Absolute Bioavailability and Disposition of (−) and (+) 2′-Deoxy-3′-Oxa-4′-Thiocytidine (dOTC) following Single Intravenous and Oral Doses of Racemic dOTC in Humans

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The purpose of this study was to characterize the pharmacokinetics and determine the absolute bioavailability of 2′-deoxy-3′-oxa-4′-thiocytidine (dOTC) (BCH-10652), a novel nucleoside analogue reverse transcriptase inhibitor, in humans. dOTC belongs to the 4′-thio heterosubstituted class of compounds and is a 1:1 mixture of its two enantiomers, (−) and (+) dOTC. Twelve healthy adult male volunteers each received oral (800-mg) and intravenous (100-mg) doses of dOTC in two study periods separated by at least 7 days. Sixteen plasma samples were obtained over 72 h and assayed for (−) and (+) dOTC, and the resultant data fit by candidate pharmacokinetic models. Data were weighted by the fitted inverse of the observation variance; model discrimination was by AIC. The pharmacokinetic model was a linear, three compartment model, with absorption occurring during one to three first-order input phases, each following a fitted lag time. The model goodness-of-fit was excellent; r² ranged from 0.995 to 1.0. The mean absolute bioavailabilities of (+) and (−) dOTC were 77.2% (coefficient of variation [given as a percentage] [CV%], 14) and 80.7% (CV%, 15), respectively. The median steady-state volume of distribution for (+) dOTC, 74.7 (CV%, 19.2) liters/65 kg, was greater than that for (−) dOTC, 51.7 (CV%, 16.7) liters/65 kg (P < 0.05). The median total clearance of (+) dOTC was less than that of (−) dOTC, 11.7 (CV%, 17.3) versus 15.4 (CV%, 18.6) liters/h/65 kg, respectively (P < 0.05). The inter-subject variability of these parameters was very low. The median terminal half-life of (+) dOTC was 18.0 (CV%, 31.5) h, significantly longer than the 6.8 (CV%, 69.9) h observed for (−) dOTC (P < 0.01). No serious adverse events were reported during the study. These results suggest that dOTC is well absorbed, widely distributed, and well tolerated. The terminal half-lives indicate that dosing intervals of 12 to 24 h would be reasonable.

Significant progress has been made in the ability to suppress human immunodeficiency virus (HIV) replication, which has led to widespread optimism in treating individuals infected with the HIV virus. However, because of drug toxicity (13, 14, 16) and the lack of a durable response (12), there is clearly a need for new compounds. Especially needed are compounds with activity against HIV isolates that are resistant to currently available therapies and compounds with beneficial pharmacokinetic profiles that allow infrequent dosing and a decreased pill burden.

The nucleoside analogue reverse transcriptase inhibitors continue to be important drugs in regimens aimed at controlling HIV replication. These drugs are generally well tolerated and are important components of combination antiretroviral regimens. 2′-Deoxy-3′-oxa-4′-thiocytidine (dOTC) (BCH-10652) is a new nucleoside belonging to the 4′-thio heterosubstituted class of nucleoside analogs and is a racemic mixture of two enantiomers (Fig. 1). Both enantiomers, (−) dOTC and (+) dOTC, exhibit activity against the HIV type 1 (HIV-1) virus, with a mean 50% inhibitory concentration of 1.76 μM for wild-type clinical isolates and of approximately 2.5 μM for clinical isolates resistant to lamivudine and azidothymidine (6). dOTC has also shown activity against clinical isolates that are resistant to lamivudine, zidovudine, saquinavir, and indinavir (J. Bedard, T. Bowlin, M. Wainberg, T. Mansour, S. Tyns, P. Williams, D. Taylor, and C. Fortier, Abstr. 12th World AIDS Conf. July 1998, abstr. 12, 1998).

dOTC used in combination with other agents in antiretroviral naïve or experienced patients is therefore expected to represent an important advance in HIV therapy. The purpose of the present study was to characterize the pharmacokinetics and absolute bioavailability of the enantiomers of dOTC in healthy, adult male volunteers.

MATERIALS AND METHODS

The study protocol was approved by the Millard Fillmore Health Systems Institutional Review Board (Buffalo, N.Y.), and written informed consent was obtained for each subject prior to participation in the study. Oral and intravenous dOTC were supplied by BioChem Pharma Inc. (Laval, Canada).

