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HEMODYNAMIC EFFECTS OF PHOSPHODIESTERASE 5 AND ANGIOTENSIN CONVERTING ENZYME INHIBITION ALONE OR IN COMBINATION IN CONSCIOUS SHR

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Non-standard abbreviations:- PDE5, phosphodiesterase type 5; NO, nitric oxide; cGMP, cyclic guanosine monophosphate, SHR, spontaneously hypertensive rat; ACE, angiotensin converting enzyme; HDAS, hemodynamics data acquisition system; LCMS, liquid chromatography-mass spectrometry; UK-357,903, 1-ethyl-4-{3-[3-ethyl-6,7-dihydro-7-oxo-2-(2-pyridylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-2-(2-methoxyethoxy)-   5-pyridylsulphonyl}piperazine

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Abstract

The regional hemodynamic responses to continuous, 4-day, infusion of UK-357,903 (266 μg kg^{-1} h^{-1}) alone, and in combination with a low dose of enalapril (10 μg kg^{-1} h^{-1}), were measured in conscious spontaneously hypertensive rats, to test the hypothesis that the renin-angiotensin system may influence the cardiovascular consequences of inhibition of phosphodiesterase 5 (PDE5) by UK-357,903, or vice versa. UK-357,903 alone, caused a fall in mean BP (–12.1mmHg), associated with vasodilatation in the mesenteric and hindquarters vascular beds. The only way in which the effects of enalapril given alone differed significantly from those of the vehicle was in causing mesenteric vasodilatation, which developed over the 4 days of infusion. UK-357,903, given in combination with enalapril, caused hypotension (-17.8 mmHg) and vasodilatation in the renal, mesenteric and hindquarters vascular beds. There was evidence of a significant interaction between the effects of the two compounds on renal Doppler shift and vascular conductance, with the combined action of the two compounds being greater than the sum of their individual effects. However, although there was a trend for the combination to have greater effects than either of the individual agents on blood pressure and mesenteric vascular conductance, there was no statistical evidence of an interaction. The results indicate that inhibition of the renin-angiotensin system uncovers additional renal vasodilator effects of UK-357,903, and/or inhibition of PDE5 enhances the renal vasodilator effects of ACE inhibition.

The effectiveness of antihypertensive therapy is influenced by the high degree of redundancy in cardiovascular control mechanisms, such that pharmacological interference with any particular system may have less functional effect than expected, due to compensation by other systems. In this context, we recently described the cardiovascular effects of a 4-day, continuous infusion of the phosphodiesterase 5 (PDE5) inhibitor, UK-357,903, in conscious spontaneously hypertensive rats (SHR) (Gardiner et al., 2004), and showed that, initially (Day 1), UK-357,903 caused modest hypotension, together with mesenteric and hindquarters vasodilatation, but no significant renal vasodilatation. On the subsequent days of the experiment, the significant effects waned, possibly due to activation of vasoconstrictor, compensatory mechanisms, one of which could have been the renin-angiotensin system (Gardiner et al., 2004). Consistent with this hypothesis, there are reports that PDE5 inhibition in man may lead to activation of the sympathetic nervous system (Phillips et al., 2000) and of the renin-angiotensin system (Chiu & Reid, 2002), although whether or not it is sympathetic activation which drives the renin-angiotensin system is unknown.

In our previous study, we concluded with the hypothesis that the lack of renal vasodilatation, and the transience of the antihypertensive effect of UK-357,903 may have been due to activation of counter-regulatory systems. Therefore, the objective of the present work was to test this hypothesis by assessing the regional hemodynamic responses to infusion of UK-357,903 alone, and in combination with the angiotensin converting enzyme (ACE) inhibitor, enalapril, to determine whether or not additional and/or more sustained effects of UK-357903 are apparent when the renin-angiotensin system is inhibited. In order to provide the best opportunity to observe whether there was an interaction between the PDE5 inhibitor and enalapril, the dose of enalapril was selected to have no overt hypotensive effect itself, in spite of resulting in plasma free drug concentrations above the Ki for ACE inhibition (Weisser & Schloos, 1991). UK-357,903 was chosen for these studies as it has higher selectivity than

sildenafil for PDE5 relative to PDE1 (Ballard et al., 1998; Gardiner et al., 2004). For

technical reasons, and to allow comparison with our previous study, the experimental

protocol ran over 4 days.

Methods

All experiments were carried out on male, SHR (Charles River U.K.), weighing between 260 and 360g (i.e., between 20 and 22 weeks of age) at the time of study. Animals were housed in a temperature-controlled environment (20-22°C), with a 12 h light-dark cycle (lights on at 06.00h), with free access to food (Beekay Rat and Mouse Diet No 1, sodium 0.18%; B&K Universal Limited, Hull, UK) and water throughout. The procedures were approved by the University of Nottingham Ethical Review Committee, and were performed under Home Office Project Licence authority.

