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RESEARCH PAPER

# Comparative pharmacokinetics and pharmacodynamics of urocortins 1, 2 and 3 in healthy sheep

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## BACKGROUND AND PURPOSE

The urocortin (Ucn) peptides are emerging as potential therapeutic targets for heart disease. However, pharmacokinetic (PK) and pharmacodynamic (PD) data are lacking. Therefore, we investigated the PK/PD for all three Ucns.

## EXPERIMENTAL APPROACH

Seven sheep received 1 $\mu g \cdot kg^{-1}$ boluses of Ucn1, Ucn2 and Ucn3. Population PK/PD models were developed to describe the time course of the haemodynamic effects.

## RESULTS

The population estimate for Ucn1 clearance (0.486 $L \cdot h^{-1}$) was lower than that for Ucn2 (21.7 $L \cdot h^{-1}$) and Ucn3 (220 $L \cdot h^{-1}$), while steady-state volumes of distribution were similar for Ucn1 and Ucn2 (~8 L) but substantially larger for Ucn3 (23.5 L). Ucn1 disposition was adequately described by a two-compartment model, with a one-compartment model required for Ucn2 and Ucn3. The half-life for Ucn1 was 2.9 h ($\alpha$ phase) and 8.3 h ($\beta$ phase), and 15.7 and 4.4 min for Ucn2 and Ucn3 respectively. All Ucns produced significant increases in heart rate, cardiac output and left ventricular systolic and mean arterial pressures, and decreases in left atrial pressure and peripheral resistance. Delayed-effect pharmacodynamic models best described the time course of haemodynamic responses, with effects more rapid and less prolonged for Ucn2 and Ucn3 than Ucn1. Similar and physiologically plausible estimated baseline ($E_0$) effects were exhibited by all Ucns, whereas $EC_{50}$ values were generally greater for Ucn1.

## CONCLUSIONS AND IMPLICATIONS

Relative to Ucn1, both the PK and haemodynamic responses to Ucn2 and Ucn3 occurred more rapidly. Our data provide important comparative information, useful to the rational design of future clinical studies.

## Abbreviations

CO, cardiac output; CRF, corticotropin-releasing factor; CTPR, calculated total peripheral resistance; CV, coefficients of variation; HPA, hypothalamus–pituitary–adrenal; HR, heart rate; LAP, left atrial pressure; LVSP, left ventricular systolic pressure; MAP, mean arterial pressure; OBJ, objective function value; PD, pharmacodynamic; PK, pharmacokinetic; Ucn, urocortin; $V_d$, volume of distribution; VPC, visual predictive check

## Introduction

The urocortin (Ucn) peptides – Ucn1, Ucn2 and Ucn3 – are a group of structurally related endogenous peptides belonging to the corticotropin-releasing factor (CRF) family (Vaughan *et al.*, 1995; Hsu and Hsueh, 2001; Lewis *et al.*, 2001; Reyes *et al.*, 2001; Fekete and Zorrilla, 2007). The more recently isolated Ucn2 and Ucn3 peptides share approximately 42% and 20% sequence homology, respectively, with Ucn1. These peptides display distinct affinities for the two G-protein-coupled CRF receptors – $CRF_1$ and $CRF_2$ (Fekete and Zorrilla, 2007; nomenclature follows Alexander *et al.*, 2011). Whereas Ucn1 binds with high affinity to both receptor subtypes, Ucn2 and Ucn3 are reported to be highly selective for $CRF_2$ receptors. This latter receptor, expressed in high concentrations throughout the cardiovascular system, mediates a range of Ucn-induced haemodynamic effects including direct vasodilation and positive cardiac inotropic, chronotropic and lusitropic actions (Parkes *et al.*, 1997; Rademaker *et al.*, 2002; 2005a; 2006; Bale *et al.*, 2004; Wiley and Davenport, 2004). Given the mounting evidence that these peptides play an important role in circulatory homeostasis (Rademaker *et al.*, 2005b; Fekete and Zorrilla, 2007), increasing interest is being focused on the Ucns as potential therapeutic agents in heart disease. In a series of studies in an experimental ovine model of heart failure, all three Ucns produced dose-dependent improvements in cardiac contractility and performance, as well as decreases in total peripheral resistance and arterial and left atrial pressures (Rademaker *et al.*, 2002; 2005a; 2006). More recently, administration of Ucn2 in humans with stable systolic heart failure has been shown to enhance left ventricular ejection fraction, cardiac output (CO) and, to a lesser extent, heart rate (HR), as well as reduce systemic vascular resistance and cardiac work (Davis *et al.*, 2007b). The beneficial findings demonstrated in these investigations have led to the current trials of Ucn2 as a short-term parenteral therapy in patients hospitalized for acute decompensated heart failure (Moral and Tomillero, 2008).

Several studies have independently reported the pharmacokinetics (PK) of Ucn1 (Davis *et al.*, 2004; 2005) and Ucn2 (Davis *et al.*, 2007a,b) in humans. Results from these investigations suggest that Ucn1 exhibits a greater volume of distribution ($V_d$) (approximately 9–14-fold) relative to Ucn2, together with a lower metabolic clearance rate (approximately 2–7-fold) and longer biological half life ($t_{1/2}$) (approximately 3.5–5-fold). In addition, lower endogenous (baseline) concentrations have been observed for Ucn1 in plasma (Davis *et al.*, 2004; 2005; Rademaker *et al.*, 2002) when compared with either Ucn2 (Davis *et al.*, 2007a,b) or Ucn3 (Takahashi, 2004). To our knowledge, there is currently no information with respect to the PK of Ucn3, or regarding the pharmacodynamics (PD) of any of the three Ucns. This information is essential given the potential clinical relevance of these peptides.