Study population. Subjects were healthy male nonsmokers between 18 and 50 years of age, each weighing ≥50 kg, with the weight being within 15% of the ideal

![FIG. 1. Molecular structure of dOTC. Asterisk denotes chiral carbon that forms the (−) and (+) enantiomers of dOTC.](image-url)

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FIG. 2. Mean concentration versus time profiles for $(-)$ and $(+)$ dOTC following 800-mg oral (A) and 100-mg intravenous (B) doses of dOTC.
body weight. Exclusion criteria included the following: a clinically relevant abnormality identified during the screening physical or laboratory examination; history of significant cardiac, renal, hepatic, neurologic, or hematologic abnormality; a history of alcohol or drug abuse within 6 months of the study; treatment with an investigational drug within 30 days prior to the first study session; use of prescription or nonprescription drugs (including vitamins and acetaminophen) within 1 week prior to or during the study; and donation of blood within 60 days prior to the first dose of study medication.

Study design. This was a randomized, open-label, two-period crossover study. The subjects, who had fasted, received, in random order, 800 mg of dOTC orally (four 200-mg hard gelatin capsules) or 100 mg of dOTC by a 30-min intravenous infusion. The oral capsules consisted of a mixture of two crystalline forms, with rapid but slightly different in vitro dissolution rates. All subjects participated in both study periods, each separated by at least a 7-day washout. Prior to dosing, a 12-lead echocardiogram (ECG) was done, vital signs were observed, and blood and urine samples were collected for safety monitoring. In both study periods, the subjects were maintained in the fasted state for 10 h prior to the administration of dOTC. The subjects did not receive food or drink for 5 h after the administration of the dose. Meals were consumed at 5 and 10 h after dosing, and the subjects did not receive caffeine- or grapefruit-containing beverages for the duration of the study period. For the oral doses, dOTC was administered with 240 ml of tepid water, and blood samples (5 ml) were drawn just prior to dosing (time zero) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, and 72 h after dosing for analysis of (−) and (+) dOTC concentrations in plasma. Intravenous doses were administered over 30 min by electronic infusion pump, and blood samples were drawn prior to the start of the infusion (time zero), immediately after the termination of the infusion (at 0.5 h), and at 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, and 72 h after the start of the infusion. Blood samples were immediately separated at 4°C by centrifugation and stored at −20°C until analyzed.

For measurement of drug concentrations, (−) and (+) dOTC were extracted from human plasma using a solid-phase extraction cartridge. Plasma drug concentrations of each enantiomer were assayed by a reverse-phase high-performance liquid chromatography method with UV detection, using 2',3'-dideoxy-cytidine as an internal standard. The internal standard, (−) dOTC, and (+) dOTC had column retention times of approximately 15.0, 20.1, and 21.5 min, respectively. For assay accuracy, (−) dOTC had a coefficient of variation (given as a percentage) (CV%) range of 2.4 to 4.5 for interassay variability and 1.0 to

![FIG. 3. Example of a fit of the pharmacokinetic model to the data of an individual subject, for comodelling of intravenous and oral data of (+) dOTC (A) and (−) dOTC (B). Circles, post-oral dose plasma drug concentrations; squares, post-intravenous dose plasma drug concentrations. The solid lines associated with each are the predicted concentrations of the pharmacokinetic model.](http://aac.asm.org/)
FIG. 4. Three-compartment pharmacokinetic model for comodelling intravenous and oral administration of dOTC. The model describes the disposition of both (−) and (+) dOTC when dOTC is administered. Intravenous doses are infused into the central compartment; oral doses are bolused into the absorptive site. Abbreviations: A, site of absorption into which oral doses are administered; $k_a$, rate constants of oral absorption; $T_{lag}$, associated lag times describing oral absorption; $D\%$, percentage of the total administered dose absorbed in each phase of oral absorption; $V_c$, volume of the central compartment; $V_{pf}$, volume of the fast equilibrating peripheral compartment; $V_{ps}$, volume of the slow equilibrating peripheral compartment; $CL_{df}$, distributional clearance between the central and fast equilibrating peripheral compartments; $CL_{ds}$, distributional clearance between the central and slow equilibrating peripheral compartments; $CL_t$, drug clearance out of the central compartment. (−) and (+) symbols refer to the (−) and (+) stereoisomers of dOTC, respectively.
The terminal half-life is difficult to determine if the intravenous dose is soon, due to a relatively small intravenous dose. Thus, the terminal profiles because the assay limit of detection is reached too slowly, terminal, log-linear elimination phase. This terminal compartment. This distributional behavior is followed by a slow distributional phase half-life; $\lambda_{1,2}$, terminal elimination half-life; $T_{\text{max}}$, time to maximum concentration of drug in serum.

