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# The Effect of Different Dosing Schedules of UCN-01 on its Pharmacokinetics and Cardiohaemodynamics in Dogs

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**Abstract**

7-Hydroxy-staurosporine (UCN-01) is now under development as a novel anticancer drug. In clinical studies, different infusion schedules are being investigated in the USA and Japan. To examine the effect of different infusion schedules on the pharmacokinetics and cardiohaemodynamics of UCN-01, dogs were treated with UCN-01 as either a 3-h or a 24-h constant intravenous infusion. Blood pressure and heart rate, together with UCN-01 concentrations during and after infusion, were monitored. To analyse the relationship between the pharmacokinetics and cardiohaemodynamics of UCN-01, the plasma concentration of UCN-01 at the end of infusion ($C_{end}$), the area under the plasma concentration versus time curves ($AUC_{0-\infty}$) and the mean residence time (MRT) were used. As indices of cardiohaemodynamic changes, the area under decreasing systolic blood pressure and increasing heart rate versus time curves ($dAUC_{pressure}$ and $AUC_{heart\ rate}$) were calculated by the trapezoidal method.

For the 3-h (0·22 and 0·65 mg kg$^{-1}$) and 24-h infusion (0·81 to 6·48 mg kg$^{-1}$), systolic and diastolic blood pressures fell after or during infusions, accompanied by a dose-dependent increase in heart rate for both infusions. During both infusion schedules, the plasma concentrations of UCN-01 gradually increased and $C_{end}$ showed a dose-proportional increase. After that, UCN-01 was eliminated bi-exponentially with an elimination half-life of $5{\cdot}14 \pm 1{\cdot}12$ to $8{\cdot}32 \pm 1{\cdot}80$ h. The total clearance ($CL_{total}$) ranged from 0·383 to $0{\cdot}666 \pm 0{\cdot}149$ L h$^{-1}$ kg$^{-1}$. There was no significant difference in these parameters among the doses in each infusion schedule, indicating that UCN-01 has a linear pharmacokinetic profile over the dose range examined for each infusion, and there were also no significant differences between the 3-h and 24-h infusion except for MRT. The pharmacokinetic parameters of $C_{end}$, $AUC_{0-\infty}$ and $slope_{0-3\,h}$ exhibited a degree of correlation with the $AUC_{heart\ rate}$ in the 3-h infusion and correlated significantly with the $dAUC_{pressure}$ in the 24-h infusion. The MRT did not correlate with cardiohaemodynamic changes during either infusion.

In conclusion, the pharmacokinetic profile of UCN-01 after the shorter infusion is similar to that after the longer one. However, a longer dosing period of UCN-01 increased the residence time in comparison with the shorter infusion. This may be due to the effect on the circulatory function in dogs.

7-Hydroxy-staurosporine (UCN-01, Figure 1), which is isolated and purified from cultured *Streptomyces* sp., is a potent inhibitor of protein kinases (Takahashi et al 1987). It has been reported to exhibit potent antitumour effects on murine and human cancer cells in-vitro (Akinaga et al 1991, 1993a; Seynaeve et al 1993) and in-vivo (Akinaga et al 1991, 1993a). Although the mechanism of its action is still not completely clear, UCN-01 acts on the cell cycle of various cancer cells, arresting progress from the $G_1$ phase to the S phase, thereby causing $G_1$ phase accumulation (Akinaga et al 1993b, 1994; Kawakami et al 1996). The $G_1$ phase accumulation has been suggested to result from the induction of cyclin-dependent kinase (CDK) inhibitor proteins, P21 and P27, and from the

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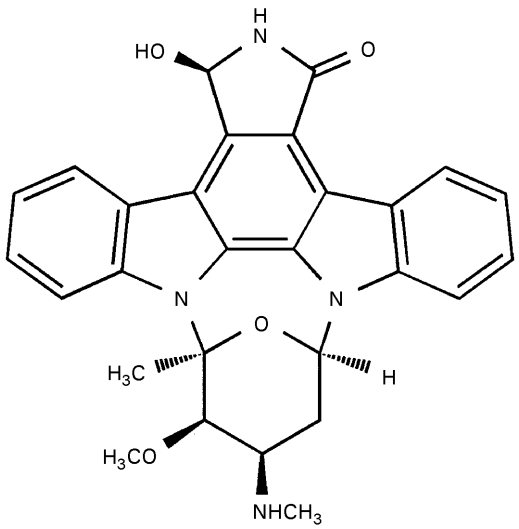


Figure 1. Chemical structure of UCN-01.

dephosphorylation of an anti-oncogene product, Rb protein, and CDK 2 protein (Akiyama et al 1997). Also, the fact that UCN-01 enhances the anti-tumour activity of several anticancer drugs in-vitro and in-vivo has attracted much interest (Akinaga et al 1992, 1993a; Bunch & Eastman 1996). UCN-01 is now under evaluation as a new type of drug with a novel mechanism of action and interesting characteristics.

