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Kenichi Yasoshima · Takashi Kuwabara · Eiichi Fuse
Tomoko Kuramitu · Noriaki Kurata
Hiroyoshi Nishiie · Takayoshi Oishi
Hiroyuki Kobayashi · Satoshi Kobayashi

Pharmacokinetics, distribution, metabolism and excretion of [^{3}H]UCN-01 in rats and dogs after intravenous administration

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Abstract *Purpose*: To evaluate the metabolic fate of UCN-01, a signal transduction inhibitor, blood and plasma concentrations, distribution, metabolism and excretion were investigated in rats and dogs after intravenous administration of [^{3}H]UCN-01. *Methods*: The radioactivity in plasma, blood and tissues was measured after intravenous administration of UCN-01. In addition, the radioactivity excreted in bile, urine and feces was also determined. *Results*: The radioactivity in rat and dog plasma disappeared triphasically with terminal half-lives of 21.3 and 27.2 h, respectively. The ratios of the blood-to-plasma concentrations ranged from 0.82 to 1.13 in rats and 0.81 to 1.73 in dogs. From 0.5 to 4 h after giving [^{3}H]UCN-01 to rats, the radioactivity in all tissues except the brain and testes was higher than in plasma. The highest concentration was observed in the lungs followed by the liver and kidneys. The radioactivity was mainly excreted in feces, reaching 96.0% of the radioactivity dose in rats and 78.4% in dogs up to 168 h after injection. Since the biliary excreted radioactivity was 67.2% over 48 h in bile duct-cannulated rats, most of the radioactivity excreted in feces was from biliary radioactivity. There were several metabolites in bile samples, but little UCN-01. *Conclusions*: UCN-01 is mainly eliminated by the liver, and there are high concentrations of radioactivity derived from [^{3}H]UCN-01 in all tissues except the brain and testes.

Key words 7-Hydroxystaurosporine (UCN-01) · Protein kinase inhibitor · Metabolism · Excretion · Pharmacokinetics

K. Yasoshima · T. Kuwabara (✉) · E. Fuse · T. Kuramitu
N. Kurata · H. Nishiie · T. Oishi · H. Kobayashi · S. Kobayashi
Drug Development Research Laboratories,
Pharmaceutical Research Institute,
Kyowa Hakko Kogyo Co., Ltd., 1188,
Shimotogari, Nagaizumi-Cho,
Sunto-Gun, Shizuoka 411-8731, Japan
e-mail: takashi.kuwabara@kyowa.co.jp
Tel.: +81-559-892021; Fax: +81-559-892073

Introduction

UCN-01 (7-hydroxystaurosporine; Fig. 1) was originally reported as a selective protein kinase C inhibitor. It exhibits potent antitumor activity in both in vitro and in vivo models [1, 2, 5]. Recent studies have revealed that UCN-01 induces G1 phase accumulation in several human tumor cells [4, 10, 15] and this effect is associated with dephosphorylation of the retinoblastoma protein and its regulatory factors including cyclin-dependent kinase 2 (CDK2) and CDK inhibitors $p21^{Cip1/WAF1}$ and $p27^{Kip1}$ [4]. UCN-01 also induces apoptosis in leukemia and colon carcinoma cell lines [9, 17, 20]. Furthermore, UCN-01 synergistically enhances the antitumor activity of cytotoxic chemotherapeutic agents such as mitomycin C, cisplatin and 5-fluorouracil with G2 abrogation [3, 12, 16]. Currently, UCN-01 is being evaluated as a new type of anticancer drug in patients with refractory neoplasms in the United States and Japan [6, 13].

We have previously reported the plasma concentration-time profiles of UCN-01 measured by HPLC in experimental animals and patients [6, 11, 13]. UCN-01 in plasma after intravenous administration disappears biphasically in mice and rats, and triphasically in dogs with elimination half-lives of about 4 h in mice and rats and 12 h in dogs. The systemic clearance (CL_{tot}) values in mice, rats and dogs are relatively high (1.93–2.64, 2.82–3.86 and 0.616 l/h per kg, respectively). The distribution volumes at steady-state (Vd_{ss}) in mice, rats and dogs are also high (7.89–8.42, 13.0–16.9 and 6.09 l/kg, respectively). No saturation in pharmacokinetics was observed in these animals [11]. On the other hand, the elimination half-lives of UCN-01 in patients is extremely long (253–1660 h) and the Vd_{ss} and CL_{tot} are very low (0.0796–0.158 l/kg and 0.0407–0.252 ml/h per kg, respectively) [6].

Protein binding studies have revealed that this marked species difference in pharmacokinetics is due, at least in part, to specifically high binding of UCN-01 to human α_1-acid glycoprotein [6, 7]. The binding affinity of

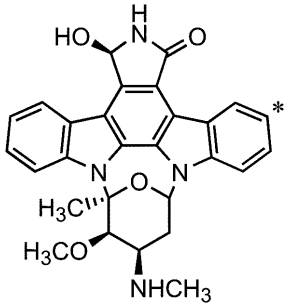

Fig. 1 Chemical structure of [^{3}H]UCN-01. The *asterisk* represents the tritium labeling position

UCN-01 to rat and dog α_1-acid glycoprotein is much weaker than to human α_1-acid glycoprotein [6, 7]. However, it would still be very useful to investigate the disposition of [^{3}H]UCN-01 in experimental animals in order to evaluate the metabolic fate of UCN-01 in the body. Because of weak protein binding, the disposition of UCN-01 in rats and dogs may reflect the disposition of "free UCN-01" in humans. Therefore, we conducted studies on the blood and plasma concentrations, distribution, metabolism and excretion of [^{3}H]UCN-01 in rats and dogs.