Therefore, we have developed population PK/PD models to describe the time course of concentration and haemodynamic effects following administration of mouse Ucn1, Ucn2 and Ucn3 in healthy sheep. Note that the mouse forms of Ucn1 and Ucn3 are identical to the ovine forms of the peptides, while ovine Ucn2 differs from the mouse form by only a single substitution of asparagine for aspartic acid at position 26 (see GenBank and Cepoi *et al.*, 1999; Rademaker *et al.*, 2005a; 2006).

## Methods

### *Surgical preparation of sheep*

All animal care and experimental protocols were approved by the local University of Otago, Christchurch, Animal Ethics Committee. The results of all studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments (McGrath *et al.*, 2010). Seven Coopworth ewes (48–67 kg) (Lincoln University Farm, Christchurch, New Zealand) were instrumented as previously described (Fitzpatrick *et al.*, 1989) via a left lateral thoracotomy under general anaesthesia (induced by i.v. thiopentone 15 $mg \cdot kg^{-1}$; maintained with 2.5% isoflurane/2 $L \cdot min^{-1}$ nitrous oxide/2 $L \cdot min^{-1}$ oxygen) and using approved peri-/post-operative antibiotics (i.v., cephazolin 20 $mg \cdot kg^{-1}$; i.v., enrofloxacin 2.5 $mg \cdot kg^{-1}$) and analgesia (intercostal, bupivacaine 0.5%/lignocaine 2%; i.v., carprofen 4 $mg \cdot kg^{-1}$; i.v., buprenorphine 0.005–0.01 $mg \cdot kg^{-1}$). The level of perioperative anaesthesia was monitored by pedal withdrawal and observation of respiration and HR. Briefly, two polyvinyl chloride catheters were inserted into the left atrium for blood sampling and left atrial pressure (LAP) determination. A Konigsberg pressure-tip transducer was inserted into the aorta to record mean arterial pressure (MAP) and into the apex of the left ventricle to measure HR and left ventricular systolic pressure (LVSP). An electromagnetic flow probe was placed around the ascending aorta to measure CO, and a Swan-Ganz cateter was inserted into the pulmonary artery for administration of the Ucn peptides.

Animals were held in metabolic cages housed in an air-conditioned, light-controlled room with free access to food (lucerne chaff and food pellets providing 75 mmol sodium and 150 mmol potassium per day) and water, and allowed to recover for at least 14 days before commencing the study protocol. Indwelling catheters were locked with heparinized saline (100 $U \cdot mL^{-1}$) to maintain patency throughout the study period. Catheters were flushed daily (with removal of the old lock solution before relocking) using aseptic techniques.

### *Experimental design*

Each sheep received a 1 $\mu g \cdot kg^{-1}$ bolus of mouse Ucn1, Ucn2 and Ucn3 administered at weekly intervals in random order via the pulmonary artery catheter.

### *Measurements made*

Blood samples for the measurement Ucn1, Ucn2 and Ucn3 concentrations in plasma were obtained at 30 min and immediately before peptide administration (baseline/endogenous levels), and then at 5, 10, 15, 30, 60, 90, 120, 180, 240 and 360 min, and 1, 2 and 3 days post bolus. Samples were taken into EDTA tubes on ice, centrifuged at 4°C and stored at –80°C until assay. All samples from individual animals were measured in the same assay to avoid inter-assay variability.

Haemodynamic measurements [HR, CO, LVSP, MAP, LAP and calculated total peripheral resistance (CTPR = MAP/CO)]

were recorded at 15 min intervals in the hour preceding Ucn1-3 administration (baseline) and then at 5, 10, 15, 30, 60, 90, 120, 180, 240 and 360 min, and 1, 2 and 3 days post bolus. Measurements were determined by online computer-assisted analysis (PowerLab Systems, ADInstruments, Dunedin, New Zealand) and made with the animals standing quietly in their metabolic crates.

### *Determination of Ucn concentrations in plasma*

*Ucn1.* Plasma Ucn1 concentrations were measured by a radioimmunoassay using an antiserum raised in rabbit to murine Ucn1 [identical to ovine Ucn1 (Cepoi *et al.*, 1999)] (The Salk Institute, La Jolla, CA, USA). Briefly, 100 µL of methanol extracted plasma or murine Ucn1 standard (Bachem, Torrance, CA, USA) were incubated with 100 µL antiserum and 100 µL radiolabelled ($I^{125}$) murine Tyro-Ucn1. Bound and free labelled Ucn1 were separated using a solid-phase second antibody. The detection limit of the assay was 3.8 pmol·$L^{-1}$. The quality controls (QCs) used were 30 and 80 pmol·$L^{-1}$, and inter-assay CVs were 14.1% and 11.9% respectively. Recovery of unlabelled Ucn1 standard was 67–76%. Cross-reactivities to ovine: CRF <0.002%, Ucn2 <0.026% and Ucn3 <0.007%. Stability studies have shown that Ucn1 in sheep plasma stored at –80°C is stable for at least 3 years.

*Ucn2 and Ucn3.* Plasma Ucn2 and Ucn3 concentrations were measured using the mouse YK190 Ucn2 and YK200 Ucn3 EIA kits respectively (Yanaihara Institute Inc., Shizuoka, Japan) – competitive enzyme immunoassays using highly specific antibodies to the murine forms of these peptides.

In the Ucn2 assay, we found the mean detection limit was 38 pmol·$L^{-1}$. The QCs used were 800 and 2700 pmol·$L^{-1}$, and the inter-assay CVs were 25% and 24% respectively. Ucn2 recovery ranged from 97% to 118%. Cross-reactivities supplied by the kit manufacturer were mouse Ucn1, 0%; mouse Ucn3, 0%; and mouse CRF, 0%.

