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Systemic haemodynamic actions of pimobendan (UD-CG 115 BS) and its *O*-demethylmetabolite UD-CG 212 Cl in the conscious pig

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1 The cardiovascular effects of the pyridazinone-derivatives pimobendan and its *O*-demethylmetabolite UD-CG 212 Cl (2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl) were studied in conscious pigs, employing consecutive intravenous 10 min infusions of 10, 25, 50 and 100 $\mu g\,kg^{-1}\,min^{-1}$ and 2, 4 and 8 $\mu g\,kg^{-1}\,min^{-1}$ respectively.

2 Pimobendan caused dose-dependent increases in LV$dP/dt_{max}$ (up to 115%) and heart rate (up to 30%), while cardiac output was slightly elevated (up to 15%) and stroke volume decreased by 12%. Left ventricular end-diastolic pressure decreased in a dose-related manner from 8.7 ± 1.0 mmHg to 2.7 ± 1.7 mmHg. Mean arterial blood pressure was not significantly affected because systemic vascular resistance decreased dose-dependently up to 15%.

3 After β-adrenoceptor blockade, the pimobendan-induced increases in heart rate and cardiac output were attenuated and the increase in LV$dP/dt_{max}$ almost abolished. The responses of left ventricular end-diastolic and mean arterial blood pressure, systemic vascular resistance and stroke volume were not modified.

4 UD-CG 212 Cl caused dose-related increases in LV$dP/dt_{max}$ (up to 100%) and heart rate (up to 25%). Cardiac output was minimally elevated (up to 8%) as stroke volume decreased dose-dependently up to 15%. As systemic vascular resistance decreased up to 12%, mean arterial blood pressure was slightly reduced (5%). Left ventricular end-diastolic blood pressure decreased dose-dependently from 9.0 ± 0.8 mmHg to 3.8 ± 1.3 mmHg.

5 After β-adrenoceptor blockade, the UD-CG 212 Cl-induced increases in heart rate and LV$dP/dt_{max}$ were attenuated and almost abolished and amounted up to 15% and 20%, respectively. The responses of the other systemic haemodynamic parameters were not significantly modified.

6 We conclude that pimobendan and UD-CG 212 Cl are compounds with marked positive inotropic and venodilator properties in the conscious pig. The attenuation of the inotropic effects by pretreatment with propranolol strongly suggests that, in the conscious pig, the β-adrenergic system is significantly involved in the positive inotropic actions. The lack of effect of β-adrenoceptor blockade on the vasodilator responses to both compounds suggest a mechanism not related to β-adrenergic activity.

Introduction

The pyridazinone-derivative pimobendan (UD-CG 115 BS) has been shown to possess veno- and arteriodilator as well as positive inotropic properties in a number of animal models (Diederen *et al.*, 1982; van Meel, 1985; Verdouw *et al.*, 1986; Duncker *et al.*, 1986). Although the precise mechanism of action of pimobendan is still largely unknown, phosphodiesterase inhibition and an increased sensitivity of contractile proteins to calcium may contribute to its cardiovascular actions (Rüegg *et al.*, 1984; Honerjäger *et al.*, 1984; Scholz & Meyer, 1986). It has also been reported that UD-CG 212 Cl (2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl), the *O*-demethylmetabolite of pimobendan, is more potent at increasing myocardial contractile force than the parent drug itself (Scholz & Meyer, 1986). Confusion therefore exists as to what

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extent the cardiovascular actions ascribed to pimobendan are in fact secondary to the presence of the metabolite. In the present study we describe the cardiovascular actions of pimobendan and UD-CG 212 Cl in the conscious pig and on the possible contribution of UD-CG 212 Cl to the actions of pimobendan. In addition, to obtain information on the relative contribution of factors other than those involving the β-adrenergic system, we also studied the effects of pimobendan and UD-CG 212 Cl after pretreatment with propranolol.