Phase I clinical studies are ongoing in the USA (Senderowicz et al 1999) and Japan (Tamura et al 1999). Different dosing schedules are being used in the clinical studies: a 72-h infusion in the USA and a 3-h infusion in Japan. UCN-01 has produced a number of toxic effects in clinical studies, involving the pulmonary, cardiac/circulatory and gastrointestinal systems as well as hyperglycaemia (Senderowicz et al 1999; Tamura et al 1999). In preclinical studies, the following toxic effects were reported when UCN-01 was administered by 72-h infusion to dogs: swelling, diarrhoea, leucopenia (neutropenia), thrombocytopenia, hepatic inflammation/necrosis, adrenal gland hypertrophy, renal necrosis/haemorrhage, intestinal necrosis and testicular hypospermia (Sausville et al 1998). UCN-01 inhibits several protein kinases (Takahashi et al 1989; Mizuno et al 1993; Seynaeve et al 1994), one of which, protein kinase C (PKC), is distributed in many organs (Huang et al 1987; Yoshida et al 1988) and has been reported to be expressed in the heart (Steinberg et al 1995; Puceat & Vassort 1996) and vascular cells (Inoguchi et al 1992). Protein kinases of this type can be roughly divided into the following 3 classes (Puceat & Vassort 1996): firstly, conventional PKCs ($\alpha$-, $\beta$- and $\gamma$-PKCs); secondly, novel PKCs ($\delta$-, $\varepsilon$-, $\theta$-, $\eta$- and $\mu$-PKCs); and thirdly, atypical PKCs ($\zeta$-, $\lambda$- and $\tau$-PKCs). Among these 3 classes, UCN-01 selectively and potently inhibits conventional PKCs (Mizuno et al 1993, 1995; Seynaeve et al 1994). It has been reported that PKC plays a role in the normal function of the myocardium (Hattori et al 1993; Rabkin 1996) and aorta (Shimamoto et al 1992; James & Hodgson 1997). UCN-01 can be classified as Type II (cell-cycle phase specific), whose antiproliferative activity is dependent on the exposure time of certain tumour cells (Seynaeve et al 1993). Therefore, the infusion schedule might be an important factor in determining its efficacy. However, as far as the toxicity of UCN-01 is concerned, the effect of the infusion schedule is unclear. To study how UCN-01 or its dosing schedule influences the circulatory function is, therefore, of great importance in the light of the aforementioned factors.

The pharmacokinetic profile of UCN-01 in patients is quite different from that in experimental animals (Fuse et al 1998; Senderowicz et al 1999; Tamura et al 1999). Clinical data have shown that the terminal half-life in plasma is extremely long, and the total plasma clearance ($CL_{total}$) and volume of distribution (Vdss) of UCN-01 in patients are very low: $0{\cdot}0796–0{\cdot}158\,L\,kg^{-1}$ and $0{\cdot}0407–0{\cdot}252\,mL\,h^{-1}\,kg^{-1}$, respectively (Fuse et al 1998). In particular, the Vdss is almost equal to the sum of the total plasma and extravascular volumes in humans. Protein-binding studies (Fuse et al 1998, 1999) have revealed that this marked species difference in pharmacokinetic profile is due to the high degree of specific binding of UCN-01 to human $\alpha_1$-acid glycoprotein ($\alpha_1$-AGP) in plasma. Moreover, the binding affinity of UCN-01 for $\alpha_1$-AGP in experimental animals is much weaker than that in humans. If the protein binding to human $\alpha_1$-AGP becomes saturated, weaker binding of UCN-01 to other proteins could occur in human plasma, as in experimental animals. The protein binding in experimental animals seems to be so weak that the total plasma concentration of UCN-01 in animals reflects the free UCN-01 concentration in human (fraction of UCN-01 unbound to human $\alpha_1$-AGP). Therefore, it would be very useful to investigate the cardiohaemodynamic effect of UCN-01 in experimental animals to analyse the relationship between its pharmacokinetics and cardiohaemodynamics.

With this in mind, we studied the dynamic effects of UCN-01 on the pharmacokinetics and cardiohaemodynamics after infusion to dogs. Moreover, considering that different infusion schedules are used in clinical studies, we also evaluated the differences in the effects of UCN-01 dosing schedules on its pharmacokinetics and cardiohaemodynamics.

## Materials and Methods

*Chemicals*

UCN-01 and staurosporine were produced by fermentation in our institute as described previously (Takahashi et al 1987). Micronomycin sulphate (Kyowa Hakko, Tokyo, Japan) was used to protect dogs from infections. Acetonitrile (HPLC grade) was purchased from Kanto Chemical (Osaka, Japan). Other reagents used were of analytical grade obtained commercially.

*Animals*

Male beagle dogs, 8·8–14·2 kg (Hazleton Research Products Inc., Kalamazoo, MI or Nippon Nosan Kogyo, Kanagawa, Japan), were used. Infusions of UCN-01 was conducted through a catheter inserted into a vein. Furthermore, to monitor blood pressure and heart rate, these dogs were equipped with a telemetry system (TL10MZ-D70-PC, Data Science International, St Paul, MN) or a transducer catheter inserted into an artery. Two to four months before the experiments, the dogs underwent an operation to implant the catheter and other equipment and, for 1 week afterwards, they were given subcutaneous micronomycin sulphate 60 mg daily. On the day before the start of the experiment, the dogs were fitted with a protection jacket, acclimatized to the environment (semi-bound condition), and then the experiment was started. The dogs were divided into the following 8 groups: 3-h infusions at doses of 0·22 and 0·65 mg kg$^{-1}$ of UCN-01 and of vehicle, and 24-h infusions at doses of 0·81, 1·62, 3·24 and 6·48 mg kg$^{-1}$ of UCN-01 and of vehicle. All dogs were accustomed to an environment involving a 12-h light–dark cycle at a controlled temperature ($23 \pm 3^{\circ}$C) and allowed free access to standard laboratory chow and water before the experiments started. All experiments were approved by the Welfare Committee for Experimental Animals in our institute.