In the Ucn3 assay, we found the mean detection limit was 20 pmol·$L^{-1}$. The QCs used were 130 and 600 pmol·$L^{-1}$, and the inter-assay CVs were 6% and 7% respectively. Ucn3 recovery ranged from 72% to 106%. Cross-reactivities supplied by the kit manufacturer were mouse Ucn1, 0%; mouse Ucn2, 0%; and mouse CRF, 0.01%.

### *Materials*

Murine Ucn1, Ucn2 and Ucn3 were obtained from Phoenix Pharmaceuticals, Inc. (Belmont, CA, USA).

Peptides were >98% purity (by HPLC analysis; correct molecular weight confirmed by mass spectral analysis) with an amidated C-terminus.

## Data analysis and statistical procedures

### *Haemodynamic statistical analysis*

Descriptive analysis of the haemodynamic measurements are expressed as mean ± SEM.

Baseline haemodynamic values represent the mean of measurements made within the hour immediately prior to treatment. The effects of Ucn1-3 treatment were determined by separate one-way ANOVA, with time as a repeated measure. Differences among Ucn1, Ucn2 and Ucn3 treatment arms were determined using a two-way ANOVA (treatment × time interactions quoted in text). Significance was assumed when $P < 0.05$.

In previous studies in similarly instrumented sheep, results from six to eight animals have usually been clear-cut under these carefully controlled experimental conditions and have provided adequate statistical power. For variables of interest including CO, LAP and MAP, this type of study design consistently give >80% power to detect >30% shifts at a significance level of $P < 0.01$.

### *PK/PD data analysis*

For each of the haemodynamic responses, PK and PD data were combined and analysed simultaneously by nonlinear mixed-effects modelling (NONMEM version 6.1, Globomax LLC, Hanover, PA) (Boeckmann *et al.*, 1994). A digital Fortran compiler was used, and the runs were executed using Wings for NONMEM. Data were analysed using the first-order conditional estimation method with interaction, and ADVAN6 was used to solve the differential equations. Models for each of the three Ucn peptides were developed independently.

Between-subject variability was calculated exponentially and was assumed to follow a lognormal distribution. Its magnitude was expressed as CV (%). Residual unexplained variability was modelled using exponential and/or additive random error.

During model building, a sequential analysis of the PK and PD data were initially performed before simultaneous PK/PD modelling. Model selection was based on visual inspection of diagnostic scatter plots, the objective function value (OBJ) computed by NONMEM and biological plausibility of parameter estimates. Statistical comparison of nested models was undertaken on the basis of a $\chi^2$ test in which a decrease in the OBJ of 3.84 units ($P < 0.05$) was considered significant.

The final model for each haemodynamic response was evaluated by performing a visual predictive check (VPC) (Karlsson and Holford, 2008). For this, 1000 data sets were simulated from the final parameter estimates using the original data as a template. The median (50th percentile), 10th and 90th percentiles of simulated concentrations were then computed and plotted against observed values. This internal method of model evaluation and graphical presentation is considered to be better than standard plots showing observed versus predicted concentration (Brendel *et al.*, 2007).

### *PK modelling*

The PK of Ucn1-3 in plasma was described by compartmental models parameterized in terms of clearance from the central compartment, apparent central and peripheral $V_d$ and inter-compartmental clearance. Exogenous Ucns were administered into the central compartment by bolus injection (over 5 s). Endogenous (baseline) Ucn concentrations in plasma were estimated as an independent parameter in the model. A forwards and backwards stepwise approach was used to

include the covariate total bodyweight into each of the PK models. This covariate was included if the parameter estimates were biologically plausible and if the decrease in OBJ was at least 3.84 units.

### PK/PD modelling

Immediate- and delayed-effect models were tested to describe the relationship between Ucn1-3 exposure and haemodynamic (HR, CO, LVSP, MAP and LAP) response. The latter includes effect compartment models, in which drug distribution into a hypothetical effect compartment accounts for any observable delay in PD (Sheiner *et al.*, 1979; Al-Sallami *et al.*, 2009). Alternative (turnover) models were also assessed to characterize the concentration–effect delay as an inhibition (or stimulation) of the input (or loss) of a physiological intermediate (Dayneka *et al.*, 1993; Krzyzanski and Jusko, 1998). Three functional models (linear, $E_{max}$ and sigmoidal $E_{max}$) were used to explore the relationship between Ucn concentration and haemodynamic effect. The $E_{max}$ or sigmoid $E_{max}$ model related to baseline effect was described by:

$$\text{Haemodynamic response} = E_0 + \frac{E_{max} \times C_p}{EC_{50}^{\gamma} + C_p^{\gamma}} \quad (1)$$

where $E_0$ is the baseline effect, $C_p$ is the concentration of Ucn in plasma, $E_{max}$ is the maximum effect, $EC_{50}$ is the concentration producing half-maximal response and $\gamma$ is the Hill coefficient defining the steepness of the concentration–response curve. The Hill coefficient for $E_{max}$ models is 1.0.

For effect compartment models, the concentration of Ucn in plasma (i.e. $C_p$ in Eq. 1) was replaced by the concentration of Ucn at the hypothetical effect site ($C_e$). A first-order equilibration rate constant ($k_{eq}$) characterizing the temporal relationship between effect compartment and plasma was parameterized as an equilibration half-time ($t_{1/2}k_{eq}$):

$$t_{\frac{1}{2}}k_{eq} = \frac{Ln(2)}{k_{eq}} \quad (2)$$

CTPR (MAP/CO) following Ucn exposure was modelled using the composite models for MAP and CO.

