In vivo effects of a novel calcium antagonist (R56865) against induced epoxyscillirosidin and tulp poisoning in sheep

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ABSTRACT

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Two anaesthetized sheep were intoxicated with epoxyscillirosidin, the main cardio-active bufadienolide, extracted from Homeria pallida (Natal yellow tulp). The epoxyscillirosidin was injected intravenously as a bolus of 50 µg/kg, followed 30 min later by a continuous infusion in a normal saline drip (0,9% NaCl) at 25 µg/kg/h. In addition, another two conscious sheep were poisoned by intraruminal dosing of 1,25 g/kg of dried H. pallida plant material. Electrocardiograms, heart and respiratory rates and venous-acid-base levels were recorded prior to and at approximately 30–60 min intervals during the course of the experiment. Additional recordings were made when animals showed signs of intoxication. R56865 (Janssen Pharmaceutica, Pty Ltd), a novel Ca++ antagonist, was administered at the first distinct signs of cardiac disturbances in the sheep given epoxyscillirosidin and after development of tachycardia and dyspnoea in those that received plant material. Activated charcoal was drenched at 3 g/kg to both sheep that received H. pallida about 1 h after the initial administration of R56865. All H. pallida sheep and one of the epoxyscillirosidin sheep survived. The signs of intoxication with H. pallida, namely groaning and tachypnoea, abated within minutes of treatment with R56865, but returned c. 30 min later in both animals. The treatment apparently had little effect on heart rate and EKG changes. One of the epoxyscillirosidin sheep was treated while exhibiting paroxysmal ventricular tachycardia. Although a transient improvement in conduction disturbance was recorded, the animal died soon afterwards. The results of this study indicate that the in vivo response of R56865 against induced bufadienolide cardiac disturbance in sheep is not as evident as that observed with R56865 against similar cardiac disturbances in vitro. The potential use of R56865 together with activated charcoal is discussed.

Keywords: In vivo effects, calcium antagonist, (R56865), epoxyscillirosidin, tulp poisoning, sheep, Homeria pallida, induced bufadienolide cardiac disturbance

INTRODUCTION

R56865 (N-[1-[4-(fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine (Wilhelm, Wilffert & Peters 1990) is a novel Ca++ antagonist that acts by selectively blocking intracellular Ca++ overload (Massingham & John 1990). In different in vitro myocardial ischaemic models, R56865 was effective in attenuating cardiac glycoside toxicity (Finet, Bravo

& Godfraind 1989; Heers, Scheufler, Wilhelm, Wermelskirchen, Wilffert & Peters 1988; Schneider, Beck & Borgers 1988; Wilhelm *et al.* 1990). In the treatment of a single case of acute digoxin toxicity in man, R56865 was also found to be effective (E.E. Carmeliet, Laboratory of Physiology, University Leuven, Belgium, personal communication 1990). Early electrophysiological studies on isolated myocytes have also confirmed the activity against the bufadienolide, proscillaridin (J.J. van Rooyen, Department Physiology, Potchefstroom University for Christian Higher Education, personal communication 1991).

In vivo efficacy studies with R56865 against tulp (*Homeria pallida*, Natal yellow tulp) intoxication, one of the five most important plant poisonings of southern Africa (Kellerman, Coetzer & Naudé 1988), were indicated to confirm these preliminary *in vitro* results. The discovery of an effective drug in the treatment of bufadienolide plant toxicity will be a major advance in the treatment of tulp poisoning and also possibly of krimpsiekte (Kellerman *et al.* 1988). The purpose of this study was to examine the effect of R56865 against induced epoxyscillirosidin and tulp poisoning in sheep.

MATERIALS AND METHODS

Three healthy SA Mutton Merino rams and one ewe of approximately 12 months of age, were used in the experiment. Cardiac glycoside intoxication was induced under pentobarbitone anaesthesia in the first two animals, sheep 1 and 2, by an initial intravenous (i/v) bolus administration of epoxyscillirosidin, the main toxic principle of *H. pallida (= H. glauca)* (Naudé & Potgieter 1971), in normal saline (0,9 % NaCl) at 50 µg/kg, followed 30 min later by a continuous i/v infusion of the toxin at 25 µg/kg/h in a normal saline drip. The other two sheep, sheep 3 and 4, were drenched with a lethal dose of 1,25 g/kg of dry, finely milled *H. pallida* plant material of known toxicity (Swan & Mülders, unpublished observations 1989).

