by the sponsor that the plasma levels of all metabolites in man were below detection limits. A metabolite, M2 (of unknown structure) is only present in the urine of mouse, and ≈10% of administered dose is excreted as M2 in the mouse.

The study using perfused rat liver (p) showed the presence of the 3 major metabolites in the bile.

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Toxicology

Note: All toxicology studies are certified by the Quality assurance units of the sponsor/labs as having been conducted in accordance with the GLP requirements of countries where the studies were conducted. Toxicology studies using i/v dofetilide are not reviewed, since this NDA is for oral formulation only. Usually, only treatment related adverse findings are described and discussed; if a parameter is not mentioned, it means there was no treatment related adverse effect on that parameter. Unless mentioned otherwise, adverse changes described in various parameters are in comparison to the values of those parameters in the control group at the same time point. Unless mentioned otherwise, organ wts are absolute organ wts.

§1: Acute Toxicity Studies in Rodents

Rat (SD, 6 week old; study # 93-68-02; study site, sponsor's labs in Japan).

5/rats/sex were administered 300 mg/kg dofetilide by gavage. Animals were observed at frequent intervals for mortality and clinical signs on the day of dosing, and twice/day for 14 days; all survivors were necropsied at the end of the study.

Results

- Mortality: There were no deaths. LD₅₀ was therefore > 300 mg/kg.
- Clinical signs: *Ptosis* appeared within 40-60 minutes post-dose in females, and within 1-2 hours post-dose in males; the sign lasted less than 24 hours. *Salivation* was observed 2-4 hours post-dose in one male and three females, and lasted for 1.5-4 hours.
- Necropsy: There were no pathological findings.

Mouse (ICR, 6 weeks old; study # 93-68-02; study site, sponsor's labs in Japan).

5/sex; same dose and methodology as in the rat study reviewed above.

Results

- Mortality: There were no deaths. LD₅₀ was therefore > 300 mg/kg.
- Clinical signs: *Decreased activity* appeared within 16-22 minutes of dosing in all animals. Soon thereafter, *ptosis* was seen in 4 males, and all females, and one female was seen in *prone* position. All signs had subsided by 4 hours post-dose.
- Necropsy: There were no pathological findings.

§2: Sub Chronic & Chronic Toxicity Studies in Rodents

Three Month Dietary Study in Mice (COBS-VAF-CD1(ICR)BR; study # 89085; study site, sponsor's labs in Amboise, France).

10/sex/group; one control (C) and three treatment groups; treatment groups L, M, and H received 5, 20, and 80 mg/kg/day dofetilide (mixed in diet) respectively. The animals were observed daily for clinical signs; body wts, food consumption, and water consumption were measured weekly. Clinical chemistry and hematology were done at the end of the study, and a complete necropsy and histopathology were done at sacrifice/death. Three satellite groups of 12 males each were used for plasma drug level determinations after dosing for 24 days; plasma levels of dofetilide were determined at 8am, 11am, and 4pm; a different subgroup of 4 males was sacrificed at each time point. At the start of the study, body wts were $\approx 26 \pm 2.2$ gm in males, and $\approx 21 \pm 1.8$ gm in females.

Results

- **Mortality & Clinical signs:** There was no mortality and there were no treatment related adverse clinical signs.
- **Body wts:** There was no treatment related adverse effect on body wts. *In males*, body wt at the end of the study was C, 35.8±2.33 gm; H, 34.8±2.9 gm; *in females*, 25.5±1.2 gm and 25.3±2.9 gm in C and H groups respectively (values are mean±SD).
- **Hematology & Clinical chemistry:** There were no treatment related adverse findings in any parameter.
- **Organ wts:** *Males: Testes*, wt was reduced ≈17% in high doses animals (p<0.01); there was a 7-8% reduction in testicular wt in mid dose animals (s-ns).
- **Histopathology:** In 3/10 high dose males, testicular atrophy was seen. Two of the animals had grade 1 atrophy (25% of tubules had degenerated germinal cells in the lumen); one had grade 3 atrophy (50% of the tubules had complete loss of germinal cells or mineralization).