## Results

### Haemodynamic data

Bolus administration of the three Ucn peptides produced significant and quantitatively similar increases in HR (maximum change: Ucn1, 69 bpm; Ucn2, 66 bpm; Ucn3, 70 bpm; all $P < 0.001$), CO (Ucn1, 1.50 L·min$^{-1}$; Ucn2, 1.36 L·min$^{-1}$; Ucn3, 1.43 L·min$^{-1}$; all $P < 0.001$), LVSP (Ucn1, 35 mmHg; Ucn2, 33 mmHg; Ucn3, 42 mmHg; all $P < 0.001$) and MAP (Ucn1, 6.6 mmHg, $P < 0.001$; Ucn2, 7.1 mmHg, $P < 0.001$; Ucn3, 5.1 mmHg, $P < 0.01$), together with falls in LAP (Ucn1, 4.4 mmHg; Ucn2, 4.4 mmHg; Ucn3, 4.4 mmHg; all $P < 0.001$) and CTPR (Ucn1, 2.6 mmHg·L$^{-1}$·min$^{-1}$; Ucn2, 3.1 mmHg·L$^{-1}$·min$^{-1}$; Ucn3, 3.7 mmHg·L$^{-1}$·min$^{-1}$; all $P < 0.001$) (Figure 1).

While the magnitude of the haemodynamic effects were comparable among the three Ucn peptides, the rapidity and duration of these responses varied markedly – with the peak effects (except for MAP) occurring at 5, 10–30 and 120 min following administration of Ucn3, Ucn2 and Ucn1 respectively. With respect to the MAP response, the maximum rise occurred later than that seen for other effects following Ucn3 and Ucn2 boluses (at 15 and 90 min, respectively), and earlier for Ucn1 (at 90 min post bolus). The duration of Ucn1-induced haemodynamic effects was appreciably longer than those of either Ucn2 or Ucn3, with all changes still significantly different from baseline at 360 min post bolus (and up to 1 day for LAP). The responses to Ucn2 and Ucn3, on the other hand, tended to last until 60–120 min (240 min for LAP) and up to 30–60 min (90 min for LAP) respectively. The pattern of HR, CO, LVSP, MAP, CTPR and LAP responses to the three Ucn peptides were all significantly different from each other (all $P < 0.001$).

### PK modelling

The time course of Ucn1 in plasma was best described by a two-compartment model with exponential residual error, an estimate of baseline Ucn1 concentration and between-subject variability on all PK parameters. In contrast, a one-compartment model adequately described the disposition of Ucn2 and Ucn3 in plasma, with between-subject variability on clearance, central $V_d$ and baseline Ucn concentrations. Exponential error models were preferred for Ucn2 and Ucn3.

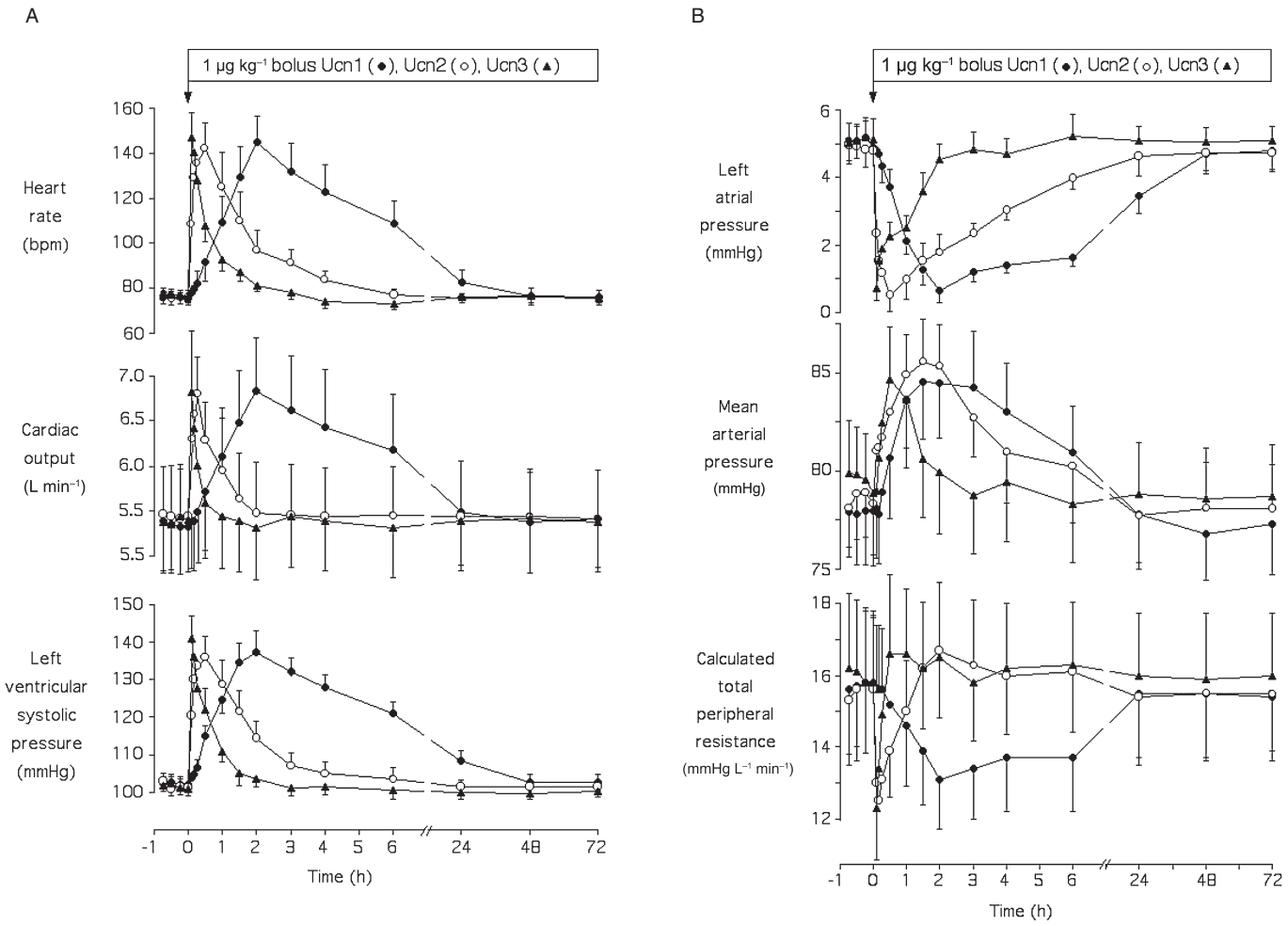
After screening the effect of bodyweight on all clearances and apparent $V_d$, no statistically significant improvements (OBJ < 3.84 units; $P > 0.05$) in the base model were found. This covariate was therefore not incorporated into the model for Ucn1-3 in plasma.

