Cancer Chemother Pharmacol (1997) 39: 532–536

ORIGINAL ARTICLE

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Preclinical pharmacokinetics of β-L-dioxolane-cytidine, a novel anticancer agent, in rats

Received: 3 May 1996/Accepted: 5 September 1996

Abstract *Purpose*: β-L-Dioxolane-cytidine (OddC), a novel L-nucleoside analog with potent cytotoxicity in vitro, appears to be a promising candidate for anticancer therapy. In this study, a high performance liquid chromatography (HPLC) analytical method was developed and the preclinical pharmacokinetics of OddC were characterized in rats. *Methods*: Adult male Sprague Dawley rats were given 10, 25, or 50 mg/kg of OddC both intravenously and orally with a 6-day washout period between doses. Each rat received one dosage level of OddC and the route of administration was assessed by a randomized crossover design. Plasma and urine concentrations were determined by HPLC. Pharmacokinetic parameters were generated by area-moment analysis. *Results*: Following intravenous administration, the plasma concentrations of OddC declined rapidly in a biexponential manner with a terminal phase half-life of 1.65 ± 1.12 h (mean ± SD). Mean total, renal, and nonrenal clearances were 1.38 ± 0.62, 0.30 ± 0.14, and 1.08 ± 0.59 l/h per kg. Approximately 22% of the administered dose was excreted unchanged in the urine. Thus, nonrenal clearance was the predominant route of elimination of OddC. The steady-state volume of distribution averaged 1.42 ± 0.66 l/kg, indicating intracellular distribution of OddC. The nucleoside analog was slowly absorbed after oral administration and bioavailability varied greatly between individual rats, averaging $41 \pm 27\%$ when calculated from urinary excretion data and $37 \pm 25\%$ when calculated from plasma OddC concentration data. *Conclusion*: The pharmacokinetics of OddC in rats were linear over the dose range studied.

Key words β-L-dioxolane-cytidine · OddC · Pharmacokinetics · Nucleoside · HPLC

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Introduction

All natural nucleosides and most biologically active synthetic nucleoside analogs occur in the β-D configuration. Recently, however, the pursuit of L-nucleoside analogs with pharmacological activity has emerged. For example, the β-L enantiomer of 2′,3′-dideoxythiacytidine (3TC) has been shown to possess greater antiviral activity and lower cytotoxicity than the β-D-enantiomer [3, 14]. Further, β-L-fluoro-5-methyl-arabinofuranosyluracil (L-FMAU) exhibits potent antiviral activity against hepatitis B virus (HBV) in vitro [15].

β-L-Dioxolane-cytidine (OddC) is another recently synthesized nucleoside analog in the L-configuration [9]. OddC exhibits potent activity against human immunodeficiency virus (HIV) and against HBV with median effective concentrations (EC_{50}) of 0.002 and 0.005 μ*M*, respectively [9]. The compound is also very cytotoxic, however, with median inhibitory concentrations (IC_{50}) ranging from 0.02 to 10 μ*M* [6, 9]. Interestingly, OddC is the first L-nucleoside analog shown to have significant cytotoxicity. For this reason, OddC is being evaluated as a potential anticancer agent. OddC has been shown to be effective against prostate tumors, hepatocellular carcinoma, colon tumors, and leukemia cells in vitro and in mice [6]. The chemical structure of this nucleoside analog is illustrated in Fig. 1.

