Plasma and intracellular exposure to ganciclovir in adult renal transplant recipients: is there an association with haematological toxicity?

Pierre-André Billat1–2*, Jean-Baptiste Woillard1–3, Marie Essig1,2,4, François-Ludovic Sauvage1,2, Nicolas Picard1–3, Sophie Alain5,6, Michael Neely7,8, Pierre Marquet1–3 and Franck Saint-Marcoux1–3

1Univ. Limoges, UMR_S 850, F-87000 Limoges, France; 2INSERM, U850, F-87000 Limoges, France; 3CHU Limoges, Service de Pharmacie, Toxiciologie et Pharmacovigilance, F-87000 Limoges, France; 4CHU Limoges, Service de Néphrologie, Dialyse et Transplantation, F-87000 Limoges, France; 5INSERM, U1092, F-87000 Limoges, France; 6CHU Limoges, Laboratoire de Bactériologie-Virologie-Hygienne, F-87000 Limoges, France; 7University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; 8Laboratory of Applied Pharmacokinetics and Bioinformatics (LAPKB), Children’s Hospital of Los Angeles, Los Angeles, CA, USA

*Corresponding author. Tel: +33-5-19-56-42-32; Fax: +33-5-55-43-59-36; E-mail: p.a@billat.org

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Objectives: Ganciclovir is the most widely used treatment for cytomegalovirus infections. However, neutropenia is a frequent associated adverse effect leading to a decrease in the ganciclovir dose or discontinuation of the therapy, thereby favouring viral resistance. In the present study, the objectives were: (i) to describe the pharmacokinetics of blood and intracellular ganciclovir and its metabolites; and (ii) to explore the relationship between exposure to ganciclovir and/or its metabolites and evolution of the neutrophil count under treatment.

Methods: Pharmacokinetic profiles (pre-dose and 1, 2, 3 and 5 h after dosing) of ganciclovir and its metabolites were measured in 22 adult renal transplant patients and further modelled by a non-parametric approach (PMetrics®). The relationship between exposure indices to ganciclovir and the slope of the neutrophil count was investigated using multiple linear regression.

Results: A four-compartment open model was able to accurately describe ganciclovir and its intracellular forms. A significant association was found between intracellular ganciclovir triphosphate concentrations (AUC0–5) and the decrease in neutrophil count over the first 3 months of treatment ($\beta = -0.0019 \pm 5 \times 10^{-4}; P<0.01$).

Conclusions: In this population of renal transplant patients, the decrease in neutrophil count, used as a surrogate marker of haematological toxicity, was associated with ganciclovir triphosphate accumulation in blood cells. Further studies are needed to test this biomarker as a predictive factor for toxicity.

Introduction

Cytomegalovirus (CMV) infection significantly increases the morbidity and mortality in solid organ transplant (SOT) patients. The two most used drugs for CMV prophylactic or curative treatment in SOT patients are ganciclovir and its prodrug valganciclovir. Because valganciclovir has better bioavailability, it is preferred for both prophylactic and pre-emptive therapies. Valganciclovir and ganciclovir pharmacodynamics are well understood. First, valganciclovir is hydrolysed into ganciclovir by intestinal and hepatic esterases. In CMV-infected cells, ganciclovir is then phosphorylated into ganciclovir monophosphate. It is known as the limiting step, because this first phosphorylation is largely dependent on the viral protein kinase, pUL97. Ganciclovir monophosphate is phosphorylated into ganciclovir triphosphate, which is the active form that incorporates into DNA.

The main side effect of ganciclovir therapy is haematological toxicity and particularly neutropenia. It can occur in up to 50% of patients given ganciclovir, leading to a decrease in the ganciclovir dose or discontinuation of the therapy, thereby favouring viral resistance. Some studies have explored the relationship between ganciclovir blood concentrations and the occurrence of neutropenia. In a study of 372 SOT patients, Wiltshire et al. only noted that higher ganciclovir exposure resulted in a weak increase of neutropenia (and leukopenia). Therefore, intracellular metabolism of ganciclovir and its metabolites could be associated with neutropenia. However, very little is known about the pharmacokinetics (PK) of intracellular ganciclovir and its metabolites in patients’ blood cells.

