A pharmacodynamic model of ganciclovir antiviral effect and toxicity for lymphoblastoid cells suggests a new dosing regimen to treat CMV infection

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Running title: PKPD model of ganciclovir

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Abstract

In bone-marrow transplantation, the efficacy of ganciclovir in CMV disease treatment or prophylaxis remains partial. Because its haematological toxicity is dose-limiting, optimisation of the dosing schedule is required for increasing its therapeutic index. The goal of our study was to describe the influence of ganciclovir concentration level and duration of exposure on cell survival and antiviral efficacy. The study was carried out in vitro on cultures of lymphoblastoid cells, infected or not by CMV AD169 reference strain, exposed to ganciclovir during 1, 2, 7 or 14 days at different concentration levels. The data were analyzed by a mathematical model that allowed a quantitative characterization of ganciclovir pharmacodynamics and its variability. Simulations of this model were undertaken to determine the optimal concentration profile for maximizing ganciclovir therapeutic index.

Ganciclovir had very little toxic and antiviral effect, even at 20 mg.l⁻¹, when the duration of exposure was less or equal to 7 days. Biologically significant effect was observed only with a 14-day exposure. Complete inhibition of viral replication was obtained at 20 mg.l⁻¹. The utility function, assuming an equal weight for antiviral effect and toxicity shows that a maximal utility was reached around 10 mg.l⁻¹. The optimal ganciclovir concentration profile consisted in maintaining the concentration at 20 mg.l⁻¹ in the intervals 0 - 2 day and 7.58 - 9.58 day, and a null concentration elsewhere. This optimal profile could be obtained by IV ganciclovir 10 mg/kg bid at day 1, 2, 8.5 and 9.5 in SCT patients with normal renal function.
Introduction

Cytomegalovirus (CMV) infection remains a major complication of bone marrow transplantation (7,11,13,17,27). The major clinical signs of CMV infection in immunocompromised patients are pneumonitis, hepatitis and intestinal disease. Ganciclovir or its prodrug valganciclovir remains the first line treatment of CMV infection [1,16]. Ganciclovir is used as a prophylactic, pre-emptive or curative treatment (16). Prophylactic and pre-emptive treatments have demonstrated similar reductions of mortality and morbidity in bone marrow transplantation (4,16,26). In allogenic bone marrow recipients receiving prophylactic ganciclovir, incidence of CMV disease varies between 30 to 60%, while incidence of death related to CMV is 25 to 30 % (26). A major risk factor for virological failure is a peak viral load >20,000 copies/mL at the onset of treatment (OR 5.88). The main risk factor for a peak viral load >20,000 copies/mL is the presence of grade II-IV acute graft-versus-host-disease (OR 16).(28)

Ganciclovir is known to have haematological toxicity, neurotoxicity and possibly hepatotoxicity (21). Haematological toxicity has been characterized in vitro on normal human haematopoietic progenitors cells. Ganciclovir inhibition was concentration dependent on both granulocyte-macrophage progenitors and erythroid progenitors (25). Haematological toxicity of ganciclovir may be enhanced by CMV itself. CMV has a particular tropism for bone marrow cells and it is toxic for these cells by direct and indirect mechanisms (13). Ganciclovir-induced neutropenia is associated with a greater risk of mortality after marrow transplantation (23), and of non viral opportunistic infections when the treatment duration is longer than four weeks (7). Hence, the efficacy of ganciclovir in CMV disease treatment or prophylaxis remains partial.

Because the haematological toxicity is dose-limiting, optimisation of the dosing schedule is
required in order to increase the therapeutic index of ganciclovir. No firm correlation has been established to date between ganciclovir exposure and antiviral efficacy or toxicity (21). The interplay between ganciclovir dosing rate and treatment duration with respect to anti-CMV efficacy has been partially characterized in immunodeficient mice (6), but the hematological toxicity has not been assessed in this study.