OddC is activated by phosphorylation by deoxycytidine kinase to its active phosphate anabolites. Additionally, it has been shown that its unnatural stereochemistry does not prevent OddC triphosphate from being incorporated into chromosomal DNA

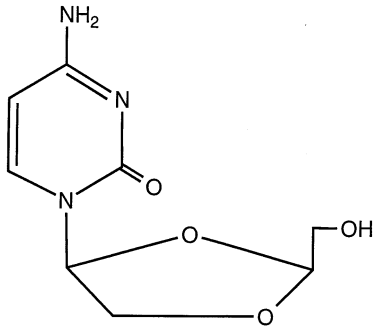

Fig. 1 Structure of β-L-dioxolane-cytidine (OddC)

where the analog acts as a chain terminator. However, like 3TC and 2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC), the L-configuration confers upon OddC a resistance to deamination by deoxycytidine deaminase, the enzyme that metabolizes deoxycytidine to its inactive uridine analog. Because the elevation of deoxycytidine deaminase levels is a mechanism by which cells become resistant to cytidine analogs, OddC may be valuable in the treatment of patients who have become unresponsive to these drugs [6, 10].

Owing to the cytotoxicity and anticancer activity of OddC in vitro and in mice, the metabolism of OddC to its phosphate anabolites and subsequent incorporation into chromosomal DNA, and the resistance of OddC to deoxycytidine deaminase, OddC appears to be a promising candidate anticancer agent. The purpose of this study was to characterize the preclinical pharmacokinetics of OddC in rats. As a prerequisite to pharmacokinetic evaluation of the compound, a high performance liquid chromatographic (HPLC) assay for the determination of OddC in biological fluids was developed. The effects of dose on the disposition of OddC were assessed following intravenous (i.v.) and oral (p.o.) administration of the L-nucleoside analog.

Materials and Methods

Chemicals

OddC was synthesized as previously described [8, 9]. The chemical purity of the compound, as determined by spectral and HPLC analysis, was greater than 99%. 2′,3′-Dideoxycytidine (ddC) was obtained from Sigma Chemical Co. (St. Louis, Mo.). Acetonitrile (HPLC grade) and all other analytical grade reagents and chemicals were obtained from J.T. Baker (Phillipsburg, N.J.).

Animals

A group of 15 adult male Sprague Dawley rats (Harland Sprague Dawley, Indianapolis, Ind.) weighing 284 ± 30 g (mean ± SD) were used for the pharmacokinetic studies. Rats were acclimatized to a 12-h light/dark cycle, controlled temperature (22 °C) environment with free access to standard laboratory chow and water for 1 week before the study. Rats were housed and all experiments were performed at the University of Georgia College of Pharmacy Animal Facility which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Animal studies were approved by the University of Georgia Animal Care and Use Committee, and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

Experimental design

External right jugular vein cannulas were surgically implanted under ketamine/acetopromazine/xylazine (50:3.3:3.3 mg/kg) anesthesia the day before the experiment. Food was withheld overnight; however, water was freely available. On the morning of the experiment, animals were placed in individual metabolism cages. Five rats each were given 10, 25, or 50 mg/kg both i.v. via the jugular vein cannula, and p.o. by oral gavage, with a 6-day washout period between doses. Each rat received one dosage level of OddC, and the route of administration was assessed by a randomized crossover design. Food was withheld for the first 4 h after dosing, but water was available ad libitum. Blood samples (0.3 ml) were collected from the cannula into heparinized tubes prior to and 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after drug administration. Blood was replaced with an equal volume of normal saline. Preliminary studies had demonstrated a lack of adsorption of OddC to the cannulas. Blood samples were promptly centrifuged, and plasma was separated and stored at −20 °C pending analysis. Urine samples were collected 24 h after drug administration, the volume was recorded, and the samples were stored at −20 °C pending analysis.

Analytical methodology

Concentrations of plasma and urine were measured by HPLC. To determine plasma concentrations of OddC, a 100-µl plasma sample, 100 µl internal standard (10 µg/ml ddC), and 50 µl 2 *M* perchloric acid were added to polypropylene microcentrifuge tubes. Tubes were thoroughly mixed, and centrifuged at 9500*g* for 10 min. Potassium hydroxide (50 µl) was added to the supernatant, and tubes were vigorously mixed and centrifuged at 9500*g* for 10 min. A 75-µl aliquot of the supernatant was injected onto the HPLC column.