**Parsimony** (9) and Akaike's Information Criterion (1). In all analyses, the two enantiomers, (+) and (-) dOTC, and oral and intravenous data were comodeled (fit simultaneously) for each individual subject. For each subject, it was assumed that dOTC disposition did not differ between the oral and intravenous study periods. Weighting was by the fitted inverse of the residual (observation) variance; standard deviation was assumed to be linear with drug concentration. The maximum concentration ($C_{\text{max}}$) and the time to maximum concentration of drug in serum ($T_{\text{max}}$) were determined by graphical inspection.

**Statistical procedures.** Apparent distribution volumes, drug clearances, $C_{\text{max}}$, and $T_{\text{max}}$ for (+) dOTC, the median $r^2$ was 0.997, with a range of 0.990 to 1.00; for intravenous (-) dOTC, the median $r^2$ was 0.997, with a range of 0.990 to 1.00. The profiles following oral doses show multiple peaks and other changes in slope during the oral absorptive phase (Fig. 3). Possible explanations for this behavior include enterohepatic or other types of recycling, or oral absorption proceeding in phases. In the postabsorptive phase, the oral profiles suggest that at least two or more, or compartments, would be needed to fit the data. The two distributive phases (fast and slow) seen in the data following an intravenous dose are less obvious in the oral data. This is because fast and slow distribution is proceeding simultaneously with oral absorption. Because the oral doses were larger than the intravenous doses, the terminal half-lives are better estimated in the oral data sets. Therefore, the intravenous data provided the most information about the distribution processes (slow and fast distributional phases), and the oral data were most informative about the terminal elimination phase. As a result, the oral and intravenous data were comodeled in each subject to allow for the most accurate assessment of all pharmacokinetic parameters.

The final pharmacokinetic model (Fig. 4) contained three compartments with oral drug bolused into an absorption site from which it is released in one to three phases, each having a separate absorptive lag time ($T_{\text{abs}}$), first-order rate constant of absorption ($k_{\text{a}}$) and percent total bioavailable dose released ($D\%$). The sum of $D\%$ were required to total 100%, and the model was constructed to allow the absorptive parameters to differ between enantiomers. Comodelling these two study periods enabled the identification of $F$, the absolute bioavailability, as one of the fitted parameters. The fitted bioavailability for (+) dOTC was allowed to differ from that of (-) dOTC.

The fit of the model to the data was excellent: for oral (+) dOTC, the median coefficient of determination ($r^2$) was 0.997, with a range of 0.987 to 1.00; for oral (-) dOTC, the median $r^2$ was 0.997, with a range of 0.990 to 1.00; for intravenous (+) dOTC, the median $r^2$ was 0.995, with a range of 0.992 to 0.999; and for intravenous (-) dOTC, the median $r^2$ was 0.995, with a range of 0.989 to 0.999. Ten of the 12 subjects required three absorption phases, and two subjects required two phases to fit the observed data. Although the model was constructed to allow absorption characteristics to differ between enantiomers, all 12 of the subjects were successfully modelled as having similar absorption characteristics for each enantiomer. An example of an individual subject fit is provided in Fig. 3.

### RESULTS

The 12 male study volunteers had a mean (CV% given in the parentheses) age of 29.5 (29.9) years, a mean weight of 80.3 (10.4) kg, and a mean serum creatinine level of 0.95 (21.3) mg/dl. No serious adverse events were noted in the study, and no significant changes in ECG results, vital signs, or clinical laboratory values were observed. The most commonly reported adverse events were gastrointestinal complaints and headache. dOTC was well tolerated following both intravenous and oral administration.