In this context, the objectives of the present study were: (i) to describe the PK of blood and intracellular ganciclovir and its metabolites in a cohort of renal transplant patients; and (ii) to explore the relationship between exposure to ganciclovir and/or its metabolites and evolution of the neutrophil count under treatment.
Methods

Population

Data were obtained from the ProGGRes (Pharmacokinetics of Ganciclovir and Valganciclovir triphosphate in Renal transplant patients) trial conducted in 22 adult renal transplant patients followed at the nephrology department of Limoges University Hospital, France. It was an ancillary study of the EPHEGREN study (no. 130-2013-30). All patients gave written informed consent. This trial complied with the Declaration of Helsinki amended in Tokyo and was reviewed and approved by the regional ethics committee of Limoges, France (no. 164-2015-01).

The patients received valganciclovir (Rovalcyte®, Roche, Switzerland) for prophylaxis of CMV infections. All patients were given valganciclovir at a dose of 450 mg once a day (19 patients) or 450 mg twice a day (3 patients) according to their creatinine clearance (as defined in the Summary of Product Characteristics), for 3 months (since transplantation). Exclusion criteria were prior valganciclovir treatment and granulocyte colony-stimulating factor intake. Demographic and clinical data for the 22 patients are summarized in Table 1.

Blood sampling and drug analysis

All patients were sampled during the third month after transplantation. Samples were collected in EDTA tubes at pre-dose and 1, 2, 3 and 5 h after the valganciclovir morning dose. Cells and plasma were immediately isolated from whole blood using a classical Ficoll–dextran method. Cells were then counted and samples stored at −20°C. For each sample, ganciclovir in plasma was measured as well as ganciclovir, ganciclovir monophosphate and ganciclovir triphosphate in PBMCs. The concentrations were obtained using a validated LC-MS/MS method and intracellular form concentrations were normalized per million PBMCs as previously described.8 Briefly, each intracellular form was isolated through a solid-phase extraction Sep-Pak Accell Plus QMA cartridge (Waters Corporation, Milford, MA, USA) using a KCl gradient. The samples were then desalted and stored at −20°C until analysis. The LC-MS/MS method used a 4000 QTrap® (Applied Biosystems, France) triple quadrupole linear ion-trap mass spectrometer.

PK modelling

All the PK profiles (plasma ganciclovir and intracellular ganciclovir, ganciclovir monophosphate and ganciclovir triphosphate) obtained from the 22 renal transplant patients were analysed using the non-parametric adaptive grid approach implemented in R software (PMetrics® version 1.3.2®). Several structural models were evaluated during the development process with and without absorption lag time. The analysis was based on a four-compartment open model: absorption of valganciclovir was modelled as a first-order process and conversion into ganciclovir in the central compartment was assumed to be instantaneous (as previously described10). As presented in Figure 1, the model included a plasmatic compartment, an intracellular ganciclovir compartment and intracellular ganciclovir monophosphate and ganciclovir triphosphate compartments.

Screening and selection of covariates were performed as part of the population PK analysis, following a classical stepwise approach. The model was parameterized in terms of the absorption rate constant (Ka), renal clearance of the drug (CL), central volume of distribution of each compartment, Ke and intercompartmental transfer rate constants (Ke′,Ke′′). The constant K0 referred to the elimination of intracellular ganciclovir triphosphate (literally from compartment 4 to compartment 0). This elimination could be made both by integration into DNA (viral or cellular) or efflux of intracellular ganciclovir triphosphate from cells.

A linear error model was used to describe the analytical variability. The performance of the model was assessed by studying its ability to estimate individual AUC values of each ganciclovir form. The mean bias between individual AUCs calculated using individual post hoc parameters and those obtained by the linear trapezoidal method was calculated.

Results

The observed concentration–time profiles of ganciclovir and its different metabolites in the 22 renal transplant patients are displayed in Figure 2. The exposure indices are given in Table 2. For each form, concentrations <5 ng/mL (the limit of quantification of the analytical method) were considered as null and intracellular

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Table 1. Demographic and clinical data for the 22 patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>57.5 (25–76)</td>
</tr>
<tr>
<td>Male/female, n/n</td>
<td>18/4</td>
</tr>
<tr>
<td>Valganciclovir dose of 450/900 mg, n/n</td>
<td>19/3</td>
</tr>
<tr>
<td>Mycophenolate mofetil yes/no, n/n</td>
<td>12/10</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min), median (range)</td>
<td>45.7 (18.8–95.7)</td>
</tr>
<tr>
<td>CMV serology +/−, n/n</td>
<td>10/12</td>
</tr>
</tbody>
</table>

