Pharmacokinetics of netivudine, a potent anti-varicella zoster virus drug, in patients with renal impairment

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The pharmacokinetics of a single oral 200 mg dose of netivudine (1-β-D-arabinofuranosyl)-5-(1-propynyl)uracil), a nucleoside analogue under development for use in varicella zoster virus infections, were studied in 12 renal failure (RF) subjects (creatinine clearance 15 ± 7 mL/min) and 12 age-matched healthy subjects with normal creatinine clearance. Blood and urine samples were collected up to nine days after drug administration. Concentrations of netivudine and of its main metabolite, the pyrimidine base 5-(1-propynyl)uracil (5 PU), were determined by a specific high performance liquid chromatography assay. The mean peak plasma concentrations of netivudine, \( C_{\text{max}} \), and volume of distribution were not significantly affected by RF. The elimination half-life of netivudine was approximately 15 h in subjects with normal renal function and 60 h in RF patients. Plasma and renal clearances of netivudine were significantly reduced in RF patients and AUC was three to four times higher in these patients. \( C_{\text{max}} \) and AUC of 5 PU were higher in RF patients, and the half-life was also significantly longer. However, the half-life of this metabolite was much lower than that of the parent compound. \( T_{\text{max}} \) and the lag time were similar in the two groups. There were highly significant correlations for netivudine and 5 PU between half-life and creatinine clearance and between renal clearance and creatinine clearance. These findings suggest that netivudine dosage may need to be reduced in patients with severe renal failure, and confirm that formation of the 5 PU is independent of the elimination of netivudine from plasma.

Introduction

Varicella zoster virus (VZV) infections can be severe with serious complications, especially in the immuno-compromised including transplanted patients. Netivudine or 882C87 (1-β-D-arabinofuranosyl)-5-(1-propynyl)uracil) (Figure 1) is a selective inhibitor of VZV with more potent activity than acyclovir: the development of netivudine has presently been discontinued. The I\( C_{50} \) values, measured by the inhibition of viral plaque formation in human MRC-5 cells infected with VZV strain G-31, are 0.4-4.1 and 10.2-17.6 \( \mu M \) for netivudine and acyclovir, respectively (Rahim et al., 1992; Purifoy et al., 1993).
Following a single oral dose of 200 mg netivudine in healthy volunteers, the peak plasma drug concentration was 9 \mu M and plasma clearance, uncorrected for bioavailability, was 0.8 mL/min/kg. The mean terminal phase elimination half-life of netivudine in plasma (T_{1/2}) was 12–13 h. The major metabolite was 5-propynyluracil (5 PU), the pyrimidine base, with a peak plasma concentration of approximately one-third that of netivudine occurring about 24 h post-dose. There was a lag-time of 5–12 h before 5 PU was detected in plasma after oral dosing (Peck et al., 1995a).

After administration of oral \textsuperscript{14}C-netivudine most of the radioactivity was recovered in urine as 5 PU or parent drug (Peck et al., 1995b). Absolute oral bioavailability, determined by comparison with iv dosing was 20–25%, total and renal clearance were 0.17 and 0.14 mL/min/kg, volume of distribution was 0.17 L/kg, half-life was 11.8 h, and after iv dosing 70–80% of the dose was recovered as unchanged netivudine in urine (Peck et al., 1995b). After oral dosing 17% of the dose was recovered as netivudine in urine with 45% recovered in urine as 5 PU. Since renal elimination is important for both the parent drug and metabolite, we performed this study to determine the pharmacokinetics of netivudine and 5 PU following a 200 mg oral dose in patients with renal failure.

\textbf{Materials and Methods}

Netivudine was supplied by Pharmaceutical Development Laboratories Wellcome, Beckenham UK, in the form of a 200 mg tablet.

