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THYMIDINE KINASE AS A CAUSATIVE FACTOR FOR TYPE 1 HERPES SIMPLEX VIRUS RESISTANCE AGAINST ACYCLOVIR

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ABSTRACT

Background: *Herpes Simplex Virus (HSV) infection demonstrates a high prevalence in the world. Acyclovir, one of guanine synthetic analogues, is commonly used to treat infections caused by HSV. HSV resistance against acyclovir may occur, especially in immunocompromised and immunocompetent patients, as the consequence of viral mutations. Thymidine kinase (TK) is an HSV tegument protein which plays an important role in HSV-1 resistance against acyclovir.* ***Purpose:*** *The purpose of this article is to review the mechanisms of TK mutation that cause HSV-1 resistance against acyclovir.* ***Review:*** *Acyclovir involves three stages of viral thymidine kinase phosphorylation to form acyclovir triphosphate. It prevents HSV replication by acting as a competitive inhibitor of viral DNA polymerase and a chain terminator in viral DNA synthesis. Resistance is associated with viral TK mutation that is encoded by U_L23 gene. Long-term use of acyclovir may promote thymidine kinase mutation in immunocompromised and immunocompetent patients via three mechanisms, namely absolute insufficiency in TK activity (TK-negative), depletion in TK synthesis, and inability in TK phosphorylation which consequently hinders the phosphorylation of acyclovir. Herpes TK gene contains a series of cytosine and guanosine, that are important for the function and the mutation of HSV by producing incomplete or fewer enzymes as the result of nucleotide addition or elimination in homopolymer process.* ***Conclusion:*** *HSV-1 resistance against acyclovir is evolved from TK mutations, in the form of TK-negative, TK low-producing, and TK altered mutants, that are unable to phosphorylate TK and accordingly disrupt acyclovir phosphorylation to convert acyclovir triphosphate.*

Keyword : *Acyclovir, Herpes simplex virus, Resistance, Thymidine kinase*

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INTRODUCTION

Herpes simplex virus (HSV) is a herpesviridae-family DNA virus that may prompt various infections in human. It is categorized into three subfamilies (α, β and γ) based on its biologic and genomic resemblance. α-Human herpes virus comprises of Herpes Simplex Virus (HSV-1, HSV-2) and Varicella Zoster Virus (VZV). The virus infects 60-95% of the population which is predominantly affected by the strains from this alpha subfamily.[1] HSV-1 is frequently related with oral and perioral infection, while HSV-2 in general will instigate infections around genital area. HSV may induce divers pathological conditions from mild to severe infection, such as cold sores, keratitis, corneal blindness, and encephalitis.[1,2]

Herpes Simplex Virus type 1 (HSV-1), an enveloped double-stranded DNA (dsDNA) virus from α-herpes virus subfamily, contains two unique regions namely long unique region (UL) and short unique regions (US) that encodes at least 84 proteins. HSV-1 genome is located inside nucleocapsid, and it is surrounded by tegument proteins. The proteins within nucleocapsid and tegument are composed of lipid and glycoprotein that is essential not only for viral adherence but also viral entry into host cells.[3-6]

HSV-1 is highly contagious and endemic around the world in which childhood infection will result in viral latency within the body. Transmission might occur through droplet or inoculation where most HSV-1 infections present as oral herpes that emerge inside or around the mouth, commonly known as oral labial, orofacial herpes.[3,5,7] The virus becomes latent in trigeminal ganglion and the infected neuron will be surrounded inherently by $CD4^+$ and $CD8^+$ T cell to control the HSV-1 reactivation. Interaction between host immunity and HSV-1 depends on the immunological response of HSV-1 specific T cell, dendritic cell, as well as $CD4^+$ and $CD8^+$ cell.[3,5]