Methods

General

After an overnight fast, Yorkshire pigs (18–20 kg, $n = 6$), pretreated with a mixture of procaine penicillin-G and benzathinepenicillin-G (Duplocillin, Gist-Brocades N.V., Delft, The Netherlands) both 300000 units i.m., were sedated with 30 mg kg^{-1} ketamine HCl i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands). The animals were intubated and connected to a respirator for artificial ventilation with a mixture of O_2 and N_2O (1:2) to which 1% halothane was added. A jugular vein and common carotid artery were cannulated for infusion of drugs and measurement of mean arterial blood pressure, respectively. The chest was opened via the left fifth intercostal space to expose the heart. A transducer ($P_{4.5}$, Konigsberg Instruments Inc. Pasadena, California, USA) was implanted into the left ventricle of the heart through its apex for recording of left ventricular pressure. The left atrium was cannulated for recording of left atrial pressure which, together with the aortic blood pressure, was used for calibration of the Konigsberg transducer signals. The aorta was approached through the third intercostal space and an electromagnetic flowprobe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. Catheters and wires were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. During the next 14 days the animals received daily intravenous bolus injections of 500 mg amoxicilline (Clamoxil; Beecham Farma B.V., Amstelveen, The Netherlands) and in addition, during the first week, 500 mg kanamycin (Kamynex; Gist-Brocades N.V., Delft, The Netherlands) to prevent infection. Daily flushing of catheters with an isotonic saline solution containing 500 iu heparin per ml (Thromboliquine; Organon Teknika B.V., Boxtel, The Netherlands) was performed to avoid clotting of blood in the lumen. After one week for recovery from surgery, at least 4 sessions were held to adapt the animals to the experimental and laboratory facilities. The experimental protocol was executed 2–3 weeks after the operation. All tracings were on a Graphtec Linearcorder (FWR 3701; Ankersmit, Breda, The Netherlands). Arterial acid-base balance and oxygenation during the experiments were not significantly different from those observed for young conscious Yorkshire pigs by Lagerwey (1973): pH = 7.41 ± 0.04, P_{CO_2} = 44 ± 4 mmHg, P_{O_2} = 87 ± 6 mmHg and HbO_2-saturation of 91 ± 2%.

Experimental protocols

Four series of experiments (6 pigs in each series) were performed. In two series, consecutive 10 min infusions of either drug were administered. For pimobendan the infusion rates were 10, 25, 50 and 100 μg kg^{-1} min^{-1} and for UD-CG 212 Cl, 2, 4 and 8 μg kg^{-1} min^{-1}. Corresponding volumes were 0.2, 0.5, 1.0 and 2.0 ml min^{-1} and 0.5, 1.0 and 2.0 ml min^{-1} for the pimobendan and UD-CG 212 Cl infusions, respectively. At the end of each 10 min infusion period, when parameters had reached a stable level, tracings of left ventricular pressure and its first derivative (LV$dP/dt$; obtained by electronic differentiation), arterial blood pressure, stroke volume and cardiac output were recorded and arterial blood samples were withdrawn for the determination of plasma concentrations of pimobendan and UD-CG 212 Cl. In the other two series of experiments the same protocols were repeated after β-adrenoceptor blockade with propranolol. The latter was dissolved in isotonic saline and administered intravenously as a bolus injection of 0.5 mg kg^{-1} (given over 2 min), immediately followed by a continuous infusion of 0.5 mg kg^{-1} h^{-1} at a rate of 0.2 ml min^{-1}. The infusions of the pyridazinone-derivatives were started 10 min after the bolus administration of propranolol. At this time haemodynamic parameters had reached a stable level. In other experiments we have shown that the isoprenaline dose-ratio for heart rate and LV$dP/dt_{max}$ for this dose of propranolol is more than 20 (unpublished data from this laboratory). Since the volume that was infused during propranolol administration was small (0.2 ml min^{-1}), isotonic saline was not administered to the animals which did not receive propranolol.