\textbf{Patients}

The study was conducted in 12 patients with renal failure (RF) defined by a 24 h urinary creatinine clearance (Cl_{ur}) <30 mL/min and 12 age and sex matched healthy volunteers with a normal creatinine clearance. All gave written informed consent to participate in the study and the protocol was approved by the Human Investigation Committee of the University of Rouen, School of Medicine. Each subject was enrolled on the basis of the medical history, a physical examination, an electrocardiogram and clinical laboratory tests (haematology, blood chemistry and urinalysis). All patients had stable renal function during the study, and RF patients were included only if their Cl_{ur} had been stable during the previous 6 months. The patients had no history of allergy, intolerance to drugs, or cardiac, liver or gastrointestinal diseases. Patients with alcohol or drug abuse, nephrotic syndrome or acute renal failure were excluded. Patients were also ineligible if they were taking phenytoin, phenobarbitone, rifampicin, antacids or calcium salts.

\textbf{Study design}

Subjects were asked to abstain from alcohol for 24 h before dosing and to fast from 10.00 p.m. the night before. All subjects remained supine and refrained from eating until the 4 h blood sample was collected after which a light lunch was served. Regular meals were resumed 8 h after dosing. Subjects remained under close observation in the study unit throughout, and no smoking or consumption of alcohol containing drinks were permitted. On the morning of the study day, each patient or volunteer received a 200 mg netivudine tablet which was taken with 120 mL water.
Pharmacokinetics of netivudine

In the healthy volunteers, blood samples (5 mL) for drug assay were taken pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 18, 24, 34, 48, 72 and 96 h. Urine was collected over the periods 0-4, 4-8, 8-12, 12-24, 24-48, 48-72 and 72-96 h after dosing. In RF patients, additional blood samples were taken for drug assay at 168 and 216 h and the 34 h sample was omitted; urine was additionally collected up to 168 h as 24 h collections, with a final collection from 168-216 h after dosing. Plasma was separated and stored in plain tubes at —20°C until analysis. The volume of each urine collection was determined by weighing, and a 10 mL aliquot was stored at —20°C until analysis. Blood samples were taken for full blood count and biochemical profile pre-dose and 48 h after dosing. Pulse and blood pressure were monitored up to 48 h after dosing.

In both healthy volunteers and RF patients 24 h urinary creatinine clearance was calculated using the 24-48 h urine collection and the 48 h plasma creatinine value.

Concentrations of netivudine and 5 PU

Concentrations of netivudine and 5 PU in plasma and urine were determined by a specific, sensitive and reproducible high performance liquid chromatography (HPLC) method with UV detection at 290 min (Buick & Fook-Sheung, 1993). The assay had a lower limit of sensitivity of 0.2 μM for netivudine and 0.4 μM for 5 PU.

Urine assays were performed using a modification of the automated sequential trace enrichment of dialysates HPLC procedure with UV detection (Cooper et al., 1993). The lower limit of sensitivity was 2.5 μM for both netivudine and 5 PU.

Measurement of protein binding of netivudine and 5 PU

Samples for protein binding were defrosted at room temperature and vortex mixed for 30 sec or until a homogenous suspension was achieved. One millilitre of plasma was transferred to a centrifree tube which was centrifuged at 2000 x g for 40 min. The filtrate collected in the bucket of the centrifree tube (Veinoject: Becton Dickinson, Rungis, France) was transferred to 0.5 mL plastic tubes, and the plasma retained in the upper chamber of the tube was removed by glass pipette to separate plastic tubes. All samples, filtrates, retentates and remaining whole plasma were returned to the —80°C freezer until analysis of netivudine or 5 PU concentrations. To calculate the percentage of bound drug, the following equation was used:

\[
\% \text{ bound drug} = \frac{\text{concentration of netivudine in whole plasma}}{- \text{concentration of netivudine in filtrate}} \times 100.
\]

Pharmacokinetic analysis

Model-independent plasma pharmacokinetic parameters for netivudine after oral dosing were determined from the data using a pharmacokinetic analysis software package (Siphar; Simed, Creteil, France). \( C_{\text{max}} \), the maximum plasma drug concentration and \( T_{\text{max}} \), the time to maximum drug concentration were taken directly from the measured values. AUC was calculated using the linear trapezoidal rule up to the last measured concentration, \( Ct \) and extrapolated to infinity from \( Ct/\lambda_s \). \( \lambda_s \), the terminal rate constant, was obtained by peeling the logarithm of the concentration-time
profile from 12 h to the last measurable concentration (96 or 216 h for healthy and RF subjects, respectively). The terminal half-life \( (T_{1/2}) \) was calculated as \( 0.693/\lambda_z \), total apparent plasma clearance, \( Cl/f \), as Dose/AUC and volume of distribution, \( V_d/f \), as \( (Cl/f)/\lambda \) and, renal clearance, \( Cl \), as the amount excreted in urine/AUC. Mean residence time was determined using statistical moments.

