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BW A256C, a chemically novel class 1 antiarrhythmic agent. A comparison of *in vitro* and *in vivo* activity with other class 1 antiarrhythmic agents

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1 BW A256C (5(3)-amino-6-(2,3-dichlorophenyl)-2,3(2,5)-dihydro-3(5)-imino-2-isopropyl-1,2,4-triazine) is a novel class 1 antiarrhythmic agent designed to combine the features of potency with reduced central nervous system penetration.

2 BW A256C reduced the maximum rate of depolarization of guinea-pig ventricle and dog Purkinje fibres *in vitro* (EC_{50}, 2.2×10^{-6} M and 1.8×10^{-6} M, respectively), being significantly more potent than quinidine, lidocaine, disopyramide and flecainide. BW A256C was also more potent than these agents at inhibiting aconitine-induced arrhythmias in anaesthetized rats; however, unlike these agents, BW A256C was devoid of hypotensive activity at antiarrhythmic doses.

3 In anaesthetized dogs, intravenous administration of BW A256C (0.25–1 mg kg^{-1}) caused a dose-dependent suppression of ventricular arrhythmias that occurred on reperfusion of an occluded coronary artery.

4 In conscious dogs, intravenous infusion (total dose, 1.5 mg kg^{-1}) or oral administration of BW A256C (1.25–5 mg kg^{-1}) caused dose-dependent suppression of the ventricular ectopic activity that occurred following 20–24 h of permanent coronary artery ligation.

5 In the conscious dog, BW A256C was approximately 7 times more potent and was also longer acting than flecainide.

6 Administration of BW A256C was not associated with any evidence of peripheral or CNS toxicity. However, plasma levels 3–4 times greater than the antiarrhythmic levels were associated with a pro-arrhythmic activity.

Introduction

The risk of sudden cardiac death in the five years following a myocardial infarction has been demonstrated to be significantly increased in patients with complex ventricular arrhythmias (Ruberman *et al.*, 1977). Although several studies have demonstrated the efficacy of antiarrhythmic agents in suppressing a variety of ventricular arrhythmias, an antiarrhythmic regime to reduce favourably the incidence of ventricular fibrillation and mortality following a myocardial infarction has not been attained (Koch-Weser *et al.*, 1969; Graboys *et al.*, 1982; Campbell, 1984). The development of safe, effective agents for the prophylaxis of arrhythmias occurring during or after a myocardial infarction thus remains an important pharmaceutical and therapeutic goal.

Limitations in presently available antiarrhythmic therapy is a major contributing factor to the failure of the medical management of sudden cardiac death. Clinically available antiarrhythmic agents suffer serious limitations in their therapeutic utility. Primarily, the frequency of adverse drug reaction with antiarrhythmic therapy is high, thus reducing patient compliance. Agents such as quinidine, procainamide and disopyramide produce a myriad of disorders of central and autonomic function ranging from gastrointestinal intolerance, bladder, visual and sexual dysfunction to giddiness, psychosis and convulsions (Bigger & Hoffman, 1980). Furthermore, the short plasma half-life and thus the need for frequent dosing of some of the clinically available agents reduces compliance and complicates disease management. Indeed, in a recent study, the problem of inadequate blood levels and patient non-compliance for procainamide, quinidine and disopyramide was highlighted by demonstrating

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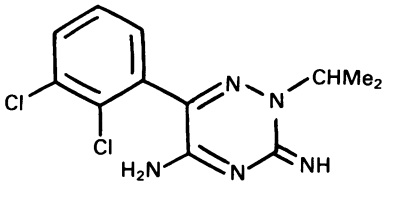
that more than 75% of a patient population receiving these agents had sub-therapeutic blood levels (Squire *et al.*, 1984). Since the continued maintenance of therapeutic blood levels is vital for achieving efficacy with any pharmacological agent, the therapeutic implications of non-compliance with antiarrhythmic agents are obvious. It is generally considered at present that the clinical success of a new antiarrhythmic agent would require a significant redress of the limitations of presently available therapy.

During the course of pharmacological studies at the Wellcome Research Laboratories, it was observed that certain phenyl diamino-1,2,4 triazines possessed anticonvulsant activity, a property attributable to the ability of these agents to modulate neuronal sodium transport (Miller *et al.*, 1984; Leach *et al.*, 1985). Preliminary evaluation demonstrated that some of these chemically novel triazines were also able to reduce, in a concentration-dependent fashion, the sodium-dependent rate of rise of phase 0 of the cardiac action potential, an action ascribed as a class 1 antiarrhythmic action (Vaughan Williams, 1981). A feature of the diamino 1,2,4 triazine moiety is that the basicity, and hence the degree of ionisation of the molecule, at physiological pH is increased significantly by alkylation of the triazine ring system thus allowing the design of agents with a potentially restricted access to the central nervous system. BW A256C (5(3)-amino-6-(2,3-dichlorophenyl)-2,3(2,5)-dihydro-3(5)-imino-2-isopropyl-1,2,4-triazine, see Figure 1) is a molecule that combines the features of antiarrhythmic potency with reduced penetration into the central nervous system, thereby potentially having a more desirable therapeutic profile. This paper describes the *in vitro* and *in vivo* activity of BW A256C and compares it with some clinically used class 1 antiarrhythmic agents.



M. wt. = 298
log P = −0.35 (pH 7.4)
pKa = 11.2

Figure 1 The chemical structure and some physicochemical properties of BW A256C.

Methods

Electrophysiological studies in vitro

Transmembrane action potentials were recorded from strips of guinea-pig right ventricle and dog isolated Purkinje fibres.

