Cardiovascular effects of UD-CG 212 CL, a metabolite of pimobendan, in anaesthetized pigs

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Systemic and regional haemodynamic effects of UD-CG 212 CL (0.5-16 μg·kg⁻¹·min⁻¹), the major metabolite of the vasodilator and cardiotonic drug pimobendan, were studied in anaesthetized pigs. The drug caused a dose-dependent decrease in left ventricular (LV) end-diastolic and arterial blood pressures while it increased systemic vascular conductance, heart rate and maxLVdP/dt. The decrease in LV end-diastolic pressure was observed at lower plasma concentrations than the increase in systemic vascular conductance. Cardiac output tended to decrease but statistical significance was achieved only with the highest concentration. These effects of UD-CG 212 CL were not altered by the blockade of β-adrenoceptors with propranolol. The vasodilator action of UD-CG 212 CL was noticed in several organs but the effects were relatively more marked (in decreasing order of magnitude) in the adrenals, kidneys, gastrointestinal tract, brain and LV epicardium. Since both arterial pressure and cardiac output decreased, the blood flow increased significantly only in the adrenals and decreased moderately in the spleen, LV endocardium and skeletal muscles. The effects of UD-CG 212 CL on the renal and skeletal muscle haemodynamics were different from those of pimobendan, which causes vasodilatation in the skeletal muscles but not in the kidneys. The results of this study show that, like the parent compound pimobendan, UD-CG 212 CL has independent cardiotonic and vasodilator actions; the latter being more pronounced on the venous side. However, the contribution of this metabolite to the overall pharmacological activity of pimobendan appears to be limited.

UD-CG 212 CL; Pimobendan; Phosphodiesterase inhibitors; Vasodilatation; Regional blood flow; β-Adrenoceptor blockade; Myocardial O₂ consumption; Tachycardia; Inotropic agents (positive); (Pig)

1. Introduction

The last decade has seen considerable attention focused on a new type of cardioactive drugs which could be useful in the treatment of congestive heart failure. These agents are thought to dilate peripheral vascular beds and increase myocardial contractility by elevating cyclic AMP levels following inhibition of phosphodiesterases (Honer-jäger et al., 1984; Scholz and Meyer, 1986). One such drug is pimobendan (UD-CG 115 BS) which in vivo experiments have shown to act more potently on the venous than on the arterial side; the positive inotropic action is only moderate (Diederan et al., 1982; Van Meel, 1985; Verdouw et al., 1986; Duncker et al., 1986c). Recently, the major metabolite of pimobendan, UD-CG 212 CL (fig. 1), was found in in vitro studies to increase cardiac contractile force (Meyer et al., 1985; Scholz...
and Meyer, 1986). The major goal of the present study was to investigate the complete systemic and regional haemodynamic profile of UD-CG 212 CL. Additionally, in view of the possible contribution of UD-CG 212 CL to the systemic haemodynamic effects of pimobendan (Meyer et al., 1985; Scholz and Meyer, 1986), we have measured the plasma concentrations of UD-CG 212 CL and compared them with concentrations achieved following infusions of the parent drug pimobendan (Verdouw et al., 1986) to assess the extent of such a contribution.