5 PU \( C_{max}, T_{max}, Cl/f \) and \( V_d/f \) were determined using similar methods to those for netivudine. The number of points used to determine \( \lambda_z \) was decided by inspection. The lag time \( (T \text{ lag}) \), the time of the latest non-quantifiable concentration in the immediate post-dose period, was taken directly from the plasma concentration data.

**Statistical analysis**

\( C_{max}, T_{max}, Cl, MRT, \) urinary recovery, \( V_d/f, Cl/f \) and lag-time were compared between groups. All parameters, except \( T_{max} \), lag-time and urinary recovery were log-transformed before analysis and were subjected to analysis of variance, taking into account sources of variation due to subject, group and age. Means and differences between means were back-transformed to give estimates on the natural scale. Urinary recovery, expressed as a percentage of the dose, was subjected to an analysis of variance without prior transformation, and means, differences in means and associated 95% confidence intervals were calculated. Differences in median \( T_{max} \) and \( T \text{ lag} \) were

![Chemical Structures](https://example.com/chemical_structures.png)

**Figure 1.** Chemical structures of netivudine and of the main metabolite 5 PU. Netivudine triphosphate is the active form of the drug.
estimated using the Wilcoxon signed rank test, except that for 5 PU it was inappropriate to determine estimates of differences between subject groups, since $T_{\text{max}}$ was observed late after dosing when sampling was infrequent. Instead, the number of subjects with $T_{\text{max}}$ values at each scheduled time was recorded by subject group. The relationship between $Cl_{\text{r}}$ and creatinine clearance was investigated by linear regression.

**Results**

All subjects completed the study according to the protocol. There were no clinically significant changes in pulse, blood pressure, full blood counts, plasma biochemistry or urinalysis. The only adverse experience reported was diarrhoea in I subject. It was mild, not serious and not considered likely to be attributable to netivudine administration.

RF subjects included ten males. Mean ($\pm$SD) age, height and weight were $64.0 \pm 6.6$ years, $168 \pm 7.5$ cm, $76 \pm 12.8$ kg, respectively. Mean $Cl_{\text{r}}$ at study entry was $15.1 \pm 7.3$ mL/min (range 9.0-32.0). Twelve age and sex matched healthy volunteers were recruited of mean age, height and weight of $61 \pm 5.9$ years, $171 \pm 7.8$ cm and $72 \pm 9.5$ kg, respectively. The mean $Cl_{\text{r}}$ of the healthy volunteers was $99.2 \pm 26.3$ mL/min (53.3-133.7). All subjects were Caucasian.
Mean plasma concentrations of netivudine (Figure 2(a)) and 5 PU (Figure 2b) were generally higher in RF patients than in healthy volunteers, although $C_{\text{max}}$ for netivudine was not significantly different (Table I). Mean values for $Cl/f$ and $Cl$, of netivudine in the RF group were about one-third of those in the healthy controls, and $Vd/f$ was slightly higher contributing to the almost four-fold increase in $T_{1/2}$ and MRT in RF patients. Interval renal clearances showed no time or concentrations related trends. There was a highly significant correlation between renal clearance of netivudine and $Cl_a$ (Figure 3).

Mean AUC for 5 PU was more than two-fold higher in the RF group and $T_{\text{max}}$ was about twice that in healthy controls (Table II). The shape of the plasma concentration profiles were similar in the two groups with $T_{\text{max}}$ occurring between 24 and 34 h in most subjects (median = 29.0 h). Renal clearance and urinary recovery of 5 PU were significantly reduced in RF patients, and there was a highly significant correlation between $Cl$, of 5 PU and $Cl_a$ (Figure 4).