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First blood sample after drug introduction | Blood sample at PK sampling

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin concentration (g/L), median (range)</td>
<td>11.55 (7.85–13.7)</td>
<td>12.05 (9.29–15.6)</td>
</tr>
<tr>
<td>Platelets (10^12/L), median (range)</td>
<td>198 (117–532)</td>
<td>204 (139–337)</td>
</tr>
<tr>
<td>Lymphocytes (10^12/L), median (range)</td>
<td>1.63 (0.35–5.44)</td>
<td>1.51 (0.28–3.59)</td>
</tr>
<tr>
<td>Neutrophils (10^9/L), median (range)</td>
<td>5.31 (1.78–8.88)</td>
<td>3.85 (0.6–8.04)</td>
</tr>
</tbody>
</table>
concentrations were normalized per million PBMCs. There was no correlation between the exposure indices to plasma ganciclovir (AUC₀–₅) and those of intracellular ganciclovir triphosphate (Spearman coefficient $r = 0.35; P = 0.11$). Moreover, the valganciclovir dose was not correlated with any intracellular ganciclovir triphosphate exposure indices (data not shown).

A model using 13 parameters plus a lag time to describe the absorption phase was necessary to determine the PK of plasma ganciclovir and intracellular ganciclovir, ganciclovir monophosphate and ganciclovir triphosphate. The PK parameters are given in Table 3. Figure S1 (available as Supplementary data at JAC Online) illustrates the goodness of fit.

The relative bias between the reference (trapezoidal rule) and model estimated AUCs were $-5.1\%$ (minimum–maximum range: $-34\%$–$-42\%$), $-2.8\%$ ($-26\%$–$-124\%$), $-0.6\%$ ($-60\%$–$-95\%$) and $-9.6\%$ ($-38\%$–$-74\%$) for plasma ganciclovir and intracellular ganciclovir, ganciclovir monophosphate and ganciclovir triphosphate, respectively.

The results of the multiple linear regression analysis investigating the relationships between individual covariates and evolution of the neutrophil count are given in Table 4. The three exposure indices for intracellular ganciclovir triphosphate were significant in the univariate analysis. In the multivariate analysis, only the AUC₀–₅ of intracellular ganciclovir triphosphate was associated with the slope of the neutrophil count. The effect on the slope was $\beta = -0.0019 \pm 5 \times 10^{-6}$ ($P < 0.01$) with a correlation $r_{\text{Spearman}} = -0.64$. This last result is illustrated in Figure 3.

![PK model](https://example.com/pk_model.png)

**Figure 1.** PK model. GCV, ganciclovir; GCV-MP, ganciclovir monophosphate; GCV-TP, ganciclovir triphosphate.

![PK profiles](https://example.com/pk_profiles.png)

**Figure 2.** PK profiles. Plasma ganciclovir (a), intracellular ganciclovir (b), intracellular ganciclovir monophosphate (c) and intracellular ganciclovir triphosphate (d). Crosses represent observed ganciclovir concentrations. Continuous lines represent the median lines, indicating the general data trend, and broken lines represent the 10th and 90th percentiles.

The relative bias between the reference (trapezoidal rule) and model estimated AUCs were $-5.1\%$ (minimum–maximum range: $-34\%$–$-42\%$), $-2.8\%$ ($-26\%$–$-124\%$), $-0.6\%$ ($-60\%$–$-95\%$) and $-9.6\%$ ($-38\%$–$-74\%$) for plasma ganciclovir and intracellular ganciclovir, ganciclovir monophosphate and ganciclovir triphosphate, respectively.

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Valganciclovir dose (mg) & intracellular ganciclovir triphosphate (per 10^6 PBMCs) & intracellular ganciclovir monophosphate (per 10^6 PBMCs) & plasma ganciclovir (per 10^6 PBMCs) & trough level & C_{max} & T_{max} (h) & Final model parameter estimate, median (range) \\
450 once & 450 twice & 450 once & 450 twice & 450 once & 450 twice & 450 once & 450 twice & \\
4.87 (10–50) & 1.28 (0.1–6.5) & 1.48 (1–6) & 8.74 (2–12) & 1.79 (0.2–2.5) & 1.48 (0.05–1.0) & 0.58 (0.4–1.8) & 32 (fixed parameter) & 40.17 (37–42) & 0.016 (0.01–0.09) & 72.96 (20–85) & 22.55 (12–25) & 1.48 (0.2–2.5) & 1.18 (0.1–10) & 1.48 (1–6) & 8.74 (2–12) & 1.85 (10–50) & 1.28 (0.1–6.5) & 0.0735 (0–0.94) \\

AUC value (2.82 ng·h/mL/10^6 cells) had a gain of 10% in the neutrophil count, while the one having the highest AUC value (29.3 ng·h/mL/10^6 cells) had severe neutropenia (decrease of 87% in the neutrophil count).