Final parameter estimates from the PK models of all three Ucn peptides are summarized in Table 1. The estimate for Ucn1 clearance (0.486 L h$^{-1}$) was approximately 45- and 450-fold slower than that of Ucn2 and Ucn3 respectively. In addition, while similar steady-state volumes of distribution ($V_c + V_p$) were calculated for Ucn1 and Ucn2 (~8 L), that for Ucn3 was approximately threefold higher. The $t_{1/2}$ values for Ucn1 were 2.9 h (α phase) and 8.3 h (β phase), while that for Ucn2 and Ucn3 were 15.7 and 4.4 min respectively. Furthermore, endogenous (baseline) concentrations of Ucn1 were considerably lower than the estimates obtained for Ucn2 and Ucn3.

### PK/PD modelling

For all three Ucn peptides, immediate-effect models were inadequate and statistically inferior (OBJ > 3.84 units; $P > 0.05$) to delayed-effect models. Effect compartment sigmoid $E_{max}$ models best described the Ucn1 exposure–response for HR, CO and MAP, with turnover models required for LAP and LVSP. All haemodynamic effects for Ucn2 and Ucn3 were adequately described by effect compartment $E_{max}$ models. For these Ucn peptides, the inclusion of a sigmoidicity parameter (Hill coefficient) in Eq. 1 was not statistically significant.

The final parameter estimates for all haemodynamic responses to Ucn1–3 are presented in Table 2. As expected, estimated baseline ($E_0$) effects for all Ucns were comparable and physiologically plausible. In addition, $EC_{50}$ values for




## Figure 1

Time course of haemodynamic responses following a 1 µg·kg$^{-1}$ bolus of Ucn1,Ucn2 and Ucn3 to seven conscious sheep. Data represent mean ± SEM. (A) Heart rate cardiac output and left ventricular systolic pressure. (B) Left atrial pressure, mean arterial pressure and the calculated total peripheral resistance.

## Table 1

Population parameter estimates from the final PK models for Ucn1-3

| Urocortin | Structural model | Population parameter estimate[a] *CL* (L h$^{-1}$) | *$V_c$* (L) | *Q* (L h$^{-1}$) | *$V_p$* (L) | *BL* (nmol L$^{-1}$) | Error model *Exponential* (CV %) |
|---|---|---|---|---|---|---|---|
| Ucn1 | Two compartments | 0.486 (34.1) | 5.81 (37.4) | 0.732 (43.7) | 3.07 (28.2) | 0.018 (20.5) | 9.80 |
| Ucn2 | One compartment | 21.7 (53.9) | 8.19 (41.1) | – | – | 0.330 (102) | 15.9 |
| Ucn3 | One compartment | 220 (31.8) | 23.5 (27.3) | – | – | 0.155 (29.5) | 11.4 |

[a]Population parameter estimates are listed together with their between-subject variability (expressed as CV %) in parenthesis.
*CL*, clearance from the central compartment; *$V_c$* and *$V_p$*, apparent volumes of distribution of the central and peripheral compartments, respectively; *Q*, inter-compartmental clearance; *BL*, baseline (endogenous) Ucn concentration.