Protein binding of netivudine and 5 PU was determined 4, 48 and 72 h after dosing. There were no apparent differences in protein binding of netivudine. Protein binding was 91–98% except in one patient with a mean value of 48%. The protein binding for 5 PU was highly variable with values from 14% to >95%.

Discussion

Plasma concentrations of netivudine in the healthy volunteers in this study were higher than those previously found in young adult volunteers but lower than those in the healthy elderly (Wood et al., 1993; Peck et al., 1995a). The mean age of our healthy volunteers was 61 years (range 53–72) and their values of creatinine clearance were intermediate between those of the young and elderly in the previous studies. Renal clearance of unchanged netivudine is known to be its main route of elimination, with 70–80% of an iv dose recovered in urine (Peck et al., 1995b). Consequently, the observed changes in netivudine pharmacokinetics in RF are expected with significant increases in AUC, $T_{1/2}$ and MRT, and correspondingly lower $Cl$, and $Cl/f$. The changes in $Cl$, are approximately proportional to $Cl_a$. Since not all of an iv dose of netivudine is recovered unchanged in urine there must be some non-renal clearance. This probably becomes relatively more important as renal function declines, which is compatible with the reduced urinary recovery of unchanged netivudine in RF subjects. Alternatively, reduced urinary netivudine recovery could be due to reduced absorption. This would also tend to increase the values of $Vd/f$ and $Cl/f$, possibly masking the effects of a true reduction in clearance. However, $Vd/f$ was not significantly higher in RF patients, and, although the reduction in $Cl/f$ was proportionally less than that of $Cl$, it is more likely this represents the effects of increased importance of non-renal clearance rather than a significant fall in bioavailability.

The concentration-time profile of netivudine in RF patients shows multiple peaks over the first 24–48 h. The lack of similar multiple peaks after iv administration of netivudine (Peck et al., 1995b) suggests that these are not due to biliary excretion and re-absorption of netivudine, and it is more likely that they are due to slow continued absorption of netivudine from the gastrointestinal tract.

The protein binding of netivudine in healthy volunteers has previously been reported as 88% (Peck et al., 1995a). In this study, protein binding of netivudine in RF patients was >84% in all but one and >91–98% in six patients out of nine. However, protein binding was not determined in the healthy control group, and it is not possible to draw
### Table 1. Pharmacokinetic parameters (geometric means) of netivudine in RF patients and healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ ($\mu M$)</th>
<th>$T_{\text{max}}^*$ (h)</th>
<th>AUC (µM.h)</th>
<th>$T_{1/2}$ (h)</th>
<th>$V_{d/f}$ (L)</th>
<th>$V_{d/f}$ (L/kg)</th>
<th>$Cl/f$ (mL/min)</th>
<th>$Cl/f$ (mL/min/kg)</th>
<th>$CLr$ (mL/min)</th>
<th>$CLr$ (mL/min/kg)</th>
<th>MRT (h)</th>
<th>% dose in urine</th>
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<td>Renal failure</td>
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<td>subjects</td>
<td>8.5</td>
<td>6.5</td>
<td>731</td>
<td>57.1</td>
<td>78.2</td>
<td>1.04</td>
<td>15.8</td>
<td>0.21</td>
<td>0.61</td>
<td>0.0081</td>
<td>84.5</td>
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<td>10.9</td>
<td>5.3</td>
<td>245</td>
<td>15.0</td>
<td>61.2</td>
<td>0.86</td>
<td>47.1</td>
<td>0.66</td>
<td>5.97</td>
<td>0.083</td>
<td>23.4</td>
<td>12.3</td>
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<tr>
<td>healthy</td>
<td>0.76</td>
<td>1*</td>
<td>2.88</td>
<td>3.84</td>
<td>1.33</td>
<td>0.35</td>
<td>0.09</td>
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<td></td>
<td>3.63</td>
<td>5.7*</td>
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<td>95% confidence</td>
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<tr>
<td>intervals</td>
<td>0.49–1.19</td>
<td>0–3</td>
<td>1.91–4.34</td>
<td>3.15–4.68</td>
<td>0.87–2.06</td>
<td>0.23–0.21</td>
<td>0.03–0.21</td>
<td></td>
<td></td>
<td></td>
<td>2.99–4.41</td>
<td>1.2–10.1</td>
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*Median values.