Electrocardiographic (EKG) recordings were made before and after induction of toxicity. Recordings commenced immediately after administration of epoxyscillirosidin and approximately 2 h after the *H. pallida* plant material had been drenched. Heart and respiratory rates were monitored by auscultation at 30–60 min intervals throughout the examination period. Venous blood was collected anaerobically from the jugular vein in 2,5-mℓ heparinized, disposable syringes for determination of blood pH and acid-base balance at similar intervals before and after induction of toxicity. The blood was kept on ice and blood-gas analysis was performed within an hour of sample collection with the use of a blood-gas/acid-base analyser (ABL3, Radiometer). Animals were observed throughout each trial period for any clinical signs of toxicity.

R56865 (Lot No 90 H 29/12) was administered i/v at the first distinct signs of cardiac-conduction disturbances in the case of the epoxyscillirosidin animals and following persistent tachycardia and signs of respiratory distress in the two dosed with *H. pallida*. Sheep 1 received an initial dose of *c.* 0,16 mg/kg (1-mℓ ampoule of 5 mg R56865) slowly over a period of 1 min, followed by a second ampoule 4 min later. Infusion of toxin was stopped 5 min after the initial treatment. Five ampoules (approximately 0,75 mg/kg) were administered to sheep 2 over a period of 5 min while toxin infusion was continued. In the case of sheep 3 (plant material), an initial i/v bolus of two ampoules of R56865 (0,54 mg/kg) was given, which was repeated approximately 1 h later. When no immediate EKG response was noted after 2 min, an additional 2 ampoules were administered. Activated charcoal at 3 g/kg was dosed to the animal just prior to the additional treatments with R56865. Sheep 4 (also plant material) was injected at onset of tachycardia with two ampoules of R56865 (0,31 mg/kg) which was repeated after 4 min. Activated charcoal at 3 g/kg was dosed 1 h after the initial R56865 treatment.

RESULTS

The two sheep (sheep 1 and 2) which had received epoxyscillirosidin while anaesthetized, exhibited signs of acute tulp poisoning at 2 and 24 min, respectively, following the start of the continuous infusion of the toxin. Severe supraventricular tachycardia (up to 200 beats per min) and irregular respiration were observed. Initial treatment with R56865 in sheep 1 was started 1 h 24 min after the bolus toxin injection. At that stage the animal showed signs of gastro-intestinal (groaning and bloat) and respiratory (tachypnoea and forced expiration) distress and on EKG a ventricular tachycardia was present. Within minutes after R56865 administration, the groaning subsided and the animal appeared to have recovered clinically. However, except for a slight reduction in cardiac rate, conduction disturbance remained unaltered and even appeared to worsen. Conduction disturbances improved over a period of 15 min following the second treatment and cessation of toxin infusion. The animal recovered fully within 1 h of the last treatment.

Sheep 2 was treated with a large dose of R56865 (0,76 mg/kg, five ampoules) in a terminal state of intoxication, when it was already showing signs of paroxysmal ventricular tachycardia, which had been preceded by a severe bradycardia. Only slight clinical signs of toxicity were observed prior to the onset of ventricular arrhythmia. Within minutes after administration of R56865, the conduction disturbances appeared to improve, and EKG recordings showed

short periods of normality. Nevertheless, the sheep died after 7 min.

The animals that had been dosed with *H. pallida* plant material (sheep 3 and 4) showed the first signs of toxicity after approximately 1,5 and 2 h, respectively. Early signs of respiratory distress, abdominal pain, bloat and supraventricular tachycardia were observed. R56865 was administered to sheep 3 after the heart rate had remained at 200 beats per min for more than 30 min and the QRS amplitude had increased from 1,5 to 2,8 mV. Within a few minutes following treatment, respiration improved and signs of abdominal pain disappeared. Heart rate and QRS amplitude were reduced over a period of 40 min. The animal then appeared to suffer a relapse and was treated with activated charcoal followed by two doses (four ampoules) of R56865. Severe conduction disturbances, including ventricular tachycardia and atrioventricular dissociation, and respiratory distress were recorded immediately following the administration of activated charcoal. However, the animal started improving within 2–3 min and, apart from groaning, all other symptoms abated within 5 min and the sheep made an uneventful recovery.

In the case of sheep 4, R56865 was administered following distinct clinical respiratory (tachypnoea and forced expiration) and gastro-intestinal (abdominal pain) signs of toxicity and a persistent mild tachycardia with QT depression. As in sheep 3, clinical signs of toxicity abated within a few minutes of R56865 administration but returned after *c.* 30 min. Cardiac rate increased slightly after treatment. Following activated charcoal administration, a transient severe respiratory distress was observed after which the animal recovered fully.

Two animals (sheep 1 and 3) developed a respiratory acidosis following intoxication. The blood pH decreased to below 7,3 and venous partial pressure of carbon dioxide (pCO_3) increased slightly. Treatment with R56865 had no effect on blood pH and acid-base- balance changes.