Determination of plasma concentrations

The plasma concentrations of pimobendan and UD-CG 212 Cl were determined by use of an h.p.l.c. assay with fully automated drug preconcentration on solid support (Roth, 1983). Briefly, the drugs were extracted on a reverse phase column and simultaneously preconcentrated after injection of whole plasma. The compounds were measured by means of fluorescence detection (332 nm/405 nm) after h.p.l.c separation on reversed phase ODS-hypersil (particle size; 5 μm). The eluent composition was methanol/water (590/460, v/

v) + 2.5 g ammonium acetate per litre eluent (total amount 2.625 g). Post column, a mixture of methanol/orthophosphoric acid 85%/water (300/100/100,v/v/v) was added with a flow rate of 0.2 ml min^{-1} via a T-fitting in order to optimize the fluorescence (increase in fluorescence by a factor of 2). The lower limit of detection for both compounds was about 1 ng ml^{-1}. Pimobendan and UD-CG 212 Cl themselves were used as external standards.

Statistical analysis

Data have been presented as mean of 6 experiments ± s.e.mean. Statistical analysis was performed by use of a parametric two-way analysis of variance (randomized block design), followed by the Duncan new multiple range test (Steel & Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

The only substances used were propranolol hydrochloride (ICI-Pharma, Rotterdam, The Netherlands), pimobendan and its *O*-demethylmetabolite (2-(4-hydroxy-phenyl)-5-(5-methyl -3-oxo-4,5-dihydro-2H-6- pyridazinyl)benzimidazole HCl). Both of the latter compounds were kind gifts from Dr Karl Thomae GmBH, Biberach a/d Riss, FRG and they were dissolved in a mixture of polyethylene glycol 200 and saline (1:1).

Results

Plasma concentrations of pimobendan and UD-CG 212 Cl

Although duration and rate of infusion of pimobendan were the same as for the anaesthetized pigs (Verdouw *et al.*, 1986), the arterial plasma concentrations of pimobendan were considerably less (30–50%) in the conscious animals. On the other hand, the concentrations of UD-CG 212 Cl were very similar in the two preparations. With the lowest two infusion rates of UD-CG 212 Cl, UD-CG 212 Cl plasma concentrations were similar to the UD-CG 212 Cl plasma concentrations measured after the highest two infusion rates of pimobendan (Table 1).

Effects of pimobendan

Pimobendan caused a mild increase in cardiac output (up to 15%) due to a moderate tachycardia (heart rate increased up to 30%), as stroke volume decreased by 12% (Figure 1). LV$dP/dt_{max}$ increased dose-dependently and was more than doubled after the highest infusion rate. Since mean arterial blood pressure was unchanged in the presence of an increased cardiac output, systemic arterial vasodilatation (reflected by a decrease in systemic vascular resistance up to 15%) must have occurred. The effect on the systemic venous

Table 1 Plasma concentrations of pimobendan and UD-CG 212 Cl after continuous intravenous 10 min infusions of pimobendan and UD-CG 212 Cl in conscious pigs

		Pimobendan (μg kg^{-1} min^{-1})			
		10	25	50	100
Total dose administered (μg kg^{-1})		100	350	850	1850
Plasma concentration (ng ml^{-1})					
Pimobendan	−	65 ± 3*	157 ± 7*	364 ± 16*	828 ± 32*
Pimobendan	+	66 ± 3*	172 ± 6*	399 ± 17*	918 ± 35*
UD-CG 212 Cl	−	0 ± 0	9 ± 1*	14 ± 2*	22 ± 4*
UD-CG 212 Cl	+	1 ± 1	7 ± 2*	13 ± 2*	20 ± 3*
		UD-CG 212Cl (μg kg^{-1} min^{-1})			
		2	4	8	
Total dose administered (μg kg^{-1})		20	60	140	
Plasma concentration (ng ml^{-1})					
UD-CG 212 Cl	−	14 ± 1*	29 ± 1*	62 ± 2*	
UD-CG 212 Cl	+	18 ± 2*†	33 ± 1*†	70 ± 2*†	

Values are given as mean of 6 experiments ± s.e.mean; (−) indicates that propranolol was not present and (+) that the animals were pretreated with propranolol (0.5 mg kg^{-1} + 0.5 mg kg^{-1} h^{-1}); *$P < 0.05$ versus each of the lower plasma concentrations in the same series of experiments. †$P < 0.05$ versus plasma level at comparable infusion rate without β-adrenoceptor blockade.