*Median differences.
any conclusions about changes in protein binding in RF. In the present study $Cl_e$ was approximately 6% of $Cl_a$ in RF patients. Since this is similar to the likely unbound fraction for netivudine, we suggest that renal clearance may be due mostly to glomerular filtration.

5 PU is probably formed within the lumen of the gastrointestinal tract from unabsorbed netivudine (Peck et al., 1995a,b). It is then absorbed and eliminated largely as unchanged drug in urine. Thus, the increases in $C_{max}$ and AUC with a corresponding reduction in $Cl$, as $Cl_a$ falls are as expected. The constant ratio of AUC for 5 PU to that of netivudine in the two groups suggests there is no major change in the extent of conversion of unabsorbed netivudine to 5 PU. The reduced recovery of 5 PU in RF patients is most likely, therefore, to be due to increased importance of non-renal clearance of 5 PU in such patients. In healthy volunteers, $T_{1/2}$ for 5 PU is similar to that of netivudine, which suggested formation-rate-limited pharmacokinetics. However, in RF, $T_{1/2}$ for 5 PU is much less than that of netivudine, which would be impossible if 5 PU formation is dependent upon netivudine elimination from the body. This confirms that the elimination of absorbed netivudine is independent of the formation of 5 PU from unabsorbed netivudine. In healthy volunteers the similarity of $T_{1/2}$ for 5 PU and netivudine is coincidental and does not mean that 5 PU pharmacokinetics are necessarily formation-rate-limited. The rate limiting step in 5 PU pharmacokinetics in subjects with normal renal function could be its formation from unabsorbed netivudine, which is known to be a slow process, its absorption or its elimination. This could only be established by a study of oral and iv administration of 5 PU.

In conclusion, a single oral dose of netivudine 200 mg was well tolerated in RF patients. $Cl/f$ and $Cl_e$ of netivudine and 5 PU were significantly lower in these patients with corresponding increases in plasma concentrations and half-lives. There were strong correlations between renal clearances of netivudine and 5 PU with $Cl_e$. The half-life of the main metabolite 5 PU was less than that of netivudine in RF patients confirming that 5 PU pharmacokinetics are not rate-limited by the elimination of netivudine from the body. If netivudine were to be given to patients with RF an approximately three-fold reduction in dose or an increase in dosing interval might be required.
Table II. Pharmacokinetics parameters (geometric means) of 5 PU in RF patients and healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>T_{eq}</th>
<th>C_{max}</th>
<th>T_{max}</th>
<th>AUC</th>
<th>T_{1/2}</th>
<th>Clr</th>
<th>Clr</th>
<th>MRT</th>
<th>% dose in urine</th>
<th>AUC 5PU/ AUC netivudine</th>
</tr>
</thead>
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<tr>
<td>Renal failure subjects</td>
<td>3.7</td>
<td>5.8</td>
<td>31.3</td>
<td>374</td>
<td>29.3</td>
<td>4.94</td>
<td>0.066</td>
<td>61.4</td>
<td>14.9</td>
<td>0.51</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>5.9</td>
<td>3.6</td>
<td>25.7</td>
<td>133</td>
<td>13.6</td>
<td>33.3</td>
<td>0.47</td>
<td>37.3</td>
<td>36.2</td>
<td>0</td>
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<tr>
<td>Mean ratio</td>
<td>1.68</td>
<td>2.98</td>
<td>2.19</td>
<td>0.14</td>
<td>1.67</td>
<td>22.8^*</td>
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<td>renal failure:healthy</td>
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<td>95% confidence intervals</td>
<td>1.19–2.36</td>
<td>2.02–4.40</td>
<td>1.75–2.75</td>
<td>0.10–0.21</td>
<td>1.39–2.00</td>
<td>11.5–34.0</td>
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*Median values.
*Median difference.
Figure 4. The relationship between creatinine clearance and renal clearance of 5 PU in healthy volunteers (▼) and renal failure patients (●) including the 95% confidence interval (—) for the fitted regression line (r² = 0.87, P < 0.001).

References


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