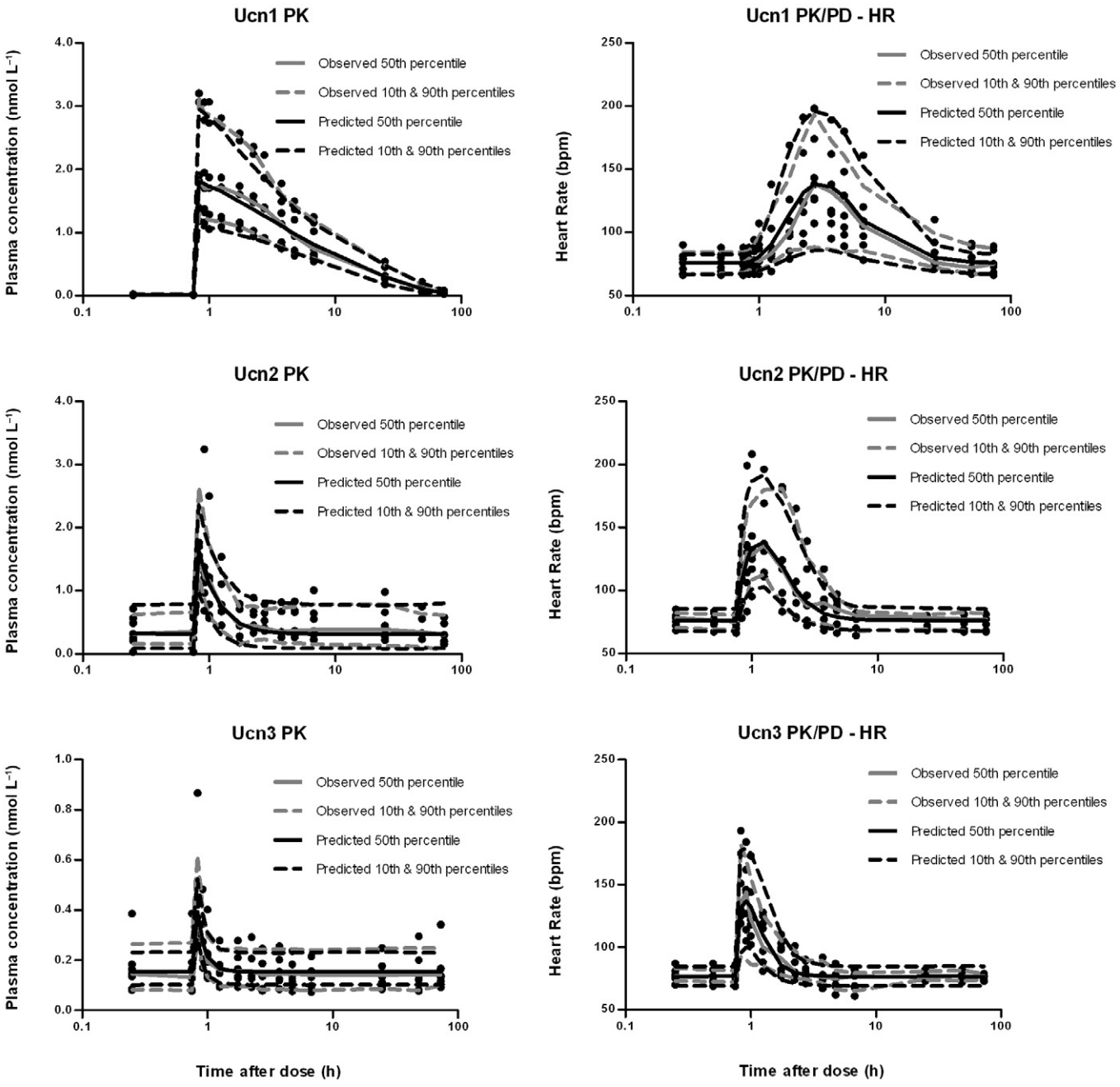
Ucn2 and Ucn3 were generally smaller than that for Ucn1. With the exception of MAP, equilibration into the hypothetic effect compartment was slowest for Ucn1 and fastest for Ucn3. Long Ucn2 and Ucn3 equilibration half-times ($t_{1/2}k_{eq}$) for MAP could be explained by a prolonged return to baseline effect. The change in LVSP and LAP response following Ucn1 exposure was described by complete inhibition ($E_{max}$ = 1.0 from the model fit) in the loss or production of a physiological intermediate. For all models, the parameter values after simultaneous PK/PD analysis were comparable (<15%

## Table 2

Population PK/PD model parameter estimates for the relationship between Ucn1-3 exposure and haemodynamic response

| PD effect | Structural model | Urocortin | Population parameter estimate[a] | | | | | | Error model | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $E_0$[b] | $E_{max}$[b] | $EC_{50}$ (nmol $L^{-1}$) | $t_{1/2}k_{eq}$ (h) | γ (–) | $k_{out}$ ($h^{-1}$) | *Exponential* (% CV) | *Additive* [b] |
| HR | Effect compartment | Ucn1 | 76.0 (8.6) | 222 (–) | 1.88 (30.8) | 0.794 (–) | 2.59 (41.6) | – (–) | 4.90 | – |
| | Effect compartment | Ucn2 | 76.2 (7.6) | 320 (–) | 1.96 (38.6) | 0.361 (53.9) | – (–) | – (–) | 4.80 | – |
| | Effect compartment | Ucn3 | 76.8 (5.4) | 139 (–) | 0.15 (81.9) | 0.176 (77.4) | – (–) | – (–) | 5.70 | – |
| CO | Effect compartment | Ucn1 | 5.25 (19.8) | 2.71 (–) | 1.34 (45.3) | 0.803 (–) | 2.93 (37.8) | – (–) | 2.30 | – |
| | Effect compartment | Ucn2 | 5.32 (20.4) | 1.57 (–) | 0.31 (110) | 0.041 (–) | – (–) | – (–) | 1.90 | – |
| | Effect compartment | Ucn3 | 5.27 (20.7) | 3.10 (–) | 0.38 (45.6) | 0.020 (–) | – (–) | – (–) | 1.50 | – |
| MAP | Effect compartment | Ucn1 | 77.4 (8.2) | 22.5 (–) | 1.95 (59.2) | 0.580 (42.9) | 2.55 (–) | – (–) | 1.80 | – |
| | Effect compartment | Ucn2 | 78.0 (7.6) | 14.4 (–) | 0.23 (–) | 1.76 (62.2) | – (–) | – (–) | – | 2.29 |
| | Effect compartment | Ucn3 | 78.9 (9.5) | 60.0 (–) | 2.41 (161) | 0.525 (–) | – (–) | – (–) | – | 2.48 |
| LVSP | Turnover model[c] | Ucn1 | 101 (4.8) | 1.0 (–) | 3.75 (16.1) | – (–) | 1.25 (30.2) | 1.03 (–) | 2.50 | – |
| | Effect compartment | Ucn2 | 102 (5.9) | 175 (–) | 1.67 (54.1) | 0.471 (87.5) | – (–) | – (–) | 2.20 | – |
| | Effect compartment | Ucn3 | 101 (3.9) | 108 (–) | 0.20 (66.3) | 0.191 (107) | – (–) | – (–) | 3.10 | – |
| LAP | Turnover model[d] | Ucn1 | 5.0 (22.1) | 1.0[d] (–) | 0.64 (72.9) | – (–) | 1.67 (33.0) | 1.08 (28.1) | – | 0.472 |
| | Effect compartment | Ucn2 | 4.7 (20.0) | –6.6 (–) | 0.19 (44.0) | 0.972 (31.8) | – (–) | – (–) | – | 0.457 |
| | Effect compartment | Ucn3 | 5.0 (18.9) | –4.1 (–) | 0.01 (67.9) | 0.296 (–) | – (–) | – (–) | – | 0.535 |