Surgical preparation

Surgery was performed in two stages, under general anesthesia (fentanyl and medetomidine, 300 µg kg^{-1} of each, i.p.). The first stage involved implantation of miniaturised, pulsed Doppler flow probes around the left renal artery, the superior mesenteric artery, and the distal abdominal aorta (below the level of the ileocecal artery, to monitor flow to the hindquarters). The second stage involved placement of catheters in the distal abdominal aorta (*via* the caudal artery) to monitor mean arterial blood pressure (BP) and heart rate, and in the right jugular vein for drug administrations. After each surgical stage, anesthesia was reversed, and analgesia provided, with atipamezole and nalbuphine, respectively (1 mg kg^{-1} of each, s.c.). The two surgical stages were separated by at least 10 days and, prior to the second stage, the fitness of all animals was certified by the named veterinary surgeon.

After catheterisation, double-channel, fluid-filled swivels (Blair et al., 1980) were used to allow overnight i.v. infusion of drugs or saline (0.4 ml h^{-1}) and i.a. infusion of heparinised (15 U ml^{-1}, 0.4 ml h^{-1}) saline to maintain catheter patency. Experiments began 24 h after

catheterization, when the animals were fully conscious, freely moving, and with access to food and water *ad libitum.*

Cardiovascular recordings

Continuous recordings of cardiovascular variables were made using a customized, computer-based system (Hemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht, The Netherlands) connected to the transducer amplifier (Gould (Ohio, USA) model 13-4615-50) and the Doppler flowmeter (Crystal Biotech (Holliston, USA) VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high velocity (HVPD-20) modules). Raw data were sampled by HDAS every 2 ms, averaged every cardiac cycle, and stored to disc at 5 s intervals. Data were analysed offline using software (Datview, University of Limburg, Maastricht, The Netherlands) which interfaced with HDAS.

Experimental protocol

There were 4 groups of animals, and the study ran over several weeks with, typically, data from equal numbers of rats from each treatment group being collected each week. After a control period of at least 90 min baseline recording on Day 1, rats were randomised to receive vehicle (isotonic saline adjusted to pH 4 with HCl, n = 7), enalapril (10 μg kg^{-1} h^{-1}, n = 8), UK-357,903 (266 μg kg^{-1} h^{-1}, n = 8) or UK-357,903 + enalapril (doses as above, n = 8). Thereafter, drugs or vehicle were administered continuously until the end of the experiment. Cardiovascular data were collected for 7h after the onset of drug administration on Day 1, and for periods of 7h on Days 2-4.

Arterial blood samples were collected prior to any intervention on Day 1 and after the recording period of each experimental day for analysis of plasma drug levels, renin activity and cGMP.

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Biochemical and drug analyses

The concentration of cGMP in plasma was determined by enzyme immunoassay, using Kit RPN 226 from Amersham Pharmacia Biotech UK Ltd (Little Chalfont, Bucks, UK) as described in Gardiner et al. (2004). Plasma renin activity was determined by monitoring the formation of angiotensin I from endogenous plasma angiotensinogen using the Angiotensin I [^{125}I] Radioimmunoassay Kit NEA104,105 (Perkin Elmer Life Sciences, Inc. Boston, MA). The procedure was as described in the kit protocol, except volumes for the angiotensin I generation step were scaled down to accept 100μl of plasma.

Plasma concentrations of UK-357,903 and enalaprilat (active metabolite of enalapril rapidly formed in rat plasma) were determined by LCMS and converted to free (unbound) levels by correcting for plasma protein binding as described previously (Gardiner et al., 2004). As plasma concentrations of enalaprilat were approaching the limit of assay sensitivity, it was necessary to pool samples from 2 or 3 animals in order to obtain sufficient for analysis.

Cardiovascular data analysis

Details of the procedures for data analysis have been described previously (Gardiner et al., 2004). Briefly, the baseline was taken as the 30-45 min period prior to drug administration on Day 1, when the animals were settled. For graphical representation, post-dosing data are expressed as 3 sequential averages (~ 140 min) on Day 1, and as 4 sequential averages (~105 min) on Days 2-4, relative to the original baseline (i.e., on Day 1) However, for statistical analyses, post-dosing data were averaged across the entire 7h recording period for each day. The average data for each day were subjected to analysis of covariance (ANOCOVA) (heart rate, mean blood pressure, free plasma cGMP), allowing for potential week-to-week differences and for differences at baseline or analysis of variance (ANOVA) (% changes in

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Doppler shift and vascular conductance), allowing for potential week-to-week differences. The possibility of a statistical interaction between enalapril and UK-357,903 was assessed using the models described. This interaction can be considered as a comparison of whether the combined action of the two compounds is greater than the sum of the individual compound effects. Estimated treatment differences are also presented from these analyses. These reflect the differences between each treatment group and vehicle, compensated for differences at baseline and week-to-week variation. The cGMP and renin data were analysed on a log scale and, therefore, comparisons of treatment groups are represented by treatment ratios, reflecting ratios of estimated means.