Male guinea-pigs (Hall, albino), weighing 300–500g, were killed by a sharp blow to the base of the skull and their hearts were rapidly excised and placed in cold oxygenated (95% O_2, 5% CO_2) Tyrode solution. The free wall of the right ventricle was dissected from the rest of the heart and cut into three strips along the direction of orientation of the endocardial muscle bundles. The strips were pinned around their edges to the silicone rubber base of a perspex chamber and superfused with warm (37°C) Tyrode solution at a rate of 7 ml min^{-1}. The composition of the Tyrode solution was (mM): NaCl 123.9, KCl 5.4, $MgCl_2$ 1.05, $NaHCO_3$ 25.0, NaH_2PO_4 0.42, $CaCl_2$ 1.8, D-glucose 5.0. When gassed, the pH of the solution in the tissue bath was 7.38–7.45.

The tissue was allowed to equilibrate at rest for at least 2 h, following which one strip was selected and stimulated at a frequency of 1 Hz via punctate, bipolar, silver-wire electrodes, insulated with Teflon except at their tips. Stimulus pulses, from a Grass S88 stimulator and S1U5 isolation unit, were of 1 ms duration and (initially) 10 V intensity.

Purkinje fibres were obtained from the left or right ventricles of hearts removed from dogs anaesthetized with pentobarbitone sodium. The hearts were placed in cold Tyrode solution and both ventricles were opened. Free running Purkinje fibres were removed with a small amount of ventricular muscle left attached at each end to allow pinning to the base of the tissue bath. In the bath the fibres were superfused with warm (37°C) Tyrode solution of the composition described above. Electrical stimuli (as above) were applied to one end of the fibre.

Intracellular recordings were made from single guinea-pig ventricular muscle fibres and dog Purkinje fibres using a high input impedance preamplifier and glass micro-electrodes filled with 3 M KCl (resistance = 10–20 MΩ); a Ag/AgCl electrode in the bath served for reference. Action potentials and the output of an analogue differentiator ($^{dV}/dt$) were displayed on a digital oscilloscope from which stored records could be written to a pen recorder. A sample and hold circuit in the differentiator allowed for measurement of the maximum rate of depolarization during phase 0 of the action potential (V_{max}).

The following parameters were measured from the records: 'diastolic' membrane potential (DMP) immediately before the action potential, V_{max}, action potential duration (APD) to 100%, 90%, 50% and 30% repolarization (APD_{100}, APD_{90}, APD_{50}, APD_{30}),

latency from stimulus to beginning of the action potential, threshold voltage, and the effective refractory period (ERP) measured by applying extra stimuli (at twice threshold voltage) at progressively later times after normal stimuli, and observing the pulse separation at which the first extra action potential was initiated.

Results were taken from experiments in which a stable recording was made from a single cell during a 45–60 min control period and then during superfusion with BW A256C, flecainide, quinidine, disopyramide or lidocaine at 5 or 6 progressively higher concentrations. Each concentration of test drug was applied for a 20 min period with readings of the action potential parameters being made during the final 2 min of each period. Drugs were initially dissolved in small volumes of Tyrode solution (BW A256C, quinidine), distilled water (flecainide) or 0.1 M HCl (lidocaine, disopyramide) and subsequently diluted in Tyrode solution.

The mean and standard error for each parameter at each concentration was calculated. The EC_{50} for V_{max} reduction was calculated by computer fitting curves to individual dose-V_{max} relationships by a least squares technique. From these curves the EC_{50} values were obtained, and the mean EC_{50} for each compound calculated.

The mean EC_{50} for each class 1 agent was compared with that for BW A256C using Student's t test.

Inhibition of aconitine-induced arrhythmias in anaesthetized rats

Male Wistar rats weighing 200–300g were used. Anaesthesia was induced with a halothane:air mixture and maintained by intravenous administration of an α-chloralose and pentobarbitone sodium mixture (9.5 mg ml^{-1} α-chloralose and 3 mg ml^{-1} pentobarbitone sodium) after insertion of a femoral vein cannula. A tracheal cannula was inserted and the animal was ventilated via a Palmer small animal respiration pump (72 strokes min^{-1} and approximately 1 ml 100g^{-1}). Rectal temperature was thermostatically maintained at 37°C throughout the experiment. Cannulae were placed in a jugular vein for administration of aconitine and in a carotid artery for continuous measurement of blood pressure. Subdermal needle electrodes were inserted for the recording of the lead II electrocardiogram (ECG) throughout the experiment.

When the blood pressure of the animal had stabilized for 10–15 min, aconitine was infused via the jugular vein cannula at a rate of 1 μg min^{-1} (0.03 ml min^{-1}). The total dose of aconitine required to produce ventricular tachycardia or ventricular fibrillation of at least 1 s duration was taken as the endpoint of the assay.

Antiarrhythmic activity was assessed by pretreating animals randomly with drug or vehicle 15 min prior to aconitine infusion then comparing the dose of aconitine necessary to induce ventricular arrhythmias in treatment groups with that of control groups. All drugs were used in their salt forms and were prepared in either 5% dextrose or 0.9% saline immediately before use, with the exception of lidocaine which was prepared in propylene glycol. Drugs were administered via a femoral venous cannula in a dose volume of 0.5 ml over a period of 5 min.

Acute coronary artery occlusion – reperfusion-induced arrhythmias in anaesthetized dogs

Adult beagles of either sex and weighing between 10–12 kg were used. Anaesthesia was induced with sodium thiopentone (approximately 30 mg kg^{-1} i.v.) and maintained with a mixture of α-chloralose and pentobarbitone sodium after cannulation of a femoral vein. Animals were placed on an operating table and rectal temperatures maintained at 38–39.5°C using an underbody heating mat and infra red lamps. A femoral artery was cannulated to allow measurement of blood pressure and the withdrawal of blood samples for analysis of pH, blood gases and haematocrit values. The trachea was cannulated and the lungs ventilated with room air using a Starling pump (rate 10 strokes min^{-1} and tidal volume about 15 ml kg^{-1}) adjusted if necessary to maintain blood gases within normal limits. An external jugular vein was cannulated for drug administration. The lead II ECG was recorded via intradermal needle electrodes.