**Discussion**

To our knowledge, we report the first study describing the PK of ganciclovir, including intracellular forms, in renal transplant patients given valganciclovir. To better explore the relationships between ganciclovir in plasma and its intracellular outcome, including the factors that may contribute to any interindividual variability, a population PK model was developed. Additionally, a significant relationship between intracellular ganciclovir triphosphate levels—i.e. the active intracellular form—and the decrease in the neutrophil count was reported.

In our population of 22 adult renal transplant patients, important interindividual variability in the exposure to valganciclovir was calculated. The observed T_{max} was reached at \( \sim 1.7 \pm 0.9 \) h with a concentration of \( \sim 5900 \pm 3600 \) ng/mL for plasma ganciclovir and a mean trough concentration of 1000 \( \pm 1200 \) ng/mL. These values were consistent with those previously reported in similar populations\(^{11,13}\), or in a review article that reported a ganciclovir maximum plasma concentration of 3100 \( \pm 800 \) ng/mL after a dose of 450 mg and of 6600 \( \pm 1900 \) ng/mL after a dose of 900 mg with a T_{max} of 3.0 \( \pm 1.0 \) h.\(^{13}\)

A non-parametric approach was chosen to describe the PK of both the plasmatic and intracellular forms of ganciclovir. Briefly, this approach does not make the assumption that PK parameters are normally distributed (or that any bimodal distribution could exist), but provides an estimate of the whole probability distribution of these PK parameters. It is recognized that this approach better describes the population PK parameters than parametric...


Table 4. Multiple linear regression investigating the effect of covariates on the slope of the neutrophil count (univariate analysis)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Slope of the neutrophil count ($\beta \pm SD$)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age per year</td>
<td></td>
<td>$-6.0 \times 10^{-7} \pm 6 \times 10^{-4}$</td>
<td>0.34</td>
</tr>
<tr>
<td>Creatinine clearance ml/min</td>
<td></td>
<td>$-3.2 \times 10^{-5} \pm 5 \times 10^{-4}$</td>
<td>0.95</td>
</tr>
<tr>
<td>CMV seropositivity yes versus no</td>
<td></td>
<td>$-3.4 \times 10^{-3} \pm 0.015$</td>
<td>0.82</td>
</tr>
<tr>
<td>Mycophenolate mofetil dose mg</td>
<td></td>
<td>$-1.54 \times 10^{-3} \pm 2 \times 10^{-5}$</td>
<td>0.93</td>
</tr>
<tr>
<td>Valganciclovir dose 450 mg/day</td>
<td></td>
<td>$-1.5 \times 10^{-5} \pm 5 \times 10^{-5}$</td>
<td>0.76</td>
</tr>
<tr>
<td>Plasma ganciclovir</td>
<td>AUC₀⁻⁵ ng·h/mL</td>
<td>$1.1 \times 10^{-7} \pm 7 \times 10^{-7}$</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>C_{max} ng/mL/10⁶ cells</td>
<td>$1.2 \times 10^{-3} \pm 2 \times 10^{-6}$</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>C_{trough} ng/mL/10⁶ cells</td>
<td>$-5.8 \times 10^{-7} \pm 6 \times 10^{-6}$</td>
<td>0.34</td>
</tr>
<tr>
<td>Intracellular ganciclovir</td>
<td>AUC₀⁻⁵ ng·h/mL/10⁶ cells</td>
<td>$4.0 \times 10^{-7} \pm 0.003$</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>C_{max} ng/mL/10⁶ cells</td>
<td>$1.01 \times 10^{-3} \pm 0.01$</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>C_{trough} ng/mL/10⁶ cells</td>
<td>$-0.015 \pm 0.005$</td>
<td>0.05</td>
</tr>
<tr>
<td>Intracellular ganciclovir monophosphate AUC₀⁻⁵ ng·h/mL/10⁶ cells</td>
<td>$1.1 \times 10^{-4} \pm 9 \times 10^{-4}$</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{max} ng/mL/10⁶ cells</td>
<td>$2.1 \times 10^{-3} \pm 0.003$</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>C_{trough} ng/mL/10⁶ cells</td>
<td>$-0.035 \pm 0.02$</td>
<td>0.05</td>
</tr>
<tr>
<td>Intracellular ganciclovir triphosphate AUC₀⁻⁵ ng·h/mL/10⁶ cells</td>
<td>$-1.9 \times 10^{-3} \pm 5 \times 10^{-4}$</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{max} ng/mL/10⁶ cells</td>
<td>$-6.5 \times 10^{-3} \pm 0.002$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>C_{trough} ng/mL/10⁶ cells</td>
<td>$-0.011 \pm 0.005$</td>
<td>0.03</td>
</tr>
</tbody>
</table>