*Pharmacokinetics*

For pharmacokinetic studies of UCN-01 following infusion, the dosing solutions were prepared by dissolving UCN-01 at appropriate concentrations in citric acid hydrate (1·2 mg mL$^{-1}$) and 5·6 mg mL$^{-1}$ disodium hydrogenphosphate dodecahydrate and 2·0 mg mL$^{-1}$ lactose. UCN-01 was administered to dogs via the catheter inserted into the foreleg vein. Blood samples were collected with a heparinized disposable syringe, via a catheter inserted into the contralateral foreleg vein for dosing, at 0·5, 1, 2, 3, 3·25, 3·5, 4, 6, 10, 24, 48 and 72 h after the start of the 3-h infusion, and at 3, 6, 12, 18, 24, 24·5, 25, 26, 28, 32, 48 and 72 h after the start of the 24-h infusion. Experiments were conducted in experimental groups of 1–4 animals each. Plasma was obtained by centrifugation (1500 *g*) of blood, and stored at −80°C until assay.

*Cardiohaemodynamics*

Blood pressure (systolic and diastolic) and heart rate were monitored in the same conscious animals at the same time as the determination of plasma concentrations. The animals were placed in metabolic cages. In the dogs equipped with the monitoring system to record blood pressure and heart rate, signals sent out by the implanted transmitter were recorded on a thermal array recorder (RTA-1200M and WS-681G, Nihon Koden, Tokyo, Japan) and analysed using haemodynamic analysis system (SBP-8, Softron, Tokyo, Japan). The monitoring of blood pressure and heart rate was conducted up to 72 h after the start of UCN-01 administration.

*HPLC*

According to the previously described method for determining UCN-01 in human plasma (Kurata et al 1998), UCN-01 concentrations were determined by HPLC using a fluorescence detector. There were no interfering peaks in plasma obtained from dogs given no drug, and the standard curves were linear. The range for quantitation was 0·2–100 ng mL$^{-1}$. The accuracy and precision of the intra- and inter-day assays were within ± 15%.

*Pharmacokinetic analysis*

Pharmacokinetic parameters were estimated in a model-independent manner (Gibaldi & Perrier 1982). The plasma concentrations were converted into logarithmic form and plotted against time, and then the slope (elimination rate constant, k) of the terminal phase was calculated using the linear least-squares method. The elimination half-life ($t_{\frac{1}{2}\beta}$) was calculated from 0·693/k, and the area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal method. The AUC infinity ($AUC_{0-\infty}$) was calculated by extrapolation using k. The total plasma clearance ($CL_{total}$) was calculated by Dose/$AUC_{0-\infty}$. The mean residence time (MRT) and steady-state volume of distribution (Vdss) following infusion (Watari & Benet 1989) were calculated by the equations 1 and 2.

$$MRT = \left( \int_0^\infty t \times Cpdt \Big/ \int_0^\infty Cpdt \right) - (\text{Infusion time}/2) \quad (1)$$

$$Vdss = MRT \times CL_{total} \quad (2)$$

where t indicates the time after the start of infusion and Cp the concentration at time t.

*Pharmacokinetic/cardiohaemodynamic analysis*

The systolic blood pressure and the heart rate were used to analyse the relationship between the pharmacokinetics and cardiohaemodynamics. The reason for using systolic blood pressure as a parameter to monitor the blood pressure change was that the systolic blood pressure change was clearer than that of diastolic blood pressure. The area under the decreasing systolic blood pressure and the increasing heart rate versus time curves ($dAUC_{pressure}$ and $AUC_{heart\ rate}$) were calculated by the trapezoidal method. To examine the relationship between $dAUC_{pressure}$ or $AUC_{heart\ rate}$, and the pharmacokinetics of UCN-01, the plasma concentration of UCN-01 at the end of infusion ($C_{end}$) and $AUC_{0-\infty}$ were used as an index of the degree of exposure to UNC-01. MRT was also used to study the exposure time to UCN-01. For regression analysis, $C_{end}$ and $AUC_{0-\infty}$ were converted to their logarithms and plotted against the cardiohaemodynamic parameters. Moreover, the initial increasing rate of plasma concentrations up to 3 h after the start of infusion ($slope_{0-3\,h}$) was calculated by the linear least-squares method to examine the effect of the size of the dose on cardiohaemodynamics.

*Statistical analysis*

Statistical analysis was conducted using the SAS system, release 6.12, for Windows (SAS Institute, Cary, NC). A comparison of the pharmacokinetic parameters among doses or between two different infusions was carried out by one-way analysis of variance and Tukey's test. A correlation analysis between the pharmacokinetic parameters and $dAUC_{pressure}$ or $AUC_{heart\ rate}$ was conducted by the *t*-test with Fisher's z-transformation. $P < 0.05$ was considered significant.