Materials and methods

Chemicals

UCN-01 (7-hydroxystaurosporine) was produced by fermentation in our institute as described previously [18]. [^{3}H]UCN-01 (Fig. 1) was synthesized by Amersham International (Little Chalfont, UK) with a specific activity of 548 GBq/mmol (TRQ 6529) or 307 GBq/mmol (TRQ7253). [^{3}H]UCN-01 was purified by HPLC to a radiochemical purity of more than 96% just before use. Acetonitrile (HPLC grade) was purchased from Kanto Chemical (Tokyo, Japan). Other reagents used were of analytical grade obtained commercially.

Preparation of dosing solutions

Purified [^{3}H]UCN-01 was dried under nitrogen and dissolved together with unlabeled UCN-01 in 1.2 mg/ml citric acid hydrate with disodium hydrogenphosphate dodecahydrate (5.6 mg/ml) and sodium chloride (6.0 mg/ml). Solutions of [^{3}H]UCN-01 at concentrations of 1.75 mg/ml (1.05–5.95 MBq/ml) were prepared for rats, and 1.0 mg/ml (0.98 MBq/ml) for dogs. The solutions were administered at doses of 2 ml/kg (rats) and 0.5 ml/kg (dogs).

Animals

Male Sprague Dawley rats were obtained at 6 weeks of age from Nihon SLC (Shizuoka, Japan), and used at 7 weeks of age when they weighed 200–270 g. Male beagle dogs were obtained from HRP (Kalamazoo, Mich.), and used at 3 years of age when they weighed 10 to 11 kg. All animals were accustomed to a 12-h light/dark cycle under controlled temperature and humidity conditions, with free access to standard laboratory chow and water before the start of the experiments. Food and water were given ad libitum starting 6 h after dosing. In the bile excretion study, saline including 5% sucrose was given instead of water. All experiments were approved by the Welfare Committee for Experimental Animals of our institute.

Blood and plasma concentration and excretion in rats

For the kinetic studies, [^{3}H]UCN-01 at 3.5 mg/kg was administered to four male rats via the femoral vein. Blood samples (0.2–0.3 ml) were serially collected from the tail vein at 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h after dosing into heparinized capillary tubes (Drummond Scientific, Broomall, Pa.). A portion of each blood sample was used for the measurement of radioactivity directly, and plasma samples were obtained by centrifugation of the remainder (11,000 *g*, 10 min, 4 °C). For the excretion studies, after intravenous administration of [^{3}H]UCN-01 to another five male rats, the rats were retained in metabolic cages. Urine and feces samples were collected during the following periods up to 168 h after dosing: 0–4, 4–8, 8–12, 12–24 and every 24 h for urine, and 0–12, 12–24 and every 24 h for feces. The feces samples were homogenized with water. After sampling, the rats were killed with diethylether. The carcasses were placed in 750 ml of 0.5 *N* sodium hydroxide solution at 50 °C for a few days and then homogenized. The interiors of the cages were washed with 50% methanol and the radioactivity in the washings was also determined.

Blood and plasma concentration and excretion in dogs

[^{3}H]UCN-01 was administered at a dose of 0.5 mg/kg to two male dogs via the foreleg vein. Blood samples (5 ml) were collected from the contralateral foreleg vein using a heparinized syringe at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h after dosing. A portion of each blood sample was used for the measurement of the radioactivity directly. Also, an aliquot of blood was used to measure the hematocrit. Plasma samples were obtained by centrifugation of the remainder (2000 *g*, 10 min, room temperature). The dogs were housed in metabolic cages and the spontaneously excreted urine and feces were collected up to 168 h during the following periods: 0–6, 6–12, 12–24 and every 24 h for urine, and every 24 h for feces. At 6 and 12 h, urine in the bladder was also collected using a catheter. The cages were washed with 50% methanol and the washings collected after the experiment. The feces samples were homogenized with water.

Biliary excretion in rats

Under light anesthesia with diethylether, the bile ducts of male rats were cannulated with a PE-10 polyethylene tube (Becton Dickinson, Parsippany, N.J.). After recovery from the anesthesia, [^{3}H]UCN-01 at a dose of 3.5 mg/kg was administered to four male rats via the femoral vein. The rats were kept in restraining cages and bile, urine and feces were collected during the following periods: 0–2, 2–4, 4–6, 6–8, 8–12, 12–24 and 24–48 h for bile, 0–12, 12–24 and 24–48 h for urine, and 0–24 and 24–48 h for feces. After sampling, the rats were killed with diethylether and the carcasses were treated as described above. The feces samples were homogenized with water.

Metabolites in rat plasma, bile and urine

In order to investigate the metabolism of [^{3}H]UCN-01, rat plasma, bile and urine samples were subjected to HPLC analysis. Plasma samples were deproteinized with an equal volume of ice-cold acetonitrile and, after centrifugation, the supernatants were subjected to HPLC. The bile samples were diluted with mobile phase (A), described below, before HPLC. The HPLC system consisted of a solvent module 126 pump and a 171 radioisotope detector (Beckman, Fullerton, Calif.) equipped with an AM-312 ODS column (150 × 6.0 mm i.d.; YMC, Kyoto, Japan). The mobile phases were 0.05 *M* phosphate buffer containing 1% triethylamine (pH

7.3) and acetonitrile at a ratio of 9:1 (A) or 4:6 (B). The fraction of mobile phase (B) was increased linearly from 0 to 100% over 20 min and held there for the following 10 min. The total flow rate was set at 1 ml/min. The radioactivity in the eluate was measured with a radioisotope detector after mixing with Scintisol EX-H (Wako Pure Chemical, Osaka, Japan) at a flow rate of 4 ml/min.