Figure 2 displays the mean logarithmic concentration-time profiles across all subjects after oral and intravenous doses. Inspection of individual concentration-time profiles revealed several features that the pharmacokinetic model had to accommodate. The profiles following intravenous administration show three decay phases. The first two are fast and slow distributional phases, representing equilibration between the central plasma compartment and fast and slow peripheral tissue compartments. This distributional behavior is followed by a slower, terminal, log-linear elimination phase. This terminal elimination phase is poorly discerned in most of the intravenous profiles because the assay limit of detection is reached too soon, due to a relatively small intravenous dose. Thus, the terminal half-life is difficult to determine if the intravenous study periods are considered alone.

### TABLE 1. Pharmacokinetic parameters of (–) and (+) dOTC

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>$V_a$ (liters/65 kg)</th>
<th>$CL_s$ (liters/h/65 kg)</th>
<th>$\lambda_{1,2}$ (h)</th>
<th>$\lambda_{2,1}$ (h)</th>
<th>$\lambda_{1,2}$ (h)</th>
<th>$C_{\text{max}}$ (µg/ml/65 kg)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$CL_{\text{ef}}$ (liters/h/65 kg)</th>
<th>$CL_{\text{kw}}$ (liters/h/65 kg)</th>
<th>$F$</th>
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<tbody>
<tr>
<td>(+) dOTC</td>
<td>74.7</td>
<td>11.7</td>
<td>0.21</td>
<td>2.49</td>
<td>18.0</td>
<td>4.7</td>
<td>1.4</td>
<td>19.3</td>
<td>1.63</td>
<td>76.3</td>
</tr>
<tr>
<td>CV%</td>
<td>19.2</td>
<td>17.3</td>
<td>56.6</td>
<td>15.7</td>
<td>31.5</td>
<td>23.7</td>
<td>44.6</td>
<td>34.5</td>
<td>22.8</td>
<td>13.6</td>
</tr>
<tr>
<td>(–) dOTC</td>
<td>51.7</td>
<td>15.4</td>
<td>0.19</td>
<td>2.03</td>
<td>6.80</td>
<td>4.2</td>
<td>1.3</td>
<td>23.6</td>
<td>1.58</td>
<td>78.2</td>
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<tr>
<td>Mean</td>
<td>54.8</td>
<td>15.3</td>
<td>0.28</td>
<td>2.15</td>
<td>8.92</td>
<td>4.4</td>
<td>1.2</td>
<td>22.5</td>
<td>1.56</td>
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<tr>
<td>CV%</td>
<td>16.7</td>
<td>18.6</td>
<td>62.7</td>
<td>24.4</td>
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<td>25.8</td>
<td>47.8</td>
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$\lambda_{1,2}$, fast distributional phase half-life; $\lambda_{2,1}$, slow distributional phase half-life; $\lambda_{1,2}$, terminal elimination half-life; $T_{\text{max}}$, time to maximum concentration of drug in serum.

4.2 for intraassay variability. For (+) dOTC, the CV% range was between 2.4 and 3.9 for interassay variability and between 1.3 and 2.7 for intraassay variability. The lower limit of quantitation for both enantiomers was 3.0 ng/ml, and no interference from endogenous human plasma components was found. A linear response was obtained over the range of 3.0 to 1,000 ng/ml for both enantiomers. Concentrations above 1,000 ng/ml were diluted to obtain a concentration within the linear portion of the calibration curve and reanalyzed. Quantitation was performed using the peak height ratio method, and samples were assayed in randomized order.
dOTC were well absorbed, with the fitted absolute bioavailability ranging from 60 to 100% and 65 to 105%, respectively. The bioavailability estimates were similar to those obtained by noncompartmental methods (data not shown).

Statistical comparisons demonstrated significant differences between the pharmacokinetic parameters of the two enantiomers. When (+) was compared to (−) dOTC, median $V_{ss}$ (74.7 versus 51.7 liters/65 kg, $P < 0.001$), $C_L$ (11.7 versus 15.4 liters/h/65 kg, $P < 0.001$), terminal half-life (18.0 versus 6.80 h, $P < 0.001$) and $F$ (76.3 versus 78.2%, $P = 0.021$), all differed significantly. The magnitude of difference in $F$ is unlikely to be of any practical importance. The results of this study are consistent with a previously conducted single-oral dose, dose-rising study of healthy adult volunteers (P. F. Smith, C. H. Ballow, A. Forrest, D. E. Martin, C. Fortier, and L. Proulx, Abstr. 6th Conf. Retrovir. Opportun. Infect., abstr. 596, 1999).

