BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 21(2), 170-172 (2013)

Pharmacokinetics of Uridine Following Ocular, Oral and Intravenous Administration in Rabbits

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Abstract

The pyrimidine nucleoside uridine has recently been reported to have a protective effect on cultured human corneal epithelial cells, in an animal model of dry eye and in patients. In this study, we investigate the pharmacokinetic profile of uridine in rabbits, following topical ocular (8 mg/eye), oral (450 mg/kg) and intravenous (100 mg/kg) administration. Blood and urine samples were serially taken, and uridine was measured by high-performance liquid chromatography-tandem mass spectrometry. No symptoms were noted in the animals after uridine treatment. Uridine was not detected in either plasma or urine after topical ocular administration, indicating no systemic exposure to uridine with this treatment route. Following a single intravenous dose, the plasma concentration of uridine showed a bi-exponential decay, with a rapid decline over 10 min, followed by a slow decay with a terminal half-life of 0.36 ± 0.05 h. Clearance and volume of distribution were 1.8 ± 0.6 L/h/kg and 0.58 ± 0.32 L/kg, respectively. The area under the plasma concentration-time curves (AUC) was 59.7 ± 18.2 μg·hr/ml, and urinary excretion up to 12 hr was ~7.7% of the dose. Plasma uridine reached a peak of 25.8 ± 4.1 μg/ml at 2.3 ± 0.8 hr after oral administration. The AUC was 79.0 ± 13.9 μg·hr/ml, representing ~29.4% of absolute bioavailability. About 1% of the oral dose was excreted in the urine. These results should prove useful in the design of future clinical and nonclinical studies conducted with uridine.

Key Words: Uridine, Pharmacokinetics, Topical ocular, Bioavailability, Rabbit

INTRODUCTION

The pyrimidine nucleoside uridine has been shown to rapidly restore the health of the ocular surface in dry eye patients, possibly because of an increase in hyaluronic acid synthesis, a reduction in degradative enzymes such as matrix metalloproteinase-9, or an enhancement of the number of goblet cells (Oh *et al.*, 2007; Chang *et al.*, 2009).

The pharmacokinetic behavior of uridine has been reported only in a few papers. A time profile of plasma uridine concentration was determined following intravenous administration in two rabbits (Peters *et al.*, 1987); the relative bioavailability of oral uridine was compared with subcutaneous injection in mice (Klubes *et al.*, 1986); and 5-(phenylthio)acyclouridine, a uridine phosphorylase inhibitor, was shown to enhance uridine oral bioavailability in mice (Al Safarjalani *et al.*, 2001).

In the present work, we investigated the pharmacokinetic profile of uridine following oral and intravenous administrations in rabbits. Additionally, in an attempt to mimic the ocular application of uridine, an 8% ocular solution was dropped into the rabbits' eyes and the systemic exposure of uridine was monitored.

MATERIALS AND METHODS

Materials

Uridine and methaqualone (internal standard, IS) were purchased from Sigma (Seoul, Korea), and methanol was obtained from J.T. Baker (Seoul, Korea). All other chemicals and solvents were of the highest analytical grade available.

Animal and housing conditions

Eighteen male New Zealand White rabbits weighing 2-2.5 kg were supplied by OrientBio, Ltd. (Suwon, Korea). The animal room was maintained at a temperature of 23 ± 3°C, a relative humidity of 50 ± 10% with 10-20 air changes/h, and a light intensity of 150-300 Lux with a 12-h light/dark cycle. This

Open Access http://dx.doi.org/10.4062/biomolther.2012.103

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Received Dec 26, 2012 **Revised** Mar 13, 2013 **Accepted** Mar 14, 2013

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study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Biotoxtech (Osong, Korea). All animals used in this study were cared for in accordance with the principles outlined in the National Institute of Health "Guide for the Care and Use of Laboratory Animals".

During all study periods, the rabbits were individually housed in a stainless steel wire cage (175×240×1,450 mm, W/L/H), which were labeled to show the study number and animal number, and fed once daily with a standard certified commercial rabbit chow (300 g of DS-A; Oriental Yeast Co., Ltd., Tokyo, Japan). Analytical results regarding possible contaminants in the feed was provided by the vendor and collected as raw data. UV- irradiated and filtered tab water was provided *ad libitum*. The water was analyzed according to standard operating procedures, and no heavy metals, microbial contamination, or insecticide residues above the permitted limits were noted.

Pharmacokinetic studies

Rabbits were randomly grouped based on the body weight. Each animal was identified by means of a tattoo on the ventral aspect of one pinna and an individual identification card on the cage. Uridine was dissolved at 450 mg/ml in normal saline for intravenous injection and oral administration, and at 80 mg/ml for topical ocular bolus.