Urine samples were diluted 50-fold with distilled deionized water. To 100 µl of the diluted urine sample, 100 µl of internal standard (10 µg/ml ddC) was added and mixed well, and 75–125 µl was injected onto the HPLC column. The amount of OddC recovered unchanged in the urine was calculated by multiplying drug concentration by urine volume.

Chromatographic separations were achieved with an octadecanoyl sulfate (ODS) reversed-phase column (5 µm particle size; 15 × 0.46 cm; Alltech Associates, Deerfield, Ill.) protected by an online C_{18} guard column. The mobile phase consisted of 1.8% (v/v) acetonitrile in 50 m*M* potassium phosphate with 2 m*M* triethylamine at pH 7.8. The flow rate of the mobile phase was 1.5 ml/min and the eluant was monitored at a UV wavelength of 270 nm.

The standard curve concentrations of OddC prepared in blank rat plasma ranged from 0.125 to 50 µg/ml. Standard curves for the analysis of urine samples were prepared over the range of 1 to 125 µg/ml OddC in water. Sample OddC concentrations were determined from the slope of standard curves of the peak area ratio (drug/internal standard) versus standard drug concentrations. Slopes were determined by linear regression analysis with a weighting factor of $1/x^2$.

The analytical recovery, precision, and accuracy were assessed at 0.25, 7.5, and 50 µg/ml OddC. The peak areas of six extracted plasma samples and six direct injections of the same amount of drug were

compared and percent recovery determined from peak $\text{area}_{\text{extracted drug}}$/mean peak $\text{area}_{\text{direct injection}} \times 100$. The intra- and interday precision of the assay was determined by the analysis of six quality control samples and the accuracy was determined by comparing the results of the precision study with the known plasma OddC concentrations.

Pharmacokinetic analysis

Pharmacokinetic parameters were generated by area-moment analysis. The area under the plasma concentrations versus time curve (AUC) and the area under the first moment curve (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time [13] with extrapolation to time infinity using the least-squares terminal slope (λ_z). The elimination half-life ($t_{1/2}$) was calculated from $0.693/\lambda_z$, mean residence time (MRT) from AUMC/AUC, and steady-state volume of distribution (V_{ss}) from dose $\times$ AUMC/AUC^2. The urinary recovery of unchanged OddC (f_e) was calculated from A_u/dose, where A_u is the amount of OddC excreted unchanged in the urine. Total clearance (CL_T) was determined from dose/AUC, renal clearance (CL_R) from $f_e \times CL_T$, and nonrenal clearance (CL_{NR}) from $CL_T - CL_R$. The oral bioavailability (F) of OddC was calculated from $A_{u,po}/A_{u,iv}$ and from AUC_{po}/AUC_{iv}.

The pharmacokinetic parameters obtained for the three dosage levels were compared for statistical significance via one-way analysis of variance. A probability level of < 0.05 was considered statistically significant.

Results and discussion

Sample chromatograms from blank rat plasma, rat plasma spiked with drug and internal standard, and a rat plasma sample after i.v. administration of 25 mg/kg OddC are shown in Fig. 2. The retention times of OddC and internal standard were 4.5 and 7.5 min, respectively. There were no interfering endogenous peaks observed in the blank rat plasma. The standard curves in plasma were linear over the concentration range 0.125 to 50 μg/ml with a lower limit of quantification of 0.125 μg/ml. The extraction recovery of OddC and ddC was greater than 89%. The precision of the analytical methodology, assessed by intra- and interday relative standard deviations at low, medium and high concentrations of OddC, were less than 10%, and the accuracy of the assay was greater than 92%.