The main goal of our study was to describe the influence of ganciclovir concentration level and duration of exposure on cell survival and antiviral efficacy. The study was carried out in vitro on cultures of lymphoblastoid cells. The data were analyzed by a mathematical model that allowed a quantitative characterization of ganciclovir pharmacodynamics and its variability. Simulations of this model were undertaken to determine the optimal concentration profile for maximizing ganciclovir therapeutic index.
Material and methods

Chemicals

GCV (9-(1,3-dihydroxy-2-propoxymethyl) guanine) was provided by Roche Laboratories (Neuilly sur Seine, France). RPMI 1640 medium was purchased from Eurobio (Courtaboeuf, France), minimum essential medium (MEM) was from LONZA laboratories (Verviers, Belgium). Fetal calf serum was from Perbio Science (Bezons, France). Phosphate buffered saline medium was from Jacques Boy (Reims, France). Penicillin/streptomycin (10,000U/ml) and amphotericin B were purchased from Bio Whittaker Europe (Verviers, Belgium) and Bristol-Myers Squibb (Rueil Malmaison, France), respectively. Cyclosporin A was from Sigma (Steinheim, Germany). High pure viral nucleic acid kit was provided by Roche Diagnostic Laboratories (Mannheim, Germany).

The human cytomegalovirus (HCMV) strain AD 169 (ATCC VR-358) was propagated in human embryonic fibroblast (MRC5 cell line, RD biotech, Besançon France) and stored at -80°C. This virus stock was thawed ten days before lymphoblastoid cell infection. After 2 passages at low virus to cell ratios, HCMV was titrated by real time quantitative PCR (18). Experiments in this study used a final stock of virus with a titer of $10^9$ pfu/ml. After the preparation and titration of virus, it was immediately used to infect lymphoblastoid cells.

Infection of lymphoblastoid cell culture and exposure to ganciclovir

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood (10 cm$^3$) of healthy paediatric donors by Ficoll density gradient centrifugation. B lymphoblastoid cell line (BLCLs) were established from PBMCs by ex vivo infection with a laboratory strain of Epstein Barr virus (EBV 95-8), an effective procedure for inducing the long term growth of certain human B lymphocyte (19). The BLCLs were then grown as suspension cultures in
RPMI 1640 medium supplemented with 12% fetal calf serum, penicillin, streptomycin and amphotericin B. The BLCL culture was performed at 37°C in a humidified atmosphere of 5% CO2. Cells were kept in the exponential phase over the course of the culture (14 days). BLCLs (10^7 cells/ml) were infected with HCMV 0.01 pfu/cell. The cell culture was incubated for 2 h at 37°C. After infection, cells were washed in phosphate buffered saline medium (PBS) three times to remove unattached and passively adsorbed virus. Cells were counted and resuspended in RPMI (10^6 cells/ml). To control the efficiency of the washing procedure, viral detection was performed by PCR after the last washing. Uninfected cells were treated in the same way as infected cells. GCV was added just after the last washing to infected and non-infected cells.

GCV stock solution was prepared at 1 mg/ml and diluted in physiological serum before the assay to obtain a range of GCV concentrations in BLCL culture medium from 1 to 20 mg/l. The duration of GCV exposure was 1, 2, 7 and 14 days for each concentration (1, 5, 10 and 20 mg/l) with three consecutive washings applied to completely remove extracellular GCV. The total culture duration, independent of the GCV exposure periods, was 14 days to determine the long term effects of the antiviral drug after removal from cell culture.

All experiments were done in triplicate.

Evaluation of ganciclovir toxicity and antiviral activity

For evaluation of toxicity, total cell number was determined using a Coulter particle counter and size analyser (Beckman Z2, Fullerton, CA, USA) after 1, 2, 7 and 14 days of cell culture, independent of GCV exposure durations.

Viable cell number was determined using an ADAM Counter (Labtech; Palaiseau, France). Samples were stained with fluorescent dye (propium iodide), which intercalated DNA to stain the nucleus of target cells, and fluorescent images were taken automatically and processed by ...
image analysis software. Measurements were performed at day 7 (for the 1-, 2- and 7-day GCV exposure) and at day 14 (for the 1-, 2-, 7- and 14-day GCV exposure).