Comments: AUCs (for the 8 hour time interval) are reported as 251, 410, and 1384 ng*h/ml for L, M, and H groups respectively. Since rodents eat at night, but the AUCs were determined during an 8 hour period of day time, the reported AUCs must be under estimates.

One Month Oral (gavage) Toxicity Study in the Rat (●:COBS-CD(SD)BR; study # 87016; study site, sponsor's labs in Ambois, France).

10/sex/group; one control (C) and three treatment groups; treatment groups L, M and H received 1, 6, and 40 mg/kg/day dofetilide (by gavage) respectively; C received vehicle; volume of fluid administered was 10 ml/kg for all groups; rest of the methodology was the same as for the mouse study reviewed above. Satellite groups of 5 males/dose were used for plasma levels of dofetilide after 9 days of dosing.

Results

- **Clinical signs & Mortality:** There were no treatment related clinical signs or mortality.
- **Body wt:** There was no treatment related adverse effect on body wt.
- **Hematology & Clinical Chemistry:** There were no treatment related adverse effects on any hematology parameter. *Clinical chemistry:* There was a 3-4% s-s increase in mean serum Ca in mid and high dose males (p<0.01) and high dose females (p<0.05), but the mean values were not > than the mean of 270 control animals in the lab's data base.
- **Organ wts:** There was a s-s 12% reduction in mean left testicular wt of high dose males (p<0.01), and a s-s 8% and 15% reduction in the mean right testicular wts of mid and high dose males respectively (p<0.05, and p<0.001 respectively).
- **Gross Pathology:** One low dose and one high dose male had marbled appearance of the kidneys.
- **Histopathology: Kidneys:** One low dose and 2 high dose males had moderate hyaline droplet formation in the proximal renal tubules (2/3 of these animals had marbled appearance of the kidneys described above). There was also a higher incidence of tubular basophilia in mid and high dose groups; 1/20 in C, and 5/20 each in mid and high dose groups.

Comments: According to the sponsor, basophilia of renal tubules is fairly common in untreated rats in their labs. Hyaline droplets are stated to have been described in the proximal tubules of untreated male rats, and these contain $\alpha 2\mu$ globulin. This protein is not found in man. The renal findings described here are therefore not considered to be of any toxicological significance. This seems to be a reasonable conclusion.

- Toxicokinetics: C_{max} and AUC_{0-5hr} increased dose relatedly. Table below shows pharmacokinetic parameters; values are mean \pm SE; range (n=5 each).

Parameter	1 mg/kg/day	6 mg/kg/day	40 mg/kg/day
C_{max} (ng/ml)	20 \pm 3.61; 9-31	189 \pm 18; 132-239	1225 \pm 142; 966-1771
AUC_{0-5hr} (ng*h/ml)	65 \pm 13.84; 25-105	622 \pm 60; 405-736	4433 \pm 480; 3471-6205
t_{max} (hr)	1-3	1-3	1-3

Note: t_{max} range was 1-3 hours in all dose groups.

Comments: The only adverse effect was a dose related decrease in testicular wt in the dose range 6-40 mg/kg/day.

Three Month Dietary Toxicity Study in the Rat COBS-CD(SD)BR; study # 89064; study site, sponsor's labs in Ambois, France).

10/sex/group; 3 treatment groups and one control (C) group; treatment groups L, M, and H received 10, 30, and 80 mg/kg/day dofetilide (mixed with diet) respectively. Satellite groups of 9 males/group were used for determining plasma drug levels; drug levels were determined on day 21 at 8am, 11am, and 4pm. Rest of the methodology was the same as in the three month dietary mouse study. Wts of the animals at the start of the study were, 163 \pm 8 gm in males, and 119 \pm 10 gm in females.

