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# Mycophenolate mofetil strongly potentiates the anti-herpesvirus activity of acyclovir

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**Abstract**

We demonstrate that the novel immunosuppressive agent mycophenolate mofetil (MMF), that has been approved for use in kidney transplant recipients, strongly potentiates the antiviral activity of acyclovir in murine models for herpesvirus infections. Hairless mice that were infected intracutaneously with herpes simplex virus type 1 were treated systemically with ACV (20 mg/kg per day) and topically with 5% MMF. Combined use of both drugs resulted in an almost complete protection, whereas single use of either compound had virtually no effect. When athymic-nude mice were infected with an ACV-resistant ($ACV^r$)–thymidine kinase-deficient ($TK^-$) HSV-2 strain, combined use of systemically administered ACV (100 mg/kg per day) and topically applied MMF (5%) protected 60% of the animals against the infection, whereas all mice treated with either drug alone succumbed. Since transplant recipients under MMF therapy may develop opportunistic herpesvirus infections, requiring treatment with acyclovir (or valaciclovir), our findings have important implications for the treatment of these herpesvirus infections. 

*Keywords:* Acyclovir; Herpesvirus; Mycophenolate mofetil (MFF); CellCept®; Immunosuppression

The immunosuppressive agent mycophenolate mofetil (MMF) has been approved (as CellCept®) for use in kidney transplant recipients. The compound, in conjunction with cyclosporin and corticosteroids, appears to be superior over azathioprine as a post-transplant immunosuppressant (Lipsky, 1996). MMF is converted into its active metabolite mycophenolic acid which is a potent inhibitor of IMP dehydrogenase, resulting in a depletion of the intracellular dGTP pools. This diminishes the proliferation of T and B lymphocytes, decreases the generation of cytotoxic T cells and suppresses antibody formation (Ransom, 1995). Patients that develop opportunistic herpesvirus infections as a result of the immunosuppressive action of MMF require treatment with anti-herpesvirus agents. We have re-

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Table 1
Inhibitory effects of systemically administered acyclovir and topically applied mycophenolate mofetil on HSV-1-induced skin lesions and associated mortality in hairless mice

| | No. with lesion[a] | MDLA[b] | Survivors[a] | MDD[c] |
|---|---|---|---|---|
| Control | 10/10 | $4.8 \pm 0.6$ | 0/10 | $7.2 \pm 2.0$ |
| 5% MMF | 10/10 | $5.6 \pm 0.7$* | 0/10 | $9.3 \pm 1.9$ |
| 20 mg/kg ACV | 10/10 | $6.0 \pm 1.5$* | 1/10 | $10.0 \pm 1.6$** |
| 20 mg/kg ACV + 5% MMF | 1/10*** | 7 | 9/10*** | 9.0 |

For MDLA and MDD differences between multiple groups were analyzed by one-way ANOVA, indicating a significant statistical difference for MDLA ($P<0.05$) and MDD ($P<0.01$). Statistical difference between a particular group and the control was assessed by the two-tailed Student's $t$-test with Bonferroni correction.
[a] As evaluated at 60 days post-infection.
[b] Mean day of lesion appearance.
[c] Mean day of death.
*$P<0.025$; **$P<0.005$; ***$P<0.001$.

cently demonstrated that MMF markedly potentiates the anti-herpes virus activity of acyclovir (ACV), ganciclovir (GCV) and penciclovir (PCV) (Neyts et al., 1998). The reason for this potentiation is that the lower intracellular concentration of the natural substrate dGTP favors the inhibitory effect of the acyclic nucleoside triphosphates on the viral DNA polymerase (Neyts et al., 1998). Here we demonstrate that the combined use of systemically administered ACV and topically applied MMF (both at doses that singly afford little or no protective activity) is highly effective in animal models for herpesvirus infections. This is an important point when treating herpesvirus infections in (kidney) transplant recipients.

The origin of herpes simplex virus type 1 (strain KOS) has been described before (De Clercq et al., 1980). The $ACV^r$–$TK^-$ HSV-2 strain is a plaque-purified TK-deficient strain isolated from a patient refractory to ACV treatment (Snoeck et al., 1994). Acyclovir (ACV) was from Glaxo Wellcome (Aalst, Belgium) and mycophenolate mofetil (MMF) was provided by Roche (Palo Alto, CA). Hairless mice or athymic nude (nu/nu) mice (Charles River Breeding, Sulzfeld, Germany) were inoculated intracutaneously (i.c.) at the lumbosacral area (by scratching the skin with a scarificator) with either HSV-1 (KOS) or $ACV^r$–$TK^-$ HSV-2 at $10^4$ PFU/0.05 ml per mouse. The mice were then treated for 5 days (hairless mice) or 8 days (athymic-nude mice) starting at 2 h after the infection. MMF (5%) was applied topically twice a day in a volume of 0.05 ml DMSO over an area of 1.5 $cm^2$; ACV (in PBS) was given twice daily via subcutaneous injection. Control animals and the 'ACV-only' group were treated topically with DMSO only; the control group and the 'MMF-only' group were injected with PBS as placebo. Mice were monitored daily for the development of herpetic skin lesions and mortality. Statistically significant differences for mean day of lesion appearance (MDLA) and mean day of death (MDD) between the multiple groups was analyzed by means of one-way ANOVA. Where statistical significant differences between the multiple groups were found, the two-tailed Student's $t$-test with Bonferroni correction was used to calculate statistical significance between two particular treatment groups. Statistical significance of the number of mice developing lesions and long-term survivors was assessed by means of the $\chi^2$-test with Yates' correction.