For evaluation of ganciclovir antiviral activity, the method of virus quantification was the real time PCR as previously described (18). “High pure viral nucleic acid Kit” was used to extract HCMV DNA and Apparatus 7500 real time PCR system (Applied Biosystem) was used to quantify HCMV DNA. Viral quantification was performed on virus stocks obtained on MRC5 cells (before BLCLs infection) and on infected cells on day 0, day 1, day 2, day 7 and day 14 post-infection, independently of GCV exposure duration.

Pharmacodynamic model for cell cultures without virus

The typical profile of the cell number versus time curve showed an initial decay until a nadir, followed by a S-shaped growth phase, and a final plateau phase. To describe the full profile, the variation of viable cell number with time, N(t), was modelled by the following equation:

\[
\frac{dN(t)}{dt} = K_{max} \cdot (1 - \alpha_1 \cdot GCV(t)) \cdot Tr \cdot \exp(1 - Tr) \cdot N(t)
\]

where

\[
Tr = \frac{(t - T_{lag})}{(T_{max} - T_{lag})}
\]

In this equation, \(K_{max}\) is the maximal growth rate constant that may be achieved during the 14 days experiment, in days\(^{-1}\). GCV(t) is ganciclovir concentration (mg.l\(^{-1}\)). The parameter \(\alpha_1\) (l.mg\(^{-1}\)) is the growth inhibition constant of ganciclovir. A value of zero means no inhibition of cell growth by ganciclovir. \(Tr\) is the dimensionless reduced time. \(T_{lag}\) is the delay before growth begins, in days. \(T_{max}\) is the time at which the maximal growth rate is observed, in days.
Pharmacodynamic model for cell cultures with virus

The structure of the infection model is described in figure 1. The model assumes that the non-infected cells (N) are infected by extracellular virion particles and by transmission of virus from mature infected cells. The intensity of this process is characterized by two infectivity rate constant, $\beta_1$ and $\beta_2$ respectively. The infected cells go through a number of states (I1 to I4) accounting for the maturation of virus, i.e. the delay between cell infection and cell death. The rate constant for each step is $k_j$, in days$^{-1}$. Hence, the mean maturation time is $4/k_1$. In the state I4, the virus is released with a rate constant $m$, in days$^{-1}$. The number of virion particles released by infected cell (the so-called reproductive number) is $R = m/k_1$. The extracellular virion particles (V) are eliminated with a rate constant $K_v$. Finally, an exponential decrease of the infectivity had to be introduced in the model to account for the late decay of viral load in spite of constant cell concentration in the control experiment with no ganciclovir. This exponential decrease was characterized by its half-life $T_{inf}$, in days.

The set of differential equations was as follows:

\[
\frac{dN(t)}{dt} = K_{max} \cdot (1 - \alpha_{t \cdot GCV(t)} \cdot Tr \cdot \exp(1 - Tr) \cdot N(t)) - Inf(t)
\]

where \(Inf(t) = [\beta_1 \cdot N(t) \cdot V(t) + \beta_2 \cdot N(t) \cdot I_4(t)] \cdot \exp(-0.693 \cdot t / T_{inf})\)

\[
\frac{dI_1(t)}{dt} = \beta_1 \cdot N(t) \cdot V(t) \cdot \exp(-0.693 \cdot t / T_{inf}) - k_1 \cdot I_1(t)
\]

\[
\frac{dI_j(t)}{dt} = k_j \cdot I_{j-1}(t) - k_j \cdot I_j(t) \quad \text{for } j = 2 \text{ to } 4 \text{ (see fig 1).}
\]

\[
\frac{dV(t)}{dt} = m \cdot I_4(t) - \beta_2 \cdot N(t) \cdot V(t) \cdot \exp(-0.693 \cdot t / T_{inf}) - K_v \cdot V(t)
\]