Anti-HSV drug has been developed for more than 60 years where acyclovir and its derivate are currently prescribed as the first line therapy for the management of HSV infection.[7-13] Acyclovir (*acycloguanosine*) is a synthetic drug from *guanosine* analog that is ten times clinically more effective against HSV-1 and HSV-2 than for being a Varicella Zoster Virus (VZV) treatment. Acyclovir entails three stages of phosphorylation to convert acyclovir triphosphate that functions in inhibiting viral DNA synthesis and replication.[14] However, acyclovir treatment as long-term prophylaxis and curative treatment may generate resistance in HSV. Acyclovir resistance in both immunocompetent and immunocompromised patients will result in more chronic and threatening viral infection.[1,8,14-17]

Resistance towards acyclovir is evolved through a change in thymidine kinase (TK) or DNA polymerase.[8,14,15] Thymidine kinase (TK) is a gene encoded by HSV-1 unique region23 (U_L23), one of HSV tegument protein, which is responsible for thymidine synthesis and HSV-1 unique region30 (U_L30) which encodes HSV DNA polymerase to initiate replication.[4,15] Acyclovir resistance is established via viral TK mutation.[7,8,10,12,18,19] The prevalence for HSV resistance towards acyclovir are varied between 3.5% to 10%, with higher prevalence observed in immunocompromised patients and hematopoietic stem cell transplant recipients.[7-13] Ninety five percent cases exposed HSV isolates that was clinically resistant towards acyclovir in relation with viral TK gene mutation, and the nucleotide mutation was apparently more frequent in HSV-1 gene than HSV-2 TK.[11,13]

This article aims to discuss the mechanism of TK mutation that instigates HSV-1 resistance towards acyclovir.

LITERATURE REVIEW

Acyclovir (acycloguanosine) is a synthetic drug from guanosine analog that possesses three hydroxyl groups on the third chain.[15] Clinically, this drug is effective against herpes simplex virus (HSV) type 1 through selective activation of acyclovir monophosphate by HSV thymidine kinase upon acyclovir triphosphate so that the accumulation of active metabolites only occur inside the infected cell.[14,15,20] Acyclovir triphosphate inhibits viral DNA synthesis through two mechanisms, competition with deoxyguanosine triphosphate (deoxyGTP) and removal of viral DNA polymerase in the irreversible complex of DNA that cause premature termination of viral DNA. The lack of 3'-hydroxyl group in acyclovir triphosphate enable the compound to bind with viral DNA as a competitor as well as a chain terminator.[14,20]

The bioavailability of acyclovir is quite low (15-20%) and the drug becomes ineffective when concurrently consumed with food. Two hundred milligrams (five times daily) or four hundred milligrams (thrice daily) of acyclovir is the therapeutic dosage for primary herpetic infection that has been proven to significantly reduce the frequency/the severity level, the symptoms and the healing time of infection; therefore, it may suppress the rate of transmission. Acyclovir is a potent prophylaxis in both immunocompromised and immunocompetent patiens against HSV infection. Recurrence may prevail immediately upon the halt of acyclovir prescription.[14]

Oral acyclovir is known to be beneficial as HSV treatment. It consistently lessens painful sensation, accelerates healing, and confers as a long-term prescription for prophylaxis in immunocompetent and immunosuppressed patients. The mechanism of acyclovir resistance itself has been investigated in vivo and in vitro. Resistance towards acyclovir is rarely observed in immunocompetent patients but it may cause an extensive muco-cutaneous disease as well as the occurrence of meningo-encephalitis, pneumonitis, or visceral diseases.[15] Recurrence after the halt of acyclovir prescription in immunocompetent patients is commonly initiated by

heterogeneous virus that is resistant against acyclovir.[21]