2. Materials and methods

2.1. Experimental set-up

After a 24 h fast, Yorkshire pigs (23-28 kg) were sedated with 120 mg azaperone (Stresnil®) i.m., anaesthetized with 150 mg metomidate (Hypnodil®), intubated and ventilated with a mixture of O₂ and N₂O (1:2). Respiratory rate and tidal volume were set to keep arterial blood gases, measured with an ABL-3 (Radiometer, Copenhagen, Denmark), within normal limits (7.35 < pH < 7.45; 35 mmHg < PCO₂ < 45 mmHg; 90 mmHg < PO₂ < 150 mmHg). Catheters placed in the superior vena cava were used for administration of α-chloralose (100 mg·kg⁻¹) and pentobarbital sodium (5 mg·kg⁻¹·h⁻¹) for anaesthesia, Haemaccel® (Behringwerke AG, Marburg, FRG) to replace blood loss, and UD-CG 212 CL. An 8F catheter was positioned in the descending aorta for withdrawal of blood samples. Left ventricular and aortic blood pressures were obtained with 8F Millar microtipped catheters (Millar Instruments Houston, Texas, USA). Prior to exposing the heart via a midsternal split, 4 mg of the muscle relaxant pancuronium bromide (Pavulon®) was administered. Ascending aortic blood flow was measured with an electromagnetic flow probe (Skalar, Delft, The Netherlands). Blood samples were collected from the great cardiac vein for the determination of haemoglobin and O₂ saturation. Myocardial O₂ consumption was calculated by multiplying left ventricular blood flow by the arterial-coronary venous O₂ content difference.

The distribution of cardiac output was determined using the radioactive microsphere technique. Microspheres of 15 ± 1 μm (mean ± S.D.) diameter labeled with either ¹⁰³Ru, ¹¹³Sn, ⁴⁶Sc, ⁹⁵Nb or ¹⁴¹Ce (NEN Chemicals GmbH, Dreieich, FRG), were injected in random order via a cannula inserted into the left atrial appendage. Flow measurements were calibrated by withdrawal of an arterial reference blood sample at a rate of 10 ml·min⁻¹ starting just before and continuing for 1 min after each injection of microspheres. The animal was killed at the end of the experiment and several organs and tissues were excised and treated as described elsewhere (Saxena and Verdouw, 1985). The data were processed using computer programmes developed for the purpose (Saxena et al., 1980).

2.2. Experimental protocols

Two series of experiments were performed. In both series pre-drug systemic haemodynamic data were collected after a stabilisation period of 30-45 min. In the first group (n = 9) this was followed by six consecutive 15 min infusions of 0.5, 1, 2, 4, 8 and 16 μg·kg⁻¹·min⁻¹ of UD-CG 212 CL.
Measurements were repeated at the end of each infusion at a given rate. Radioactive microspheres were injected at baseline and at the end of the four highest 'infusion rates' (2, 4, 8 and 16 μg·kg\(^{-1}\)·min\(^{-1}\)), because of the limited number of isotopes available. Since increases in heart rate and maxLVdP/dt in these experiments were accompanied by a fall in mean arterial blood pressure, the same infusion rates were repeated in a second group of 5 animals after pretreatment with propranolol (0.5 mg·kg\(^{-1}\) followed by an infusion of 0.5 mg·kg\(^{-1}\)·h\(^{-1}\)) to exclude effects of a direct or indirect β-adrenoceptor mechanism. Regional blood flows and plasma concentrations were not determined in this series of experiments. An earlier study from our laboratory (Wolffenbuttel and Verdouw, 1983) had shown that the above-mentioned dose regimen for propranolol provides adequate β-adrenoceptor blockade and that systemic haemodynamic parameters change less than 5% over a period of 90 min.

2.3. Determination of plasma drug concentrations

The concentration of UD-CG 212 CL in the plasma was measured using high-performance liquid chromatography (HPLC). The details of the HPLC assay have been described earlier (Roth, 1983; Verdouw et al., 1986). The lower limit for detection of the compound is 1 ng·ml\(^{-1}\).

2.4. Statistical analysis

Data are presented as means ± S.E.M. Statistical analysis was performed by use of a parametric two-way analysis of variance (randomized block design), followed by Duncan’s new multiple range test (Steel and Torrie, 1980). Statistical significance was accepted at P < 0.05 (two-tailed).

2.5. Drugs

The substances used were the anaesthetics, Haemaccel, propranolol hydrochloride (ICI-Pharma, Rotterdam, The Netherlands) and UD-CG 212 CL (Dr. Karl Thomas GmbBH, Biberach a/d Riss, FRG). The latter was dissolved in a mixture of polyethylene glycol and saline, such that the infusion rates of polyethylene glycol ranged between 0.5 and 1.0 ml·min\(^{-1}\). The infusion of the solvent of these rates has no cardiovascular effects (Verdouw et al., 1983).