Results

- Clinical signs & Mortality: There were no treatment related clinical signs or mortality.
- Body wt: Males: Mean body wt of high dose group was s-s lower than that of control group from day 8 onwards ($p<0.001$ for almost all time points). Wt was 6% lower on day 8; 10% at the end of one month; 13% at the end of two months, and 15% from 2 ½ months till the end of the study. Mid dose: mean body wt of this group was 8% lower than that of control group from day 75 till the end of the study (s-s; $p<0.05$). Females: mean body wt of the high dose group was no different from control group at any time point.
- Food consumption: Males: Mean food consumption of high dose group was s-s ($p<0.01$ or $p<0.001$) lower than that of control group throughout the study; food consumption was lower by 11-13% for almost all time points except day 57 and 64, when differences from control were \approx 9% and s-ns. Mid dose: Mean food consumption of this group was sporadically s-s ($p<0.05$) lower than that of control group by \approx 9%. Low dose: mean food consumption of this group was s-s ($p<0.05$) lower by 6-7% on 4 occasions. Females: There was no treatment related adverse effect on food consumption of females.
- Water consumption: Males: Water consumption of the high dose group was s-s lower than that of control at a few time points; differences were as much as 20-27%.
- Clinical chemistry: Males: In the high dose group, mean alkaline phosphatase was increased by 32% (s-s, $p=0.03$, s-rev). Females: Serum Ca was increased \approx 4% (s-s) in all treatment groups.

Comments: The sponsor does not report the increase in alkaline phosphatase as s-s. The small increase in serum Ca in females was within 1SD of the control group value, and is not dose related. Therefore, as stated by the sponsor it probably has no toxicological significance.

- Organ wts: Males: In high dose group, wt of both Kidneys was reduced by \approx 15% (s-s). Wt of both testes was reduced by \approx 40% (s-s). In mid dose group, wt of both testes was reduced by \approx 28% (s-s).
- Gross Pathology: Testes: 2 high dose males had small, flaccid testes.
- Histopathology: Males: 10/10 high dose animals had testicular atrophy; 2, grade 1; 5, grade 2, and 3, grade 3. Testes of 2/3 animals that had grade 3 atrophy, were flaccid and small at gross pathology. Mid dose: 4/10 animals had testicular atrophy; 2, grade 1, and 2, grade 2.

- Toxicokinetics:**

	<u>L (n=3)</u>	<u>M (n=3)</u>	<u>H(n=3)</u>
C _{max} (ng/ml)	47±15	188±23	442±3
AUC _{0-16hr} (ng*h/ml)	311	947	2924

Note: Since plasma drug levels were determined at 8, 11, and 16 hours, C_{max} and AUC under-estimate drug exposure.

Comments: Treatment related adverse findings were seen only in mid and high dose males. Besides a dose related lower body wt gain, there was a dose-related increase in incidence and severity of testicular atrophy in these groups. High dose males also had: 1) A s-s reduction in absolute kidney wts, but not in relative kidney wts; reduction in kidney wts, therefore probably does not have any toxicological significance. 2) Increase (s-s, s-rev) in alkaline phosphatase.

Six Month Oral Toxicity Study in Rat COBS-VAF-CD(SD)BR; study # 88082; study site, sponsor's labs in Ambois, France).

20/sex/group; treatment groups L, M, and H received 0.5, 5, and 40 mg/kg dofetilide respectively by gavage; control group (C) received vehicle. Satellite groups of 10/sex/group were used for toxicokinetics; plasma levels of drug were determined at 1, 3, and 5 hours post dose. Animals were observed daily pre-dose and post-dose for any adverse clinical signs; body wts and food consumption were recorded weekly; water consumption was measured at 1, 3, and 5 months over 24 hour periods. Clinical chemistry and hematology were done at 2, 4, and 6 months. Full necropsy and histopathology were done at sacrifice/death.

Results

- Clinical signs & mortality:** There were no treatment related clinical signs or mortality.
- Body wt:** There were no treatment related adverse effects on body wts.
- Water consumption:** In mid and high dose females, water consumption was reduced by 25% and 18% respectively during the 5th month measurement (s-s).
- Hematology:** Treatment related adverse effects (s-s) which occurred only in males, are shown below. There were no adverse effects in the low dose group at any time point.