The observed cell number, C, is the sum of non-infected and infected cells concentrations. The observed viral load, VL, is approximated as the sum of the extracellular virus, the virus in cells I1, and R times I4.
The intensity of the antiviral effect of ganciclovir was described as a linear function of its concentration. The effect of ganciclovir on cells and virus was assumed instantaneous, because the steady-state of ganciclovir triphosphate intracellular concentration is reached in less than 6h (S. Cohen and J. Guitton, unpublished data). Several submodels were tested to determine the site of action of ganciclovir (with respect to our model of infection): inhibition of infectivity ($\beta_1$ or $\beta_2$ were multiplied by $1 - \alpha_2 \cdot \text{GCV}(t)$), inhibition of maturation ($m$ was multiplied by $1 - \alpha_2 \cdot \text{GCV}(t)$), stimulation of virus elimination ($K_v$ was multiplied by $1 + \alpha_3 \cdot \text{GCV}(t)$).

Parameter estimation and model building

To account for inter-assay variability, the model was written as a mixed-effects model. Each parameter of the model was assumed to follow a lognormal distribution. The parameters to be estimated were the median of all parameters and the variance of $K_{\text{max}}$, $T_{\text{max}}$, $\beta_1$, $\beta_2$, $k_1$, $m$, $T_{\text{inf}}$ and $K_v$. All these parameters were estimated by non-linear regression with NONMEM VII with the so-called FOCE method (2).

The strategy for model building was as follows. In the first step, the model was fitted to the cell number versus time data from cell cultures with no virus. In the second step, the model was fitted to the cell number and viral load versus time data from cell cultures with virus, by fixing $\alpha_1$ to the value estimated in the former step, and $\alpha_2$, $\alpha_5$ to zero. In this way, the antiviral effect of ganciclovir was not accounted for in the model. The posthoc estimates of $K_{\text{max}}$, $T_{\text{max}}$, $\beta_1$, $\beta_2$, $k_1$, $m$, $T_{\text{inf}}$ and $K_v$ were plotted against the concentration and the AUC of ganciclovir, in order to detect any influence of ganciclovir exposure on these parameters. In the last step, the relationships between the parameters and ganciclovir concentration were included in the model, i.e. $\alpha_2$ and $\alpha_3$ were estimated.
Hypothesis testing for e.g. fixing a parameter to zero, was based on the likelihood ratio test. A p-value less than 0.05 was considered as significant.

Simulation-based diagnostic tools were used for final model evaluation. Thousand individual simulated profiles were obtained by sampling in the distribution of the random effects of the mixed-effects model. A visual predictive check allowed to compare the 90% prediction interval to the experimental data (30). Normalized prediction distribution errors (a criterion with high power to detect departures from the model) were calculated and plotted against time and ganciclovir concentration (3). These approaches do not rely on any approximation.

Simulations

Using the final mixed-effects model, 400 cell number and viral load profiles of the complete design (ganciclovir concentration equal to 0, 1, 2, 5, 10 or 20 mg.L⁻¹, exposure to ganciclovir during 0, 1, 2, 7 or 14 days) were simulated by sampling in the distribution of the random effects. For each simulation, several metrics were calculated. To characterize the variation of cells during the 14 days of the experiment, the following ratio was defined:

\[ \text{AUC}_{CN} = \frac{\int_0^{14} N(t) \, dt}{\int_0^{14} N(0) \, dt} \]

The numerator is the AUC of non-infected cells, while the denominator is the AUC that would be observed if the cell number remained to the initial value during 14 days. A value of \( \text{AUC}_{CN} \) greater than 1 indicates cell reproduction.

A similar index was calculated to characterize the replication of CMV:

\[ \text{AUC}_{VN} = \left( \frac{\int_0^{14} VL(t) \, dt - \text{AUC}_{min}}{\int_0^{14} VL(0) \, dt - \text{AUC}_{min}} \right) \]

In this equation, \( \text{AUC}_{min} \) is the AUC of viral load that would be observed if there was no replication of the virus. In this case, \( VL(t) \) would decrease exponentially from \( VL(0) \) to zero and the corresponding \( \text{AUC}_{min} \) is equal to \( VL(0)/K_v \). A value of \( \text{AUC}_{VN} \) equal to zero means a
complete inhibition of viral replication by ganciclovir. A value greater than 1 indicates net replication of the virus.