To assess the treatment effects over time an analysis of the post-dosing data was performed using a linear mixed model with a Greenhouse-Geisser correction for the repeated measures. This also allowed for potential week-to-week differences and for differences at baseline (heart rate, mean blood pressure, free plasma cGMP measures only). For measures where there was no evidence of differences in treatment effects over time, overall treatment comparisons (i.e., comparisons averaged over time) were performed and reported, in addition to the separate daily analyses.

All analyses were carried out using GenStat for Windows, version 6.1. A P-value ≤ 0.05 was taken as significant.

Drugs

Fentanyl citrate was from Janssen-Cilag (High Wycombe, U.K); medetomidine

hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer

(Sandwich, Kent, U.K.), nalbuphine hydrochloride (Nubain) was from Bristol Myers Squibb

(Hounslow, U.K.). UK-357,903, 1-ethyl-4-{3-[3-ethyl-6,7-dihydro-7-oxo-2-(2-

pyridylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-2-(2-methoxyethoxy)-5-

pyridylsulphonyl}piperazine was supplied by Pfizer and enalapril was purchased from Sigma

(Poole, Dorset, U.K.) Drugs and vehicle were infused at a rate of 0.4 ml h^{-1}.

Results

Baseline cardiovascular variables

Resting cardiovascular variables in the 4 groups of rats prior to drug or vehicle administration are shown in Table 1. There were no statistically significant differences between the groups.

Figures 1a-3a show the mean BP and heart rate (Figure 1a), % changes in Doppler shift (Figure 2a), and % changes in vascular conductances (Figure 3a), and Figures 1b-3b show the corresponding treatment effects (i.e., adjusted mean differences from vehicle) on each of the 4 study days.

Blood pressure

In rats treated with enalapril, there was a trend for BP to be lower than in vehicle-treated animals (maximum –7.1mmHg on day 4), but the differences were not statistically significant (Figure 1b). In contrast, rats treated with UK-357,903 alone, or in combination with enalapril, showed significant reductions in blood pressure up to a maximum of -14.5 mmHg and –23.0mmHg, respectively (Figure 1b). The falls in BP were significant on Days 1, 3 and 4 in the group treated with UK-357903 alone, and on all four days in the group treated with the combination of drugs (Figure 1b). Despite the observation that blood pressure falls tended to be greatest for each treatment on days 3 and 4, a repeated measures analysis did not provide statistical confirmation that treatment effects were changing with time. Therefore overall BP falls for the 4 days were estimated, providing values for enalapril, UK-357,903 and combination groups of -2.4 mmHg (not significant), -12.1mmHg ($P<0.05$) and –17.8mmHg ($P<0.05$), respectively. There was no statistical evidence of an interaction between UK-357903 and enalapril on BP, i.e., the combined effect was not significantly greater than the sum of the individual effects ($P = 0.157$).

Heart rate

Only the group treated with the combination of UK-357903 and enalapril showed any apparent change in heart rate which differed from that seen with the vehicle, and the effect was small (+17 beats min^{-1}) and only significant on Day 1 (Figure 1b). Furthermore, the repeated measures analysis indicated that, for the 4-days overall, there was no significant effect of any treatment on heart rate.

Renal Doppler shift and vascular conductance

In rats treated with enalapril alone, or with UK-357,903 alone, there were no changes in renal Doppler shift or vascular conductance which differed significantly from those seen with the vehicle (Figures 2 & 3). However, in the group treated with the combination of drugs, there was an increase in the % change in renal Doppler shift (significant on Day 2, 10% increase) and vascular conductance (significant on Days 1 to 4, from 9% increase on Day 1 to 34% increase on Day 4). There was evidence for a statistical interaction between UK-357,903 and enalapril on renal Doppler shift and vascular conductance, since the combined effects of the drugs exceeded the sum of the individual effects (significant on Day 2, $P<0.05$ for Doppler shift and $P<0.01$ for conductance). Although not statistically significant, the lower resting renal vascular conductance in the group treated with the combination of drugs (Table 1) could have influenced the vasodilator effects observed. However, consideration of individual responses showed no systematic association between resting renal vascular conductance and response to UK-357,903 plus enalapril. Furthermore, consideration of individual responses within the groups showed that animals having similar baseline values for renal vascular conductance, nonetheless showed larger increases in the combination group than in any other.

Mesenteric Doppler shift and vascular conductance

In all three treatment groups, there were increases in mesenteric Doppler shift and vascular conductance which differed from those seen with the vehicle. For the group treated with

enalapril, the changes were significant on Days 2 (Doppler shift) and 4 (Doppler shift and vascular conductance). For the group treated with UK-357,903, the changes in vascular conductance were sustained (~ 20% increase on Days 1 to 4), although the changes in Doppler shift were significant on Days 1 and 2 only. For the group treated with the combination of drugs, the changes in Doppler shift and vascular conductance were significant throughout the experiment, with the latter increasing from ~ 25% increase on Day 1 to ~ 48% increase on day 4 (Figures 2 & 3). There was no evidence of a statistical interaction between UK-357,903 and enalapril on mesenteric Doppler shift or vascular conductance.