	Males:		
	Control	Medium	High
67 days:			
WBC (x10 ³)	10.89±2.24	13.72±3.1*** (↑26%)	12.72±2.3* (↑17%)
Neutrophils (x10 ³)	1.302±0.479	1.630±0.667	1.674±0.738* (↑29%)
Lymphocytes (x10 ³)	9.512±2.041	11.933±2.924** (↑25%)	10.872±2.016* (↑14%)
130 days			
WBC (x10 ³)	11.03±2.11	12.69±2.87* (↑15%); n=19	12.72±2.38* (↑15%); n=19
Neutrophils (x10 ³)	1.4009±0.7184	1.3843±0.7095; n=19	1.8604±0.5932* (↑33%); n=19
Lymphocytes (x10 ³)	9.4142±2.0561	11.0483±2.6461* (↑17%); n=19	10.5862±2.3451; n=19
PCV (%)	45.56±3.14	44.92±2.52	43.40±1.71* (↓3.4%)
Platelets (x10 ³)	1056.6±169.4	976.1±99.1; n=19	1153.1±184* (↑9%); n=19
186 days			
Platelets (x10 ³)	990±154.8	947.7±87.5	1089.7±167.3* (↑9%)

Note: '↑' is increase; '↓' is decrease; n=20, unless specified otherwise; values are mean±SD; '**', '***', and '****', are p<0.05, 0.01, and 0.001 respectively

Comments: Increase in WBCs in the mid and high dose groups, and decrease in PCV in the high dose group, were not present at the end of the study and were therefore of no toxicological significance. The small increase in platelet count in the high dose group, though present at 4 and 6 months, does not seem to be of any toxicological significance.

- Clinical chemistry:** s-s adverse changes vs C are shown below. Values are mean±SD. In the low dose group, on day 130, there were s-s increases in creatinine and glucose by ≈11% (creatinine, 0.62±0.07 mg/dL v 0.56±0.06 mg/dL in C (n=19); glucose, 138.5±14 mg/dL v 125.3 ±19.8 mg/dL in C (n=19))

	Males			Females		
	Control	Medium	High	Control	Medium	High
Day 67						
Ca (mg/dL)	10.42±0.49	10.77±0.44 [*] ; ↑3.4%	10.71±0.47 [*] ; ↑2.8%	10.29±0.5	10.56±0.44	10.73±0.63 ^{**} ; ↑4.3%
Triglycerides (mg/dL)	88.2±61.9	88.2±48.3	124.8±78.4	32.7±10.4; n=19	39.6±13.6	46.0±12.1 ^{***} ; 41%
Day 130						
Ca (mg/dL)	10.42±0.43	10.58±0.28	10.66±0.48	10.46±0.42; n=19	10.58±0.37	10.62±0.39
Day 186						
Ca (mg/dL)	10.61±0.26	10.79±0.33	10.92±0.36 ^{**} ; ↑2.9%	10.71±0.44; n=18	10.72±0.35	10.98±0.42 [*] ; ↑2.5%; n=19
Triglycerides (mg/dL)	173.4±91.9	155.9±77.1	234.1±122.5 [*] ; ↑35%	57.5±33.3; n=19	69.3±37.3	83.7±41.3; n=19
Urea (mg/dL)	26.4±3.1	27.4±3.9	29.3±3.9 [*] ; ↑11%	31.5±3.5; n=18	31.2±5.2	31.5±4.6; n=19

Note: n=20, unless specified otherwise. '↑' is increase; '↓' is decrease; '*', '**', and '***', are p<0.05, 0.01, and 0.001 respectively.

Comments: The changes that were present at study end were: a small (≈3%), s-s increase in serum Ca in high dose animals; s-s, 35% increase in triglycerides in high dose males; and s-s, ≈11% increase in serum urea in high dose males. Toxicological significance, if any, of these findings is not known.

- Organ wts:** Testicular wts were dose relatedly decreased in mid and high dose males.

	C	L	M	H
Left testes	1.836±0.142	1.825±0.143	1.676±0.202 ^{**} ; ↓9%	1.262±0.169 ^{***} ; ↓31%
Right testes	1.826±0.142	1.890±0.373	1.684±0.180 ^{***} ; ↓8%	1.198±0.191 ^{***} ; ↓34%

Note: '***r', s-rev; the sponsor does not report this reduction as s-s.