Thymidine kinase is a β-gene family which is encoded by HSV-1 gene, unique long 23 (U_L23). It is classified as a tegument protein that is responsible for thymidine synthesis into thymidine monophosphate (TMP) and inhibits deoxycytidine kinase (dCK) activity for deoxycytidine kinase (dCK) phosphorylation into dCyd monophosphate. TK is also in charge of acyclovir phosphorylation to convert acyclovir triphosphate, a competitive DNA in viral replication as well as a suppressor for viral polymerase DNA activity, thus validating TK as an important target for the development of acyclovir antiviral therapy.[2,11] The structure of HSV-1 TK consists of 376 amino acid proteins transcripted by U_L23 within the frame shift. Important parts that is included in the enzyme activity are ATP binding site (aa51-63), nucleoside binding site (aa168-176), and cysteine site at codon 336 that functions in the three-dimensional structure maintenance of the active sites.[13,19] Other than that, there are six conserved regions that facilitate TK mutation. These regions are located in amino acid 56 to 62 (site 1), 83 to 88 (site 2), 162 to 164 (site 3), 171 to 173 (site 4), 216 to 222 (site 5), and 284 to 289 (site 6). TK structure is arranged of 15 α-helices and 7 β-sheets. Five stranded parallel β sheets from the main protein specifically contain the active sites.[13,19s]

As a part of β gene family, thymidine kinase regulates HSV-1 TK gene by following the time and the cascading effect. Immediate-early gene (IE or α), early gene (E or β), and late gen (L or γ) are three common genes found in HSV-1. In viral infection process, these gene functional expressions are controlled by α protein and depend on RNA polymerase II (RNA Pol II) transcription. Five important proteins of alpha gene are ICP0, ICP4, ICP22, ICP27, and US1.5. ICP0, ICP4, ICP22, and ICP27 are believed as the main regulator protein for HSV-TK gene, especially ICP4 that is required for the activation of TK gene transcription. ICP27 protein, a TK gene expression post-transcription regulator, increases DNA binding activity of ICP4. ICP0 demonstrates huge influence towards transcription rate of all beta genome, therefore ICP0 protein inactivation may significantly reduce TK enzyme regulation in mRNA level.[4,6,11,13]

Stages of HSV replication is initiated by HSV adherence on host cell and followed by viral protein binding with glycosaminoglycan (GAG) or heparan sulfate. Viral proteins that act in the adhesion process are glycoprotein C (gC) and glycoprotein B (gB). Further, glycoprotein D (Gd) will fusionally aid viral entry into host cells. Viral fusion with cell membrane will be assisted by gD through one of the three receptors binding with viral glycoprotein namely nectin 1, nectin 2, and herpes virus entry mediator (HVEM) or 3'-O-Sulfat-Ed Heparan Sulfat.[4,6] Viral fusion into host cell will be followed by the entry of viral tegument and capsid inside cytoplasm. The capsid of HSV migrates into microtubules and transfers viral DNA into host cell nucleus with the support of viral protein 16 (Vp16).[4-6] After the entry of viral DNA within the nucleus, the translation and the transcription process of viral genome will occur.

The replication of viral DNA and the assembly of new capsid take place inside host nucleus. Vp16 as a *viral host shutoff* (VHS) protein is mobilized to the nucleus causing degradation in host messenger RNA (mRNA). Host DNA will later be translated by RNA Polymerase II (RNA Pol II) into α messenger RNA (α mRNA).[6] HSV genome is composed of DNA that also produce viral RNA molecule during infection cycle as the consequence of RNA molecule transcription when it is transformed as tridimensional mRNA that can be identified by body sensor.[10] α messenger RNA and α gene (Immediate-Early gene) are early components transcripted without the presence of viral protein synthesis de novo. α protein transactivates β gene (delayed-early gene) into β protein to replicate viral DNA molecule since several β gene products are known as DNA binding enzymes and protein. The synthesis of viral DNA molecules stimulates γ gene expression that results in γ protein synthesis used for the arrangement of capsid, nucleus and modified membrane in new virion formation. After the viral assembly has been accomplished, virus will be released to infect other host cells.[6]

Various types of HSV genes are affecting the regulation of each gene transcription interchangeably. α protein activates β gene transcription, while the inhibition of β gene transcription may lower the rate of α protein synthesis. Activation of γ gene transcription may terminate β protein expression on the later stage. In viral infection process, functional gene expression is regulated by α protein and depends

on the mechanism of RNA polymerase II (RNA Pol II) transcription along the transcription process.[6,13]