Hindquarters Doppler shift and vascular conductance

In the group treated with enalapril alone, there were no changes in hindquarters Doppler shift or vascular conductance that differed from those seen with the vehicle. In the group treated with UK-357,903 alone, there were increases in hindquarters Doppler shift and vascular conductance and, although the estimated treatment differences were approximately constant across the 4 days (supported by repeated measures analysis), they were only significant on Days 1 and 2 due to an increase in the variability thereafter (Figures 2 & 3). The repeated measures analysis showed that overall, for the 4-day treatment period, the effect of UK-357,903 was significant for both Doppler shift (13% increase) and hindquarters conductance (22% increase). In rats treated with the combination of drugs, increases in hindquarters vascular conductance were different from vehicle on Days 1, 2 and 4, although the increase in Doppler shift was only significant on Day 1. The overall treatment effect was significant for vascular conductance (24% increase), but not for Doppler shift. There was no evidence of an interaction between the effects of UK-357,903 and enalapril on hindquarters vascular conductance.

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Plasma cGMP concentrations and renin activity levels

UK-357,903 alone caused a significant rise in plasma cGMP to approximately 2- to 3-fold vehicle level (Figure 4). Cyclic GMP was similarly increased in the combination group (~2-fold, Figure 4). In contrast, enalapril alone was without significant effect (treatment ratios ~1; Figure 4).

Plasma renin activity levels (geometric mean in ng ml^{-1} h^{-1} with [95% CI]) determined on day 2 of the study were similar for vehicle (n=7) and UK-357,903 (n=6) treatment groups (2.5 [2.1, 3.1] and 2.4 [1.9, 3.0], respectively). In contrast, renin activity was significantly elevated to approximately twice the vehicle level in both enalapril alone (n=6) and combination groups (n=6) (4.8 [3.0, 7.7] and 5.67 [4.1, 7.9], respectively).

Plasma drug levels

Plasma concentrations of free UK-357,903 (geometric mean in nM with [95% confidence intervals]) were relatively stable across the 4 experimental days in both the UK-357,903 alone (range 7.1 to 10.1nM) and in the combination treatment groups (range 6.6 to 10.9nM; Table 2). Similarly, plasma concentrations of free enalaprilat ranged from 9.8 to 15.2nM in the enalapril alone group and from 9.3 to 20.1nM in the combination group (Table 2). There was therefore no evidence that concentrations of either UK-357,903 or enalaprilat were affected when both compounds were administered together. The free levels of UK-357,903 were approximately 5 to 8-fold the IC$_{50}$ for inhibition of PDE5 (1.3nM; Gardiner et al., 2004).

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Discussion

In our previous study (Gardiner et al., 2004) we found that, in conscious SHR, UK-357,903 infused for 4 days at doses of 133 and 1330 $\mu g \ kg^{-1} \ h^{-1}$, had modest hypotensive effects, accompanied by mesenteric and hindquarters vasodilatations that were not dose-related and tended to wane after the first day of infusion. Since the plasma drug levels and cGMP concentrations increased with increasing dose of UK-357,903 and were sustained, we hypothesised that the cardiovascular effects of UK-357,903 might have been influenced by activation of compensatory systems (Gardiner et al., 2004). Here we tested the hypothesis that there was an interaction between the effects of ACE inhibition by enalapril, and PDE5 inhibition by UK-357,903. This was found to be the case, but only for the renal vascular bed. Here, UK-357,903 had no detectable effect on vascular conductance and, while enalapril showed a trend to increase both flow and conductance, this was not significant. However, in combination, UK-357,903 and enalapril caused hyperemic renal vasodilatation that was significantly greater than the sum of the effects of the individual drugs. This finding is consistent with the renin-angiotensin system opposing the renal vasodilator action of UK-357,903, and/or UK-357,903 augmenting a renal vasodilator effect of enalapril.

The dose of enalapril was chosen to have sub-maximal cardiovascular effects when given alone, but to provide drug levels within the clinical range. Actual free concentrations in SHR were similar to those found in man at the 24h trough after a 10mg dose which is commonly used to treat hypertension (~25nM based on 83% free fraction; Weisser & Schloos, 1993). In our earlier study (Gardiner et al., 2004) we used a dose of enalapril which was 100-fold higher than that used here, and it caused a substantial fall in blood pressure (-39 mmHg at the end of 4 days). In pilot studies (n = 3) we found a 10-fold higher dose than that used in the present study still caused a marked fall in mean BP (-36 mmHg at the end of 4 days). The

dose of enalapril finally chosen for this study caused significant mesenteric vasodilatation

and showed trends to progressively lower blood pressure and increase renal vascular

conductance. Although the apparent changes in blood pressure and renal conductance were

not statistically significant (mean differences from vehicle –7.1 mmHg and +14% after 4

days), the chosen dose was ideal for the purpose of the study, inasmuch as it was

pharmacologically active (significant mesenteric vasodilatation and increase in plasma renin

activity), but left scope for demonstrating interaction. It was surprising to us that the

mesenteric vascular bed showed more consistent vasodilatation in response to enalapril than

the renal vascular bed, since, using higher doses, we (Gardiner et al., 2004) and others (Smits