The median and percentiles of AUC_{CN} and AUC_{VN} were calculated from the distribution of the 400 values for each set of conditions (ganciclovir concentration, duration of exposure).

The median was taken as the point estimate of the effect of a given concentration of ganciclovir on cells and viral load for a given duration of exposure. These point estimates were used to calculate the fraction of maximal effect as a function of ganciclovir concentration, GCV:

\[
\text{Antiviral effect}(GCV) = 1 - \frac{\text{AUC}_{\text{VN}}(GCV)}{\text{AUC}_{\text{VN}}(GCV = 0)}
\]

\[
\text{Toxic effect}(GCV) = 1 - \frac{\text{AUC}_{\text{CN}}(GCV)}{\text{AUC}_{\text{CN}}(GCV = 0)}
\]

All the simulations were carried out with NONMEM VII.

**Optimization of drug concentration profile**

Because ganciclovir toxic and antiviral effect are both concentration dependent, an optimal ganciclovir concentration profile that maximizes antiviral effect while minimizing toxicity was calculated. To avoid confusion, the concentration profile of ganciclovir will be referred to as the dosing schedule, D. Our approach was based on the concept of utility function U \((20)\), which is a weighted sum of antiviral activity and non-toxicity evaluated over 14 days. In order to account for the variability of the cell and viral load profiles under a given dosing schedule, the mean utility is to be maximized with respect to D. The mean utility is computed as the arithmetic mean of 400 values, obtained by generating 400 profiles of C(t) and VL(t) for a given D, using the mixed-effects model. In mathematical terms, U is defined as follows for the j-th simulated profile of C(t) and VL(t):

\[
U_j(D) = w.A_j + (1 - w).(1 - T_j)
\]

\(j = 1\) to 400
A and T are the antiviral effect and the toxic effect associated with a given dosing schedule:

\[
A_j = 1 - \frac{AUC_{VNj}}{\max(AUC_{VN})} \quad \text{and} \quad T_j = 1 - \frac{AUC_{CNj}}{\max(AUC_{CN})}
\]

max(AUC_{VN}) and max(AUC_{CN}) are the maximal value that may be observed among the 400 profiles when no ganciclovir is added. (1-T) may be regarded as the safety. In this way, A and (1-T) are constrained to be in the range 0 to 1. W is the weight attributed to the antiviral effect, on a scale from 0 to 1 while (1-w) is the weight attributed to safety. By construction, U_j is in the range 0 to 1 also. In our study, w was fixed to 0.5.

The strategy to find the optimal dosing schedule comprised two steps. In the first step, the 14 days period was splitted into 7 consecutive periods of 2 days each. The mean utility was maximised with respect to the ganciclovir concentration in each period. In the second step, the 14-day AUC of ganciclovir was constrained to the value obtained in step 1, but the mean utility was maximised with respect to the time of onset of each period. All these calculations were done with NONMEM VII, using the LIKELIHOOD option.

Finally, the performances of two ganciclovir concentration profiles were compared with respect to their utility, AUC_{CN} and AUC_{VN}. The first profile was the optimal profile; the other one was a constant concentration profile with the same ganciclovir 14-day AUC.
Results

Parameter estimation and model building

The model was first fitted to the cell number versus time data from cell cultures with no virus.

The growth model fitted well the data (data not shown) and the value of $\alpha_1$ was estimated at 0.026 with a relative SE of 6%. The model was then fitted to the cell number and viral load versus time data from virus infected cell cultures, by fixing $\alpha_1$ to 0.026, and $\alpha_2, \alpha_3$ to zero.