- Gross Pathology:** Testes of 1 mid dose and 7 high dose animals were small and/or soft.
- Histopathology:** Table below shows lesions (other than testicular and epididymal lesions, which are shown in a separate table); n=20, unless stated otherwise. When incidence of a lesion in a treatment group exceeded that in the control group by >1, it is underlined; in the high dose group, such incidences are shown in bold.

Lesion	Males				Females			
	Control	Low	Medium	High	Control	Low	Medium	High
Kidney:								
Basophilic tubules	10	<u>15</u>	10	9	0	2	0	2
Hydronephrosis	1	1	0	5	1	1	1	3
Hyaline droplets	3	<u>5</u>	<u>5</u>	5	0	0	0	0
Liver: Cetrilobular fat dep	2	1	0	4	0	0	0	0
Pancreas: Ch. Inflam.	1	1	<u>3</u>	0	0	<u>2</u>	0	2
Adrenal Cortex								
Focal cellular alteration	1	1	0	0	0	0	0	2

Incidences of testicular and epididymal lesions are shown below.

	Control	Low	Medium	High
Testes				
Focal atrophy, grade 1	11	6	10	6
Focal atrophy, grade 2	0	0	1	10
Focal atrophy, grade 3	0	0	0	1
Diffuse atrophy	0	0	1	2
Grades (2 & 3) & Diffuse	0	0	2	13 ^{****r}
Epididymis, abnormal content	0	0	1	2

Note: ^{****r}, p<0.0001 (Fishers' exact test, s-rev).

The sponsor has defined various grades of testicular atrophy as follows: *grade 1*, rare sections of tubules affected; *grade 2*, small numbers of tubules in the peripheral part affected; *grade 3*, many tubules affected; *diffuse*, more than 80% of tubules affected. According to the sponsor, affected tubules showed total loss of germinal cells; Sertoli cells seemed unaffected, and there was no evidence of any changes in Leydig cells.

- **Toxicokinetics:** C_{max} , and AUC_{0-5hr} in different groups are shown below.

Dose	Males			Females		
	0.5mg/kg/day	5 mg/kg/day	40 mg/kg/day	0.5mg/kg/day	5 mg/kg/day	40 mg/kg/day
C_{max} (ng/ml)	30±10; 20-40;	280±60; 200-330	2028±188; 1490-2550	40±10 30-50	570±120 .. 380-690	2546±130; 2410-2760;
AUC_{0-5hr} (ng*h/ml)	90±20	730±170	6120±1250	100±20	1050±230	7360±1060
t_{max} (hr)	1 (in all)	1 (in all)	1,1,1,2,2	1 (in all)	1 (in all)	1,1,1,1,2

Note: n=5 in all cases. Values are: C_{max} , 'mean±SD; ranges'; AUC, mean±SD; for t_{max} , individual values are given.

Discussion: The only treatment related adverse effects were: 1) Reduced testicular wt, and testicular atrophy of severity >grade 1 in mid and high dose males (grade 1 atrophy was seen in all groups). In the mid dose group, incidence of testicular atrophy was s-ns. However, since testicular wt was s-s reduced in this group, atrophy > grade 1 is most probably a treatment effect. 2) Incidence of hydronephrosis is s-s increased in the high dose group, when males and females are combined (p=0.044, Fishers' exact test, s-rev). The mechanism of this finding (if it is a drug effect, and not a false positive) is not understood. The sponsor does not comment on this finding.

12 Month Dietary Study in the Rat ████████ COBS-VAF-CD(SD)BR; study # 91086; study site, sponsor's labs in Ambois, France).

20/sex/treatment group; treatment groups L, M, and H received 2, 6, and 20 mg/kg/day dofetilide (mixed with diet) respectively; one control (C) group. Rest of the methodology was the same as in the other chronic studies reviewed above, except that food consumption was measured monthly after the first six months, and water consumption was measured monthly.