Tissue distribution in rat

To determine the tissue radioactivity, rats were killed by bleeding from the femoral artery 0.5, 1, 4, 8 and 24 h after administration of [^{3}H]UCN-01 via the contralateral femoral vein at a dose of 3.5 mg/kg. Three rats were used for each time-point. Blood was collected into heparinized tubes and the plasma was obtained as described above. An aliquot of blood was also used to measure the hematocrit. The tissues indicated in the Table 4 were removed and the wet weight was measured. The brain, kidneys, spleen, pancreas, testes, stomach, small intestine and large intestine were homogenized with an appropriate volume of saline. The radioactivity in these tissues and tissue homogenates was determined.

Determination of radioactivity in the samples

Total and nonvolatile radioactivity were measured in the blood, plasma, urine, bile, feces homogenate, carcass homogenate and cage washings. The volatile radioactivity was also calculated by subtracting nonvolatile radioactivity from total radioactivity. The total radioactivity in the plasma, urine, bile and cage washings was determined after emulsifying with liquid scintillator (Scintisol EX-H or Ultima Gold; Packard, Meriden, Ct.). The blood, feces homogenate and carcass homogenates were dissolved in tissue dissolver (Solvable; Daiichi Pure Chemicals, Tokyo, Japan), treated overnight with hydrogen peroxide, and then mixed with liquid scintillator (Hionic Fluor, Packard). For the determination of nonvolatile radioactivity, the samples were dried in a freeze-dryer to remove the volatile radioactivity, and then treated as described above. The tissues and tissue homogenates were dried in a freeze-dryer, dissolved in Solvable at 50 °C for 3 h, and then mixed with Hionic Fluor. For all samples, radioactivity was measured using a liquid scintillation counter (LSC4530 or Tri-Carb 300 or 2200, Packard, LS6500; Beckman).

The counting efficiency was automatically corrected using an external standard. Samples from control animals treated by the same methods were used to obtain the background counts. The radioactivity detection limit was defined as twice the value of the corresponding background count. The radioactive concentration in blood, plasma and tissue samples was calculated as the equivalent concentration of [^{3}H]UCN-01 from the specific radioactivity of each dosing solution. The excreted radioactivity in bile, urine and feces was expressed as a percentage of the dosed radioactivity. The ratio of the radioactivity in erythrocytes to that in blood (RBC) was calculated from the following equation:

$$\mathrm{RBC}(\%) = (1 - (1 - \mathrm{Ht})/\mathrm{Rb}) \times 100$$

where Ht is the hematocrit and Rb is the ratio of the blood to the plasma concentration.

In this report, since the volatile radioactivity can be considered to originate from tritiated water, to avoid confusion, 'radioactivity' indicates 'nonvolatile radioactivity', unless otherwise specified.

Pharmacokinetic analysis

Individual pharmacokinetic parameters were analyzed using model-independent methods [8, 21]. The concentrations of radioactivity in plasma and blood were converted to logarithms and plotted against time. The slope of the terminal phase (elimination rate constant of the β phase) was determined by log-linear regression analysis, and those of the second and first phases (elimination rate constant of the α and π phase) were determined by the method of residuals. In this study, the time-points of the β phase were designated at and after 24 h and those of the α phase at and after 2 h. The elimination half-lives ($t_{1/2}\pi$, $t_{1/2}\alpha$ and $t_{1/2}\beta$) were calculated by dividing 0.693 by the elimination rate constant of the respective phase. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule, and extrapolated to infinity. The systemic clearance (CL_{tot}) was calculated as dose/$AUC_{0-\infty}$.

Results

Blood and plasma radioactivity concentration-time profiles

Figure 2 shows the radioactive concentration-time profiles in plasma and blood after intravenous administration of UCN-01. After intravenous administration of [^{3}H]UCN-01 at 3.5 mg/kg to rats, the blood concentration of radioactivity was 0.975 ± 0.213 μg equivalents/ml (mean ± SD of four rats) at 0.05 h, and then disappeared biphasically to below the detection limit after 48 h. No