Heparinized blood (0.5 ml) was serially collected from the marginal ear vein before treatment and at 1, 5, 10, and 30 min, and 1, 2, 3, 4, 6, and 12 h following intravenous administration of 100 mg/kg uridine via the central ear vein. After a single oral administration of 450 mg/kg, blood samples were collected at 15 and 30 min, and 1, 1.5, 2, 3, 4, 6, 8, and 12 h. For ocular administration, 8 mg of uridine were dropped into each eye, and blood was collected at 5, 15, 30, and 45 min, and 1, 2, 3, 4, and 6 h later. The plasma was separated by centrifugation at 12,000 rpm for 3 min and stored frozen until analyzed.

Urine was collected for the periods of 0-3, 3-6, 6-9, and 9-12 hr following uridine administration. The total urine volume for each period was recorded, and 1 ml of each urine sample was frozen and stored until analyzed.

Determination of uridine in plasma and urine

Uridine concentrations in plasma and urine were quantified by high-performance liquid chromatography-tandem mass spectrometry with an API 4,000 LC/MS/MS System (Sciex Division of MDS, Inc., Toronto, Canada) equipped with an electrospray ionization interface used to generate $[M-H]^-$ ions for uridine and $[M+H]^+$ ions for methaqualone (internal standard) (Kang, 2012). One milliliter of internal standard methanol solution (10 ng/ml) was added to 100 μl of plasma or urine samples, and the samples were mixed by vortexing, followed by centrifugation at 13,200 rpm for 10 min. A 2 μl sample of the supernatant was injected and separated on a reversed-phase column (Zorvax Eclipse C18, 3 μm, 2.1×100 mm; Agilent, Wilmington, DE, USA) with an isocratic mobile phase consisting of methanol and 0.1% aqueous formic acid (4:1, v/v) at a flow rate of 0.2 ml/min, using an HP 1,100 series pump (Agilent). Quantitation was performed by multiple reaction monitoring of the protonated precursor ion and the related product ion for uridine, using an internal standard method with peak area ratios. The mass transitions used for uridine and internal standard were m/z 243.0→199.6 and 251.1→131.9, respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with WinNonlin® (Standard ver., Pharsight Corporation, Mountain View, USA) by a model independent analysis. The time course of the plasma uridine concentration was used to determine the maximum plasma concentration (C_{max}) and the time (T_{max}) to reach C_{max}. The elimination rate constant (k_e) was obtained by linear regression of the terminal phase, and the calculated elimination half-life ($t_{1/2}$) was 0.693/k_e. The area under the plasma concentration-time curve (AUC_{last}) was calculated using the trapezoidal rule and extrapolated to infinity for AUC. Area under the moment curve (AUMC) was estimated based on the moment theory, and mean residual time (MRT) was obtained by AUMC/AUC. Total clearance (Cl) was calculated as Dose/AUC. A 2-compartment model was used to fit the plasma uridine concentration-time curve following intravenous administration. Distribution (α) and elimination (β) rate constants, and their corresponding y-intercepts, A and B were determined, respectively. Apparent volume of distribution was calculated as Dose/(A+B).

RESULTS

No symptoms were noted in any of the rabbits that received uridine treatment. The mean plasma concentration-time profile of uridine after a single intravenous or oral administration in the rabbits is illustrated in Fig. 1A. Table 1 lists the pharma-

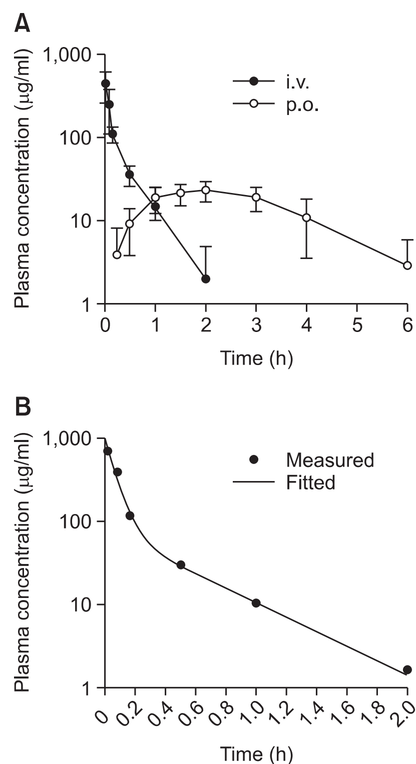

Fig. 1. (A) Time courses of plasma uridine concentrations after a single intravenous (100 mg/kg) and oral (450 mg/kg) administration in rabbits (n=6). (B) A representative fit of plasma uridine concentration-time profile in a rabbit obtained by a 2-compartment model.