A left thoracotomy was performed via the fourth or fifth intercostal space, the lungs were displaced and a pericardial cradle formed in order to expose the heart. The left atrium was retracted and the left anterior descending coronary artery (LAD) dissected free distal to the bifurcation of the circumflex branch and septal artery but proximal to all other major diagonal branches. A size 0 suture was placed under the artery and an occlusive polyethylene snare was placed around the vessel. Acute myocardial ischaemia was induced by occluding the LAD for 60 min, after which time the snare was removed and the artery was allowed to reperfuse for a further 2 h.

The arterial blood pressure and ECG were recorded continuously on a Beckman R611 Dynograph. A control group of animals ($n = 22$) was untreated whereas 3 experimental groups ($n = 5–6$) were given BW A256C at doses of 0.25, 0.5 and 1 mg kg^{-1} by bolus intravenous injection immediately before removal of the occluding snare. The total number of ventricular ectopic beats occurring during the 2 h reperfusion phase was counted and the data expressed as a median with a range. Statistical comparisons between control and test groups were carried out using

non-parametric analysis (Mann Whitney U test).

Acute coronary artery occlusion-induced arrhythmias in the conscious dog

Healthy beagle dogs of either sex and weighing 8–14 kg were used. Severe ventricular arrhythmias were induced by surgical occlusion of a coronary artery.

Before surgery, animals were given procaine penicillin and streptomycin (Glaxovet Streptopen, 500 mg i.m.). They were tranquillized with acepromazine (0.5 mg kg^{-1} i.m.) and anaesthesia was induced by using sodium thiopentone (18–25 mg kg^{-1} i.v.) and maintained with pentobarbitone sodium (10–20 mg kg^{-1} i.v.). The dogs were intubated and ventilation was maintained with room air via a constant volume pump. Using aseptic techniques, a left thoracotomy was performed in the 4th or 5th intercostal space and the heart exposed. The pericardium was opened and the LAD dissected free from the myocardium just distal to the first major diagonal branch. The LAD was completely occluded using a single-stage ligation procedure. The pericardium was repaired and the chest closed in layers with appropriate sutures. To allow subsequent measurement of aortic blood pressure, a polythene or polyvinyl plastic catheter was inserted in the left carotid artery to the level of the aortic arch or into the descending aorta. Cannulae were filled with heparin-saline (1000 u ml^{-1}), sealed and exteriorized at the back of the neck. All wounds were sutured, covered with sterile dressings and the animals allowed to recover from anaesthesia. Pethidine (10 mg kg^{-1} s.c.) and diazepam (0.5 mg kg^{-1} i.m.) were given at intervals during the 8 h following surgery to ease the pain and restrict mobility.

About 20 h following surgery, the animals were conscious and exhibited severe multifocal ventricular arrhythmias, with no, or very few, normal sinus beats in evidence. For the study conscious animals were supported in restraint slings within the laboratory. Aortic blood pressure was measured by connecting a Statham P23Gb or Kulite pressure transducer to the indwelling catheter. Lead II ECG was measured via subdermal needle electrodes. Continuous recordings of both variables were made on a Beckman R611 Dynograph.

Heart rate was measured at intervals by counting ventricular depolarizations over 1 min periods. Additionally, the chart speed was increased at intervals to allow analysis of the arrhythmias and for measurements of ECG segment intervals (P-R, QRS and QT) of normal sinus beats.

An intravenous cannula was inserted percutaneously into either a cephalic or saphenous vein for intravenous drug administration. Blood samples for plasma drug analysis were removed from the indwelling arterial cannula and assayed for BW A256C by an h.p.l.c. method, to be described elsewhere (Buick & Parsons).

Seven groups ($n = 5–8$) of experimental animals were used. In one group, BW A256C was administered by intravenous infusion (0.01 mg kg^{-1} min^{-1}) until ventricular ectopic activity was suppressed. In a further 5 groups of animals, BW A256C was administered by oral gavage at doses of 1.25, 1.75, 2.5, 3.5 and 5 mg kg^{-1}. The remaining group of animals received an intravenous infusion of flecainide (0.1–0.25 mg kg^{-1} min^{-1}) until ventricular ectopic activity was suppressed. Ventricular ectopic activity was measured over a 5 min period, at least every 15 or 30 min by counting ectopic beats and expressing these as a percentage of the total ventricular rate. Mean ± s.e.mean values were calculated and the treatment values were compared with initial values using paired *t* tests. Aortic blood pressure and ECG segment intervals (P-R, QRS and QT; when sinus beats appeared) were also measured at 15 or 30 min intervals.

Drugs used

BW A256C was prepared as a mesylate salt in the Department of Medicinal Chemistry of the Wellcome Research Laboratories. Flecainide acetate was a gift from Riker Laboratories. Quinidine hydrochloride, procainamide hydrochloride and disopyramide (base) and lidocaine (crystalline) were obtained from the Sigma Chemical Company.

Results

Electrophysiological studies in vitro

(a) *Comparison with other agents* In the guinea-pig ventricle, BW A256C and the four class 1 agents; quinidine, lidocaine, disopyramide and flecainide produced concentration-dependent decreases in V_{max} (Figure 2). BW A256C was significantly the most potent compound (Table 1). The log EC_{50} for BW A256C was -5.66 ± 0.06 M; the EC_{50} was 2.2×10^{-6} M.