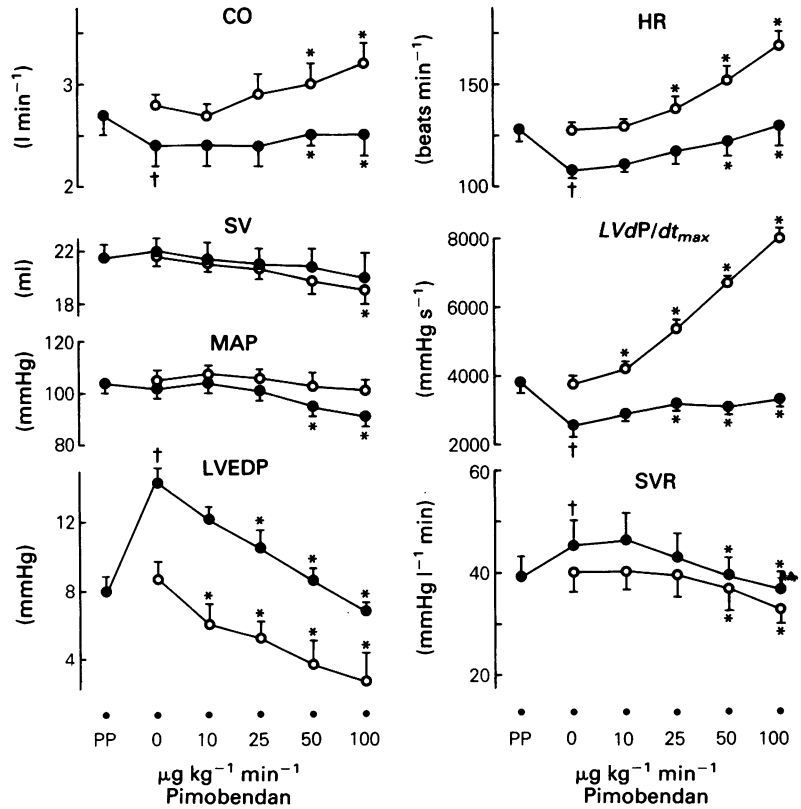

Figure 1 Effects of continuous intravenous 10 min infusions of pimobendan, before (○) or after (●) β-adrenoceptor blockade with propranolol, on systemic haemodynamics in conscious pigs. CO = cardiac output; HR = heart rate; SV = stroke volume; MAP = mean arterial blood pressure; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance. Data have been presented as mean of 6 experiments with s.e.mean shown by vertical lines; †$P < 0.05$ vs pre-propranolol (PP) values; *$P < 0.05$ vs baseline (0).

vasculature was, however, much more pronounced as left ventricular end-diastolic pressure was reduced from 8.7 ± 1.0 mmHg to 2.7 ± 1.7 mmHg.

Pretreatment with propranolol attenuated the increases in heart rate and cardiac output, almost abolished the response of $LVdP/dt_{max}$ but had no effect on the reductions in pre- and afterload (Figure 1).

Effects of UD-CG 212 Cl

UD-CG 212 Cl (0–60 ng ml^{-1}) produced cardiovascular effects similar, both qualitatively and quantitatively, to pimobendan (0–900 ng ml^{-1}), as shown in Figure 2. After β-adrenoceptor blockade the UD-CG 212 Cl-induced increases in heart rate and $LVdP/dt_{max}$ were attenuated and almost abolished, respectively, whereas there was no effect on the changes in systemic vascular resistance, mean arterial blood pressure or left ventricular end-diastolic pressure.