If numerous studies explored ganciclovir concentrations in plasma, little is known about the intracellular outcome of ganciclovir. For intracellular ganciclovir triphosphate, we observed that $t_{max}$ was reached at $\sim 3$ h with a concentration of $\sim -6$ ng/mL and a mean trough concentration of $\sim 1.5$ ng/mL per million PBMCs. However, a huge interindividual variability was observed.

Through multiple linear regression analysis, we found an association between the decrease in the neutrophil count during the first 3 months of treatment and intracellular ganciclovir triphosphate exposure. Precisely, the main exposure indices to intracellular ganciclovir triphosphate were significant in the univariate analysis ($C_{trough}$, $C_{max}$ and AUC₀⁻⁵) and, finally, the single significant covariate was AUC₀⁻⁵ in the multivariate analysis. This was not the case for exposure to plasma ganciclovir, as previously reported.

Thirty years ago, Smee et al. observed in vitro that intracellular ganciclovir triphosphate could persist in cells for $18$ h after exposure to ganciclovir and that ganciclovir excretion from cells was very slow. These authors wrote that ‘presumably, higher toxicity in these cell lines [HET and MET cell lines] may be correlated with higher levels of intracellular nucleoside triphosphate’. Moreover, in cells transfected with the viral thymidine kinase treated with ganciclovir, Halloran and Fenton showed that the phosphorylated form of ganciclovir is incorporated into the cellular DNA and arrests the cell cycle. Our result supports these hypotheses.

Therefore, the correlation between the AUC₀⁻⁵ and neutrophil slope values is quite poor. For example, several patients have an AUC₀⁻⁵ of $\sim 30$ ng·h/mL/10⁶ cells with a high variability in the slope of the neutrophil count (decreasing from 0.02 to 0.1 G/L/day). It is likely that this biomarker is not the main one and other variables may play also a role in the decrease of the neutrophil count.

More precisely, the role of ganciclovir and intracellular ganciclovir triphosphate membrane transporters is probably important and is certainly involved in this variability among patients. Adachi et al. reported that multidrug resistance protein 4 (MRP4) is involved in intracellular ganciclovir efflux. In a population of 206 renal transplant patients, we have identified an SNP known to decrease MRP4 activity and that favours ganciclovir accumulation in neutrophils. In that study, it was observed that the decrease in neutrophil count was more pronounced in patients carrying this SNP.
This study has some limitations. It is a monocentric study that included only 22 renal transplant patients. More patients would certainly be required to reach more meaningful conclusions concerning the correlation between AUC values and the slope of the neutrophil count. Additionally, while the neutrophil count was measured many times in each patient, exposure to ganciclovir was measured only during the third month after drug introduction and the ganciclovir dose, adapted according their creatinine clearance, may have changed during the treatment. This change was not reported and only the morning ganciclovir dose before the PK sampling was taken into account. A longitudinal follow-up with multiple measurements of ganciclovir exposure should be performed in further studies.

While valganciclovir is widely used as a CMV prophylactic or curative treatment, its haematological toxicity limits its efficacy. A classical therapeutic drug monitoring based on the measurement of plasma ganciclovir is probably unable to detect this toxicity. The results of the present study suggest that the measurement of intracellular ganciclovir triphosphate could better anticipate a decrease in the neutrophil count. The combination of this biomarker together with pharmacogenetic information about the transporters involved in the accumulation of ganciclovir in cells would probably be helpful.

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**Transparency declarations**

None to declare.

**Author contributions**


**Supplementary data**

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**