## Results

*Pharmacokinetics*

The plasma concentration versus time curves during and after 3-h and 24-h infusions of UCN-01 to dogs are shown in Figures 2A and 2B, respectively. During the 3-h infusion at doses of 0·22 and 0·65 mg kg$^{-1}$, the plasma concentrations of UCN-01 increased gradually reaching a maximum at the end of infusion, and exhibited a dose-proportional increase (Figure 2A and Table 1). After that, UCN-01 was eliminated bi-exponentially with an elimination half-life of 5·14 ± 1·12 and 7·30 ± 1·79 h at the respective doses. The total clearance ($CL_{total}$) was 0·666 ± 0·149 and 0·564 ± 0·161 L h$^{-1}$ kg$^{-1}$, respectively. The steady-state volume of distribution (Vdss) and mean residence time (MRT) were almost constant, indicating that UCN-01 has a linear pharmacokinetic profile over the dose range examined in the 3-h infusion. For the 24-h infusion at doses of 0·81–6·48 mg kg$^{-1}$, the plasma concentration reached a plateau after 12 h of administration at the low doses, but continued to increase gradually at the higher doses during infusions, as

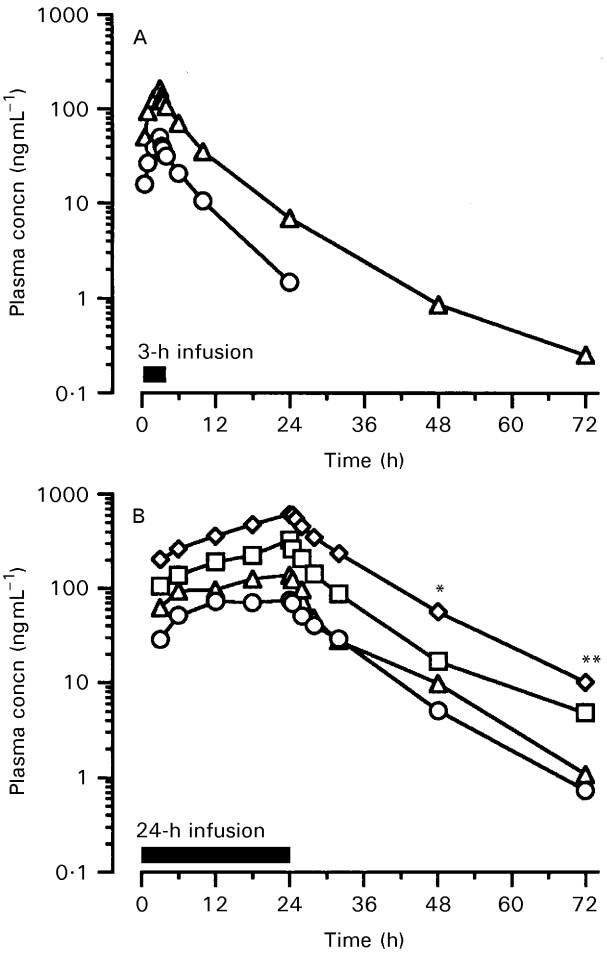


Figure 2. Mean plasma concentration–time profiles of UCN-01 during and after 3-h (A) or 24-h (B) constant intravenous infusion to conscious dogs. A. Dose of UCN-01: ○, 0·22 mg kg$^{-1}$ (n = 4); △, 0·65 mg kg$^{-1}$ (n = 4). B. Dose of UCN-01: ○, 0·81 mg kg$^{-1}$ (n = 1); △, 1·62 mg kg$^{-1}$ (n = 3); □, 3·24 mg kg$^{-1}$ (n = 3); ◇, 6·48 mg kg$^{-1}$ (n = 4). *n = 2, **n = 1. n = number of animals.

Table 1. Pharmacokinetic parameters of UCN-01 during and after 3-h or 24-h intravenous infusion to conscious dogs.

| Dose ($mg\,kg^{-1}$) | $C_{end}$ ($ng\,mL^{-1}$) | $t_{1/2\beta}$ (h) | $AUC_{0-\infty}$ ($ng\,h\,mL^{-1}$) | $CL_{total}$ ($L\,h^{-1}\,kg^{-1}$) | Vdss ($L\,kg^{-1}$) | MRT (h) |
|---|---|---|---|---|---|---|
| 3-h infusion | | | | | | |
| 0·22 (n = 4) | 50·0 ± 13·4 | 5·14 ± 1·12 | 343 ± 76 | 0·666 ± 0·149 | 3·69 ± 0·90 | 5·57 ± 1·01 |
| 0·65 (n = 4) | 165 ± 26 | 7·30 ± 1·79 | 1230 ± 360 | 0·564 ± 0·161 | 3·62 ± 0·51 | 6·71 ± 1·58 |
| 24-h infusion | | | | | | |
| 0·81 (n = 1) | 75·1 | 7·46 | 2120 | 0·383 | 3·24 | 8·47 |
| 1·62 (n = 3) | 139 ± 27 | 7·94 ± 0·87 | 3340 ± 810 | 0·503 ± 0·110 | 3·93 ± 1·00 | 7·78 ± 0·54 |
| 3·24 (n = 3) | 329 ± 6 | 8·32 ± 1·80 | 6890 ± 1580 | 0·489 ± 0·123 | 4·48 ± 0·61 | 9·42 ± 1·89* |
| 6·48 (n = 4) | 614 ± 64 | 7·59 ± 1·02 | 14600 ± 2200 | 0·452 ± 0·070 | 5·02 ± 1·50 | 10·9 ± 1·6†‡ |