Plasma OddC concentration versus time curves following i.v. and p.o. administration of 10, 25, and 50 mg/kg to rats are shown in Fig. 3. Following i.v. administration, plasma concentrations of OddC declined rapidly in a biexponential manner and fell below the limit of quantification in samples obtained 8 h after drug administration. OddC was slowly absorbed after p.o. administration with maximum plasma OddC concentrations of approximately 1.5, 2, and 4 μg/ml after administration of 10, 25 and 50 mg/kg, respectively. The plasma concentrations of OddC obtained after i.v. and p.o. administration of the three doses were greater than the IC_{50} values [6] of the compound against nasopharyngeal, prostate, and hepatocellular carcinomas and against leukemia (IC_{50} ranging from 0.005 to 0.023 μg/ml).

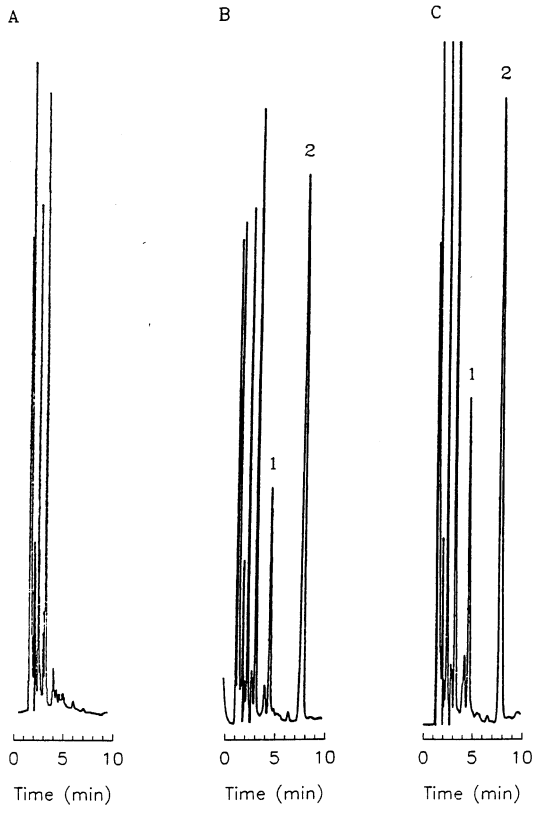

Fig. 2A–C Chromatograms for OddC (*1*) and ddC internal standard, (*2*) obtained from **(A)** blank rat plasma, **(B)** blank rat plasma spiked with OddC, and **(C)** a rat plasma sample obtained after i.v. administration of 25 mg/kg of OddC

The pharmacokinetic parameters for OddC derived from area-moment analysis are shown in Table 1. Following i.v. administration, the AUC of OddC increased proportionally with dose, and there were no statistically significant differences in other parameters as a function of dose. Thus, the pharmacokinetics of OddC in rats were independent of concentration over the dose range 10 to 50 mg/kg. The $t_{1/2}$ averaged 1.65 ± 1.12 h. The CL_T of OddC was moderate, averaging 1.38 ± 0.62 l/h per kg. The proportion of the dose of OddC recovered unchanged in the urine was $22 \pm 11\%$ and CL_R was 0.30 ± 0.16 l/h per kg. The CL_R of OddC was approximately equal to the glomerular filtration rate (0.27 l/h per kg) in rats [11]. The hydrophilicity of the compound probably precludes tubular reabsorption. Therefore, the low CL_R of OddC suggests that, unlike most nucleoside analogs [1, 2, 4, 7, 12, 15], the compound does not appear to undergo extensive active tubular secretion. CL_{NR} was the prevalent method of elimination for OddC, approximating 78% of total clearance. The CL_{NR}, averaging 1.08 ± 0.59 l/h per kg, was moderate compared to hepatic blood flow (2.9 l/h per kg) in rats [5].