The posthoc estimates of $K_{\text{max}}, T_{\text{max}}, \beta_1, \beta_2, m, T_{\text{inf}}$ and $K_v$ were plotted against the concentration and the AUC of ganciclovir, in order to detect any influence of ganciclovir on these parameters. It was observed that $\beta_1, \beta_2,$ and $m$ were approximately linearly related with ganciclovir concentration. The other parameters showed no trend with respect to ganciclovir exposure. In particular, there was no visible relationship between the rate constant of viral elimination ($K_v$) and the exposure. Therefore, in the last step, $\alpha_3$ remained fixed to zero while the relationships between $\beta_1, \beta_2,$ and $m$ and ganciclovir concentration were included in the model. Because exposure to ganciclovir at 20 mg/l during 14 days resulted obviously in a complete inhibition of viral replication, $\alpha_2$ was fixed to 0.05 l.mg$^{-1}$. Finally, because the uncertainty about $k_1$ and $m$ estimate was large, these parameters were fixed to 4 and 60 respectively. In this way, the mean maturation time $4/k_1$ was equal to 1 day (according to (9)) and the reproductive number $m/k_1$ was equal to 15 (according to (10)). This final model fitted well the data, as shown by the visual predictive check (fig 2) and the other criteria (not shown).

Simulations

The median and percentiles of $\text{AUC}_{\text{CN}}$ and $\text{AUC}_{\text{VN}}$, calculated from the distribution of 400 replications for several sets of conditions showed that ganciclovir had very little toxic and
antiviral effect, even at 20 mg.l\(^{-1}\), when the duration of exposure was less or equal to 7 days. Biologically significant effect was observed only with a 14-day exposure. The effect of exposure to different concentrations of ganciclovir during 14 days on the normalized AUC of viable cells, and the normalized viral load is shown in fig 3. Although the inter-assay variability is quite high, complete inhibition of viral replication is obtained at 20 mg.l\(^{-1}\). The fraction of maximal effect as a function of exposure to a constant ganciclovir concentration during 14 days is shown in fig 4A. The corresponding utility function, assuming an equal weight for antiviral effect and toxicity, is plotted in fig 4B. This plot shows that a maximal utility of ca. 0.9 is reached around 10 mg.l\(^{-1}\). Lower concentrations lead to low antiviral effect, while higher concentrations lead to unacceptable toxicity. Therefore, it makes sense to search an optimal concentration profile aiming at maximizing the utility function.

**Optimization of drug concentration profile**

In the first step, the optimization procedure (i.e. maximizing the mean utility function with respect to the ganciclovir concentration in each two-day period) showed that the optimal profile consisted in maintaining ganciclovir concentration at 20 mg.l\(^{-1}\) in the first and the fifth two-day period, i.e. in the intervals 0 - 2 day and 8 - 10 day, and a null concentration elsewhere. In the second step, the optimal time for onset of each of these two periods was found to be zero and 7.58 days respectively. The optimal profile was finally: ganciclovir concentration at 20 mg.l\(^{-1}\) in the intervals 0 - 2 day and 7.58 - 9.58 day, and a null concentration elsewhere. The AUC of the optimal profile is equivalent to that of a constant concentration profile at 5.71 mg.l\(^{-1}\) during 14 days. The performances of the optimal profile and the constant profile, evaluated by simulation, are compared in table II. The optimal profile was significantly better on all criteria: higher mean utility, lower cell toxicity, higher viral reduction. The kinetic profile of cell and viral load under both dosing schedule is shown in fig
5, and compared to the profiles without treatment by ganciclovir. The optimal schedule results in almost no toxicity but in a marked antiviral effect.

Discussion

In this study, a pharmacodynamic model was derived and fitted to cell number and viral load under different concentrations of ganciclovir and durations of exposure. The model is similar to models developed previously for HIV infection (12) and HCV infection (5) but the cell growth function of our model is more elaborate. In our conditions, the cells were asynchronie at the beginning of the experiment, the cell count reached a plateau due to consumption of nutrients (the medium was not renewed during the experiment), and the cells were quiescent at the end of the experiment. These characteristics mimic the clinical situation of a primary infect in a bone marrow recipient. The antiviral drug concentration range was 0 – 20 mg/L. This range is to be compared with the mean (SD) maximal concentration of 10 (2) mg/L observed in SCT patients receiving IV ganciclovir 5 mg/kg bid (29). Higher ganciclovir concentrations may have been studied in our in vitro experiments, to observe the entire toxicity versus concentration profile. This approach could have resulted in a different equation for the toxicity versus concentration model. Hence, the limit of our current model is that it is not suitable for extrapolation beyond 20 mg/L.