Each value represents the mean ± s.d. or the value (n = 1). *$P = 0.0332$ vs $0.22\,mg\,kg^{-1}$ in 3-h infusion. †$P = 0.0015$ vs $0.22\,mg\,kg^{-1}$ in 3-h infusion. ‡$P = 0.0109$ vs $0.65\,mg\,kg^{-1}$ in 3-h infusion.

for the 3-h infusion (Figure 2B). However, the $C_{end}$ showed an almost dose-proportional increase (Table 1), followed by bi-exponential elimination with an elimination half-life of 7·46–8·32 h. The $CL_{total}$ ranged from 0·383 to $0.503 \pm 0.110\,L\,h^{-1}\,kg^{-1}$. There was no significant difference in the Vdss and MRT among doses. The pharmacokinetic parameters for the 24-h infusion were almost constant over the dose range examined, indicating that the plasma UCN-01 profile was also linear following 24-h infusion. The MRT in the 24-h infusion tended to be longer than that in the 3-h infusion, and significantly different values were obtained at the higher doses (Table 1). Except for MRT, the pharmacokinetic parameters obtained for the 3-h and 24-h infusions were very similar.

*Cardiohaemodynamics*

Blood pressure and heart rate during and after infusion of UCN-01 were monitored. In the 3-h infusion at doses of 0·22 and $0.65\,mg\,kg^{-1}$, the systolic blood pressures fell slightly after the end of administration (Figure 3A, with insert indicating the short time scale), and the diastolic blood pressures also fell slightly (data not shown). However, the blood pressure changes were not clearly proportional to dose. These reductions in blood pressure appeared to vanish 6 h after the end of the UCN-01 infusion.

In the 24-h infusion, a dose-proportional reduction in systolic blood pressure was observed (Figure 3B), and the diastolic blood pressures also fell (data not shown). At a dose of $3.24\,mg\,kg^{-1}$ or higher, a marked decrease in systolic blood pressure was observed, and the systolic blood pressure at $3.24\,mg\,kg^{-1}$ tended to recover 12 h after the end of administration. However, UCN-01 at a dose of $6.48\,mg\,kg^{-1}$ had the most potent effect on cardiohaemodynamics of all the doses tested in the 24-h infusion, with a reduction in blood pressure persisting after the end of infusion and no sign of recovery, even 24 h after the end of administration. In both infusions, the control groups receiving no drug treatment exhibited a slight decline in blood pressure after the start of experiments. This was presumed to be due to the experimental conditions, such as noise and operation, because there were significant differences in the reduction in blood

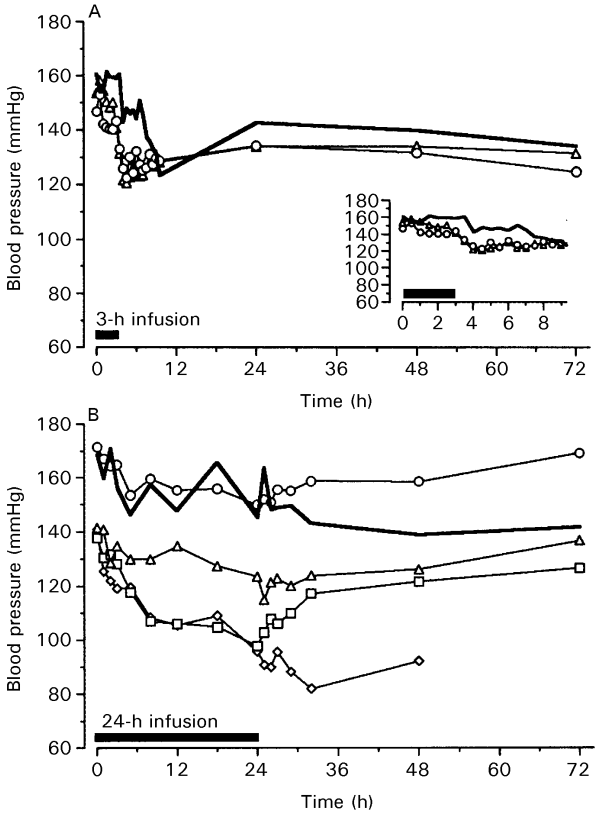


Figure 3. Effects of UCN-01 on systolic blood pressure during and after 3-h (A) and 24-h (B) constant intravenous infusion to conscious dogs. A. Dose of UCN-01: ○, $0.22\,mg\,kg^{-1}$ (n = 4); △, $0.65\,mg\,kg^{-1}$ (n = 4). B. ○, $0.81\,mg\,kg^{-1}$ (n = 1); △, $1.62\,mg\,kg^{-1}$ (n = 3); □, $3.24\,mg\,kg^{-1}$ (n = 3); ◇, $6.48\,mg\,kg^{-1}$ (n = 4). —, Vehicle (A, n = 4; B, n = 2). The inset in A indicates the early time-scale at 0 to 9 h after the start of the 3-h infusion. n = number of animals.

pressure between the groups, with and without the drug treatment.