& Strujker-Boudier, 1984; Lappe et al., 1985; Kanagawa et al., 1997) found the vasodilator

effects of ACE inhibition in SHR to be most marked in the kidney. It is possible that, at the

low concentration of enalapril used here, given as an infusion, selective inhibition of local

angiotensin II production in the vascular walls occurred, and that this was counteracted by

autoregulatory mechanisms in the kidney (but see below). Certainly, plasma renin activity

was increased, and this would have led to increased angiotensin I levels that might have

overcome partial inhibition of ACE, particularly in regions such as the kidney, where ACE

activity levels are high. Alternatively, since there was some evidence that the effect of

enalapril was increasing with time, it is possible that an effect on renal vascular conductance

is slow to develop at the low dose and might have become significant if treatment had

continued beyond 4 days.

In this study, the effects of UK-357,903 administration alone were interesting against the

background of our earlier study. Thus, the previous results (Gardiner et al., 2004) showed

modest hypotensive and mesenteric and hindquarters vasodilator effects of UK-357,903 (133

and 1330 μg kg^{-1} h^{-1}) that were not dose-dependent, and which waned after Day 1. In the

light of those findings, we chose an intermediate dose of UK-357,903 (266 μg kg^{-1} h^{-1}),

which resulted in plasma free drug levels that were 5- to 8-fold the IC$_{50}$ for inhibition of

PDE5 (see Gardiner et al., 2004), and more closely equated to the relative Cmax free

concentrations of sildenafil observed in man after doses that are clinically effective in erectile

dysfunction (15 to 30nM; 4 to 8-fold PDE5 IC50; Ballard et al., 1998). Here we confirmed

our earlier observations on the regional heterogeneity of the effects of UK-357,903 (Gardiner

et al., 2004), in that it caused vasodilatation in the mesenteric and hindquarters vascular beds,

while the renal vascular bed appeared to be relatively resistant. However, the effects of the

intermediate dose of UK-357,903 used in the present study were more persistent than those of

the higher dose used previously. We have no ready explanation for this, unless, at the higher

dose, additional effects of UK-357,903 gave rise to activation of more powerful

compensatory mechanisms. In this context, one possible explanation is that accumulation of

cGMP, in response to UK-357,903, causes up-regulation of ACE (Saijonmaa and Fyhrquist,

1998). However, we did not measure serum or tissue ACE, so cannot confirm whether or not

this happened in our experiments.

It is notable that, when UK-357,903 was given in combination with enalapril, hyperemic

renal vasodilatation occurred that exceeded the sum of effects of the individual agents.

Previously, we discussed possible reasons for the lack of effect of UK-357,903 on the renal

vascular bed (Gardiner et al., 2004), and predicted that "in the presence of renin-angiotensin

blockade, mesenteric vasodilator effects of UK-357,903 might be enhanced and renal

vasodilatation might be revealed". The present findings clearly fit with our prediction, at

least as far as the renal vascular bed is concerned. Although measurements of plasma renin

activity in samples taken at the end of Day 2 in the present study showed no significant

influence of UK-357,903, this does not preclude a functional involvement of the renin-

angiotensin system in renal vascular responses to the drug. Thus, it is feasible that the renal

vascular bed is less sensitive than other regions to the vasodilator effects of the nitric oxide

(NO)-cGMP system, but that removal of a vasoconstrictor influence of angiotensin can

uncover an effect. Although there was a trend for blood pressure reduction to be greater in the

combination group than with UK-357,903 alone, this was not statistically significant. It

would be of interest to determine whether a longer treatment period might reveal a significant

difference between the combination and single agents, although in such a protocol it would

not be possible to collect detailed hemodynamic data of the sort reported here.

It is feasible that our results, rather than reflecting an influence of the renin-angiotensin

system on the vasodilator effect of UK-357,903, were due to an influence of UK-357,903 on

responses to enalapril. For example, if a component of the hemodynamic response to ACE

inhibition is NO-mediated vasodilatation secondary to increased bradykinin levels (Gohlke et

al., 1994; Gainer et al., 1998; Kohno et al., 1999; Squire et al., 2000), this effect would be

amplified by PDE5 inhibition. Although there was no evidence that ACE inhibition

enhanced the increase in plasma cGMP caused by UK-357,903, this does not preclude subtle

local effects on cGMP levels that might be physiologically relevant.