Ganciclovir toxicity for lymphoblastoid cells in absence of virus could be characterized in spite of the complex shape of the cell growth curve. At the highest concentration (20 mg/L), the growth rate constant was reduced by 52 %. Hence, ganciclovir IC₅₀ is about 20 mg/L for these cells. This value is higher than that observed with granulocyte-macrophage progenitors (0.7-4.8 mg/L) and erythroid progenitors (0.4-7.4 mg/L) (25).
Ganciclovir appeared to exert its antiviral effect by decreasing the infectivity and the release rate constant of CMV. This is consistent with the molecular mechanism of action of ganciclovir regarding inhibition of viral replication (15). A complete inhibition of viral replication by ganciclovir was observed at 20 mg/L. These results were obtained with the CMV AD169 strain, whose IC$_{50}$ is typically 0.9 mg/L. The IC$_{50}$ of clinical strains is typically 0.7 mg/L (range 0.2 to 1.9 mg/L, depending on the assay) (21). Hence, a similar pattern of activity should be observed with clinical strains, although the extra genes present in the clinical strains may alter their cell entry and possibly replication efficiency. Clinically, in case of secondary infection, the immune reaction is susceptible to increase viral degradation (parameter K$_{v}$) and decrease infectivity (parameters $\beta_1$ and $\beta_2$) and the reproductive number ($m/k_1$). If the IC$_{50}$ is unchanged, a greater efficacy of the treatment is expected, as observed by Emery et al (10).

Because our model handles drug action by an empirical model, our approach is not restricted to a specific drug action mechanism, and may possibly be applied to other drugs. For example, maribavir (24) and AIC246 (letermivir) (14) are new antiviral drugs with anti-CMV activity, that are respectively in phase III and phase II of their clinical development. Their mechanism of action is different from that of ganciclovir. Their toxicity seems very low and the target organs are different from those of ganciclovir. Our model may be applied to maribavir and letermivir by setting $\alpha_1 = 0$ (no toxicity for lymphoblastoid cells), and adjusting $\alpha_2$ to a suitable value, to account for the fact that the IC$_{50}$ of these drugs are lower than that of ganciclovir.

The kinetics of the viral load consisted in an initial decline, due to penetration of virus into cells, followed by a rebound, due to production and release of new virus. According to the model simulation, the rebound reached its maximum about 8 days after the beginning of the infection. The rate of increase in CMV load in our in vitro model was consistent with the
doubling time of CMV load in the blood of bone marrow transplant recipients after allogeneic transplantation (9). The optimal ganciclovir concentration profile consisted in maintaining the concentration at 20 mg.l\(^{-1}\) in the intervals 0 - 2 day and 7.58 - 9.58 day, and a null concentration elsewhere. Hence, the model-based optimisation suggests that a high concentration of ganciclovir should be applied at the time at the onset of infection and at the time of viral rebound, in order to maximize efficacy and minimize toxicity. This optimal profile could be obtained by IV ganciclovir 10 mg/kg at time 0, 12, 24, 36, 180, 192, 204 and 216 h in SCT patients with normal renal function.

The feasibility of a short treatment at high dose is partly supported by the clinical study reported by Saleh (22). In the latter study, valganciclovir was given at 900 mg bid during one or two weeks to HSCT CMV-positive patients. Complete responses rate was over 92% while severe neutropenia requiring granulocyte-colony stimulating factor occurred in 8 among 61 episodes (13%). In another study with the same dosing regimen, the mean (SD) peak concentration of ganciclovir was 8.8 (2.4) mg/L (8). This value is comparable to that of the constant concentration profile at 5.7 mg.l\(^{-1}\) during 14 days equivalent to the AUC of the optimal profile determined in our study.