Table 1. Pharmacokinetic parameters of uridine in rabbits

Oral (450 mg/kg, n=6)		Intravenous (100 mg/kg, n=6)	
Parameter	Mean ± s.d.	Parameter	Mean ± s.d.
C_{max} (μg/ml)	25.8 ± 4.1	V_d (L/kg)	0.85 ± 0.32
T_{max} (h)	2.3 ± 0.8	Cl (L/h/kg)	1.82 ± 0.60
AUC (μg·h/ml)	79.0 ± 13.9	AUC (μg·h/ml)	59.7 ± 18.2
AUMC (μg·h^2/ml)	186.2 ± 78.1	A (μg/ml)	463.3 ± 268.4
MRT (h)	2.39 ± 0.65	B (μg/ml)	87.3 ± 37.4
$t_{1/2}$ (h)	1.38 ± 0.41	α (h^{-1})	14.2 ± 4.3
		β (h^{-1})	1.8 ± 0.5

cokinetic parameters. Following a single intravenous dose of uridine (100 mg/kg), the plasma uridine concentration demonstrated a bi-exponential decay, with a rapid decline over 10 min, followed by a slow decay with a terminal half-life of 0.41 ± 0.14 h. Mean clearance, volume of distribution and AUC were 1.82 ± 0.60 L/h/kg, 0.85 ± 0.32 L/kg and 59.7 ± 18.2 μg·h/ml, respectively. A representative fit for the time course of plasma uridine concentration was shown in Fig. 1B. Following oral administration of 450 mg/kg uridine, the plasma uridine concentration reached a peak of 25.8 ± 4.1 μg/ml at 2.3 ± 0.8 h. The AUC was 79.0 ± 13.9 μg·hr/ml, representing ~29.4% of the absolute bioavailability.

Total urinary excretion of urine following intravenous administration was 19.4 ± 7.4 mg, which was equivalent to 7.7% of the dose. Only about 1% (11.8 ± 5.3 mg) of the dose was excreted in the urine during the 12 hr following oral administration.

Uridine was not detected in either the plasma or urine following topical ocular administration, indicating that no systemic exposure occurred after administration by this route.

DISCUSSION

In the present study, we investigated the pharmacokinetic profiles of intravenously, orally, and ocularly administered uridine in rabbits. Initially, tritium-labeled uridine was used to distinguish between administered and endogenous uridine in the plasma (Peters *et al.*, 1987). However, the concentration of the commercially available standard stock solution was too low to be useful. Consequently, only unlabeled uridine was administered.

No reports on the bioavailbility of uridine have appeared in the literature, other than that by Klubes *et al.* (1986). They reported 7% bioavailability of orally administered uridine relative to subcutaneously injected uridine in mice; the absolute bioavailability is likely to be even lower. In the present study, the absolute bioavailability of orally administered uridine in rabbits was 29.4%. The apparent difference between these results may be attributable to differences in the gastrointestinal tract structure between mice and rabbits, or to differences in the methods of uridine administration.

Peters *et al.* (1987) reported the time course of plasma uridine concentrations in rabbits for up to 5 h following an intravenous administration of 400 mg/kg uridine in two rabbits. However, because the first blood sample was not taken until 30 min after dosing, the AUC and volume of distribution appeared to be under- and over-estimated, respectively. In addition, the clearance was much faster than that seen in our study. As shown in Fig. 1, uridine undergoes bi-exponential decay, and the AUC between 0 and 30 min reaches 65% of the total AUC. Thus, it is important to measure plasma concentrations at early time points after administration, especially following intravenous injection.

After oral administration of uridine, the plasma concentration slowly increased for up to 2 h and then decayed with a half-life of 1.4 ± 0.4 h. The half-life is much longer than that following intravenous administration (0.36 ± 0.05 h), which may be attributable to flip-flop kinetics and/or the enterohepatic circulation.

About 7.7% of the uridine dose was recovered in the urine after intravenous injection, whereas only 1% of orally administered uridine was excreted in the urine. This difference is probably due to biotransformation during the absorption process.

One main purpose of this study was to investigate the absorption and systemic distribution of the topical ocular uridine preparation. For topical ocular administration, the maximum concentration tolerated without serious irritation to the eyes was 8% uridine (80 mg/ml) in normal saline (Oh *et al.*, 2007), and 0.1 ml was the maximum volume that could be administered. There was no elevation of uridine levels in the plasma and urine following a dose of 16 mg topical ocular uridine, indicating that there was no systemic exposure or distribution to other organs after topical ocular administration at this dose.

To our knowledge, the present work represents the first report of the pharmacokinetic behavior of uridine, including the determination of absolute bioavailability, in rabbits. These results should prove useful in the design of future clinical and nonclinical studies conducted with uridine.

ACKNOWLEDGMENTS

This research was supported by Yeungnam University research grant in 2011 (211-A-380205).

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