Results

- **Clinical signs & mortality:** There were no treatment related clinical signs. One animal from each group was sacrificed moribund on days 212 (H), 240 (M), 316 (L), and 344 (C).
- **Body wt:** There was no treatment related adverse effect on body wt (mean body wt of high dose males was 3-5% lower than mean wt of control group, but difference was s-ns at any time).
- **Hematology & clinical chemistry:** *High dose:* Serum phosphate (Pi) was s-s increased in males & females and serum Ca was s-s increased in females; values of Ca and Pi (mean±SD) in C and H groups are shown below.

	Males		Females	
	Control (n=19)	High (n=19)	Control (n=20)	High (n=20)
Ca (mg/dL)	10.51±0.36	10.73±0.35	10.61±0.44	10.93±0.4 [*] ↑3%
P _i (mg/dL)	5.65±0.39	6.39±0.49 ^{***} ↑13%	4.62±0.49	5.19±0.68 ^{**} ↑11%

- **Organ wts:** *Males:* Spleen wt was reduced in mid and high dose groups by 14% and 12% respectively (s-s). Testes wt was reduced in mid and high dose groups by 7% and ≈28% respectively (s-s; p<0.05 for mid dose, and <0.001 for high dose groups). *Females:* Adrenal wts were reduced in the high dose group by 15-18% (s-s for the right but s-ns for the left adrenal).
- **Gross Pathology:** Both testes were soft and/or reduced in size in 3/20 mid dose, and 12/20^{***} high dose males (p <0.001, Fishers' exact test, s-rev).
- **Histopathology:** Table below shows lesions (other than testicular & epididymal lesions, which are shown separately); n=20, unless stated otherwise. When incidence of a lesion in a treatment group exceeded that in the control group by >1, it is underlined; in the high dose group, such incidences are shown in bold.

Lesion	Males				Females			
	Control	Low	Medium	High	Control	Low	Medium	High
Kidney: Ch. nephropathy	19	18	20	17	9	<u>12</u>	<u>11</u>	<u>11</u>
Liver								
Eosinophilic foci	2	1	2	4	1	0	0	0
Focal vacuolation	0	0	0	<u>2</u>	0	0	0	0
Pancreas: Focal atrophy	4	5	2	<u>6</u>	2	<u>4</u>	<u>4</u>	1
Lung: Foam cell foci	5	5	5	6	5	4	6	7
Spleen: Hemosiderosis	3	1	1	<u>5</u>	5	5	4	6
Pituitary: Cyst	0	1	0	<u>2</u>	0	0	0	<u>2</u>
Adrenal cortex								
Cystic degeneration	1	0	0	0	11	<u>14</u>	<u>11</u>	<u>13</u>
Focal degeneration	0	0	0	0	0	2	3	2
Brain: (Basophilic bodies)	0	1	1	<u>2</u>	0	0	0	1
Mammary g. hyperplasia	0	0	0	0	3	1	3	5
Sternal chondropathy	5	1	1	3	2	2	1	5

Incidences of testicular & epididymal lesions are shown below

Testes	Control	Low	Medium	High
Atrophy of < 10% tubules (grade 1)	6	4	3	<u>12</u>
Atrophy of 10-30% tubules (grade 2)	0	0	0	1
Atrophy of 30-60% tubules (grade 3)	0	0	0	<u>2</u>
Atrophy of 60-90% tubules (grade 4)	0	1	0	1
Atrophy of >90% tubules (grade 5)	1	0	0	0
Total numbers with atrophy	7	5	3	<u>16^{**r}</u>
Numbers with atrophy ≥ grade-2	1	1	0	4
Epididymis				
Abnormal content ¹	1	1	0	<u>8^{**r}</u>

Note: ¹, Abnormal content is described as presence of necrotic eosinophilic cells, and decreased number of spermatozoa. ^{**r}, p <0.01 (Fishers' exact test, s-rev).

Discussion

The small changes in serum Ca in high dose females are probably of no toxicological significance; the mechanism and toxicological significance (if any) of the somewhat larger increase in P_i in high dose animals is not understood. Increases in incidences of lesions other than testicular and epididymal lesions are very small and probably of no toxicological significance.