The effects of UCN-01 on heart rate after 3-h and 24-h infusions are shown in Figures 4A and 4B, respectively. In the 3-h infusion, the maximum heart rate was 136 ± 8% of the pre-treatment value at 0·5 h after the start of infusion at 0·22 mg kg$^{-1}$, and 184 ± 12% at 3·5 h at 0·65 mg kg$^{-1}$. The increase in the heart rate was dose proportional; afterwards, the heart rate decreased, and showed almost the same value as the pre-treatment level 48 h after the start of infusion. On the other hand, there was a difference in the time-course of the heart rate increase in the 24-h infusion between the low and high doses. At the low doses (0·81 and 1·62 mg kg$^{-1}$), the heart rate gradually increased after the start of infusion, reaching a maximum value of 117 ± 5% at the end of infusion and 148 ± 11% 2 h after the end of infusion, respectively. The heart rate increased sharply after the start of infusion at higher doses (3·24 and 6·48 mg kg$^{-1}$), with the maximum value (151 ± 15% and 161 ± 9·3%) 5 h after the start of infusion.

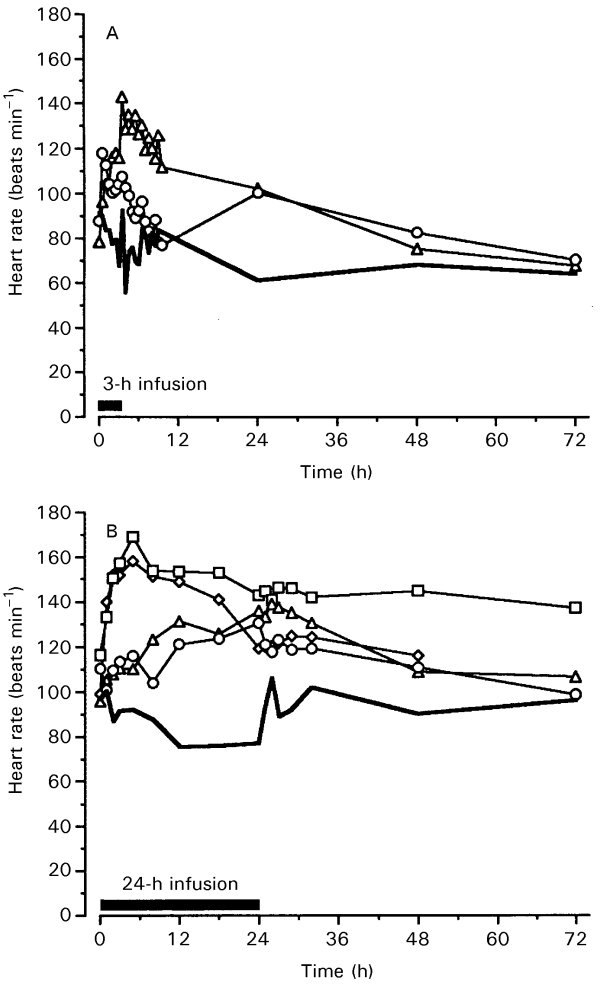


Figure 4. Effects of UCN-01 on heart rate during and after 3-h (A) or 24-h (B) constant intravenous infusion to conscious dogs. A. Dose of UCN-01: ○, 0·22 mg kg$^{-1}$ (n = 4); △, 0·65 mg kg$^{-1}$ (n = 4). B. Dose of UCN-01: ○, 0.81 mg kg$^{-1}$ (n = 1); △, 1·62 mg kg$^{-1}$ (n = 3); □, 3·24 mg kg$^{-1}$ (n = 3); ◇, 6·48 mg kg$^{-1}$ (n = 4). —, Vehicle (A, n = 4; B, n = 2). n = number of animals.

*Pharmacokinetic/cardiohaemodynamic analysis*

The relationship between pharmacokinetics and cardiohaemodynamics is shown in Table 2. The area under the systolic blood pressure and heart rate versus time curves ($dAUC_{pressure}$ and $AUC_{heart\ rate}$) were used as index parameters of the cardiohaemodynamics. The increase in the plasma concentrations of UCN-01 after the start of infusion ($slope_{0-3h}$) of 0·22 mg kg$^{-1}$ in the 3-h infusion (15·7 ± 4·2 ng mL$^{-1}$ h$^{-1}$) was almost the same as that of 1·62 mg kg$^{-1}$ in the 24-h infusion (20·9 ± 3·7 ng mL$^{-1}$ h$^{-1}$). Similarly, the $slope_{0-3h}$ at 0·65 mg kg$^{-1}$ in the 3-h infusion (52·1 ± 8·4 ng mL$^{-1}$ h$^{-1}$) was within the range of those at 3·24 and 6·48 mg kg$^{-1}$ in the 24-h infusion (35·5 ± 4·2 and 67·9 ± 24·9 ng mL$^{-1}$ h$^{-1}$, respectively). In the 3-h infusion, the $dAUC_{pressure}$ was not markedly dependent on $slope_{0-3h}$, $C_{end}$ or $AUC_{0-\infty}$ (Table 2). On the other hand, the $dAUC_{pressure}$ in the 24-h infusion correlated significantly with $C_{end}$ and $AUC_{0-\infty}$ with $P = 0.0185$ and 0·0218, respectively, and there was a trend to a correlation with $slope_{0-3h}$. There was no clear correlation between $dAUC_{pressure}$ and MRT in each infusion (Table 2). In the case of the heart rate increase, by contrast, there was a degree of correlation between $slope_{0-3h}$, $C_{end}$ or $AUC_{0-\infty}$ and $AUC_{heart\ rate}$ in the 3-h infusion rather than in the 24-h infusion, but this was not statistically significant. As far as the MRT was concerned, there was no correlation between MRT and $AUC_{heart\ rate}$ in both the 3-h and 24-h infusions, like the $dAUC_{pressure}$.

