論文内容の要旨

論 文 題 目 : Drug-Resistant Herpes Simplex Virus Infections in Immunocompromised Patients: Study on its Pathophysiology and Development of a Rapid Drug-Sensitivity Assay System

和訳：免疫不全症患者における薬剤耐性単純ヘルペスウイルス感染症の病態に関する研究と迅速薬剤感受性試験法の開発

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平成１９年４月進学

保健学博士課程

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ABSTRACT

The primary infection with herpes simplex virus type 1 (HSV-1) causes acute gingivostomatitis, which is usually benign in children. After the primary infection, HSV-1 establishes latent infection in the neuronal ganglia. HSV-1 latently infected in the neuronal ganglia reactivates from latency and causes skin infections such as herpes labialis. However, HSV-1 infections in immunocompromised patients are severe,

intractable, and frequently recurrent. Chronic and intractable HSV-1 infections of immunocompromised patients require long-term acyclovir (ACV) administration, resulting in ACV-resistant (ACV^r) HSV-1 infections in some patients. Most of the ACV^r HSV-1 are due to mutations in the viral thymidine kinase (vTK), which is one of the target proteins for antiherpetic drugs. It was reported that vTK-deficient (vTK^-) HSV-1 could establish latency in mouse trigeminal ganglia, but it could not reactivate from latency. The mechanism of the reactivation of TK^- HSV-1 in humans is still unclear. The precise sensitivity assay of HSV-1 isolates to various vTK-associated drugs such as ACV, ganciclovir (GCV), and brivudine (BVdU) provides an useful information for proper treatment. A plaque reduction assay (PRA) is the most popular and the standard for an evaluation of the sensitivity of HSV-1 to antiviral drugs. HSV-1 is considered to be ACV^r, if the 50% effective concentration (EC_{50}) shows >2.0 μg/ml. The PRA-based sensitivity system requires virus isolation, titration of the isolate, and PRA in the process of the assay, and thus takes a long period of time to obtain the results. This is a disadvantage in the treatment of HSV-1 infections in immunocompromised patients and in patients with severe forms of infection. Therefore, development of a rapid and reliable sensitivity assay system has been desired.

In the first chapter of this study, one of the mechanisms for the reactivation of TK^- HSV-1 in humans was discussed by the clinical observation of and virological analyses on a child with congenital immunodeficiency for about 10 years from the primary infection at the age of 3 to the fatal infection, progressive multifocal encephalopathy, due to JC virus at the age of 13. The child suffered from a severe infection due to ACV^r HSV-1 at the age of 8 (Age-8 ACV^r infection). He suffered from recurrent ACV-sensitive (ACV^s) and ACV^r HSV-1 infections before and after this episode. HSV-1 isolates recovered from this patient were virologically analyzed. All the ACV^r HSV-1 isolates were revealed to be TK^- due to a single cytosine (C) deletion within the 4-C residues (positions 1061 to 1064) in the TK gene. Furthermore, TK^-/ACV^r HSV-1 with this mutation existed in the total populations of ACV^s HSV-1 isolates recovered before and after the Age-8 ACV^r infection, but not in that of ACV^s HSV-1 isolate recovered at the primary infection. These results suggest that TK^-/ACV^r HSV-1 reactivated with that of TK^+/ACV^s HSV-1, and that TK^-/ACV^r HSV-1 might reactivate using the TK activity induced by the latently co-infected TK^+/ACV^s HSV-1 when it reactivated.

In the second chapter of this study, a novel drug-sensitivity assay system was developed. The 293T cells transiently expressed with vTK by transfection of the cells with the expression vector were infected with TK^-/ACV^r HSV-1 (HSV-1 TAR), and

were then cultured in maintenance medium with or without designated concentration of ACV, GCV and BVdU. The replication of HSV-1 TAR was strongly inhibited by ACV, GCV and BVdU in 293T cells expressed with vTK of ACV^s viruses, while it was not inhibited in those expressed with vTK of highly resistant and intermediately ACV^r viruses. The inverse correlation was demonstrated between EC_{50}s and inhibitory effect of these compounds on the replication of HSV-1 TAR. The HSV-1 isolates' solution recovered from 5 ACV-therapy resistant subjects with HSV-1 keratitis was sent from Chile in an inactivated form. Out of 5 isolates, one was demonstrated to be ACV, GCV, and BVdU-resistant and the rest were to be sensitive to these compounds. The time required for the measurement of the inhibitory effect could be minimized by the introduction of quantitative real-time PCR. The sensitivity of HSV-1 to antiviral drugs is usually determined by plaque reduction assay, which usually requires more than 10 days. However, the time required for the novel sensitivity assay system was less than 4 days, making it possible to treat patients with drug-resistant HSV-1 infections more properly.

In summary, the possible mechanism of the reactivation of the vTK^-/ACV^r HSV-1 in humans was discussed. Furthermore, a novel and rapid sensitivity assay system for HSV-1 to vTK-associated drugs was developed.