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SHORT COMMUNICATION

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Disposition in mice of 7-hydroxystaurosporine, a protein kinase inhibitor with antitumor activity

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Abstract UCN-01, a hydroxylated derivative of staurosporine, was selected for study because of its promising antitumor activity. For mice dosed intravenously, subcutaneously, or by oral gavage with this compound, the maximum tolerated doses (MTD) were 20, 10, and >100 mg/kg, respectively. UCN-01 was stable in mouse and dog plasma, but in human plasma it was converted to a metabolite in a process not inhibited by standard protease and esterase inhibitors. Following an intravenous dose of 10 mg/kg UCN-01, the half-lives for the initial ($t_{1/2\alpha}$) and terminal ($t_{1/2\beta}$) exponential phases of elimination were 10 and 85 min, respectively; the area under the plasma concentration-time curve (AUC value) was 117 μg min ml^{-1}. In mice dosed by oral gavage with 10 mg/kg, the calculated value for the half-life of the elimination phase was 150 min. The AUC value was 15 μg min ml^{-1}, giving a value for bioavailability of 13%. After subcutaneous dosing with 10 mg/kg, the calculated values for half-lives for the distribution and elimination phases were 23 and 130 min, respectively; the AUC value was 113 μg min ml^{-1}. Since this value is equivalent to that obtained for intravenous dosing, administration of UCN-01 by the subcutaneous route may be an alternative to intravenous dosing in preclinical and clinical trials.

Key words Disposition · 7-Hydroxystaurosporine · Protein kinase

Introduction

UCN-01 (Fig. 1) is a hydroxylated derivative of staurosporine isolated from a strain of *Streptomyces*. This compound, the stereochemical structure of which has been determined [7], is a potent inhibitor of protein kinase C and has considerable activity against protein kinase A and p60 v-*src* tyrosine-specific kinase [6]. UCN-01 inhibits the growth of breast-cancer cells in culture; in one cell line, it blocks progression of the cell cycle in the G_1 phase [4]. Injected intraperitoneally into mice, it has marginal but definite activity against P388 leukemia [6], two oncogene-activated murine fibrosarcoma models, and three human tumor xenografts carried in mice [1]. The present effort was undertaken to evaluate the disposition of UCN-01 given to mice by the intravenous, oral, and subcutaneous routes. Since intraperitoneal administration is not appropriate for larger experimental animals or for humans, the subcutaneous route was chosen as an alternative. The studies were designed to compare three routes of administration of UCN-01 and, thus, to provide information to be used in preclinical toxicity evaluations and in the development of clinical trials for this compound.

Materials and methods

For analysis of UCN-01, plasma samples were diluted with equal volumes of acetonitrile and centrifuged; urine samples were diluted 1:9 (v/v) with 0.1 *M* sodium acetate (pH 5): acetonitrile (1:1, v/v) and centrifuged. Solutions and samples were protected from light. Samples were analyzed isocratically by high-performance liquid chromatography (HPLC) with a Nucleosil C18 reverse-phase column

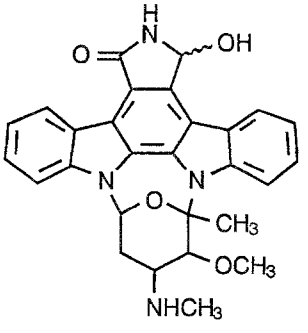

Fig. 1 Structure of UCN-01 and UCN-02. The compounds are isomers with the hydroxyl group in the 7-position (indicated by a *wavy line*) either above (UCN-01) or below (UCN-02) the plane of the ring as shown

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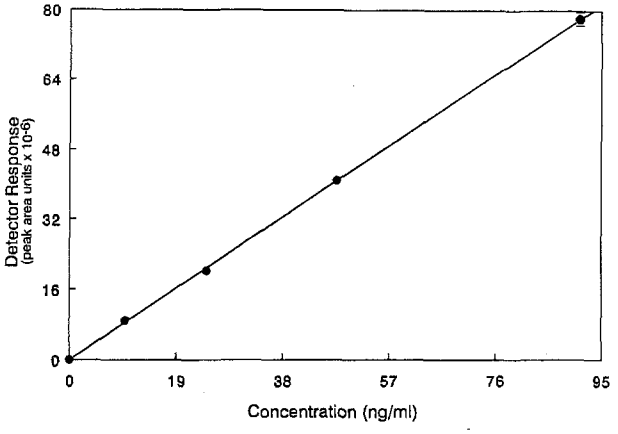