## Discussion

In this study, we investigated the pharmacokinetics and cardiohaemodynamics of UCN-01 in dogs. In clinical studies, UCN-01 is administered as a 3-h infusion in Japan (Tamura et al 1999) and a 72-h infusion in the USA (Senderowicz et al 1999). To examine the effects of different dosing schedules on the pharmacokinetics and cardiohaemodynamics, dogs were given UCN-01 over different infusion periods, namely, 3-h and 24-h infusion. The pharmacokinetic profile was almost linear over the dose range examined in each infusion schedule.

Table 2. Correlation analysis between the pharmacokinetic parameters of UCN-01 and cardiohaemodynaemic parameters following intravenous infusions of UCN-01 to conscious dogs.

| | 3-h infusion (n = 8) Regression line | r | *P* | 24-h infusion (n = 11) Regression line | r | *P* |
|---|---|---|---|---|---|---|
| $dAUC_{pressure}$ | | | | | | |
| $Slope_{0-3\,h}$ | $y = -8{\cdot}67x - 1038$ | 0·2612 | 0·5321 | $y = -18{\cdot}77x - 283{\cdot}5$ | 0·5983 | 0·0518 |
| $C_{end}$ | $y = -523{\cdot}8\log(x) - 310{\cdot}9$ | 0·2289 | 0·5855 | $y = -1739\log(x) + 3214$ | 0·6913 | 0·0185 |
| $AUC_{0-\infty}$ | $y = -327{\cdot}2\log(x) - 416{\cdot}0$ | 0·1508 | 0·7215 | $y = -1758\log(x) + 5661$ | 0·6781 | 0·0218 |
| MRT | $y = 198{\cdot}3x - 2551$ | 0·4033 | 0·3218 | $y = -216{\cdot}3x + 989{\cdot}1$ | 0·4750 | 0·1398 |
| $AUC_{heart\ rate}$ | | | | | | |
| $Slope_{0-3\,h}$ | $y = 20{\cdot}51x - 126{\cdot}6$ | 0·6499 | 0·0811 | $y = 6{\cdot}909x + 1428$ | 0·1592 | 0·6401 |
| $C_{end}$ | $y = 1335\log(x) - 2034$ | 0·6133 | 0·1059 | $y = 992{\cdot}5\log(x) - 724{\cdot}9$ | 0·2853 | 0·3951 |
| $AUC_{0-\infty}$ | $y = 1276\log(x) - 3005$ | 0·6183 | 0·1023 | $y = 780{\cdot}8\log(x) - 1271$ | 0·2178 | 0·5200 |
| MRT | $y = 184{\cdot}3x - 563{\cdot}1$ | 0·3941 | 0·3341 | $y = 49{\cdot}02x + 1248$ | 0·0779 | 0·8200 |

y represents the cardiohaemodynamic parameter, $dAUC_{pressure}$ or $AUC_{heart\ rate}$. x represents the pharmacokinetic parameter, $slope_{0-3\,h}$, $C_{end}$, $AUC_{0-\infty}$ or MRT.

Furthermore, the pharmacokinetic parameters obtained in this study were almost equal to those after bolus intravenous administration of UCN-01 to dogs (Kurata et al 1999). However, the MRT in the 24-h infusion at 3·24 and 6·48 mg kg$^{-1}$ tended to be longer than that in the 3-h infusion. This prolonged MRT suggests that a longer dosing schedule for UCN-01 influences the residence time due to the effect of UCN-01 on circulatory function in dogs. In fact, the reaction of systolic blood pressures after the infusion of UCN-01 varied with the duration of the infusion. It took a longer time for the systolic blood pressure to recover following the 24-h infusion than following the 3-h infusion, even though the plasma concentrations were almost the same. For example, $C_{end}$ was $165 \pm 26$ and $139 \pm 27$ ng mL$^{-1}$ at 0·65 mg kg$^{-1}$ in the 3-h infusion and 1·62 mg kg$^{-1}$ in the 24-h infusion. Moreover, the plasma concentration at 3 h after the start of the 24-h infusion at 3·24 mg kg$^{-1}$ was $106 \pm 13$ ng mL$^{-1}$. Considering that the blood pressure reduction occurred after the end of dosing in the 3-h infusion, while it occurred during dosing in the 24-h infusion, it might be expected that the effect on blood pressure would be observed when the plasma concentration of UCN-01 was maintained at over 100 ng mL$^{-1}$. On the other hand, the total dose and $AUC_{0-\infty}$ in the 24-h infusion were much higher than in the 3-h infusion. A dose of over 0·5 mg kg$^{-1}$, given by bolus intravenous administration, is lethal to dogs. Therefore, the dose of 0·65 mg kg$^{-1}$ would be the highest dose tolerated by the animals in the 3-h infusion. In the 24-h infusion, it was considered that a dose of 6·48 mg kg$^{-1}$ would also be the highest dose that dogs would be able to tolerate because the animals seemed to be getting weak and, in fact, 48 h after the start of infusion 2–4 animals had died. These results suggest that a longer dosing schedule would enable a higher dose to be given with greater exposure to UCN-01 due to the lower plasma concentrations.