In a first set of experiments (Table 1), hairless mice were infected in the lumbosacral area with herpes simplex virus ([HSV-1 (KOS)) and were treated with either ACV (20 mg/kg per day, divided over two doses) via subcutaneous (s.c.) injection for 5 consecutive days, or a 5% MMF ointment (twice daily for 5 consecutive days), or the combination ACV (s.c.) + MMF (ointment), twice daily for 5 consecutive days. A suboptimal dose of ACV (two times 10 mg/kg per day) was chosen so that the compound alone would have

Table 2
Inhibitory effects of systemically administered acyclovir and topically applied mycophenolate mofetil on ACV$^r$ HSV-2-induced skin lesions and associated mortality in athymic-nude mice

| | No. with lesion[a] | MDLA[b] | Survivors[a] | MDD[c] |
|---|---|---|---|---|
| Control | 10/10 | $6.8 \pm 1.3$ | 0/10 | $29.8 \pm 4.5$ |
| 5% MMF | 10/10 | $9.1 \pm 1.9$* | 0/10 | $30.2 \pm 7.8$ |
| 100 mg/kg ACV | 10/10 | $7.6 \pm 1.7$ | 0/10 | $27.6 \pm 7.1$ |
| 100 mg/kg ACV + 5% MMF | 5/10** | $16.1 + 5.1$ | 6/10** | $31 \pm 6.7$ |

For MDLA and MDD differences between multiple groups were analyzed by one-way ANOVA, indicating a significant statistical difference for MDLA ($P < 0.001$) but not MDD. Statistical difference between a particular group and the control was assessed by the two-tailed Student's $t$-test with Bonferroni correction.
[a] As evaluated at 60 days post-infection.
[b] Mean day of lesion appearance.
[c] Mean day of death.
*$P < 0.016$; **$P < 0.001$.

no protective effect but, when combined with MMF, would afford marked protection. MMF by itself had little or no effect on lesion development. This is in agreement with our previous findings (Neyts et al., 1998) that MMF and MPA have little or no effect on HSV-1- and HSV-2-induced cytopathogenic effect (CPE) progression and virus yield in cell culture assays. As shown in Table 1, neither ACV nor MMF, when used alone, reduced the number of animals that developed lesions or that eventually succumbed to the infection, although there was some minor delay in the mean day of lesion appearance (MDLA) and the mean day of death (MDD). However, when the systemic administration of ACV was combined with the topical administration of MMF, 90% of the animals remained lesion-free and survived the infection. In addition, no drug-related side effects were noted with MMF, and the scarified area healed rapidly.

In a second set of experiments (Table 2), athymic-nude mice were infected at the lumbosacral area with an ACV$^r$–TK$^-$ HSV-2 strain that was isolated from a patient with an ACV-refractory mucocutaneous lesion (Snoeck et al., 1994). Animals were treated with either ACV at 100 mg/kg per day (twice daily for 8 consecutive days) via s.c. injection, or a 5% MMF ointment (twice daily for 8 consecutive days), or the combination ACV (s.c.) + MMF (ointment) (for 8 consecutive days). Lesions induced by TK$^-$ HSV-2 in nu/nu mice developed somewhat slower than HSV-1-induced lesions in hairless mice. For this reason treatment was continued for 8 consecutive days, i.e. until almost all animals in the control and the ACV- or MMF-only groups had developed lesions. Also for this experiment we chose a dose of ACV (100 mg/kg per day) that as such has no protective action against the ACV$^r$–TK$^-$ HSV-2 infection but that results in marked protection when combined with MMF. Neither ACV nor MMF, when used alone, reduced the number of mice developing lesions as compared to the untreated controls. However, combined use of both drugs caused a pronounced protection. Fifty percent of the animals remained lesion-free or survived the infection, and in those mice that developed lesions, the MDLA was delayed by 10 days as compared to the controls, although this was not reflected in a delay in the MDD. We have as yet no explanation for this latter observation. Use of the combination ACV + MMF for a longer period of time (i.e. $> 8$ days) may be expected to result in an even more pronounced protection. The observation that MMF potentiates the action of ACV against the ACV$^r$–TK$^-$ HSV-2 strain implies that at least trace amounts of ACV-TP are formed in the virus-infected cells. These traces then appear sufficient to inhibit the viral DNA polymerase in the presence of reduced levels of dGTP; levels of which are depleted by the action of MMF. We have demonstrated earlier that guanosine reverses the potentiating effect of MMF on the activity of ACV against TK$^-$

HSV strains (Neyts et al., 1998). The monophosphorylation of ACV in cells infected with $TK^-$ HSV strains can be either accomplished by (i) some residual activity of the defective viral TK, or (ii) cellular TK and/or (iii) 5′-nucleotidase.

Thus, as demonstrated here, the combined use of ACV and MMF, given via different routes (i.e. systemically or topically, respectively) results in a markedly increased antiviral efficacy of ACV. The use of MMF in transplant recipients may thus be considered as a double-edged sword. On the one hand, it may precipitate the reactivation of opportunistic herpesvirus infections. On the other hand, once the patient receives ACV (or its prodrug valaciclovir) for this infection, the synergistic action between the two compounds may compensate for the increased risk of herpesvirus infections.

Prophylactic use of ACV for the prevention of cytomegalovirus (CMV) infections has received considerable attention (Kletzmayr et al., 1996; Patel, 1996; Zaia, 1996; Badley et al., 1997). Since we found that MMF also potentiates the in vitro anti-CMV activity of ACV (Neyts et al., 1998), it would be worth investigating whether MMF increases the anti-CMV activity of ACV in this cohort of patients.

Our present findings also suggest that the combination of ACV with MMF should be pursued in the treatment of ACV-resistant (muco)cutaneous herpesvirus infections in transplant recipients. ACV (or valaciclovir) could be administered systemically to prevent dissemination of the herpesvirus infection, whereas MMF could be applied topically to the affected area.

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