(b) *Effects of BW A256C on intracellular action potential recordings from guinea-pig ventricle and dog Purkinje fibres*

(1) *Rate of rise of phase 0* (V_{max}) BW A256C caused a concentration-dependent reduction of V_{max} in both tissues. The EC_{50} for BW A256C was not significantly different between the two tissues (guinea-pig ventricle 2.2×10^{-6} M, dog Purkinje fibre 1.8×10^{-6} M).

(2) *Diastolic membrane potential* BW A256C

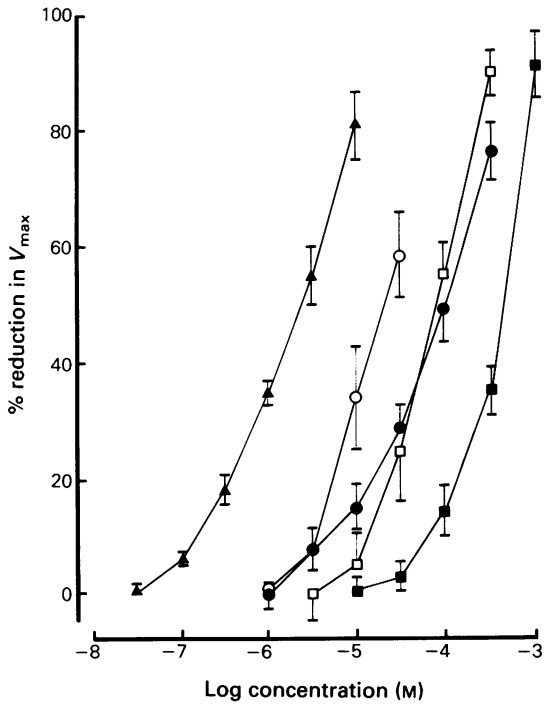


Figure 2 The effect of BW A256C (▲), flecainide (○), disopyramide (●), quinidine (□) and lidocaine (■) on the V_{max} of guinea-pig right ventricular action potentials. Tissue was stimulated at a frequency of 1 Hz. $n = 5–6$.

caused no significant change in DMP in guinea-pig ventricle up to 10^{-6} M, but did cause a small hyperpolarization (3mV) at 3×10^{-6} and 10^{-5} M. In Purkinje fibres no significant changes in DMP were observed at these concentrations.

(3) *Action potential amplitude* Amplitude decreased in a concentration-related way in both tissues, but the changes were greater in Purkinje fibres than in ventricle. For example, at 10^{-6} M BW A256C, amplitude was reduced by 7.8% in Purkinje fibres but only by 1.3% in guinea-pig ventricle (Table 2).

(4) *Action potential duration* In the guinea-pig ventricle, the values for APD_{100}, APD_{90}, APD_{50} and APD_{30} were all shortened by BW A256C at a concentration of 10^{-6} M or greater. However the changes were small, in the order of 5%.

In contrast to guinea-pig ventricle, the changes in APD in Purkinje fibres were marked. APD_{100}, APD_{90} and APD_{50} showed concentration-dependent reductions in the range 10^{-7} to 10^{-5} M BW A256C. At 10^{-6} M BW A256C, the reductions were 20.3, 24.5 and 34.3%, respectively. In terms of shape of the action potential, this was observed as a progressive loss of the plateau (phase 2), the action potential becoming more triangular in shape. The APD_{30} showed progressive reduction up to 10^{-6} M, but at 3×10^{-6} and 10^{-5} M there was no mean decrease (in 3/6 preparations, APD_{30} increased). The two highest doses of BW A256C caused total loss of the fast overshoot, and thus the APD_{30} was measured to a relatively much more negative membrane potential, thereby accounting for the increase in APD_{30} observed in the three preparations.

(5) *Effective refractory period* In the presence of BW A256C, the ERP did not change significantly in the ventricle, nor did the ratio of APD_{90}:ERP.

In contrast, in Purkinje fibres, the ERP decreased markedly when BW A256C was applied. This was mostly due to the shortening of the APD, however, the ratio APD_{90}:ERP was not significantly altered.

(6) *Latency* The latency increased in a concentration-related way in both tissues. At 10^{-6} M BW A256C

Table 1 EC_{50} values (50% depression of V_{max}, expressed as log [M] ± s.e.mean) in the guinea-pig ventricle *in vitro*, for BW A256C, quinidine, lidocaine, flecainide and disopyramide

Antiarrhythmic agent	EC_{50}
BW A256C	−5.66 ± 0.06
Quinidine	−4.27 ± 0.07*
Lidocaine	−3.42 ± 0.03*
Flecainide	−4.69 ± 0.08*
Disopyramide	−4.05 ± 0.10*

*Significantly different ($P < 0.01$) when compared to value for BW A256C. $n = 5–6$.

Table 2 The effects of BW A256C (10^{-6} M) on cardiac action potential parameters of the guinea-pig ventricle (GPV) and dog Purkinje fibre (DPF)

Parameter	*% change in GPV*	*DPF*
V_{max}	−34.8 ± 2.0	−36.1 ± 4.4
DMP	+1.4 ± 1.0	+0.4 ± 1.0
AP amplitude	−1.3 ± 1.0	−7.8 ± 2.4
APD_{90}	−5.3 ± 1.8	−24.5 ± 2.6
ERP	−2.2 ± 4.0	−18.2 ± 5.8
Latency	+32.1 ± 7.0	+34.8 ± 12.0

Data show mean % change ± s.e.mean. $n = 6$ (GPV); 5 (DPF). V_{max} = maximum rate of depolarization during phase 0 of the action potential (AP); DMP = diastolic membrane potential immediately before the action potential; APD_{90} = action potential duration to 90% repolarization and ERP = effective refractory period.