Dietary Toxicity Study in Rat to Determine Reversibility of Testicular Atrophy (COBS-VAF-CD(SD)BR; study # 90086; study site, sponsor's labs in Ambois, France)

10 males/group; one control (C) and 3 treatment groups; treatment groups low, medium and high received 5, 10, and 50 mg/kg/day dofetilide (mixed with diet) respectively for six months; three satellite groups of 10/group were treated with 5, 10, and 50 mg/kg/day dofetilide for 2 and 1/2 months and used for toxicokinetics. Two supplementary groups of 20/group received 0 and 50 mg/kg/day dofetilide for 3 months; 10/group were sacrificed at 3 months, and 10/group at the end of a treatment free period of 5 months. At study end, plasma electrolytes (except P_i) and FSH concentrations were determined, and pathology and histopathology of testes was done.

Results

Note: For all results, *, p<0.05; **, p<0.01; ***, p<0.001; all comparisons v Control (t-tests, s-rev)

- Ca and FSH values are shown below; values are mean±SD; n=10 for each group (electrolytes other than Ca were not affected by treatment)..

	Six months		Three months		5 months' recovery	
	Ca (mg/dL)	FSH (ng/dL)	Ca (mg/dL)	FSH (ng/dL)	Ca (mg/dL)	FSH (ng/dL)
Control	10.05±0.34	2.815±2.381	10.51±0.23	7.855±1.597	10.46±0.25	0.449±0.647
High	10.42±0.36*; ↑4.1%	2.213±0.377	11.02±0.25***; ↑4.8%	8.391±1.099	10.51±0.242	0.338±0.286
Medium	10.31±0.47	2.181±0.404				
Low	10.29±0.31	1.831±0.443				

- Testes:

Testes wts (gm) are shown below.

	Six months		Three months		5 months' recovery	
	Left	Right	Left	Right	Left	Right
Control	1.86±0.19	1.89±0.17	1.77±0.17	1.78±0.19	1.88±0.28	1.90±0.29
High	1.07±0.31***; ↓42%	1.01±0.28***; ↓47%	1.26±0.19***; ↓28%	1.25±0.22***; 30%	1.52±0.47 ¹ ; ↓19%	1.53±0.47 ² ; ↓19%
Medium ²	1.65±0.19 ¹ ; ↓13% ²	1.65±0.17 ² ; ↓13%				
Low	1.71±0.42	1.70±0.47				

Note: ¹, p=0.053 (t-tests, s-rev); ², the sponsor does not report the ↓ in wt in this group as s-s.

Gross Pathology: Incidences of small and/or soft testes are shown below:

	Control	High	Medium	Low
Six months	0/10	10/10***	0/10	1/10
Three months	0/10	5/10*		
5 months' recovery	0/10	6/10**		

Histopathology: In this study, atrophy has been graded as follows: Grade-1, 5-20% tubules affected; grade-2, 20-50% tubules affected; grade-3, >50% tubules affected; grade-4, diffuse, almost all tubules affected. One low dose animal had grade-4 atrophy, and one mid dose animal had grade-1 atrophy. Incidences of atrophy of various grades of severity in control & high dose groups are shown below.

	Control; 50 mg/kg/day		
	6 months	3 months	Recovery
Grade-1 (5-20% tubules affected)	C, 2 ¹ ; 0; H, 3 ¹ ; 2	C, 0; H, 6 ¹ ; 5	C, 0; H, 7 ¹ ; 5
Grade-2 (20-50% tubules affected)	C, 0; H, 0	C, 0; H, 0	C, 0; H, 0
Grade-3 (> 50% tubules affected)	C, 0; H, 0	C, 0; H, 2	C, 0; H, 1
Grade-4 (Diffuse; almost all tubules affected)	C, 0; H, 7 ^{**}	C, 0; H, 1	C, 0; H, 2
Grades 3-4	C, 0; H, 7 ^{**}	C, 0; H, 3	C, 0; H, 3

Note: ¹: In individual animal listings, two 6 month C, one 3 month H, and two recovery H animals are reported as having a few peripheral tubules devoid of germinal cells; 'n¹' is the incidence when these animals are included, and 'n' is the incidence when they are excluded, as the sponsor has done in the summary table.