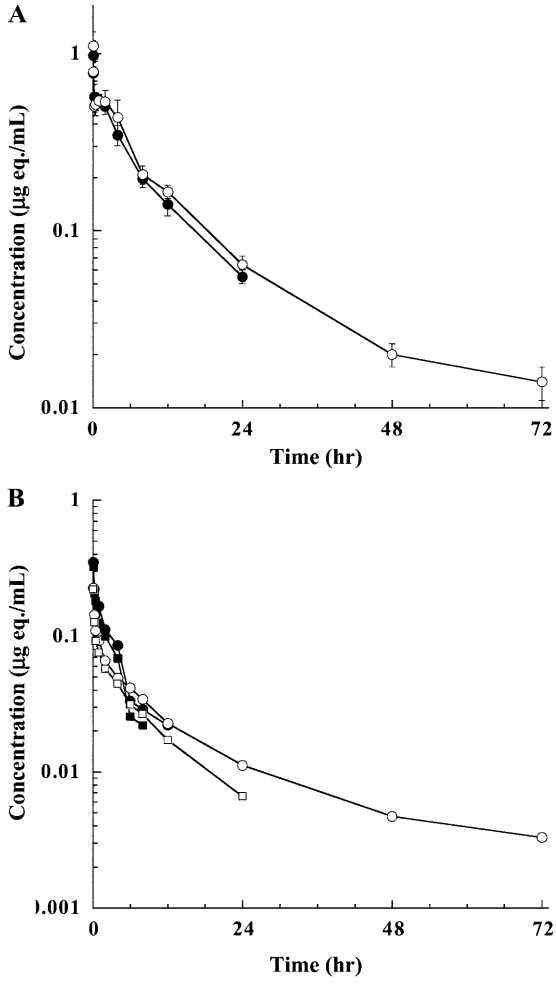

Fig. 2 **A** Blood (●) and plasma (○) radioactive concentration time-profiles after intravenous administration of [^{3}H]UCN-01 at a dose of 3.5 mg/kg to rats. Each point represents the mean ± SD from four rats. **B** Individual blood (●, ■) and plasma (○, □) radioactive concentration time-profiles after intravenous administration of [^{3}H]UCN-01 at a dose of 0.5 mg/kg to two dogs

Table 1 Pharmacokinetic parameters of [^{3}H]UCN-01 in blood and plasma after intravenous administration of [^{3}H]UCN-01 at doses of 3.5 mg/kg to rats and 0.5 mg/kg to dogs. For rats each value is the mean ± SD ($n = 4$); for dogs each blood value is a mean ($n = 2$), and each plasma value is an individual value

	$t_{1/2}\pi$ (h)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg equivalents · h/ml)	CL_{tot} (l/h/kg)
Rat					
Blood	0.37 ± 0.21	7.17 ± 0.17	–[a]	5.40 ± 0.45	0.652 ± 0.056
Plasma	0.02 ± 0.02	4.08 ± 0.68	21.3 ± 2.3	7.53 ± 0.68	0.468 ± 0.042
Dog					
Blood	0.33	3.29	–[a]	0.804	0.635
Plasma					
No. 1	0.33	4.18	27.2	1.21	0.412
No. 2	0.33	7.20	–[a]	0.71	0.703

[a] Could not be calculated

terminal elimination phase (β phase) was observed in contrast to plasma (Fig. 2A). The plasma radioactivity at 0.05 h (1.10 ± 0.22 μg equivalents/ml) was very similar to the level in blood (Fig. 2A), and disappeared triphasically to 0.014 ± 0.003 μg equivalents/ml at 72 h with a terminal half-life of 21.3 ± 2.3 h (Table 1). The AUC values for blood and plasma were 5.40 and 7.53 μg equivalents · h/ml, respectively. The blood to plasma concentration ratios (Rb) were almost constant, ranging from 0.82 to 1.13, and were independent of the time after injection. Calculated RBC values were 31.6% to 52.9%.

After intravenous administration of [^{3}H]UCN-01 at 0.5 mg/kg to dogs, the blood radioactivity was 0.337 μg equivalents/ml (mean of two dogs) at 0.083 h and disappeared biphasically. At 12 h after dosing, the radioactivity in one dog was 0.022 μg equivalents/ml and that in the other was below the detection limit (Fig. 2B). No terminal elimination phase (β phase) was observed in either dog. The plasma radioactivity in one dog was 0.225 μg equivalents/ml at 0.083 h and disappeared triphasically to 0.0033 μg equivalents/ml at 72 h with a terminal half-life of 27.2 h, which was comparable with that in rats (Table 1). In the other dog, the plasma radioactivity was 0.220 μg equivalents/ml at 0.083 h, and then disappeared biphasically to below the detection limit after 24 h. The distribution of radioactivity into erythrocytes (RBC) in dogs was higher than in rats. That is, Rb values up to 4 h after dosing were 1.51–1.73, and then decreased to 0.81–0.97 at later time-points. RBC values were about 65% up to 4 h. In both rats and dogs, significant levels of volatile radioactivity were found in blood and plasma samples at time-points in the elimination phase (data not shown).

Table 2 Cumulative excretion of nonvolatile radioactivity in urine and feces after an intravenous administration of [^{3}H]UCN-01 to rats (3.5 mg/kg) and to dogs (0.5 mg/kg). Values are percent of dose. For rats each value is the mean ± SD ($n = 5$), and for dogs each value is the mean ($n = 2$)

Time period (h)	Rat		Dog	
	Urine	Feces	Urine	Feces
0–4	0.5 ± 0.1	–[a]	–[a]	–[a]
0–8	1.1 ± 0.0	–[a]	0.3[b]	–[a]
0–12	1.5 ± 0.1	2.8 ± 3.3	0.5	–[a]
0–24	1.8 ± 0.2	57.3 ± 14.6	0.6	50.9
0–48	1.9 ± 0.2	91.6 ± 4.8	0.8	67.0
0–72	2.0 ± 0.2	94.9 ± 3.3	0.8	73.1
0–96	2.0 ± 0.3	95.5 ± 3.2	0.9	76.6
0–120	2.0 ± 0.3	95.8 ± 3.2	0.9	77.5
0–144	2.1 ± 0.3	95.9 ± 3.1	0.9	78.1
0–168	2.1 ± 0.3	96.0 ± 3.1	0.9	78.4