Fig. 2 Standard curve for analysis of UCN-01 in mouse plasma. For most of the points, the standard deviation is too small to be displayed

(150 × 4.6 mm). The injection volume was 100 µl; the mobile phase was 5 m*M* ammonium phosphate (pH 2.8): acetonitrile (70:30, v/v); and the flow rate was 1 ml/min. Detection was accomplished by fluorescence, with excitation at 375 nm and measurement of emission at 409 nm. UCN-01 eluted with a retention time (R_T) of about 8 min. In study samples, UCN-01 was quantified by reference to a recovery curve for which quadruplicate samples at all concentrations were spiked and prepared in the same manner as study samples. No internal standard was used. After analysis of each sample, the column was washed with 5 m*M* ammonium phosphate (pH 2.8): acetonitrile (1:1, v/v).

To assess the stability of UCN-01 at various pH values, the compound (100 ng/ml) was dissolved in solutions of citric acid (5 mg/ml) adjusted to different pH values with sodium hydroxide. These preparations were analyzed immediately and at 16 h after preparation.

To establish that in human plasma the formation of a product was time-dependent, UCN-01 (100 ng/ml) was added to plasma, and the preparation was incubated at room temperature. At selected times, including time zero, portions were taken and added to an equal volume of acetonitrile. The samples were centrifuged, and the soluble portions were analyzed by the HPLC procedure described above.

Mice were handled in accordance with the standards incorporated in the *Guide for the Care and Use of Laboratory Animals* (revised in 1985; DHHS, USPHS) in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). During quarantine, which lasted for at least 1 week, these animals were housed in microisolator cages lined with hardwood chips; during the experimental period, each mouse was housed individually.

Neither food nor water was withheld prior to dosing. CD_2F_1 male mice, approximately 7 weeks old and weighing 21–26 g, were dosed either intravenously via a tail vein, by oral gavage with a feeding needle, or subcutaneously at a dorso-lateral site. For this purpose, UCN-01 was dissolved in 0.1 *M* sodium acetate buffer (pH 5.0). The volumes of dosing solution were: intravenous and subcutaneous, 10 ml/kg; and oral, 20 ml/kg.

To determine the toxicity of UCN-01, various amounts of UCN-01 were given by these routes to groups of five or six mice. In disposition studies, mice were dosed with 10 mg/kg UCN-01 and were euthanized in groups of three at 5, 15, 30, 60, 120, 180, 360, and 1440 min after dosing. Blood was collected into tubes containing heparin and centrifuged to obtain plasma. For each route of administration, three to five additional mice were dosed for 24-h urine specimens, which were collected over dry ice.

Pharmacokinetic parameters were estimated with a modified form of NONLIN [2] and C-STRIP [3]. The data were fitted ton one-, two-, and three-compartment open models. A model was accepted as the best fit if an additional term, or compartment, failed to reduce significantly ($P < 0.05$) the weighted sums of squared errors as estimated by the *F* test with appropriate degrees of freedom. In all cases, a two-compartment model was selected. Statistical weights were determined from the measured concentrations.

