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Short communication

Combinations of ganciclovir and antibody for experimental CMV infections

Frans S. Stals[a,*], Sjoerd Sc. Wagenaar[b], Jeroen S. Kloover[a], Ward Y.R. Vanagt[a], Cathrien A. Bruggeman[a]

[a]*Department of Medical Microbiology, University of Limburg, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands*
[b]*Department of Pathology, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands*

Abstract

The effect of combined treatment with ganciclovir and hyper immune serum (HIS) was evaluated in three animal models. It concerned a generalized CMV infection model, a meningo-encephalitis model and an interstitial lung disease (ILD) model in immunocompromised rats. In the generalized model, the ganciclovir and HIS had a moderate synergistic effect on survival and greatly decreased virus titers in internal organs. In contrast, in the meningoencephalitis model, combined treatment had no effect on the local virus titers and the histopathology. Combined treatment with ganciclovir and HIS, however, effectively abolished CMV-induced ILD.

Keywords: Cytomegalovirus; Combination-therapy; Ganciclovir; Hyper-immune-serum; Animal

1. Introduction

Combined treatment with ganciclovir and hyper immune serum (HIS) has been widely used for the treatment of cytomegalovirus (CMV) infections in man, and seems especially appropriate for the treatment of CMV infection in allogeneic bone marrow transplant recipients C. Emanuel et al., 1988, Reed et al., 1988). Because of many confounders, such as concomitant opportunistic infections and differences in the type of immunosuppression, however, it is difficult to measure the effect of anti-CMV treatment in immunocompromised man. Therefore, we performed experimental studies on the combined effect of ganciclovir and HIS in three rat models, using a rat-specific cytomegalovirus (RCMV, strain Maastricht)

The first model was used to study the treatment of generalized CMV infections as prevalent in perinatally infected neonates and in patients with the acquired immunodeficiency syndrome (AIDS). Life threatening meningo-encephalitis was in-

* Corresponding author.

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duced in a second model. The third model concerned CMV-induced ILD, as prevalent in allogeneic BMT recipients. We studied the combined action of ganciclovir and HIS on the outcome of disease under these circumstances.

2. Materials and methods

2.1. Infection models

Animals were infected with rat CMV (RCMV) (Bruggeman et al., 1983). In the generalized model RCMV infection was established in immunosuppressed male Brown Norway (BN) rats as described previously (Stals et al., 1990). The effect of treatment was studied with respect to survival, reduction of virus titers and histopathology in the internal organs. The minimal effective dose, the duration of treatment were optimized for each drug as described elsewhere (Stals et al., 1990, 1991).

In the meningo-encephalitis model, rats received intracerebral infection with 10^5 plaque forming units (PFU) of RCMV after they had received 5 Gray total body irradiation for immunosuppression. Survival was recorded for 21 days. At 8 days p.i., virus titers were determined and brain tissue was studied histologically.

To induce CMV-induced ILD, rats received an allogeneic bone marrow transplantation after otherwise lethal total body irradiation, immediately followed by intraperitoneal inoculation of 10^5 PFU RCMV (Stals et al., 1994). The presence of infectious virus and histopathology in the lung were evaluated at 8 days p.i., as described elsewhere (Stals et al., 1994).

2.2. Treatment

The neutralization titer (NT_{50}) of the HIS preparation was 640, whereas the NT_{50} of a control serum (CIS) was below 10. One ml of HIS, diluted in tissue culture medium, was administered intravenously at 6 hours p.i. Ganciclovir was administered at varying dosages administered from 6 h. p.i. twice daily for 5 days. For combined treatment the same regimen were used.

3. Results

3.1. Generalized RCMV infection

3.1.1. Survival

As depicted from Fig. 1, the minimal effective dose (ED_{50}), or the daily dose at which at least 50% of the animals survived was 20 mg/kg/day (twice daily for 5 days) for ganciclovir and one bolus HIS of 40 times the NT_{50}. The combined effect of ganciclovir and HIS was calculated from the fractionary effective dose ($FED = (ED^{\text{in combination}}_{\text{ganciclovir}}/ED^{\text{alone}}_{\text{ganciclovir}}) + (ED^{\text{in combination}}_{\text{HIS}}/ED^{\text{alone}}_{\text{HIS}}) = (3.9/16.8) + (3.9/15.0)$) to be 0.49, which indicated a moderate synergistic effect. Treatment with CIS did not affect survival, neither administered as a single drug, nor during combined treatment. When combined treatment was delayed until 3 days p.i., it had no effect on survival.