Table 3 The effect of pretreatment with quinidine, procainamide, lidocaine, flecainide and BW A256C on the dose of aconitine required to induce ventricular arrhythmias in anaesthetized rats

Antiarrhythmic agent	(n)	*Dose* ($mg\,kg^{-1}$)	*Dose of aconitine to elicit VT/VF* ($\mu g\,ml^{-1}$)	*% increase from control*	*% change in diastolic BP*
Control	(56)		19.9 ± 0.6		
Quinidine	(5)	10	21.6 ± 1.5	8.4	−43 ± 6
Procainamide	(5)	20	22.2 ± 2.5	11.4	−22 ± 9
Lidocaine	(4)	10	24.0 ± 1.0	20.3	−14 ± 6
Flecainide	(6)	5	40.3 ± 3.0	102.2	−26 ± 8
	(4)	10	43.5 ± 2.8	168.1	−34 ± 11
BW A256C	(7)	0.5	54.6 ± 9.4	173.7	0 ± 7
	(6)	1	119.5 ± 12.7	498.9	−13 ± 4

the increases were equivalent in the two tissues (32.1%, ventricle; 34.8%, Purkinje fibres, Table 2).

(7) *Threshold* The threshold voltage increased in a concentration-related way in both tissues. There was considerable variability of the percentage increase. However, at a concentration of 10^{-6} M BW A256C, 4/5 of Purkinje fibres and 6/6 ventricle preparations showed an increase in threshold voltage compared to control.

Inhibition of aconitine-induced arrhythmias in anaesthetized rats

Intravenous administration of aconitine to the anaesthetized rat rapidly elicited ventricular extrasystoles of both monofocal and multifocal origin. All animals succumbed to a rapid ventricular tachycardia or ventricular fibrillation. The dose required to elicit ventricular tachycardia (10 consecutive beats) or ventricular fibrillation in control animals was $19.95 \pm 0.6\,\mu g\,kg^{-1}$ ($n = 56$). Pretreatment of anaesthetized rats with either quinidine, procainamide, lidocaine or flecainide, administered by intravenous infusion, resulted in an increase in the amount of aconitine required to elicit ventricular arrhythmias (Table 3). Moreover, pretreatment of anaesthetized rats with the above agents also resulted in drug-induced reduction in the diastolic blood pressure, suggesting a significant cardiovascular depression associated with the antiarrhythmic efficacy of these agents (Table 3). Intravenous administration of BW A256C ($0.5-1\,mg\,kg^{-1}$) caused a dose-dependent increase in the dose of aconitine required to elicit ventricular arrhythmias but with only minimal alteration in resting diastolic blood pressure (Table 3). Of all the agents tested in the anaesthetized rat preparation, BW A256C was the most potent. When compared with flecainide, it was demonstrated that for equieffective antiarrhythmic doses of drug there was greater cardiovascular depression, as indicated by a reduction in blood pressure, heart rate and atrioventricular conduction, associated with the administration of flecainide (Table 4). Antiarrhythmic efficacy of BW A256C in the anaesthetized rat was associated with a prolongation of conduction time across the myocardium which was observed as an increase in the P-R interval on the lead II ECG. Increases of 11–35% in the P-R interval were observed following pretreatment of anaesthetized rats with BW A256C at doses displaying antiarrhythmic activity.

Acute coronary artery occlusion/reperfusion-induced arrhythmias in the anaesthetized dog

Immediately after the occlusion of the LAD, T wave voltage increased and the ST segment was elevated. These changes were succeeded by the occurrence of multiple ventricular tachyarrhythmias at 5–10 min following occlusion. A second surge of ventricular arrhythmias occurred 12–20 min following occlusion. This pattern of ventricular arrhythmias occurring after coronary occlusion was essentially similar to that described by previous workers (Harris, 1950; Kaplinsky *et al.*, 1979). On reperfusion of the LAD there was an immediate phase of ventricular tachyarrhythmias

Table 4 The effect of approximately equiactive antiarrhythmic doses of BW A256C and flecainide on diastolic blood pressure (BP), heart rate and atrioventricular conduction time P-R interval in anaesthetized rats

	BW A256C ($0.5\,mg\,kg^{-1}$)	*Flecainide* ($10\,mg\,kg^{-1}$)
Diastolic BP (% change)	0	−34 ± 11
Heart rate (% change)	0	−26 ± 5
P-R interval (% change)	+24 ± 2	+59 ± 3

Antiarrhythmic doses of BW A256C and flecainide were derived from the aconitine-induced arrhythmia model.

which lasted for approximately 5 min. There was a resurgence of ventricular arrhythmias 15–20 min following reperfusion which was succeeded by a relatively quiescent period for the remainder of the reperfusion period.

The incidence of arrhythmias in all groups of animals was not significantly different during the occlusion period. However, there was a dose-dependent decrease in the frequency of ventricular arrhythmias during the reperfusion phase in groups of animals receiving BW A256C. Statistical evaluation of the data revealed that significant suppression (81% reduction) of ventricular ectopic activity occurred at a dose of 1 mg kg^{-1} (Figure 3). Intravenous administration of BW A256C, 0.25–1 mg kg^{-1}, produced a dose-dependent prolongation of the P-R interval (8.5–31% increase). However, no other discernible haemodynamic changes were noted following drug administration.