3. Results

3.1. Cardiovascular actions of UD-CG 212 CL without β-adrenoceptor blockade

3.1.1. Plasma concentrations of UD-CG 212 CL

As the infusion rates (0.5, 1, 2, 4, 8 and 16 μg·kg\(^{-1}\)·min\(^{-1}\)) of UD-CG 212 CL were increased, the plasma concentrations reached levels of 9 ± 1 ng·ml\(^{-1}\); 21 ± 2 ng·ml\(^{-1}\); 44 ± 5 ng·ml\(^{-1}\); 87 ± 8 ng·ml\(^{-1}\); 170 ± 16 ng·ml\(^{-1}\) and 361 ± 32 ng·ml\(^{-1}\), respectively. The latter was approximately 20 times the highest UD-CG 212 CL concentration obtained in pimobendan infusion experiments (Verdouw et al., 1986).

![Fig. 2. Systemic haemodynamics at increasing UD-CG 212 CL plasma concentrations. HR, heart rate; maxLVdP/dt, maximal rate of rise of left ventricular pressure; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; CO, cardiac output; SVC, systemic vascular conductance. Data are presented as means ± S.E.M. * P < 0.05 vs. pre-drug values.](image-url)
3.1.2. Systemic haemodynamics

UD-CG 212 CL caused dose-related decreases in mean arterial blood pressure (fig. 2), without affecting pulse pressure (not shown). Although the hypotensive action of UD-CG 212 CL was accompanied by a positive chronotropic action (heart rate increased up to 40%) it was not sufficient to prevent a fall in cardiac output, as stroke volume decreased dose dependently from a pre-drug value of 29 ± 2 to 17 ± 2 ml (not shown). For concentrations less than 30 ng · ml⁻¹ this decrease in stroke volume was primarily due to a reduced left ventricular filling (left ventricular end-diastolic pressure decreased up to 50%), as the increases in systemic vascular conductance (flow/pressure) by 10% and maxLVdP/dt (15%) would facilitate left ventricular emptying. No additional effects on left ventricular end-diastolic pressure were seen for concentrations higher than 50 ng · ml⁻¹, while maxLVdP/dt increased gradually up to 60% and systemic vascular conductance increased by 45% (fig. 2).

3.1.3. Regional blood flows and vascular conductances

Because of the limited number of microspheres available with different radioactive labels, regional blood flows were determined before the start of the infusion of UD-CG 212 CL and at the end of the four highest infusion rates. Figure 3 shows that the decrease in cardiac output was not equally distributed over all organs. Blood flow to the adrenals was increased at each plasma concentration (up to 100%). Renal blood flow initially tended to increase, but started to decrease at concentrations higher than 40 ng · ml⁻¹ and had fallen to below pre-drug values at 360 ng · ml⁻¹. At concentrations higher than 40 ng · ml⁻¹, splenic (up to 45%), skeletal muscle (up to 40%) and left ventricular (up to 20%, for further details see below) blood flow decreased but no significant changes were observed at any concentration in

![Fig. 3](image)

Fig. 3. Effects of i.v. infusions of UD-CG 212 CL on regional blood flows. Data are presented as means ± S.E.M. * P < 0.05 vs. pre-drug values.

![Fig. 4](image)

Fig. 4. Effects of i.v. infusions of UD-CG 212 CL on regional vascular conductances. Data are presented as means ± S.E.M. * P < 0.05 vs. pre-drug values.
any of the other organs studied (stomach, small intestine, brain, liver and skin (not shown)). Dose-related increases in vascular conductance were observed in the adrenals (up to 300%), stomach (up to 100%), brain (up to 60%) and small intestine (up to 60%; fig. 4). The increase in conductance in the renal bed was independent of the dose, whereas vasodilatation in the spleen only occurred at the lowest two doses. The changes in vascular conductance in skeletal muscle, liver and skin (not shown) did not reach significance.