Discussion

In a number of animal models, pimobendan has been shown to dilate the venous and arterial vasculature, as well as exert positive inotropic and chronotropic effects. However, differences in the potency of this drug with respect to the vasodilator and cardiac stimulatory effects have been reported, which may be due to the absence or presence of anaesthesia as well as to differences in species. Diederen *et al.* (1982) reported that in conscious dogs there was a more potent effect on the myocardium than on the vasculature, while in anaesthetized baboons prominent effects on both the vasculature and the myocardium were observed. Van Meel (1985) described in anaesthetized cats a strong venodilator effect besides a potent positive inotropic action. In anaesthetized pigs, pimobendan proved to be a more potent vasodilator, in particular of the venous bed, than a positive

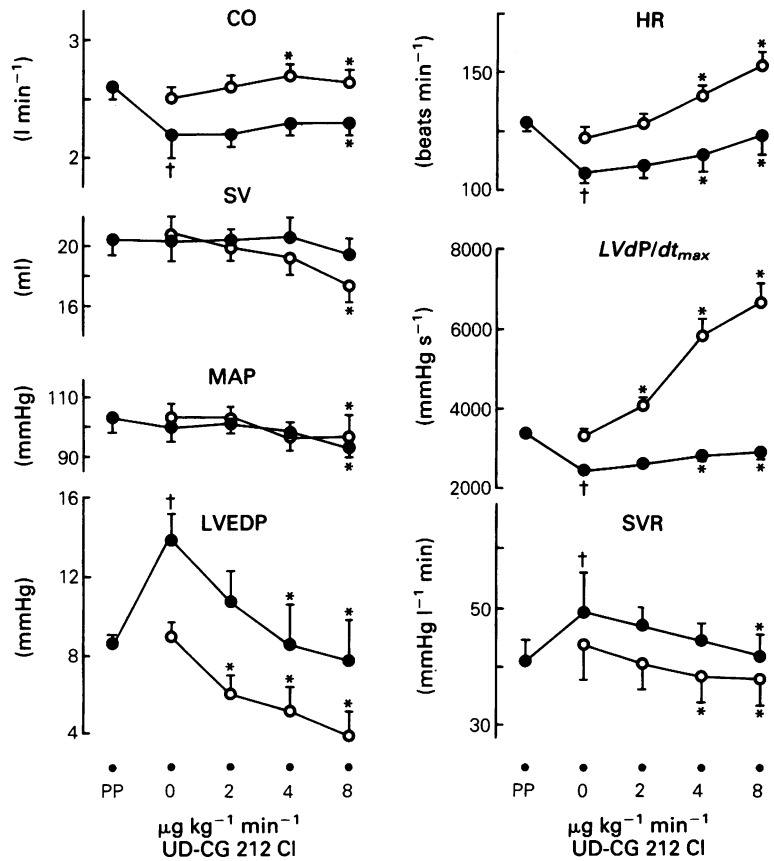

Figure 2 Effects of continuous intravenous 10 min infusions of UD-CG 212 Cl, before (○) or after (●) β-adrenoceptor blockade with propranolol, on systemic haemodynamics in conscious pigs. CO = cardiac output: HR = heart rate; SV = stroke volume; MAP = mean arterial blood pressure; $LVDP/DT_{max}$ = maximal rate of rise of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; SVR = systemic vascular resistance. Data have been presented as mean of 6 experiments with s.e.mean shown by vertical lines; †$P < 0.05$ vs pre-propranolol (PP) values; *$P < 0.05$ vs baseline (0).

inotropic agent (Verdouw *et al.*, 1986; Duncker *et al.*, 1986). In the present study pimobendan caused a dramatic increase in $LVdP/dt_{max}$, while the vasodilator effect was primarily confined to the venous vasculature.

The use of $LVdP/dt_{max}$ as an index of myocardial contractility is often subject to criticism because of its dependence on heart rate, preload and afterload (Mason, 1969). The question therefore remains to what extent the increase in $LVdP/dt_{max}$ induced by these pyridazinone derivatives reflects true positive inotropy. In anaesthetized pigs we have shown that increasing heart rate, by atrial pacing, from 100 beats min^{-1} to 160 beats min^{-1} has no effect on $LVdP/dt_{max}$ (Scheffer & Verdouw, 1983). Although we have no such data in the conscious animal, it appears unlikely that $LVdP/dt_{max}$ would more than double when heart rate increases only by 30%. Furthermore, the reduction in left ventricular filling pressure leads to a underestimation of myocardial contractility changes by using $LVdP/dt_{max}$.

