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Ganciclovir (GCV) resistance due to a new mutation in the UL97 gene product in a HCMV isolate from an AIDS patient.

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HCMV GCV-resistance may be due to mutations in both UL97 (phosphotransferase) and UL54 (DNA polymerase) gene products. Mutations in UL97 impair the drug anabolysm and are far more frequently detected in the clinical settings. A patient with AIDS and HCMV retinitis was submitted to virological follow-up and monitoring of antiviral treatment by quantitation of antigenemia, viremia and leuko-DNAemia for a period of 14 months (March 1994 to May 1995). Due to retinitis relapses, the patient was given 2 GCV IV induction treatments (Apr. 94, Sept. 94) each followed by standard maintenance treatment. The sharp drop in viral load documented a good response to therapy. A third induction course of GCV (Feb. 95) was ineffective in reducing the viral parameters and a GCV-resistant isolate was recovered from blood culture (VR5406, GCV ID50 = 18 uM). Sequencing analysis of UL97 revealed a GGT->AGC change in codon 598 of the gene leading to a Gly-> Ser substitution. This amino acid change was not present in pre-therapy sensitive isolates, which showed a silent GGT->GGC nucleotide change in the same codon. Mutations (substitutions or deletions) in positions 460, 520, 590-595, 607 of the gene have already been described as responsible for GCV-resistance in clinical isolates or laboratory-induced mutants. The Gly598->Ser change here described suggests that a larger portion of the UL97-encoded protein carboxy terminus is involved in the drug recognition and binding. The infrequent detection of Gly598->Ser change seems likely to be attributable to the double nucleotide substitution required. The biological significance of this mutation is currently under evaluation by GCV-phosphorylation analysis and marker transfer experiments.

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Ganciclovir (GCV) resistance caused by oral GCV in an heart transplant recipient with multiple HCMV strains in blood.

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An heart transplant recipient, seronegative for HCMV, was monitored for HCMV infection since Apr. 95. On May 95, the patient developed a primary infection which was treated on a preemptive basis with 2 courses of IV GCV (10 mg/Kg/d) with good virological response, as documented by reduction or disappearance of antigenemia, viremia and DNAemia. On June 95, the patient received a blood transfusion due to a severe anemia episode (Hb, 7.8/dL) associated with the presence of parvovirus DNA in plasma. A third induction treatment with IV GCV was administered in Aug. 95 and was followed by a 2-month maintenance treatment with oral GCV (3g/d). Although at a very low level, antigenemia and DNAemia were always positive during the oral GCV treatment. The viral isolates VR5549 (May, pretherapy), VR5611 (July 95), VR5712 (Oct. 95) showed GCV-sensitivity (ID50, <3uM). A relapse of HCMV infection occurred during oral GCV treatment. Despite the subsequent administration of IV GCV the virological parameters increased in level. The viral isolate VR5747 (Nov. 95) was GCV resistant (ID50, 25uM). The patient was then shifted to PFA (180 mg/d) with sharp reduction of all virological parameters. The isolate VR5878 obtained in Jan 96, was sensitive to GCV (ID50, <3uM). UL97 gene sequencing showed a C607=>Y change in VR5747. In addition, restriction analysis of multiple genome regions as well as the comparison between silent changes in UL97 gene from the sequential isolates showed the presence of two viral variants: the first lasting till Oct 95, and the second present afterwards. Thus: i) GCV resistance may arise in the transplantation settings; ii) oral GCV treatment may select resistant mutants; iii) multiple infections may occurr following organ transplantation and/or blood transfusion; iv) a disappearance of the UL97 mutation C607=>Y conferring GCV resistance may be possible after treatment change.

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Dose- and Treatment Duration-Dependence of Ganciclovir Against Murine Cytomegalovirus Infection in Severe Combined Immunodeficient Mice---Experimental and Clinical Implications

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The present study examines the clinical relevance of the murine cytomegalovirus (MCMV) infection in severe combined immunodeficient (SCID) mouse model in terms of dose- and treatment duration-dependent effects of ganciclovir (GCV). It also seeks the optimal treatment duration for drug screening in this model. Animals inoculated intraperitoneally with MCMV at a titer of $10^{3.8}$ plaque-forming units per mouse developed typical wasting syndrome rapidly and died around day 12 post inoculation. Subcutaneous injection of GCV once daily for 5 days dose-dependently delayed MCMV-induced wasting syndrome and mortality at the dose-range of 1 to 80 mg/kg/day, while the 160 mg/kg/day dose induced toxic effects as revealed by body weight loss. The GCV effect on mean death day was linearly correlated with reduction of viral titers from the lung ($r=0.969$, $p<0.05$). Treatment duration-dependence at the optimal dose of GCV at 80 mg/kg/day for 1, 5, 8 and 12 days demonstrated a consistent protection in delaying viral replication, wasting syndrome and death (3-4 days plus the treatment duration). At a moderately effective dose of GCV at 10 mg/kg/day, maximum protection was achieved with a 8 day treatment regimen. Prolongation to 12 days failed to further delay wasting syndrome (starting at day 10 for both 8 and 12 days treated groups) and animal death, indicating insufficient suppression of viral replication. A single day of GCV treatment showed that the minimum effective dose (80 mg/kg/day) was very close to the toxic dose (160 mg/kg/day). These results demonstrate that the described model mimics clinical observations in terms of sustained effects of optimal GCV treatment and relapses of CMV infection upon termination of the treatment. It also suggests that 5-8 days treatment duration may be the optimal balance between maximizing the opportunity of identifying active anti-CMV compounds and speeding up the turnover rate of drug screening.

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Lethal Hepatitis During Acute MCMV Infection; Analysis and Treatment With Ganciclovir and HPMPC.

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We have investigated the relationship between mortality, elevation of serological markers of hepatitis and viral replication in the liver in the weanling BALB/c mouse following acute intraperitoneal infection with murine cytomegalovirus (MCMV). MCMV (Smith Strain, salivary gland passed) infection produced an inoculum-dependent mortality, weight loss, elevation of serological markers of hepatitis (plasma liver transaminases ALT: alanine transaminase and AST: aspartate transaminase) and organ burdens of MCMV. The %-mortality and mean death day following an inoculum of $7x10^4$ plaque forming units (pfu) of MCMV was 90% and 4.1±0.2 days post inoculation. Three days post inoculation the percent mean weight loss and fold elevation of ALT and AST were 19±2%, 24 fold and 15 fold respectively. Organ titers (log pfu/g tissue) of MCMV in the liver, spleen and lung were 6.16, 6.05 and 4.14 respectively. There was an excellent correlation ($r=0.98$) between liver titers of MCMV and elevation of either ALT or AST. At an inoculum of $3.5x10^3$ pfu there was 12±3% weight loss, no mortality and serological markers were maximally elevated 2 days post inoculation. The elevation of serological markers lasted for up to seven days prior to returning to normal, coincident with normalization of MCMV induced weight loss. Treatment with ganciclovir either via the intraperitoneal, subcutaneous or oral route produced a dose-dependent reduction of weight loss, mortality, serological marker elevation and liver titers of MCMV; the ED_{50} values for each of these processes being in good agreement with each other. This was also true for HPMPC which was ~40 fold more potent than GCV. These results strongly suggest that acute intraperitoneal infection of weanling BALB/c mice with MCMV results in a hepatitis which contributes to overall disease and mortality and can be reversed by inhibition of viral replication in the liver.