3.1.4. Coronary circulation

As shown in fig. 5 the blood flow to the two atria and the right ventricle did not change after the administration of UD-CG 212 CL. Since a decrease in arterial blood pressure was observed, vascular conductance in these organs increased dose dependently. Transmural left ventricular blood flow decreased by 12% when UD-CG 212 CL reached arterial plasma levels of 40 ng·ml$^{-1}$. A further decline (28%) was observed at 170 ng·ml$^{-1}$ (fig. 5). The decreases were confined to the subendocardial layers, yielding a moderately decreased endo-epi blood flow ratio (from 1.23 ± 0.04 to 0.95 ± 0.03, P < 0.05, not shown). Vascular conductances of the subendocardial and subepicardial layers were, respectively, virtually unchanged and increased dose dependently (up to 55%).

The O$_2$ saturation in the great cardiac vein increased from 15 ± 2 to 21 ± 1% (P < 0.05) at 40 ng·ml$^{-1}$ but was not further affected at higher

![Figure 5](image-url)

Fig. 5. Myocardial blood flows and vascular conductances with increasing rate of UD-CG 212 CL i.v. infusion. The plasma concentrations at baseline and at the end of the infusions at each rate were: 0 ng·ml$^{-1}$, 44 ± 5 ng·ml$^{-1}$, 87 ± 8 ng·ml$^{-1}$, 170 ± 16 ng·ml$^{-1}$ and 361 ± 32 ng·ml$^{-1}$, respectively. Data are presented as means ± S.E.M. * P < 0.05 vs. pre-drug values.
concentrations (not shown). Myocardial O\textsubscript{2} consumption therefore decreased gradually from 5.1 ± 0.4 μmol·min\textsuperscript{-1}·g\textsuperscript{-1} to 3.6 ± 0.5 μmol·min\textsuperscript{-1}·g\textsuperscript{-1} (P < 0.05; not shown).

3.2. Cardiovascular actions of UD-CG 212 CL after β-adrenoceptor blockade

When UD-CG 212 CL was infused at rates up to 8 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} neither the responses of heart rate nor those of maxLVdP/dt, mean arterial blood pressure, cardiac output and left ventricular end-diastolic pressure were significantly modified by the presence of propranolol (not shown).

4. Discussion

In the study just described UD-CG 212 CL dilated both arterial and venous vascular beds and increased myocardial contractility. The data in fig. 2 reveal that at concentrations lower than 30 ng·ml\textsuperscript{-1} UD-CG 212 CL is primarily a venodilator (decrease in left ventricular end-diastolic pressure) and that the arterial vasodilator and maxLVdP/dt increasing properties only become apparent at higher concentrations. Because maxLVdP/dt depends on heart rate and pre- and afterload, its use as an index of inotropy demands caution. In this study the heart rate increased, which could be a factor contributing to the augmentation of maxLVdP/dt (Higgins et al., 1973). However, in the anaesthetized pig, maxLVdP/dt is not significantly affected by a heart rate in the range of 100-150 beats·min\textsuperscript{-1} (Scheffer and Verdouw, 1983). Furthermore, the decreases in diastolic arterial blood pressure and left ventricular end-diastolic pressure tend to reduce maxLVdP/dt (Mason, 1969). It thus seems that the enhancement of maxLVdP/dt by UD-CG 212 CL represents its positive inotropic effect. The increases in heart rate and maxLVdP/dt induced by UD-CG 212 CL were not modified by propranolol. We therefore conclude that these effects are not mediated by β-adrenoceptors, either directly or via enhancement of sympathetic nerve activity (due to baroreceptor reflex) but the possibility of withdrawal of vagal tone cannot be excluded. With respect to the positive inotropic effects of UD-CG 212 CL as well as of pimobendan, evidence from in vitro studies suggests that inhibition of phosphodiesterase is involved, but the extent of this involvement is less than in the case of bipyridine derivatives such as amrinone and milrinone (Scholz and Meyer, 1986).