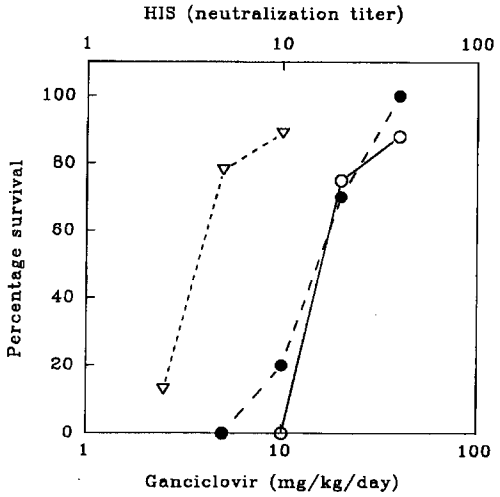

Fig. 1. The combined effect of ganciclovir and HIS treatment on survival from generalized RCMV infection. Groups of 12 BN rats each received 5.0 Gy TBI 24 h before either intraperitoneal inoculation with 10^5 RCMV. Survival was recorded for 21 days p.i. Ganciclovir was administered intravenously at different dosages twice daily for 5 days, started 6 h p.i. (– ● –). HIS 1 ml with a neutralization titer of 640 was diluted and administered in one single dose 6 h p.i. (– ○ –). The corresponding titer after dilution is presented on the superior X-axis. Combined treatment was started at 6 h p.i. as indicated on both X-axis (– ▽ –).

3.1.2. Virus titers

The effect treatment on the virus titers in several organs was determined at 8 days p.i. Ganciclovir at 20 mg/kg/day reduced RCMV titers in the liver and lungs by 3 log values versus untreated controls ($P < 0.01$). Lower dosages of ganciclovir did not reduce virus titers in any organ. Administration of HIS (at an NT_{50} of 160) caused a significant reduction of virus titers in the liver and lungs as compared to untreated controls ($P < 0.01$). Combined treatment with ganciclovir and HIS significantly reduced virus titers in lungs and liver to below detection level ($P < 0.01$).

3.2. Intracerebral CMV infection

At 8 days p.i., high virus titers (6.1–6.3 log PFU of RCMV) were obtained by plaque assays from brain tissue. The infection was established mainly in the leptomeninges and in the lateral ventricles.

Animals treated with 1 ml of HIS intravenously and rats treated with ganciclovir at 80 mg/kg/day for 5 days did not survive intracerebral infection. In addition, combined treatment with both drugs did not survive. Neither virus titers in the brain tissue nor the number of antigen harbouring cells were reduced. In addition, histopathologic changes, induced by RCMV infection were not affected by any treatment regimen.

3.3. CMV-induced ILD

3.3.1. Virus titers and histopathology

CMV-infected animals developed a severe ILD, characterized by high infectious virus titers in the lung and viral antigens in the interstitial septa, with concomitant inflammatory response in the perivascular and interstitial region of the lung (Fig. 2). Virus titers were decreased by ganciclovir, but the inflammatory response was hardly influenced. HIS affected both virus titers and inflammatory response, but treatment with CIS did not. Combined treatment completely reduced both virus titers and interstitial infiltrates. Interestingly, the combined treatment with ganciclovir and CIS did slightly effect the virus titers in the lung, but effectively abolished the inflammatory response in the interstitial septa.

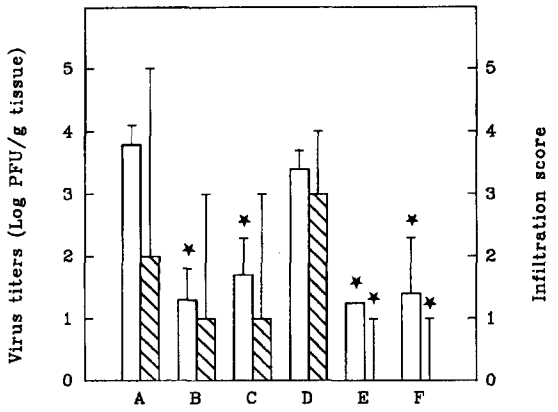

Fig. 2. The effect of combined treatment with ganciclovir and HIS on the infectious virus titers and inflammatory response in the lungs of allogeneic bone marrow transplant recipient rats. Virus titers were expressed as mean (open bars) and standard deviation. The infiltration of mononuclear cells in the alveolar septa was scored on a scale from 0 (absent) to 3 (maximum) and expressed as the median (dashed bars) and range. These parameters were studied in the following groups of rats: Untreated animals (A); animals treated with ganciclovir 20 mg/kg/day in two daily doses for 5 days (B); animals treated with 1 ml of HIS at a neutralization titer of 160 (C); control immune serum at a neutralization titer below 10 (D); combined treatment of ganciclovir and HIS at corresponding dosages (E); combined treatment with ganciclovir and control immune serum (F). *Differences by t-test (titers) and Krusskal wallis two way ANOVA (infiltration score) vs. group A, $P < 0.05$.

4. Discussion

These data clearly demonstrated that combined treatment with ganciclovir and HIS has a moderate synergistic effect on survival from life threatening generalized CMV infections, and that it reduced the viral load in the internal organs. This supports the idea that clinical trials on the combined effect of HIS and ganciclovir should be encouraged in patient groups with immature or impaired immunity.

Combined systemic administration of ganciclovir and HIS for the treatment of CMV-induced meningo-encephalitis had no beneficial effect. The

alternative of local administration should be explored.

Combined treatment with ganciclovir and HIS greatly improved the outcome of ILD in allogeneic BMT recipient rats. In addition, the use of HIS seems to be superior above randomly chosen intravenous immunoglobulin preparations.

From these results, we recommend the combined treatment with ganciclovir and HIS for CMV infections in allogeneic BMT recipients and the investigation on combined treatment for generalized CMV infections in other patients with impaired immunity, such as AIDS patients, perinatally infected infants and organ transplant recipients.

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