[a] Not collected
[b] 0–6 h

Urinary and fecal excretion of radioactivity

The urinary and fecal excretion of radioactivity in rats and dogs after intravenous administration of [^{3}H]UCN-01 are shown in Table 2. In rats, the radioactivity was mainly excreted in feces, that is 57.3 ± 14.6% (mean ± SD, $n = 5$) of the injected radioactivity was excreted over the initial 24 h, and 96.0 ± 3.1% over 168 h. The urinary excretion was very low, only 2.1% up to 168 h. The radioactivity remaining in the carcass was 1.4%. Total recovery of volatile and nonvolatile radioactivity was 102.2%, comprising 4.0% excreted in the urine, 94.8% excreted in the feces and 3.3% remaining in the carcass. The volatile radioactivity accounted for approximately half the total radioactivity in the urine and carcass, but there was little in the feces.

In dogs, the excreted radioactivity in the urine and feces was 0.6% and 50.9% (mean of two dogs) over the first 24 h after injection and 0.9% and 78.4% over 168 h, respectively. Fecal excretion was also the main route, as in rats. The excretion of total radioactivity (volatile and nonvolatile) in the urine and feces was 1.3% and 77.3%, respectively. The volatile radioactivity was about 0.4% in the urine and there was little in the feces.

Biliary excretion in rats

Table 3 shows the cumulative biliary excretion of radioactivity after intravenous administration of [^{3}H]UCN-01 to rats. The biliary excretion of radioactivity was 49.7 ± 5.1% (mean ± SD, $n = 4$) over the first 12 h and 67.2 ± 2.9% over 48 h. Urinary and fecal excretion of radioactivity was 4.0 ± 1.3% and 9.1 ± 5.4%, respectively. Total recovery of volatile and nonvolatile radioactivity was 104.3%, comprising 70.5% in the bile, 5.9% excreted in the urine, 9.1% excreted in the feces

Table 3 Cumulative excretion of nonvolatile radioactivity of [^{3}H]UCN-01 in bile, urine and feces after intravenous administration of [^{3}H]UCN-01 at a dose of 3.5 mg/kg to rats following cannulation of the bile duct. Values are percent of dose. Each value is the mean ± SD ($n = 4$)

Time period (h)	Bile	Urine	Feces
0–2	15.0 ± 3.8	–[a]	–[a]
0–4	28.8 ± 4.6	–[a]	–[a]
0–6	36.8 ± 5.1	–[a]	–[a]
0–8	42.5 ± 5.3	–[a]	–[a]
0–12	49.7 ± 5.1	2.0 ± 0.6	–[a]
0–24	61.5 ± 5.6	2.7 ± 0.7	3.5 ± 2.7
0–48	67.2 ± 2.9	4.0 ± 1.3	9.1 ± 5.4

[a] Not collected

and 18.9% remaining in the carcass. The volatile radioactivity in the bile was only 3.3%.

Metabolites in rat plasma, bile and urine

The rat plasma, bile and urine samples obtained from the above studies were analyzed by HPLC to elucidate the metabolism of UCN-01. Figure 3 shows typical chromatograms of a plasma sample at 15 min after dosing (A), a bile sample over the first 6 h (B) and a urine sample over the first 12 h (C). In the plasma, there were three minor unidentified peaks besides UCN-01. The ratio of UCN-01 radioactivity to total radioactivity in the sample was 58.2% at 15 min decreasing to 17.7% at 4 h (data not shown). In bile, there was no radioactivity at the UCN-01 position and several polar metabolites were observed. In urine, there were two peaks: a minor peak due to UCN-01 and a major peak ahead of it.

Tissue distribution in rats

The concentration of radioactivity in tissues after intravenous administration of [^{3}H]UCN-01 to rats is shown in Table 4. All the tissues except brain and testis showed a higher concentration of radioactivity than plasma at 0.5 to 4 h after dosing. Most tissues showed their highest concentration at 0.5 or 1 h, indicating rapid tissue distribution of UCN-01. At 0.5 h after administration, in comparison with the plasma concentration (0.468 μg equivalents/ml), the distribution to endocrinal and reticuloendothelial tissues was relatively high. The highest concentration of radioactivity was observed in the lungs (37.6 μg equivalents/g), followed by the liver (16.2 μg equivalents/g), kidneys (13.5 μg equivalents/g), thyroid gland (12.4 μg equivalents/g), pituitary gland (11.7 μg equivalents/g), adrenal gland (11.5 μg equivalents/g) and pancreas (10.6 μg equivalents/g). At 1 h after administration, the tissue concentrations of radioactivity were comparable with those at 0.5 h with a similar distribution pattern. At 4 h and thereafter, in parallel with the decrease in plasma radioactivity, the radioactivity in most tissues also decreased. At 24 h after