The precise mechanism of action of the pyridazinone derivatives is still largely unknown. However, phosphodiesterase inhibition, an increased sensitivity of contractile proteins to calcium and a prolongation of duration of the action potential, allowing more calcium to enter the cell, have been demonstrated to be involved in their actions in a number of *in vitro* preparations (Rüegg *et al.*, 1984; Honerjäger *et al.*, 1984; Berger *et al.*, 1985; Scholz & Meyer, 1986). The marked attenuation of the pyridazinones-induced increases in $LVdP/dt_{max}$ after pretreatment with

propranolol implies that in the present study the β-adrenergic system, possibly via phosphodiesterase inhibition, contributed significantly to the actions of these drugs. In contrast, in pentobarbitone-anaesthetized pigs, β-adrenoceptor blockade did not modify the responses to pimobendan (Verdouw *et al.*, 1986). One must keep in mind, however, that in the anaesthetized animals, pimobendan caused not only much smaller increases in $LVdP/dt_{max}$ compared to the effects in conscious animals, but also that $LVdP/dt_{max}$ was already severely depressed (baseline 1500 mmHg s^{-1} by the presence of pentobarbitone. Barbiturates have been reported to decrease sympathetic outflow (Roberts, 1980) and this would render phosphodiesterase inhibition less effective than in the conscious state with a higher β-adrenergic activity. Therefore, in the conscious pigs phosphodiesterase inhibition might be involved in the positive inotropic action of pimobendan and UD-CG 212 Cl. However, the increase in $LVdP/dt_{max}$ which was insensitive to β-adrenoceptor blockade in the anaesthetized animals as well as the pyridazinone-induced increase in $LVdP/dt_{max}$ after β-adrenoceptor blockade in the conscious animals, strongly suggest that other mechanisms are also involved in the inotropic actions of these drugs. It is of interest that another pyridazinone-derivative (sulmazole) increased $LVdP/dt_{max}$ by 75% from its baseline value of 2400 mmHg s^{-1} in anaesthetized pigs (Verdouw *et al.*, 1981). Surprisingly the vasodilator actions of pimobendan and UD-CG 212 Cl were not affected by propranolol suggesting that these effects are not mediated by a β-adrenergic mechanism.

UD-CG 212 Cl is more potent at increasing contractile force than its parent compound (Scholz & Meyer, 1986). In the present study UD-CG 212 Cl exerted a cardiovascular action similar to that of pimobendan, but at much lower plasma concentrations. It is therefore feasible that the metabolite contributed to the positive inotropic actions of pimobendan. During the pimobendan infusions, UD-CG 212 Cl plasma levels did not exceed 22 ng ml^{-1}. When this concentration was attained during infusion of the metabolite itself, heart rate and $LVdP/dt_{max}$ were only moderately elevated but left ventricular filling pressure was already markedly reduced. However, at the end of the first infusion period of pimobendan, there was already a pronounced reduction in left ventricular filling pressure, while UD-CG 212 Cl could not yet be detected. It therefore appears that the effects during pimobendan infusion are primarily due to the parent drug itself.

In conclusion pimobendan as well as UD-CG 212 Cl are, in conscious pigs, potent positive inotropic agents and venodilators. The vasodilator effects on the systemic arterial vasculature are much less pronounced than in anaesthetized animals. Since pretreatment with propranolol strongly attenuated, but did not abolish, the increases in myocardial contractility caused by both compounds, phosphodiesterase inhibition could well be involved but this does not account completely for their positive inotropic actions. In contrast, the vasodilator effects are not affected by the presence of β-adrenoceptor blockade and other mechanisms than those operating through the β-adrenergic system must be involved.