A dose-dependent increase in heart rate was found in both the 3-h and 24-h infusions. The increase in heart rate was considered to be a reflex reaction to the blood pressure reduction (Karasawa et al 1988). For this homoeostasis in physiological function, it was observed that there was no marked reduction in blood pressure in the 3-h infusion, and at lower doses in the 24-h infusion. However, in the longer infusion, the incremental heart-rate patterns differed between the lower and higher doses. There might be a difference in vagal reactions as a physiological homoeostatic response if the dose of UCN-01 exceed the threshold value.

The activity of PKC has been reported to play a role in signal transduction involved in myocardial function or in vascular cells (Inoguchi et al 1994). PKC inhibitors, H-7 and staurosporine, inhibited the muscle contraction induced by endothelin-1 in the left atrium (Hattori et al 1993). The contraction induced by endothelin-1 (Shimamoto et al 1992) and 5-HT (James & Hodgson 1997) in vascular smooth muscle was also inhibited by a PKC inhibitor, calphostin C. Since UCN-01 is a potent PKC inhibitor, it is possible that this drug also inhibits myocardial or peripheral vascular contraction. Furthermore, it has been reported that pre-treatment with PKC inhibitors is needed to inhibit endothelin-1-induced atrial contraction by these agents (Hattori et al 1993). This may be related to our finding that it takes several hours after administration of UCN-01 before a reduction in blood pressure is seen (Figure 3). As far as myocardial energy metabolism is concerned, many reports have described that PKC activity regulates cellular glucose transport (Henriksen et al 1989; Tanti et al 1989; Yu et al 1992). Phorbol esters which activate

PKC regulate the expression of GLUT4 in the plasma membrane (Vogt et al 1991; Galant et al 1995), and glucose transport in myocardial cells is inhibited by a PKC inhibitor, staurosporine (Russ & Eckel 1995). Moreover, it has been reported that PKC plays a role in the glycolysis system of the heart (Schaffer et al 1997), and there is increasing evidence suggesting that glucose transport and metabolism are regulated by the action of PKC on the myocardial energy metabolism. Because UCN-01 potently inhibits the activity of PKC with an IC50 of 4·1 nM (Takahashi et al 1987), it is possible that the effects of UCN-01 on circulatory function in this study were mainly induced by inhibition of PKC activity.

To estimate the effects of different dosing schedules on the relationship between the pharmacokinetics and cardiohaemodynamics of UCN-01, we conducted a separate regression analysis using data from the 3-h and 24-h infusion. The MRT of a drug having a linear pharmacokinetic profile should remain constant, independent of dose or dosing schedule. The reason for conducting a correlation analysis for MRT was simply to estimate the effects of individual variations in the MRT of UCN-01 administered by different schedules on the cardiohaemodynamics. Althoug the results show that the MRT did not correlated with the $dAUC_{pressure}$ or the $AUC_{heart\ rate}$ in either the 3-h or the 24-h infusion, it tended to be long at the higher doses in the 24-h infusion. It might be thought that the lack of correlation between the MRT and the cardiohaemodynamic parameters was simply due to individual variations in the MRT of UCN-01 rather than the effect of changes in the MRT of UCN-01 depending on the cardiohaemodynamics in dogs. On the other hand, there was a degree of correlation between the cardiohaemodynamics and $C_{end}$ or $AUC_{0-\infty}$. In addition, the dose intensity (i.e. the $slope_{0-3\,h}$) also tended to correlated with the cardiohaemodynamics. However, a correlation analysis between the pharmacokinetics and cardiohaemodynamics showed that the pharmacokinetic parameters of $C_{end}$, $AUC_{0-\infty}$ and $slope_{0-3\,h}$ tended to correlate with the $AUC_{heart\ rate}$ in the 3-h infusion and correlated significantly with the $dAUC_{pressure}$ in the 24-h infusion. These results mean that, although UCN-01 caused a reduction in blood pressure accompanied by an increase in heart rate due to a homoeostatic reaction, UCN-01 may have different cardiohaemodynamic effects if the dosing schedule was different.

In conclusion, the pharmacokinetic profile of UCN-01 administered over the shorter infusion period was almost identical to that over the longer one. However, UCN-01 had a longer residence time with the longer dosing schedule of than with the shorter one. This may be due to the effect of UCN-01 on circulatory function in dogs.

Finally, the results we obtained in this study will be very helpful in interpreting the toxicity of UCN-01 in clinical studies, and will also assist in the development of UCN-01 as an anticancer drug.

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