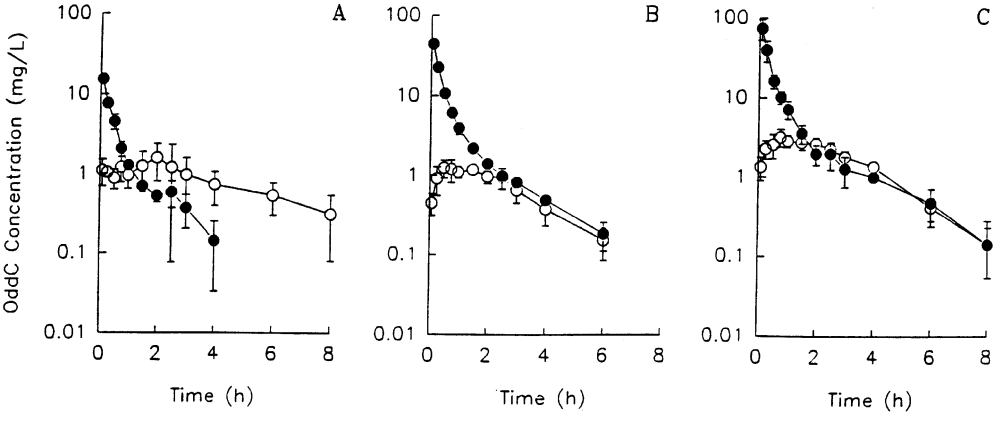

Fig. 3A–C Mean (SE) plasma concentrations of OddC as a function of time following i.v. (●) or p.o. (○) administration of **(A)** 10 mg/kg OddC, **(B)** 25 mg/kg OddC, and **(C)** 50 mg/kg OddC to rats

Table 1 Mean (SD) pharmacokinetic parameters after i.v. and p.o. administration of 10, 25, and 50 mg/kg of OddC to rats

Parameter	Dose (mg/kg)			
	10	25	50	Overall
$t_{1/2}$ (h)	1.60 (1.65)	1.73 (0.38)	1.61 (1.04)	1.65 (1.12)
V_{ss} (l/kg)	1.51 (0.97)	1.26 (0.34)	1.48 (0.46)	1.42 (0.66)
f_e	0.29 (0.14)	0.24 (0.12)	0.15 (0.07)	0.22 (0.11)
CL_T (l/h/kg)	1.29 (0.32)	1.21 (0.31)	1.73 (1.14)	1.38 (0.62)
CL_R (l/h/kg)	0.36 (0.16)	0.28 (0.10)	0.25 (0.16)	0.30 (0.14)
CL_{NR} (l/h/kg)	0.93 (0.32)	0.94 (0.32)	1.49 (1.0)	1.08 (0.59)
F (AUC_{po}/AUC_{iv})	0.50 (0.40)	0.24 (0.06)	0.40 (0.21)	0.37 (0.25)

The V_{ss} of OddC was 1.42 ± 0.66 l/kg. This is two-fold greater than total body water (0.70 l/kg) in rats [5], and indicates intracellular distribution and perhaps intracellular entrapment of the active phosphate metabolites. Indeed, studies have shown that OddC undergoes extensive phosphorylation and is incorporated into chromosomal DNA with high levels of the di- and triphosphate forms present in vitro [6]. This is of particular interest since OddC is being evaluated as a potential anticancer agent.

Absorption of OddC following oral administration was variable. The value of F of OddC varied greatly between individual rats, ranging from 18 to 100% for the three dosage levels. Bioavailability calculated from the OddC AUC averaged 37 ± 25% and ranged from 18 to 100% following oral administration of 10 mg/kg OddC, from 18 to 34% after 25 mg/kg, and from 22 to 70% after 50 mg/kg OddC. Bioavailabilty determined from urinary excretion data was also variable, averaging 41 ± 27%. There were no statistically significant differences in the F value as a function of dose regardless of method of calculation.

In summary, the pharmacokinetics of OddC in rats were determined to be independent of concentration over the dose range 10 to 50 mg/kg. CL_T was moderate with CL_{NR} being the predominant method of elimination. The V_{ss} indicated that intracellular distribution of OddC is probable. Absorption after p.o. administration was slow and bioavailability varied greatly between individual animals.

Acknowledgements This work was supported in part by Public Health Service grants AI-32351 and AI-25899 from the National Institutes of Health.

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