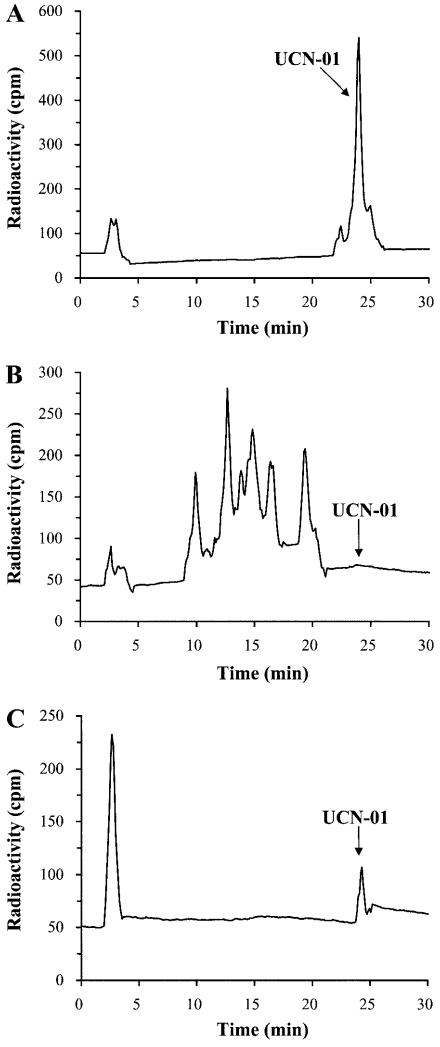

Fig. 3A–C Typical HPLC radiochromatograms of plasma at 15 min (**A**), bile over the first 6 h (**B**) and urine over the first 12 h (**C**). These samples were obtained from rats given 3.5 mg/kg [^{3}H]UCN-01

administration, the plasma concentration had fallen to 0.15 times that at 0.5 h. The ratios of tissue concentrations at 24 h to those at 0.5 h were 0.05 in the lungs, 0.08 in the liver, 0.07 in the kidneys and 0.04 in the thyroid grand. Also, the concentrations in bone marrow, in which UCN-01 exhibits dose-limiting toxicity in rats [13], decreased from 5.62 μg equivalents/g at 0.5 h to 0.590 μg equivalents/g at 24 h. However, the decrease in concentration in several tissues such as Harderian gland, testes, skin and intestinal tract were relatively slow. The slow decrease in the intestinal tract may partially have been due to biliary excretion of [^{3}H]UCN-01.

Discussion

We investigated the metabolic fate of UCN-01 after intravenous administration in rats and dogs using radiolabeled drug. [^{3}H]UCN-01 disappeared triexponentially

Table 4 Tissue distribution of radioactivity after intravenous administration of [^{3}H]UCN-01 at a dose of 3.5 mg/kg to rats. Values are concentrations (μg equivalents per ml or g tissue) of UCN-01 (means ± SD, $n = 3$)

Tissue	0.5 h	1 h	4 h	8 h	24 h
Plasma	0.468 ± 0.066	0.463 ± 0.026	0.355 ± 0.031	0.215 ± 0.034	0.0682 ± 0.0551
Blood	0.577 ± 0.039	0.542 ± 0.011	0.350 ± 0.007	0.195 ± 0.031	0.0600 ± 0.0465
Brain	0.245 ± 0.058	0.344 ± 0.068	0.153 ± 0.005	0.147 ± 0.012	0.0798 ± 0.0278
Pituitary gland	11.7 ± 0.2	11.0 ± 2.3	7.24 ± 2.60	5.48 ± 2.43	1.85 ± 1.07
Eye balls	0.862 ± 0.162	0.881 ± 0.098	0.510 ± 0.101	0.297 ± 0.063	0.119 ± 0.066
Harderian gland	3.51 ± 0.45	4.42 ± 0.27	5.85 ± 0.62	5.53 ± 0.50	3.76 ± 1.11
Submaxillary gland	8.63 ± 0.85	8.66 ± 0.60	6.64 ± 1.13	3.84 ± 0.69	0.661 ± 0.576
Submaxillaric lymph node	4.38 ± 0.38	4.70 ± 0.16	4.47 ± 0.54	3.55 ± 0.60	0.668 ± 0.479
Thyroid gland	12.4 ± 3.5	7.22 ± 0.78	4.29 ± 0.43	2.37 ± 0.70	0.516 ± 0.356
Thymus	3.21 ± 0.25	3.79 ± 0.31	3.80 ± 0.31	2.90 ± 0.56	0.680 ± 0.489
Heart	5.05 ± 0.22	3.94 ± 0.39	2.24 ± 0.14	1.09 ± 0.27	0.278 ± 0.230
Lungs	37.6 ± 2.0	35.2 ± 6.3	19.3 ± 4.1	10.2 ± 2.2	2.05 ± 1.76
Liver	16.2 ± 1.7	16.3 ± 1.1	10.7 ± 0.4	5.13 ± 0.78	1.24 ± 0.76
Kidneys	13.5 ± 1.1	11.2 ± 0.2	7.34 ± 0.89	3.57 ± 0.74	0.992 ± 0.581
Adrenal gland	11.5 ± 0.3	8.45 ± 1.45	5.55 ± 0.73	3.05 ± 0.63	0.815 ± 0.556
Spleen	7.93 ± 0.30	6.84 ± 2.32	6.06 ± 1.07	3.78 ± 0.86	1.00 ± 0.84
Pancreas	10.6 ± 1.2	8.56 ± 0.59	7.24 ± 1.46	4.95 ± 1.06	0.815 ± 0.653
Perirenal fat	1.04 ± 0.08	1.02 ± 0.09	0.776 ± 0.034	0.468 ± 0.068	0.137 ± 0.095
Brown fat	5.27 ± 0.31	4.34 ± 0.46	2.39 ± 0.20	1.65 ± 0.42	0.649 ± 0.554
Mesenteric lymph node	4.92 ± 0.23	4.89 ± 0.28	4.45 ± 0.85	3.16 ± 0.65	0.730 ± 0.477
Muscle	3.37 ± 0.31	2.69 ± 0.23	1.79 ± 0.19	0.935 ± 0.174	0.186 ± 0.145
Skin	1.60 ± 0.08	1.73 ± 0.17	2.16 ± 0.21	1.72 ± 0.25	0.431 ± 0.359
Bone marrow	5.62 ± 0.31	5.90 ± 0.37	4.81 ± 0.30	2.55 ± 0.71	0.590 ± 0.434
Testes	0.326 ± 0.059	0.353 ± 0.111	0.342 ± 0.067	0.293 ± 0.069	0.225 ± 0.036
Seminal vesicle	1.40 ± 0.10	2.01 ± 0.29	1.73 ± 0.30	1.20 ± 0.39	0.385 ± 0.339
Prostate	3.12 ± 0.26	3.73 ± 0.23	2.75 ± 0.09	1.49 ± 0.18	0.380 ± 0.284
Urinary bladder	1.94 ± 0.16	1.72 ± 0.24	1.43 ± 0.18	0.695 ± 0.106	0.212 ± 0.141
Stomach	7.72 ± 1.08	7.01 ± 1.39	3.01 ± 1.20	1.77 ± 0.28	0.937 ± 0.429
Small intestine	6.19 ± 0.84	7.07 ± 0.61	9.70 ± 0.58	5.13 ± 1.06	1.10 ± 1.11
Large intestine	3.18 ± 0.22	3.17 ± 0.42	2.37 ± 0.36	3.99 ± 0.29	2.76 ± 1.76