DISCUSSION

R56865 was developed in the search for a selective myocardial anti-ischaemic drug for use in man (Massingham & John 1990; Wilhelm *et al.* 1990). Myocardial ischaemia results in the production of energy by anaerobic glycolysis, reduction of cellular pH, depletion of high-energy phosphates, disturbance of ionic flux and Ca^{++} overload (Reimer & Jennings 1986). The loss of ionic and metabolic homeostasis results in functional and structural changes, leading to cell death (Massingham & John 1990). Several of these features are consistent with cardiac glycoside intoxication and, consequently, have led to the use of cardiac glycoside *in vitro* models for the study of anti-ischaemic drugs (Wilhelm *et al.* 1990).

When the cardiac glycoside *in vitro* model was used, R56865 was found to attenuate the effects of cardenolide cardiac glycoside intoxication (Finet *et al.* 1989; Heers *et al.* 1988; Schneider *et al.* 1988; Wilhelm *et al.* 1990). Attenuation consisted of prevention of cardiac-glycoside-induced shortening of action potential, the decrease of action-potential amplitude, the lowering of resting potential, the occurrence of delayed after-depolarizations and the increase in intracellular Ca^{++} concentration (Wilhelm *et al.* 1990). Guinea-pig hearts which were exposed to R56865 and a toxic concentration of digitoxin, were structurally comparable to that of non-intoxicated control hearts in respect of sarcolemmal Ca^{++} binding and mitochondrial Ca^{++} accumulation (Wilhelm *et al.* 1990).

The precise mechanism whereby R56865 acts is not known but, similarly to other Ca^{++} antagonists, R56865 has a net haemodynamic and electrophysiological effect (Massingham & John 1990). This includes coronary vasodilation, decreased myocardial oxygen demand, increased myocardial oxygen supply and direct cytoprotective effect against Ca^{++} overload (Vanhoutte 1988). Of these, the latter effect is most likely more important in cardiac glycoside toxicity, since Ca^{++} overload in this case results from inhibition of the Na^+-K^+-ATPase (Hoffman & Bigger 1990).

R56865 is claimed to be a selective Ca^{++}-overload blocker, particularly against cardiac-glycoside-induced Ca^{++} overloading (Massingham & John 1990). Although its pharmacological action may involve some blockade of the L-type voltage-operated channels (VOC), Ca^{++} entry into cardiac and vascular myocytes via a non-VOC pathway is more likely (Finet *et al.* 1989). Kock, Wilhelm, Wermelskirchen, Nebel, Wilffert & Peters (1989) proposed from studies in the rat aorta, that R56865 is a weak Ca^{++}-entry blocker and inhibits α_1-adrenoceptor-mediated responses by acting at sites distal to the catecholamine binding site. They also suggest an interference with intracellular Ca^{++} pools.

The results of the current study performed in sheep, indicate that R56865 might have some clinical therapeutic effect against the bufadienolide epoxyscillirosidin and against *H. pallida* intoxication. However, the response against cardiac-conduction disturbance was not as evident as that reported for *in vitro* studies. This could possibly be explained by the action of R56865 which appears to protect the heart only against Ca^{++} overload. Similarly, the difference in effectiveness of Ca^{++} antagonists in the treatment of myocardial infarction in man and in experimental myocardial ischaemia has also been explained by the fact that patients are generally treated with Ca^{++} antagonists only after symptoms of myocardial infarction have been detected (Hugtenburg, Beckeringh, Boddeke, Heijnis, Jap, Mathy & Van Zwieten 1990). Nevertheless, owing to the prevention of possible

further Ca⁺⁺ entry intracellularly, and owing to myocardial haemodynamic improvements, the drug should be effective in stabilizing the cardiac-toxic effects of cardiac-glycoside-containing plant (e.g. *H. pallida*) poisonings, and it does appear to be so.

R56865 would therefore be indicated as a premedication, prior to the administration of activated charcoal. On its own, R56865 would not be sufficient to treat cardiac glycoside plant poisoning, without the prevention of further gastro-intestinal absorption of excess residual toxin from plant material in the rumen. According to the results of this preliminary trial, the duration of effect of R56865 seems to be only *c.* 30 min. Dosing of activated charcoal at 2–3 g/kg, on the other hand, has been shown to be effective and practical in the treatment of plant-induced cardiac glycoside poisoning of livestock (Joubert & Schultz 1982a & b). However, owing to the stressful effects resulting from the administration of this large dose, some animals do not survive (Kellerman *et al.* 1988). These effects could possibly be avoided by the protective effects of R56865. Both animals, initially treated with R56865 on their developing signs of intoxication and later given activated charcoal, survived a known lethal dose of *H. pallida*. Further trials would be needed to confirm these initial observations and to explain the *in vivo* cardiac effects of the drug.

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