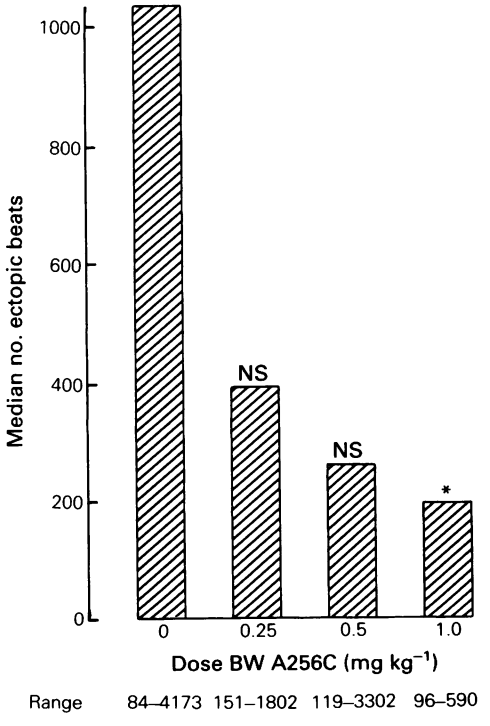


Figure 3 The effect of BW A256C on the total number of ectopic beats during 1 h reperfusion of the left anterior descending coronary artery in the anaesthetized dog. Values represent the median number and range of ectopic beats measured throughout the 1 h reperfusion period. *Significant difference between control and treatment groups ($P < 0.01$, Mann Whitney U test). $n = 23$ in the control group and 5–8 in the treatment groups. NS, no significant difference.

Acute coronary artery occlusion-induced arrhythmias in the conscious dog

Approximately 20–24 h after ligation of the LAD all dogs exhibited severe ventricular arrhythmias of predominantly multifocal origin. The frequency of ectopic activity was always greater than 80% and was sustained (> 50%) in control animals over the following 24 h.

Intravenous infusion with BW A256C (0.01 mg kg^{-1} min^{-1}) but not with the vehicle alone (5% dextrose) resulted in a gradual suppression of ectopic activity until a normal sinus rhythm was restored (Figure 4).

The total doses for 50% and 100% suppression of ectopic activity (ED_{50} and ED_{100}, respectively) were, ED_{50} 0.73 ± 0.13 mg kg^{-1} and ED_{100} 1.27 ± 0.18 mg kg^{-1} (mean ± s.e.mean; $n = 5$). The associated mean ± s.e. plasma levels (in three dogs) were 196 ± 18 ng ml^{-1} and 216 ± 12 ng ml^{-1}, respectively. The peak plasma level attained with BW A256C always preceded the peak antiarrhythmic activity. The

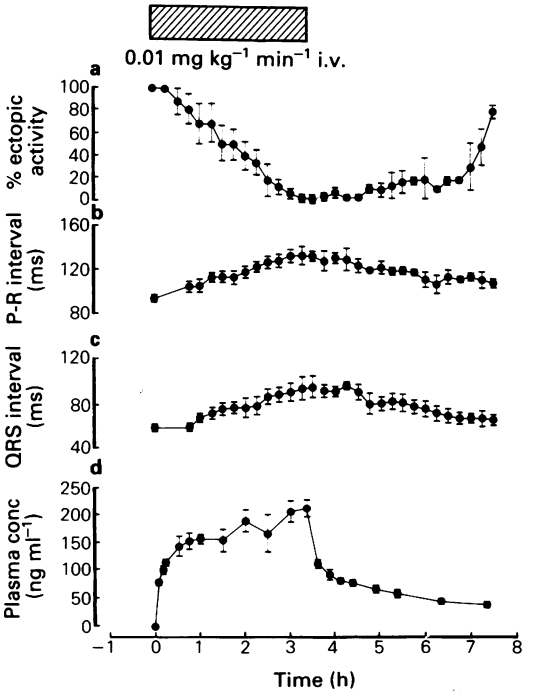


Figure 4 The effect of BW A256C (0.01 mg kg^{-1} min^{-1}, shown by hatched bar) on the frequency of ectopic activity (a) and the P-R (b) and QRS (c) intervals in the conscious dog ($n = 5$) 24 h after acute coronary artery occlusion. (d) Shows the plasma levels of BW A256C achieved with drug infusion ($n = 3$).

antiarrhythmic activity was first apparent after 30–60 min of drug infusion, attained a maximum at approximately 3 h (whereupon the drug infusion was terminated) and was maintained for a further 3–4 h. In contrast the peak plasma level was attained more rapidly (60–120 min) and declined more rapidly on termination of the infusion (Figure 4).

Intravenous infusion of flecainide (0.1–0.25 mg kg^{-1} min^{-1}) also resulted in suppression of the arrhythmias; ED_{100} was 8.8 ± 0.9 mg kg^{-1} ($n = 8$). Following restoration of a normal sinus rhythm, the infusions were terminated in a number of dogs to assess the duration of the antiarrhythmic action. With BW A256C the effect (> 50% suppression of ectopic activity) was maintained for 3–4 h whereas that following flecainide was only maintained for 0.5–1 h (Table 5).

Oral administration of all doses of BW A256C (1.25–5 mg kg^{-1}) suppressed ectopic activity and restored normal sinus rhythm. Maximum effects occurred within 0.75–5.5 h of drug administration and persisted for up to 24 h depending on the dose administered. A number of the animals given the highest doses, 3.5 and 5 mg kg^{-1}, showed up to 100% suppression of arrhythmias within 3 h of dosing but thereafter showed periods of increased arrhythmias which were attributed to a proarrhythmic activity of the drug. These arrhythmias were distinct from those apparent before drug administration, and were characterized by complete atrioventricular block and wide QRS complexes resulting in 'runs' of ventricular tachycardia. The incidence and duration of this effect varied; thus in the seven dogs given 3.5 mg kg^{-1}, the proarrhythmic activity was marked in three animals between 2–8 h of drug administration; only slight in two others at 6.5–8 h after; and absent in the remaining two dogs. Of the six dogs given 5 mg kg^{-1}, the effect was marked in four dogs between 2.5–8 h after drug administration (one subsequently died of ventricular fibrillation); only slight at 3–5 h in one dog; and absent in the other dog. Following recovery from the pro-arrhythmic activity a normal sinus rhythm was again apparent and persisted up to 24 h after drug administration.