[a]Population parameter estimates are listed together with their between-subject variability (expressed as CV %) in parenthesis. Between-subject variability for $E_{max}$ was not estimated due to implausible parameter estimates and/or model instability.
[b]Units are beats·$min^{-1}$ for HR, L·$min^{-1}$ for CO and mmHg for MAP, LVSP and LAP.
[c]LVSP turnover model is inhibition in the loss of a physiological intermediate. An $E_{max}$ of 1.0 indicates 100% inhibition.
[d]LAP turnover model is inhibition in the production of a physiological intermediate. An $E_{max}$ estimate of 1.0 indicates 100% inhibition.
$E_0$, baseline effect; $E_{max}$, maximum effect; $EC_{50}$, concentration producing half-maximal response; $t_{1/2}k_{eq}$, equilibration half-time of Ucn into hypothetic effect compartment; γ, Hill coefficient defining the steepness of the concentration–response curve; $k_{out}$, rate constant for loss of a physiological intermediate in turnover models.



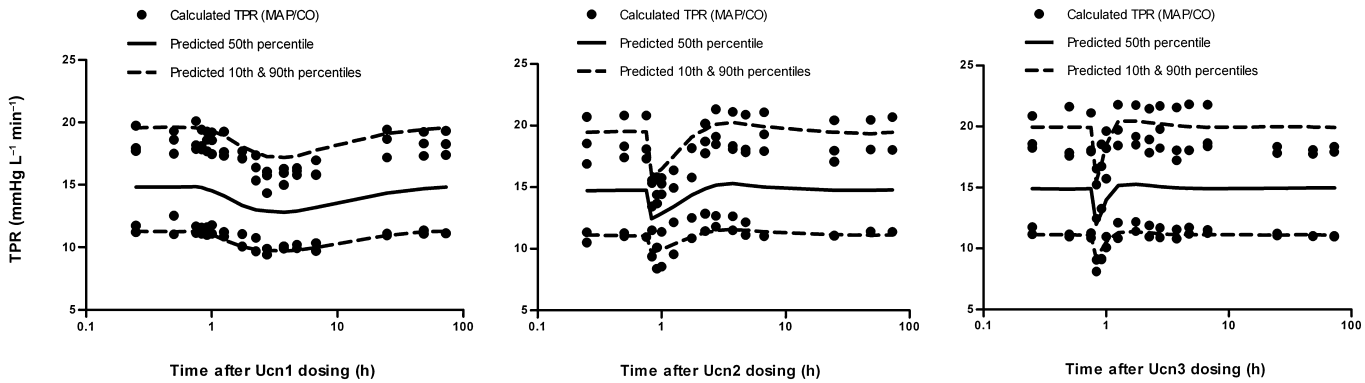

## Figure 2

Visual predictive check for Ucn1-3 in plasma (*left panels*) and corresponding changes in heart rate following Ucn dosing (*right panels*). Predicted median, 10th and 90th percentiles closely match corresponding observed percentiles, indicating the suitability of each of the developed PK/PD models. *Closed circles* represent actual observed data. For clear demonstration of the rapid PK/PD profiles for Ucn2 and Ucn3, all time axes are presented on a log scale.

deviation) with their corresponding estimates during initial sequential model building.

### *Model evaluation by VPC*

The VPC for Ucn1-3 in plasma and using HR as a representative haemodynamic effect is presented in Figure 2. All (10th, 50th and 90th) prediction percentiles closely match their corresponding observation percentiles, thus confirming the suitability of the developed PK/PD models in this population of sheep. In addition, approximately 10–20% of the data were outside of the 10th and 90th prediction percentiles, with the majority of observations evenly distributed around the predicted median.




## Figure 3

Predictive check for the change in calculated total peripheral resistance (TPR) following bolus administration of Ucn1 (*left*), Ucn2 (*middle*) and Ucn3 (*right*) to sheep. Calculated TPR values were determined from MAP and CO observations, and predicted median, 10th and 90th percentiles were obtained using a composite model for MAP and CO. For clear demonstration of the rapid PK/PD profiles for Ucn2 and Ucn3, all time axes are presented on a log scale.

### *Model for total peripheral resistance*

A model describing the change in CTPR following Ucn1-3 treatment was developed using the established composite models for MAP and CO. Final parameter estimates were comparable (<15% deviation) with those calculated from each of the individual MAP and CO models, with additive error for CTPR ranging from 0.21–0.47 mmHg·L$^{-1}$·min$^{-1}$. The VPC scatter plots (Figure 3) illustrate that while all three Ucn peptides decreased CTPR by a similar magnitude, the duration of response was longest for Ucn1 and shortest for Ucn3.

## Discussion and conclusions

Despite advances in the treatment of the causes and consequences of heart disease, this condition remains a leading cause of death in the Western world. Heart failure, in particular, continues to carry a poor prognosis (Rosamond *et al.*, 2008). In the search for novel treatments for heart disease, the Ucn peptides are emerging as potential therapeutic targets, due to their powerful cardioprotective properties and favourable cardiovascular actions (Davis *et al.*, 2004; Fekete and Zorrilla, 2007; Boonprasert *et al.*, 2008). Information detailing their respective PK/PD is essential if any one, or all of these peptides are to progress further towards development as a pharmaceutical agent.