To conclude, the model-based analysis of our \textit{in vitro} pharmacodynamic data suggests that ganciclovir therapeutic index could be increased by infusions of 10 mg/kg bid at day 1, 2, 8.5 and 9.5 in SCT patients. This prediction should be evaluated in a clinical trial.
References


Legends to figures

Fig 1: Description of the pharmacodynamic model and its parameters.

Fig 2: Visual predictive check of the model. (A) Cell number vs time. (B) Viral load vs time. The points are the observations. The line is the median profile of 400 individual simulations. The dashed lines are the 5th and 95th percentiles of the 400 simulations. The symbols are coded CL_X_Y or VL_X_Y, where X is ganciclovir concentration in mg/L and Y is exposure duration in days.

Fig 3: Effect of exposure to different concentrations of ganciclovir during 14 days on (A) the normalized AUC of viable cells, (B) the normalized viral load.

Fig 4: (A) Antiviral effect and cell toxicity vs concentration of ganciclovir, after exposure during 14 days. (B) Utility function of ganciclovir concentration.

Fig 5: Comparison of the influence of three concentration profiles of ganciclovir on (A) the median cell concentration profile, (B) the median viral load profile.
Table I: Parameters estimated in the final model

<table>
<thead>
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<th>Parameter</th>
<th>Median (RSE in %)</th>
<th>CV in % (RSE in %)</th>
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<tr>
<td>$K_{\text{max}}$ (d$^{-1}$)</td>
<td>0.234 (9)</td>
<td>-</td>
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<tr>
<td>$T_{\text{max}}$ (d)</td>
<td>2.39 (8)</td>
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<td>$\beta_1$ (d$^{-1}$ cell$^{-1}$)</td>
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<td>-</td>
</tr>
<tr>
<td>$k_1$ (d$^{-1}$)</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>$m$ (d$^{-1}$)</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>$K_v$ (d$^{-1}$)</td>
<td>1 (7)</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha_1$ (l.mg$^{-1}$)</td>
<td>0.026</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha_2$ (l.mg$^{-1}$)</td>
<td>0.050</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha_3$ (l.mg$^{-1}$)</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

RSE: relative standard error. Fixed parameters have no RSE.
Table II: Comparison of the optimal concentration profile with the constant concentration profile. The numbers are median (25-75th percentile), based on 400 individual simulations.

<table>
<thead>
<tr>
<th></th>
<th>Constant concentration profile</th>
<th>Optimal concentration profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utility</td>
<td>0.79 (0.75 – 0.83)</td>
<td>0.87 (0.83 – 0.91)</td>
</tr>
<tr>
<td>Normalized AUC of viable cells</td>
<td>1.66 (1.49 – 1.84)</td>
<td>1.83 (1.64 – 2.03)</td>
</tr>
<tr>
<td>Normalized AUC of viral load</td>
<td>0.40 (0.33 – 0.55)</td>
<td>0.10 (0.08 – 0.14)</td>
</tr>
</tbody>
</table>
Non infected cells → virus-cell infection $\beta_1$ → Extracellular virus → virus degradation

Non infected cells → replication

Infected cells I1 → cell-cell infection $\beta_2$ → infected cells I2, I3, I4

Infected cells I2 → virus maturation

Infected cells I4 → cell death

release of (m/k) virus
Fig 1: Description of the pharmacodynamic model and its parameters
Fig 2: Visual predictive check of the model. (A) Cell number vs time. (B) Viral load vs time. The points are the observations. The line is the median profile of 400 individual simulations. The dashed lines are the 5th and 95th percentiles of the 400 simulations. The symbols are coded CL_X_Y or VL_X_Y, where X is ganciclovir concentration in mg/L and Y is exposure duration in days.
Fig 3: Effect of exposure to different concentrations of ganciclovir during 14 days on (A) the normalized AUC of viable cells, (B) the normalized viral load.
Fig 4: (A) Antiviral effect and cell toxicity vs concentration of ganciclovir, after exposure during 14 days. (B) Utility function of ganciclovir concentration.
Fig 5: Comparison of the influence of three concentration profiles of ganciclovir on (A) the median cell concentration profile, (B) the median viral load profile.