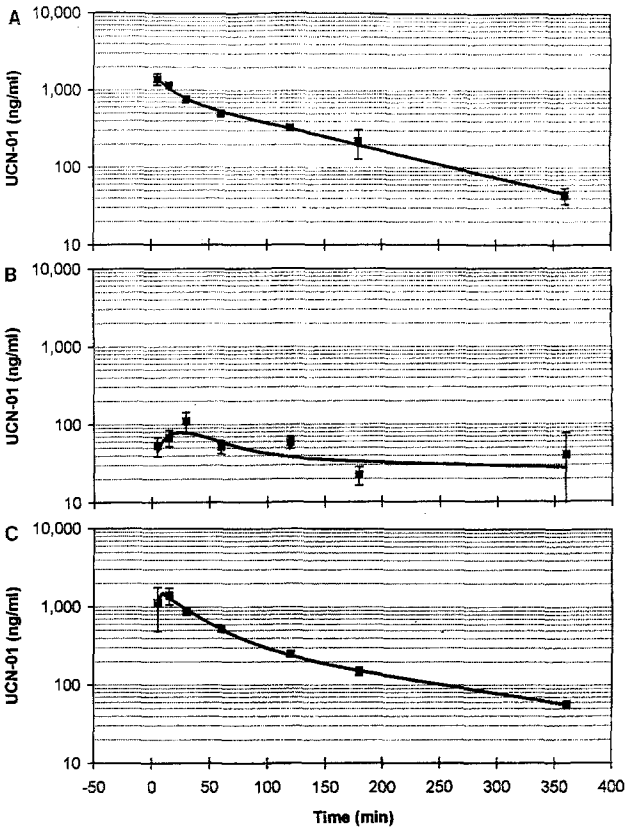

Fig. 3 Concentrations of UCN-01 in plasma from mice dosed intravenously (*top*), by oral gavage (*middle*), and subcutaneously (*bottom*) with 10 mg/kg

Results and discussion

Analysis of quadruplicate samples of mouse plasma spiked with solutions of UCN-01 over a range of 9.9–91.9 ng/ml revealed a linear regression line characterized by a correlation coefficient of 0.9995, a slope of 875,000, and a *Y*-intercept of –49,000 (Fig. 2). Similar lines for dog and human plasma were characterized by correlation coefficients of 0.9993 and 0.9985, slopes of 864,000 and 823,000, and *Y*-intercepts of 1,557,000 and –358,000, respectively. The coefficient of variation for the slopes of these curves was 2.6%. The high value of the *Y*-intercept for dog plasma was due to the presence of a small endogenous peak that eluted with the same retention time as did UCN-01. The coefficients of variation recorded for the four samples at each concentration and in all three matrices was <5%. UCN-01 was detectable at 5 ng/ml and reproducibly quantifiable at 10 ng/ml. The recoveries of UCN-01 were 89.3% ± 4.6%, 95.5% ± 6.7%, and 84% ± 5.2% for mouse, dog, and human plasma, respectively. These results demonstrate that the HPLC method was adequate for analysis of plasma samples containing UCN-01.

Concerning the stability of UCN-01, substantial conversion to UCN-02 (R_T, 4.5 min), an isomer of UCN-01 [7], was evident at pH values of 3 and 4. At pH 5, however, there was no measurable formation of UCN-02. At pH values greater than 5, there was no detectable conversion to UCN-02, but the amount of UCN-01 recovered was low due to decreased solubility. In methanol and in sodium acetate buffer (pH 5.0), solutions of UCN-01 were stable for 72 h at room temperature. The compound was also stable in mouse and dog plasma for 24 h at temperatures of 0°, 4°, 22°, and 37 °C and for 14 days at temperatures of 0° and 4 °C.

For human plasma that had been spiked with UCN-01 and allowed to stand at room temperature, an additional peak with an R_T of about 7 min was noted. The concentration of UCN-01 initially decreased with time, and formation of the product increased. No additional product, however, was formed after 3 h. Protein-free human plasma did not catalyze the reaction, and addition of acetonitrile at time zero prevented formation of the product, indicating that it represented a metabolite. Since the total area values obtained for the substrate and product peaks were essentially equal to that obtained for the initial concentration of UCN-01, the extinction coefficient recorded for the metabolite was approximately the same as that noted for UCN-01. An appropriate calculation showed that after 3 h of incubation, about 18% of the UCN-01 present was converted to the metabolite. Neither sodium fluoride, an esterase inhibitor; diisopropylfluorophosphate, an esterase and protease inhibitor; nor 1-chloro-3-tosylamido-7-amino-2-heptanone hydrochloride and L-1-*p*-tosylamino-2-phenylethylchloromethyl ketone, which are protease inhibitors, inhibited formation of the metabolite. The nature of the enzyme catalyzing the reaction remains unknown. Since no effective inhibitor could be found in this brief survey, clinicians giving UCN-01 should be aware that metabolism of this compound may continue in collected blood samples. Future work with UCN-01 could involve isolation of the metabolite and evaluation of its biological activity.