**DISCUSSION**

The plasma pharmacokinetics of (+) and (−) dOTC following intravenous and oral administration of dOTC were well described by a linear, three-compartment model, with elimination from the central compartment. Data after single oral doses showed multiple peaks and other changes in slope. This was well described by the incorporation of two or three absorption phases, in which a portion of the dose was released and absorbed, each with a separate lag time and absorption rate constant. These observations necessitated comodelling of intravenous and oral data to obtain the most valid estimates of the pharmacokinetic parameters.

The complex absorption behavior observed with dOTC may be due to either enterohepatic recycling of drug or oral absorption in phases. Enterohepatic recycling appears to be less likely, as this phenomenon was not observed with the intravenous doses. These multiple peaks also did not appear to correlate with the administration of food, as subjects remained fasted until at least 5 h after administration of the dose and the majority of multiple peaks were all seen within this time frame. The majority of drugs that do undergo enterohepatic recycling are polar molecules, with molecular masses in excess of 500 kDa. dOTC, while being a polar compound, has a small molecular mass (229 kDa) and therefore would not be considered a likely candidate to be enterohepatically recirculated. Nucleoside analogue reverse transcriptase inhibitors are not known, as a drug class, to undergo this process.

The long plasma half-life suggests that dOTC could be administered once or twice daily, pending clinical efficacy data, which would promote patient adherence with complicated drug regimens. Poor adherence has been associated with the development of viral resistance and therapeutic failure (3).

The absolute bioavailability of a nucleoside analogue is important to ensure adequate plasma drug concentrations following oral administration. dOTC was found to be well absorbed, with an absolute bioavailability of between 75 and 80% for both (−) and (+) dOTC. The intersubject variability of this parameter was also found to be small. This degree of absorption is similar to those found with other nucleoside analogues and is superior to those observed with agents such as didanosine and zidovudine, which have reported bioavailabilities of 45 and 63%, respectively (2, 7, 8, 10, 11, 15).

Achieving adequate plasma concentrations following oral absorption is an important characteristic of an effective nucleoside analogue reverse transcriptase inhibitor. It should be noted that the unbound concentration of drug in plasma and the intracellular concentration of phosphorylated drug are likely to be the most relevant considerations related to drug efficacy. In the absence of these measurements, total drug concentrations in plasma are representative of the amount of drug available to the intracellular compartment, and represent an as-yet-unidentified relationship between intracellular and plasma concentrations of dOTC. Similarly, because the site of drug action is within the cell, an observed plasma half-life may not equate to a long duration of drug action. Further studies to characterize the protein binding and intracellular pharmacokinetics of phosphorylated dOTC are underway.

Because of the limited number of drugs currently available to treat HIV infection, alternative agents for both empiric and salvage therapy are needed. Agents with long half-lives that can be dosed infrequently, and thereby improve adherence, are also desirable. The results of the present study demonstrated that dOTC is well tolerated following single oral doses of 800 mg and intravenous doses of 100 mg in healthy adult volunteers. The relatively long plasma half-lives support single daily or twice daily dosing intervals. Oral dosing achieved serum concentrations well above the in vitro 50% inhibitory concentrations reported for the HIV type 1 virus, suggesting that dOTC would be an effective agent in the treatment of HIV infection. Based on these virology studies and a beneficial pharmacokinetic profile, dOTC remains a promising compound that may offer an alternative in treating HIV infection.

**ACKNOWLEDGMENT**

This work was supported in part by a grant from BioChem Pharma Inc.

**REFERENCES**


**TABLE 2. Absorption characteristics of (−) and (+) dOTC**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
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<tr>
<td></td>
<td>$T_{lag}$ (h)</td>
<td>$k_a$ (h$^{-1}$)</td>
<td>D%</td>
<td>$T_{lag}$ (h)</td>
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<td>$n$</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Median</td>
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<td>1.03</td>
<td>31.4</td>
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<td>2.82</td>
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<tr>
<td>CV%</td>
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