The present study investigated the PK and PD of the three recently isolated Ucn peptides – Ucn1, Ucn2 and Ucn3. Administration of all three Ucn peptides in healthy sheep induced the expected increases in HR, CO, MAP and LVSP, together with decreases in LAP and CTPR. These haemodynamic actions of the Ucn peptides are identical to those reported previously in sheep (Rademaker *et al.*, 2002; 2005a; 2006), and the mechanisms underlying these effects will not be discussed in detail here. What is new in this study is that we have developed population PK/PD models to describe the time course of Ucn1-3 exposure and haemodynamic response. To our knowledge, this is the first study to report and compare the PK and PD for the three Ucn peptides.

Population PK analysis demonstrated that a two-compartment model adequately described the disposition of Ucn1 in plasma, with one-compartment models required for Ucn2 and Ucn3. Furthermore, the clearance and steady-state $V_d$ for Ucn1 and Ucn2 were broadly consistent with previously reported values (Rademaker *et al.*, 2002; Davis *et al.*, 2004; 2005; 2007a,b). More importantly, the estimated clearance for Ucn2 and Ucn3 were much more rapid (45- and 450-fold, respectively) than that calculated for Ucn1. Consequently, at the same administered dose, Ucn2 and Ucn3 will show lower total systemic exposure (defined by area under the concentration-time curve) when compared with Ucn1. This has obvious implications on frequency and size of dosing regimens required to achieve the same effect for the different Ucn peptides, particularly if bolus dosing is used.

For all three Ucn peptides, the time courses of all haemodynamic responses in the present study were described by delayed-effect models. In addition, the onset and duration of haemodynamic responses to Ucn2 and Ucn3 exposure were more rapid and less prolonged relative to Ucn1, consistent with the respective PK data. Importantly, our PD data also show generally lower $EC_{50}$ estimates for Ucn2 and Ucn3, suggesting that these peptides are possibly more potent than Ucn1, at least in regard to their haemodynamic actions. These results are consistent with reports in humans where a 1 h infusion of Ucn1 at 50 μg did not significantly alter haemodynamic parameters (Davis *et al.*, 2005), whereas Ucn2 infusion at 25 μg for the same period produced noticeable augmentation of CO and HR and reductions in blood pressure (Davis *et al.*, 2007b). The relative potency of Ucn3 in man remains unknown and is of particular interest given *in vitro* evidence suggesting that the human Ucn3 peptide may display lower affinity for the $CRF_2$ than either Ucn1 or Ucn2 (Lewis *et al.*, 2001).

By virtue of their reported specificity for $CRF_2$ receptors (Hsu and Hsueh, 2001), and thus negligible activation of $CRF_1$ receptiors and stimulation of the hypothalamus–pituitary–adrenal (HPA) axis (Fekete and Zorrilla, 2007), Ucn2 and Ucn3 are generally regarded as having greater potential as

therapeutic agents in heart disease. However, to date, only Ucn1 and Ucn2 have been investigated in humans and, surprisingly, Ucn2 administration in heart failure patients (as well as Ucn1) was associated with significant increases in plasma adrenocorticotrophic hormone levels (Davis *et al.*, 2005; 2007b) – indicating HPA activation. The effects of Ucn3 in humans in either normal subjects or heart failure patients and its effects on the HPA axis have yet to be determined. If Ucn3 does exhibit a lower propensity to activate the HPA axis, then this may be the peptide of choice as a therapeutic target. Indeed, Ucn3 is reported to produce more pronounced antiapoptotic, cardioprotective (Chanalaris *et al.*, 2003) and natriuretic peptide secretory activity (Chanalaris *et al.*, 2005) when compared with either Ucn1 or Ucn2. However, this may need to be counterbalanced against the requirement for increased frequency of dosing for Ucn3 given its more rapid clearance (and shorter half-life). Ideally, analogues would be developed with an enhanced beneficial haemodynamic/cardioprotective profile and minimal activation of the HPA, but with a more prolonged time course of action.

A possible limitation of the current study is the small numbers of animals recruited. However, the very intensive sampling of PK and PD, as well as early observations provided adequate information for the estimation of PK/PD parameters. During model building, implausible parameter estimates were obtained for some PK/PD models, particularly turnover models. Nonetheless, all final models presented in this analysis converged successfully and gave appropriately sized standard errors of the estimates. In addition, none of the final models were over-parameterized or had significant shrinkage of between-subject variability. It should also be noted that population PK–PD analyses are preferable to standard two-stage approaches, as they are able to correctly identify between-subject variability from residual unexplained variability (Patel and Kirkpatrick, 2011). Furthermore, the VPC produced during model evaluation adequately described both the central tendency and the spread of the data. A moderate between-subject variability was obtained for clearance and $V_d$ (~30%) in these healthy sheep of similar age and weight. Greater variability in these parameters might be expected in sheep with heart failure (Hansson, 2005) and must be quantified before designing dosing protocols to target specific concentrations. The latter is necessary to achieve chronic dosing or shorter maximal effect dosing schedules of a particular Ucn peptide. It is currently unknown whether chronic exposure to Ucn causes tolerance by either increasing $EC_{50}$ or changing $E_{max}$. Further modelling is warranted to investigate possible changes in PD parameters in heart failure.

In conclusion, although the known spectrum of bioactivity of the Ucns suggests potential therapeutic benefit in cardiovascular disease, there have been no formal studies comparing the PK/PD of the three peptides. Results from the present study confirm and extend previous findings that bolus injection of Ucn1 is cleared slowly from the circulation and exhibits long-lasting haemodynamic actions. In contrast, Ucn2 and Ucn3 are cleared much more rapidly, and the onset and duration of haemodynamic responses are more rapid and less prolonged than those to Ucn1. Our data provide valuable comparative information that may assist in the rational design of future clinical studies. Clearly, the efficacy and specificity of Ucn3 (in particular for the $CRF_2$ receptors) in humans needs to be established, and large prospective clinical studies are needed to confirm the therapeutic significance of all three Ucn peptides.

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## Conflict of interest

None.

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