Whatever the explanation, we were surprised that the drug interaction was (i) not seen on Day

1 and (ii) not apparent in the mesenteric vascular bed. Although plasma levels of enalaprilat

were lower on Day 1 than on subsequent days, they were above the Ki for ACE inhibition

throughout the experimental protocol. However, we cannot dismiss the possibility of an

enhanced functional effect occurring in association with the higher plasma levels of

enalaprilat seen on Days 2-4

If activation of the renin-angiotensin system by UK-357,903 occurred, then the lack of

interaction between UK-357,903 and enalapril on Day 1 suggests that the stimulus for

activation of the renin-angiotensin system cannot have been the hypotensive effect of UK-357,903, since this was already apparent on Day 1. As noted above, it is possible that UK-357,903 caused up-regulation of ACE (Saijonmaa and Fyhrquist, 1998), but that this process took time to develop. However, if our results reflected augmentation by UK-357,903 of an NO-mediated vasodilator effect of enalapril (see earlier), then slow accumulation of the effect of enalapril to enhance bradykinin levels and cause downstream increases in NO and cGMP could be the reason why the effect only became apparent on Day 2.

If the latter explanation is correct, it is not obvious why interaction between enalapril and UK-357,903 was not seen in the mesenteric vascular bed unless, for example, mesenteric vasodilator effects of ACE inhibition are more dependent on removal of the vasoconstrictor influence of angiotensin than on bradykinin accumulation. Resolution of this question will require further experiments, specifically comparing the effects of angiotensin receptor antagonism with those of ACE inhibition.

In conclusion, the present results indicate that combined administration of UK 357,903 and enalapril might improve renal perfusion over and above that achieved with ACE inhibition alone. The present experiments do not allow us to determine if the effects observed were due to a renal vasodilator effect of UK-357,903 being uncovered in the presence of enalapril, or UK-357,903 augmenting a renal vasodilator effect of enalapril. Nevertheless, if these findings extrapolate to chronic treatment in the clinical setting, it is feasible that combined treatment of the sort used here would be beneficial in patients with compromised renal blood flow.

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Footnotes

a) Financial support for this work was provided by Pfizer Limited.

b) Reprint requests to Professor SM Gardiner, Centre for Integrated Systems Biology &

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Legends for Figures

Figure 1. Heart rate and mean arterial blood pressure over a 4-day continuous infusion of

vehicle (n = 7), enalapril (10 µg kg^{-1} h^{-1}; n = 8), UK-357,903 (266 µg kg^{-1} h^{-1}; n = 8) or

enalapril together with UK-357,903 (doses as above; n = 8). Panel (a) shows values averaged

over 105 min epochs during the 7h monitoring-period on each day. Panel (b) shows the

estimated differences between each treatment group and vehicle with 95% confidence

intervals. Treatment effects are significantly different from vehicle (P<0.05) where the

confidence interval bar does not cross the zero line.

Figure 2. Changes in regional Doppler shift over a 4-day continuous infusion of vehicle (n =

7), enalapril (10 µg kg^{-1} h^{-1}; n = 8), UK-357,903 (266 µg kg^{-1} h^{-1}; n = 8) or enalapril together

with UK-357,903 (doses as above; n = 8). Panel (a) shows values averaged over 105 min

epochs during the 7h monitoring-period on each day. Panel (b) shows the estimated

differences between each treatment group and vehicle with 95% confidence intervals.

Treatment effects are significantly different from vehicle (P<0.05) where the confidence

interval bar does not cross the zero line.

Figure 3. Changes in regional vascular conductance over a 4-day continuous infusion of

vehicle (n = 7), enalapril (10 µg kg^{-1} h^{-1}; n = 8), UK-357,903 (266 µg kg^{-1} h^{-1}; n = 8) or

enalapril together with UK-357,903 (doses as above; n = 8). Panel (a) shows values averaged

over 105 min epochs during the 7h monitoring-period on each day. Panel (b) shows the

estimated differences between each treatment group and vehicle with 95% confidence

intervals. Treatment effects are significantly different from vehicle (P<0.05) where the

confidence interval bar does not cross the zero line.

Figure 4. Plasma concentrations of cGMP during continuous infusion of vehicle (n = 7),

enalapril (10 µg kg^{-1} h^{-1}; n = 8), UK-357,903 (266 µg kg^{-1} h^{-1}; n = 8) or enalapril together

with UK-357,903 (doses as above; n = 8). in conscious SHR. Panel (a) shows geometric

mean concentrations of cGMP in plasma samples taken at the end of each days monitoring.

Panel (b) shows the estimated treatment ratios, relative to vehicle, with 95% confidence

intervals. Treatment effects are significantly different from vehicle (P<0.05) where the

treatment ratio confidence interval does not cross the unity line.