The plasma drug levels associated with the antiarrhythmic effect of orally administered BW A256C are shown as the actual peak levels in Table 6. Maximum plasma drug levels were apparent within approximately 3 h of drug administration and were dose-related. In contrast to the intravenous study, the lowest dose of BW A256C administered orally which consistently produced a 50% or greater suppression of ectopic activity was 1.25 mg kg^{-1} and the associated plasma level was 93 ± 9.3 ng ml^{-1} (cf. 196 ng ml^{-1} required after intravenous administration). Peak plasma levels associated with pro-arrhythmic activity of BW A256C (290–436 ng ml^{-1}) were 3–4 times higher than those levels associated with the antiarrhythmic activity of this agent (Table 6).

Myocardial conduction, as indicated by the P-R and QRS intervals of the ECG, was prolonged in association with the antiarrhythmic action of BW A256C and flecainide. The effect with flecainide appeared less pronounced than that with BW A256C. Thus, the maximum changes in conduction associated with the suppression of ectopic activity (at the intravenous ED_{100}) were + 37 ± 3% (P-R interval) and + 44 ± 3% (QRS) with BW A256C, and + 8 ± 2% (P-R) and + 30 ± 6% (QRS) with flecainide.

Following oral administration of BW A256C, myocardial conduction was also prolonged in a dose-dependent manner. Maximum effects on conduction were coincident with the maximum antiarrhythmic action, with mean increases of 16% (P-R) and 18% (QRS) at 1.25 mg kg^{-1}, and 34% (P-R) and 51% (QRS) at 2.5 mg kg^{-1}. The effects on conduction were more marked (> 80% on P-R and > 90% on QRS) following the administration of 3.5 and 5 mg kg^{-1} and were associated with a dissociation of atrioventricular conduction.

The intravenous and oral administration of BW A256C was well tolerated since no behavioural changes were observed other than slight emetic reactions in the dogs given the highest oral dose (5 mg kg^{-1}).

Table 5 Comparison of the antiarrhythmic activity of BW A256C and flecainide in the conscious beagle dog 24 h after permanent occlusion of the left anterior descending coronary artery

Drug	*Antiarrhythmic* *ED_{100}	*Duration of action*
BW A256C	1.27 ± 0.18	3–4 h
Flecainide	8.8 ± 0.90	0.5–1 h

*Dose producing 100% suppression of ventricular arrhythmias.

Discussion

Vaughan Williams (1981) has proposed that antiarrhythmic agents be classified into four types. These are: class 1, sodium channel blockers (e.g. lidocaine, quinidine, flecainide), class 2, 'anti-sympathetic' agents (e.g. β-blockers), class 3, compounds that increase APD (e.g. amiodarone), and class 4, calcium channel blockers (e.g. verapamil).

The primary effect of class 1 agents is to cause a reduction of the fast inward sodium current which flows during phase 0 of the action potential; this effect

Table 6 The antiarrhythmic activity of BW A256C administered orally to conscious beagle dogs 24 h after permanent occlusion of the left anterior descending coronary artery

Dose ($mg\,kg^{-1}$)	n	*Antiarrhythmic activity (>50% suppression*)*	*Proarrhythmic activity* (n)	*Peak plasma drug level* (mean ± s.e.mean; $ng\,ml^{-1}$)
1.25	4	4	0	93 ± 9.3
1.75	4	3	0	194 ± 31
2.5	6	4	0	178 ± 30
3.5	7	7	5	297 ± 28
5	6	6	5	436 ± 58

*Suppression of ventricular ectopic activity by 50% or more. n = number of animals.

is observed as a reduction in the maximum rate of depolarization (V_{max}). In the experiments described in this paper, BW A256C was shown to reduce V_{max} in a concentration-dependent way, thus BW A256C can be classified as a class 1 antiarrhythmic agent. All of the other effects of BW A256C on the cardiac action potential are expected consequences of sodium channel blockade, i.e. the increase in latency (slowing of conduction), reduction of action potential amplitude and increase in threshold voltage. The differing action of BW A256C on APD in ventricle and Purkinje fibres can also be explained as a block of sodium conductance since in the ventricle, the plateau phase of the action potential is mainly due to a balance of Ca^{2+} and K^+ currents, whilst in Purkinje fibres an inward 'window' current carried by Na^+ ions, and sensitive to class 1 agents, contributes to the plateau (Attwell *et al.*, 1979; Colatsky, 1982). By reducing this 'window' current, BW A256C would shorten the plateau phase and hence the APD would decrease. The data from guinea-pig ventricle presented in this paper indicate that BW A256C neither has class 3 properties nor is it likely to have class 4 properties since the plateau duration and shape in the guinea-pig ventricle were not greatly changed even at the highest doses. By definition, class 3 agents increase APD whilst calcium antagonists have been shown to produce significant shortening of the plateau in guinea-pig ventricular action potentials (Grant & Katzung, 1976; Molyvdas & Sperelakis, 1983). No evidence is presented in this study to show whether or not BW A256C possesses class 2 activity.

The lack of an effect of BW A256C on APD_{90} and ERP in guinea-pig ventricle, has been previously described for some other class 1 agents, in particular for flecainide (Campbell, 1983). Other class 1 agents have been shown to lengthen ERP in the presence of decreased APD_{90} (e.g. lidocaine) or to lengthen both APD_{90} and ERP (e.g. procainamide, disopyramide, quinidine). On the basis of such effects it has been proposed that class 1 agents be further subdivided into 1a (quinidine-like), 1b (lidocaine-like) and 1c (flecainide-like) (Harrison *et al.*, 1981; Campbell, 1983). BW A256C could be classified tentatively as a class 1c agent using such criteria. Studies concerning the frequency-dependent actions of BW A256C will be the subject of a separate paper and will provide further evidence for this subclassification.