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References

BERGER, C., MEYER, W., SCHOLZ, H. & STARBATTY, J. (1985). Effects of the benzimidazole derivatives pimobendan and 2-(4-Hydroxyphenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl on phosphodiesterase activity and force of contraction in guinea-pig hearts. *Arzneim. Forsch./Drug Res.*, **35**(II), 1668–1673.

DIEDEREN, W., DÄMMGEN, J. & KADATZ, R. (1982). Cardiovascular profile of UD-CG 115, a new orally and long acting cardiotonic compound, not related to beta-mimetics or cardiac glycosides, *Naunyn-Schmiedebergs Arch. Pharmac.*, **321**, R36, 141.

DUNCKER, D.J., VAN DALEN, F.J., HARTOG, J.M. LAMERS, J.M.J., RENSEN, R.J., SAXENA, P.R. & VERDOUW, P.D. (1986). Usefulness of pimobendan in the treatment of heart failure. *Arzneim. Forsch./Drug Res.*, **36**(II), 1740–1744.

HONERJÄGER, P., HEISS, A., SCHAFER-KORTING, M., SCHÖNSTEINER, G. & REITER, M. (1984). UD-CG 115 - a cardiotonic pyridazinone which elevates cyclic AMP and prolongs the action potential in guinea-pig papillary muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **325**, 259–269.

LAGERWEY, E. (1973). Anaesthesia in swine for experimental purpose. *Ph.D. dissertation, University of Utrecht, The Netherlands.*

MASON, D.T. (1969). Usefulness and limitations of the rate of rise of left ventricular pressure (dp/dt) in the evaluation of myocardial contractility in man. *Am. J. Cardiol.*, **23**, 516–527.

ROBERTS, J.G. (1980). Intravenous anaesthetic agents, in the circulation. In *Anaesthesia.* ed. Prys-Roberts, C. pp. 460–489. Oxford: Blackwell Scientific Publications.

ROTH, W. (1983). Rapid, sensitive and fully automated high performance liquid chromatographic assay with fluorescence detection for sulmazole and metabolites. *J. Chromatogr. Biomed. Appl.*, **278**, 347–358.

RÜEGG, J.C., PFITZER, G., EUBLER, D. & ZEUGNER, C.

(1984). Effect on contractility of skinned fibres from mammalian heart and smooth muscle by a new benzimidazole derivative, 4,5-dihydro-6-[2-(4-methoxyphenyl)-1H-benzimidazol-5-yl]-5-methyl-3(2H)-pyridazinone. *Arzneim. Forsch./Drug Res.*, **34**(II), 1736–1738.

SCHEFFER, M.G. & VERDOUW, P.D. (1983). Decreased incidence of ventricular fibrillation after an acute coronary artery ligation in exercised pigs. *Basic Res. Cardiol.*, **78**, 298–309.

SCHOLZ, H. & MEYER, W. (1986). Phosphodiesterase inhibiting properties of newer inotropic agents *Circulation*, **73** (suppl. III), 99–108.

STEEL, R.G.D. & TORRIE, J.J. (1980). *Principles and Procedures of Statistics, A Biomedical Approach*. 2nd edn. Tokyo:McGraw-Hill Kogakusha Ltd.

VAN MEEL, J.C.A. (1985). Cardiovascular effects of the positive inotropic agents pimobendan and sulmazole in vivo. *Arzneim. Forsch./Drug Res.*, **35**, 284–288.

VERDOUW, P.D., HARTOG, J.M. & RUTTEMAN, A.M. (1981). Systemic and regional myocardial responses to AR-L 115 BS, a positive inotropic imidazo-pyridine, in the absence or in the presence of the bradycardiac action of alinidine. *Basic Res. Cardiol.*, **76**, 328–343.

VERDOUW, P.D., HARTOG, J.M., DUNCKER, D.J., ROTH, W. & SAXENA, P.R. (1986). Cardiovascular profile of pimobendan, a benzimidazole-pyridazinone derivative with vasodilating and inotropic properties. *Eur. J. Pharmac.*, **126**, 21–30.

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