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Table 1. Resting cardiovascular variables in conscious spontaneously hypertensive rats

Treatment group	Vehicle n=7	Enalapril n=8	UK-357,903 n=8	UK-357,903 + Enalapril n=8
Heart rate (beats min^{-1})	322±12	326±7	306±6	325±6
Mean BP (mmHg)	169±4	161±6	165±3	172±5
Renal DS (kHz)	7.5±0.7	6.9±1.0	7.0±0.9	5.5±0.2
Mesenteric DS (kHz)	9.1±0.8	8.9±0.8	7.5±0.8	8.8±1.0
Hindquarters DS (kHz)	3.9±0.6	4.1±0.4	4.6±0.5	4.1±0.5
Renal VC (units)	45±4	42±5	42±5	32±2
Mesenteric VC (units)	55±6	56±5	45±5	52±7
Hindquarters VC (units)	23±4	26±4	27±3	24±4

Values are mean ± S.E. for groups of 7 or 8 rats. DS = Doppler shift, VC = vascular conductance. Units for VC are (kHz mmHg^{-1}) 10^3.

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Table 2. Plasma concentrations of free UK-357,903 and enalaprilat.

Treatment	UK-357,903 (nM free)		Enalaprilat (nM free)	
	UK-357,903	UK-357,903 + Enalapril	Enalapril	UK-357,903 + Enalapril
Day 1	10.1	8.4	9.8	9.3
	(7.3, 14.2)	(6.6, 10.6)	(5.8, 16.6)	(2.8, 30.7)
Day 2	8.1	10.9	15.1	15.8
	(6.3, 10.4)	(7.9, 15.0)	(8.7, 26.3)	(12.3, 20.2)
Day 3	7.6	7.5	15.2	15.7
	(6.1, 9.4)	(5.5, 10.4)	(4.5, 53.1)	(5.4, 45.8)
Day 4	7.1	6.6	15.0	20.1
	(5.4, 9.3)	(4.9, 8.8)	(6.6, 34.2)	(8.7, 46.5)

Values are geometric means with 95% CI (in parentheses) for data from individual animals