from plasma, was highly distributed to tissues, extensively metabolized in the liver and finally excreted into the feces via the bile. Since [^{3}H]UCN-01 was used in the present study, the nonvolatile radioactivity was mainly estimated to avoid the effect of the ^3H_2O which is easily produced. The ratios of volatile to total radioactivity were low in plasma and feces. Although this ratio was 50% in urine, the urinary excretion was low. The contribution of ^3H_2O to the total metabolic fate was thought to be minimal.

The elimination half-life of plasma radioactivity in rats and dogs was 21.3 h and 27.2 h, respectively, much longer than previously reported values (4.46 h for rats and 11.6 h for dogs) [11]. Also, the AUC of the radioactivity in rats was seven times higher than that of UCN-01. Since the concentrations of radioactivity during the elimination phase in rats were more than ten times higher than the concentrations of UCN-01 determined by HPLC (about 0.003 μg/ml at 24 h after dosing [11]), the differences in the half-lives could be accounted for by the presence of metabolites in the plasma. Indeed, several metabolites were found in the plasma samples (Fig. 3A).

In both rats and dogs the main route of excretion was fecal. The urinary excretion of UCN-01 was minimal (Fig. 3C). This supports a previous finding that the hepatic clearance of UCN-01 makes a major contribution to its systemic clearance [11]. Studies with bile duct-cannulated rats have shown that most of the excreted [^{3}H]UCN-01 in the feces is via the bile, although a smaller fraction is apparently excreted by direct secretion into the gastrointestinal tract. This was supported by finding a significant level of radioactivity in the stomach (i.e. 7.72 μg equivalents/g at 0.5 h, Table 4) and in the stomach contents (data not shown). The radioactivity in the stomach and its contents was calculated to be approximately 1% of the injected dose. Most of the radioactivity in the bile was due to the metabolites (Fig. 3B). Therefore, the elimination of UCN-01 in rats was expected to be mainly due to metabolism in the liver. It is very important to determine the structures of these metabolites and the metabolic enzyme(s) involved in order to predict drug-drug interactions in clinical situations. Although the structures of these metabolites have not been elucidated, judging from their hydrophilicity in comparison with UCN-01, the metabolism could be catalyzed by cytochrome P450. Further studies are ongoing.

It has been shown that the strong protein binding of UCN-01 is specific to human α_1-acid glycoprotein but not to rat or dog α_1-acid glycoprotein [6, 7]. Considering this species difference, the results of this study cannot be directly extrapolated to predict the metabolic fate of UCN-01 in humans. Recent UCN-01 phase I studies have revealed that the peak plasma concentrations of UCN-01 (C_{max}) after infusion tend to be saturated at

higher dose levels. This nonlinear pharmacokinetics might be due to saturation of the strong binding of UCN-01 to human α_1-acid glycoprotein. At these dose levels, significant levels of "free UCN-01" are observed [14, 19]. If it is assumed that there are no species differences in tissue transfer of "free drug", "free UCN-01" would behave as it did in rats. Therefore, it is expected that "free UCN-01" from α_1-acid glycoprotein in humans may be finally metabolized in the liver and excreted into the bile in humans. Very little excretion of UCN-01 into patients' urine [19] may support this speculation.