Of the agents evaluated *in vitro*, BW A256C was the most potent, being approximately 10 times more potent than flecainide. Potency estimates of these agents were established under the conditions of 1 Hz stimulation frequency and therefore do not take into consideration the marked frequency-dependent properties of class 1 antiarrhythmic agents (Courtney, 1980).

In agreement with our *in vitro* studies was the finding that of all the agents tested in the anaesthetized rat preparation, BW A256C was the most potent, being approximately 20 times more potent than the class 1 antiarrhythmic agent flecainide. Moreover, BW A256C was devoid of hypotensive activity at doses displaying antiarrhythmic activity, whereas all the other class 1 antiarrhythmic agents studied had a significant hypotensive effect. These findings suggest that in this species, only minimal cardiovascular depression is associated with the antiarrhythmic activity of BW A256C. This desirable action of BW A256C is highlighted when compared with flecainide, where it is demonstrated that for equiactive antiarrhythmic doses of drug there is greater cardiovascular depression, as indicated by a reduction in blood pressure, heart rate and atrioventricular conduction, associated with the administration of flecainide.

Electrophysiological studies have demonstrated that cardiac arrhythmias associated with the administration of aconitine are a consequence of an increase in sodium conductance together with a delayed inactivation of the sodium current, resulting in a highly unstable membrane (Peper & Trautwein, 1967). The ability of BW A256C to delay the onset of aconitine-induced arrhythmias is thus consistent with the blockade of sodium influx during depolarization of the

cardiac muscle.

The results presented here also demonstrate the potent antiarrhythmic actions of BW A256C administered either intravenously or orally in anaesthetized and conscious dogs. In the anaesthetized dog, tachyarrhythmias were produced by reperfusion of the acutely ischaemic myocardium. Such arrhythmias have been shown to result primarily from an enhanced ventricular automaticity, whereas the arrhythmias occurring 24 h after permanent coronary artery occlusion in the dog have been demonstrated to originate in the subendocardial Purkinje network of the infarcted region as a result of both abnormal automaticity and re-entrant mechanisms (Wit & Friedman, 1975; Kaplinsky *et al.*, 1981). Regardless of whether abnormal automaticity or re-entrant mechanisms contribute to the genesis of these arrhythmias, our results with BW A256C demonstrate clearly the efficacy of a potent class 1 agent in these models.

In the conscious dog, in addition to the demonstration of antiarrhythmic efficacy of BW A256C, important information regarding systemic tolerance, time course of action and oral activity was obtained. BW A256C was more potent as an antiarrhythmic agent than flecainide in the conscious dog, a finding consistent with our results obtained in the anaesthetized rat and our *in vitro* studies. Furthermore, in the dog, BW A256C was longer acting than flecainide, the antiarrhythmic action of flecainide being fairly short lived. However, the time courses in the dog cannot necessarily be extrapolated to those in man. In man, flecainide has been found to have a long therapeutic time course due to its long plasma half-life of approximately 20 h (cf. approximately 1 h in the dog) (Somani, 1980; Conard & Ober, 1984).

When BW A256C was administered to the dog by the oral route, maximum effects occurred within 0.75–5 h of drug administration and persisted for up to 24 h, depending on the dose administered. These results demonstrate good oral bioavailability and a long time course of action. Of interest is that the antiarrhythmic activity following oral administration of drug was associated with lower peak plasma levels of drug than the corresponding effect when the drug was administered by intravenous infusion. This difference could be attributed to the formation of an active metabolite which might predominate when BW A256C is administered orally. However, this is unlikely since there is no evidence of any major metabolism in the dog (Parsons, unpublished observations). A more likely explanation is that the difference arises from the physicochemical nature of BW A256C and the differing rates of exposure of the myocardium from the two routes of administration. An important physicochemical property of BW A256C is that it has a pK_a of 11.2 and is therefore fully ionised at physiological pH. Consequently, tissue distribution would be expected to be relatively slow and the myocardium would not be expected to come into rapid equilibrium with the plasma compartment. It could therefore be argued that plasma levels following intravenous infusion of drug can rise more rapidly prior to equilibrium with the myocardial compartment, whereas those following oral administration being slower in onset allow greater time for equilibrium with the myocardium.

Although a number of clinically effective antiarrhythmic agents have been found to be effective in suppressing post myocardial infarction arrhythmias in conscious dogs, efficacy is often associated with severe central nervous system disorders (Carmeliet *et al.*, 1978; Bergey *et al.*, 1981). Whilst no specific neurological observations were carried out, no gross behavioural observations indicative of adverse effects due to central nervous system penetration were made during the experiments on conscious dogs, thus supporting our hypothesis that this agent has restricted access to the CNS.

In this study paradoxical pro-arrhythmic activity was observed following oral administration of the highest doses of BW A256C. Provocation of ventricular arrhythmias is increasingly becoming a clinically recognised feature of antiarrhythmic agents, such arrhythmias often occurring within the therapeutic plasma range of the drug (Velebit *et al.*, 1982; Goldstein *et al.*, 1984). The pro-arrhythmic activity of BW A256C, observed at plasma concentrations 3–4 fold greater than those displaying antiarrhythmic activity, further demonstrates the need for caution when using these agents in the medical management of patients with life threatening arrhythmias.

In conclusion, we have demonstrated that BW A256C is a potent class 1 antiarrhythmic agent and in this respect it is the most potent among a series of contemporary antiarrhythmic agents. The feature of potency in the dog was combined with good oral bioavailability and a long time course of action. In contrast to other class 1 antiarrhythmic agents, BW A256C was well tolerated with no evidence of central or peripheral dysfunction associated with its administration at doses which achieved antiarrhythmic efficacy.

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