for UK-357,903 (n= 5 to 8), and for pooled samples from 2 or 3 animals for enalaprilat (n=3
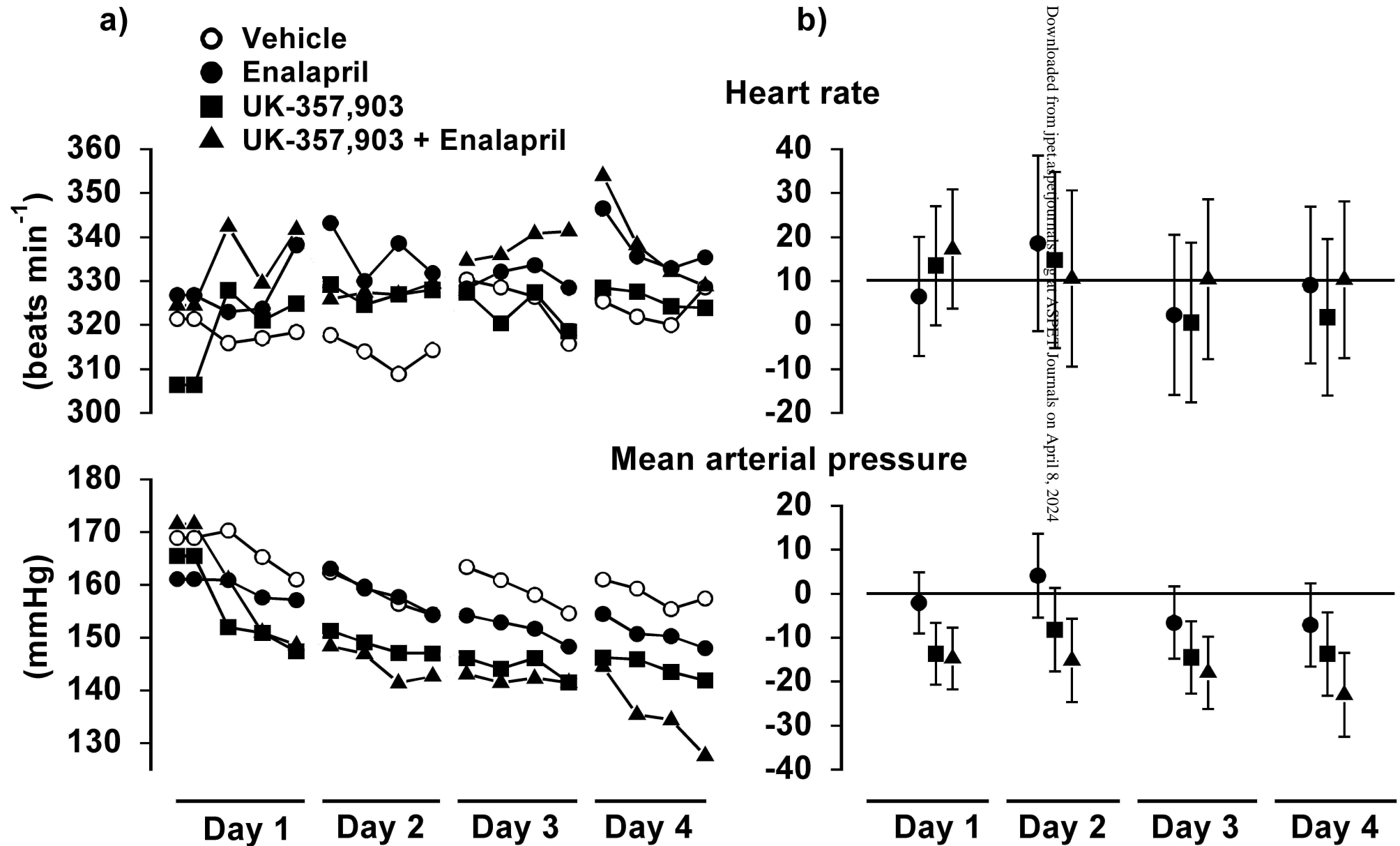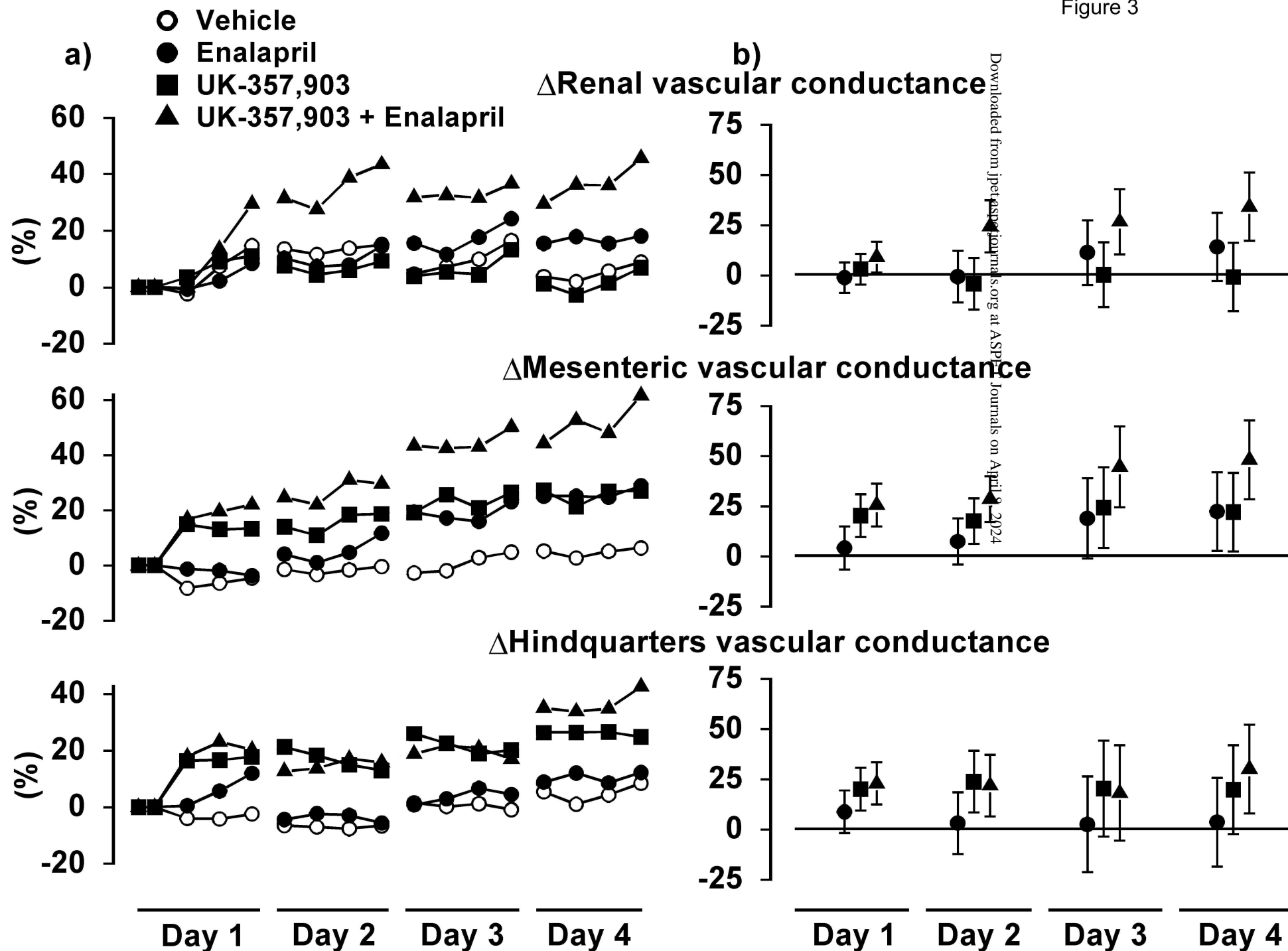
pooled samples).

Figure 1

Figure 2

a)

- ○ Vehicle
- ● Enalapril
- ■ UK-357,903
- ▲ UK-357,903 + Enalapril

(%)

Day 1   Day 2   Day 3   Day 4

b)

ΔRenal Doppler shift

ΔMesenteric Doppler shift

ΔHindquarters Doppler shift

Day 1   Day 2   Day 3   Day 4

Figure 3

Figure 4