All five of the mice dosed intravenously or subcutaneously with UCN-01 at 60 mg/kg died within 24 h, but none of the five treated orally with this dose died over a 3-week period of observation. All five of the mice dosed intravenously or subcutaneously with 30 mg/kg also died within 72 h. There was no mortality among the five mice dosed orally with 100 mg/kg. Five mice dosed intravenously with 20 mg/kg survived for a 3-week period. Two of the six mice dosed subcutaneously with 20 mg/kg died; none of the six mice dosed with 10 mg/kg died. Thus, the maximum tolerated doses (MTD) for intravenous dosing, subcutaneous dosing, and oral gavage were 20, 10, and >100 mg/kg, respectively. Administration of the buffer alone did not result in mortality.

In the 24-h urine specimens collected from mice dosed by either of these three routes with 10 mg/kg, $<1\%$ of the dose was present as unchanged UCN-01. This information was consistent with either extensive metabolism or biliary excretion of UCN-01 by these mice.

Plasma levels of UCN-01 decreased more than 10-fold over a period of 6 h after intravenous dosing with 10 mg/kg (Fig. 3); the compound was not present in detectable amounts at 24 h. In the plasma samples collected at 60–180 min after dosing, additional peaks were present; a peak eluting with an R_T of 7 min may have been the same as that observed in human plasma. Values obtained for $t_{1/2\alpha}$ and $t_{1/2\beta}$, the half-lives for the initial and terminal exponential phases of elimination, were 10 and 85 min, respectively. The AUC value (extrapolated from time zero to infinity) was 117 μg min ml^{-1}.

After dosing of mice with 10 mg/kg by oral gavage, plasma levels of the test compound increased to a maximum of about 100 ng/ml at 30 min (Fig. 3), but the compound was not present in detectable amounts at 24 h. The calculated value for the half-life for the elimination phase was 150 min. The peak with a retention time of 7 min was not measurable. The long terminal phase may be related to slow absorption of UCN-01 from the intestinal tract, where it may have precipitated in the alkaline environment. The AUC value was 15 μg min ml^{-1}, giving a bioavailability of 13%. Since the 50% growth-inhibitory concentrations (IC_{50} values) determined in five lines of murine and human tumor cells in culture are in the range of 40–157 ng/ml [1] and those obtained in five lines of human breast-cancer cells are in the range of 13–60 ng/ml [4], it is likely that larger doses of UCN-01 will have to be given by the oral route to achieve a substantial therapeutic effect.

Following subcutaneous dosing of mice with 10 mg/kg UCN-01, there was no visible tissue damage at the injection site. Plasma levels of the test compound decreased more than 10-fold over a period of 6 h (Fig. 3); the compound was not present in detectable amounts at 24 h after dosing. Calculated values for half-lives for the distribution and elimination phases were 23 and 130 min, respectively. The AUC value was 113 μg min ml^{-1}, a value equivalent to that obtained after intravenous dosing. The peak with a retention time of 7 min was not measurable. The observation that the MTD for a subcutaneous dose is lower than that for an intravenous dose could be related to the longer terminal elimination phase for the subcutaneous dose. UCN-01 may be a cell-cycle-specific compound, for which toxicity is related not to the AUC value but to the length of time for which a toxic concentration of drug remains in the plasma [5].

If response is related to the AUC value, however, the subcutaneous route of administration of UCN-01 may be useful in preclinical and clinical tests as an alternative to i. v. dosing, since the AUC value recorded for subcutaneous dosing is larger than that noted for oral dosing and equal to that observed for intravenous administration. In view of its potential as a novel anticancer agent, UCN-01 is presently being evaluated in preclinical toxicity tests.

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