UD-CG 212 CL caused a pronounced vasodilatation of the systemic arterial bed. Although vasodilatation occurred in most regional beds it was conspicuously absent in skeletal muscle and was of only limited magnitude in the left ventricle. In this respect the effects of UD-CG 212 CL are similar to those of amrinone (Hartog et al., 1986), but quite different from those of other vasodilators such as the calcium channel blockers nimodipine and nisoldipine (Duncker et al., 1986a,b; Verdouw et al., in press), the nitrate-like drug nicorandil (unpublished data from our laboratory) or even pimobendan (Verdouw et al., 1986; Duncker et al., 1986c), which were all evaluated in the same experimental model and elicited moderate to pronounced vasodilatation in these regions. The vasodilatation that occurred in the left ventricle was not sufficient to prevent a reduction in transmural blood flow. As the coronary venous O\textsubscript{2} content increased slightly it is likely that the reduction in flow reflects the diminished metabolic needs (myocardial O\textsubscript{2} consumption decreased up to 30%). The transmural vasodilatation was almost solely confined to the subepicardial layers; endocardial blood flow and the endo/epi ratio decreased. It is known that endocardial blood flow is more susceptible to a decrease in diastolic perfusion time (due to tachycardia in this case) and systemic perfusion pressure (see Feigl, 1983). Alternatively, though less likely, there is the possibility that UD-CG 212 CL has a more potent vasodilator effect on the epicardial layers whereby a ‘steal’ of endocardial blood flow may take place. Interestingly, a reduction in endo-epi ratio has also been observed for the phosphodiesterase inhibitor amrinone in the same animal model (Hartog et al., 1986), but in animals with ischaemic hearts amrinone significantly increased endocardial blood flow and the endo-epi ratio (Hartog et al., 1987). It therefore appears that the decrease in endocardial blood
flow and endo-epi ratio by UD-CG 212 CL found in this study in animals with a normal heart may not be of much consequence for the clinical situation. Furthermore, in congestive heart failure, the heart rate is usually already high and vasodilator drugs are likely to reduce heart rate, while blood pressure is maintained (due to an increase in cardiac output).

Of particular interest in the treatment of congestive heart failure are the renal and skeletal muscle blood flows. With UD-CG 212 CL vasodilatation occurred in the renal vascular bed at all concentrations. There was even an increase in blood flow at the lower doses despite the fall in arterial blood pressure and cardiac output. However, no vasodilatation occurred in skeletal muscle. In this respect the actions of UD-CG 212 CL were quite different from those of pimobendan which did not affect renal vascular conductance but increased skeletal muscle conductance by 40% at comparable changes in mean arterial blood pressure and cardiac output (Verdouw et al., 1986).

The maximum concentration of UD-CG 212 CL after administration of pharmacologically active doses (10, 25, 50 and 100 μg · kg⁻¹ · min⁻¹) of pimobendan did not exceed 20 ng · ml⁻¹ (Verdouw et al., 1986). Around this concentration, UD-CG 212 CL caused only small but significant reductions in left ventricular end-diastolic and mean arterial pressures and increases in heart rate. These effects may partly contribute to the systemic effects of pimobendan. However, in view of the much higher plasma concentrations of UD-CG 212 CL required to cause major haemodynamic effects, it would appear that most cardiovascular changes observed after the administration of pimobendan in the original study (Verdouw et al., 1986) were induced by the parent drug itself.

In summary, the present experiments show that UD-CG 212 CL is a vasodilator agent with positive inotropic actions. The vasodilator effect of the drug is more marked on the venous than on the arterial vascular bed. In addition, the positive inotropic action of UD-CG 212 CL seems to be more potent than that of its parent compound pimobendan.

References


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