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References

1. Akinaga S, Gomi K, Morimoto M, Tamaoki T, Okabe M (1991) Antitumor activity of UCN-01, a selective inhibitor of protein kinase C, in murine and human tumor models. Cancer Res 51: 4888
2. Akinaga S, Nomura K, Gomi K, Okabe M (1993) Enhancement of antitumor activity of mitomycin C in vitro and in vivo by UCN-01, a selective inhibitor of protein kinase C. Cancer Chemother Pharmacol 32: 183
3. Akinaga S, Nomura K, Gomi K, Okabe M (1993) Synergistic antitumor effect of UCN-01, a protein kinase (C) inhibitor, combined with various anti-cancer agents. Proc Am Assoc Cancer Res 33: 3072
4. Akiyama T, Yoshida T, Tsujita T, Shimizu M, Mizukami T, Okabe M, Akinaga S (1997) G1 phase accumulation induced by UCN-01 is associated with dephosphorylation of Rb and CDK2 proteins as well as induction of CDK inhibitor p21/Cip1/WAF1/Sdi1 in p53-mutated human epidermoid carcinoma A431 cells. Cancer Res 57: 1495
5. Bunch RT, Eastman A (1996) Enhancement of cisplatin-induced cytotoxicity by 7-hydroxystaurosporine (UCN-01), a new G2-checkpoint inhibitor. Clin Cancer Res 2: 791
6. Fuse E, Tanii H, Kurata N, Kobayashi H, Shimada Y, Tamura T, Sasaki Y, Tanigawara Y, Lush RD, Headlee D, Figg WD, Arbuck SG, Senderowicz AM, Sausville EA, Akinaga S, Kuwabara T, Kobayashi S (1998) Unpredicted clinical pharmacology of UCN-01 caused by specific binding to human α_1-acid glycoprotein. Cancer Res 58: 3248
7. Fuse E, Tanii H, Takai K, Asanome K, Kurata N, Kobayashi H, Kuwabara T, Kobayashi S, Sugiyama Y (1999) Altered pharmacokinetics of a novel anticancer drug, UCN-01, caused by specific high affinity binding to α_1-acid glycoprotein in humans. Cancer Res 59: 1054
8. Gibaldi M, Perrier D (1982) Noncompartmental analysis based on statistical moment theory. In: Gibaldi M, Perrier D (eds) Pharmacokinetics. Marcel Dekker, New York, p 409
9. Harkin ST, Cohen GM, Gescher A (1998) Modulation of apoptosis in rat thymocytes by analogs of staurosporine: lack of direct association with inhibition of protein kinase C. Mol Pharmacol 54: 663
10. Kawakami K, Futami H, Takahara J, Yamaguchi K (1996) UCN-01, 7-hydroxyl-staurosporine, inhibits kinase activity of cyclin-dependent kinases and reduces the phosphorylation of the retinoblastoma susceptibility gene product in A549 human lung cancer cell line. Biochem Biophys Res Commun 219: 778
11. Kurata N, Kuwabara T, Tanii H, Fuse E, Akiyama T, Akinaga S, Kobayashi H, Yamaguchi K, Kobayashi S (1999) Pharmacokinetics and pharmacodynamics of a novel protein kinase inhibitor, UCN-01. Cancer Chemother Pharmacol 44: 12
12. Pollack IF, Kawecki S, Lazo JS (1996) Blocking of glioma proliferation in in vitro and in vivo and potentiating the effects of BCNU and cisplatin: UCN-01, a selective protein kinase C inhibitor. J Neurosurg 84: 1024
13. Sausville EA, Lush RD, Headlee D, Smith AC, Figg WD, Arbuck SG, Senderowicz AM, Fuse E, Tanii H, Kuwabara T, Kobayashi S (1998) Clinical pharmacology of UCN-01: initial observations and comparison to preclinical models. Cancer Chemother Pharmacol 42: S54
14. Senderowicz AM, Headlee D, Lush R, Arbuck S, Bauer K, Figg WD, Murgo A, Inoue K, Kobayashi S, Kuwabara T, Sausville EA (1999) Phase I trial of infusional UCN-01, a novel protein kinase inhibitor, in patients with refractory neoplasms. Proc Am Soc Clin Oncol 18: 159a
15. Seynaeve CM, Stetler-Stevenson M, Sebers S, Kaur G, Sausville EA, Worland PJ (1993) Cell cycle arrest and growth inhibition by the protein kinase antagonist UCN-01 in human breast carcinoma cells. Cancer Res 53: 2081
16. Shao RG, Cao CX, Shimizu T, O'Connor PM, Kohn KW, Pommier Y (1997) Abrogation of an S-phase checkpoint and potentiation of camptothecin cytotoxicity by 7-hydroxystaurosporine (UCN-01) in human cancer cell lines, possibly influenced by p53 function. Cancer Res 57: 4029
17. Shao RG, Shimizu T, Pommier Y (1997) 7-Hydroxystaurosporine (UCN-01) induces apoptosis in human colon carcinoma and leukemia cells independently of p53. Exp Cell Res 234: 388
18. Takahashi I, Kobayashi E, Asano K, Yoshida M, Nakano H (1987) UCN-01, a selective inhibitor of protein kinase C from *Streptomyces*. J Antibiot 40: 1782
19. Tamura T, Sasaski Y, Minami H, Fujii K, Ito K, Igarashi T, Kamiya Y, Kurata T, Ohtsu T, Onozawa Y, Yamamoto N, Yamanoto Y, Watanabe Y, Tanigawara Y, Fuse E, Kuwabara T, Kobayashi S, Shimada Y (1999) Phase I study of UCN-01 by 3-hour infusion. Proc Am Soc Clin Oncol 18: 159a
20. Wang Q, Worland PJ, Clark JL, Carlson BA, Sausville EA (1995) Apoptosis in 7-hydroxystaurosporine-treated T lymphoblasts correlates with activation of cyclin-dependent kinases 1 and 2. Cell Growth Differ 6: 927
21. Watari N, Benet LZ (1989) Determination of mean input time, mean residence time, and steady-state volume of distribution with multiple drug inputs. J Pharmacokinet Biopharm 17: 593