Primary infection of HSV occurs in epithelial or mucosal cells and becomes latent in trigeminal ganglion. During the latent stage, genome transcription is inhibited by LATs (latency associated transcripts). Recurrence emerges in mucosal epithelium when the latent virus is reactivated. Immune system holds an important role to prevent the replication of HSV-1 during primary infection and inherently maintain the virus to remain dormant.[22] Equilibrium between neuron environment, LATs, and $CD8^+$ cells determines the reactivation of viral lytic gene in neuronal ganglia. A number of factors may initiate the reactivation, such as menstrual cycle and birth control drugs. Medroxy-progesteronacetate is an example of birth controls that reduces the number of $CD8^+$ in trigeminal ganglion and generates the decline of interferon γ activity. Stress may also promote the reactivation as it increases the level of glucocorticoid cortisol that will reduce the expression of interferon γ produced by T cells.[22]

DISCUSSION

The TK structure of HSV-1 is comprised of amino acid protein 376 that is coded by gene U_L23 within the frame shift. Thymidine kinase is a part of β gene family which is trans-activated by α protein. It is a delayed-early gene that forms β protein and behaves as a molecule replicator for viral DNA. Thymidine kinase presents no predominant role in the replication of HSV but it is required for acyclovir transformation into acyclovir triphosphate. As thymidine kinase is encoded by U_L23, any mutation in this gene will cause impediment in acyclovir triphosphate conversion. Mutation in TK does not hinder HSV reactivation and pathogenesis, but it particularly prevents the activation of acyclovir.[6,13]

Acyclovir targets viral thymidine kinase as it may be transformed into acyclovir monophosphate. Two additional phosphates will be earned from host cell enzymes to formulate acyclovir triphosphate that is subsequently transported inside the nucleus. Viral DNA polymerase separates pyrophosphate from acyclovir triphosphate that later will bind 2′-deoxyguanosine monophosphate of viral DNA and initiates chain terminator. Further elongation of viral DNA chain will not occur as acyclovir monophosphate does not possess 3' hydroxyl group that is essential for the entry of additional nucleotide and exonuclease. Viral DNA also demonstrates no ability to eliminate acyclovir group.[14,15]

Acyclovir-resistant HSV infection may occur in immunocompetent and immunocompromised patients. Two mechanisms are proposed to initiate such condition: first, prolonged viral replication among patients with dysregulated immune system enabling the occurrence of persistent infection. Second is disrupted and decreased body response that facilitates viral development into resistant pathogen and results in more severe symptoms. Resistance towards acyclovir among immunocompetent patients demonstrates a very low prevalence. It is not related with side effects, but more into the prolonged use of acyclovir as prophylaxis and therapy.[19] Most viral infections that are resistant toward acyclovir can be detected among AIDS patients with mucocutaneous lesions, as well as in several life-threatening HSV infections that is commenced by acyclovir-resistant mutants.[11,16] This proves that immune system is crucial in the process of viral mutation. The diversity of viral genetics is defined by various process related to virus and host, and that the viral mutation may develop as selective response against particular stressors.[23]

Mutation in U_L23 viral gene that encodes thymidine kinase (TK) enzyme and UL30 that encodes DNA polymerase enzyme may present in the form of nucleotides deletion, addition or substitution in conserved regions that modify the frame shift. U_L23 gene mutation has been reported in 95% clinical isolates that are resistant to acyclovir with high level of polymorphism. Nucleotides addition or deletion has been confirmed as the cause of those reported cases, especially the homopolymer repetition of guanine (G) or cysteine (S) as hot spot resistance.[11,13]

Six conserved regions that promote viral mutation are located in amino acid 56 to 62 (site 1), 83 to 88 (site 2), 162 to 164 (site 3), 171 to 173 (site 4), 216 to 222 (site 5), and 284 to 289 (site 6). TK structure is arranged of 15 α-helices and 7β-sheets. Five stranded parallel β-sheets from the main protein contain the active sites.[13,19] Addition or deletion in these sites might change the frame shift reading that result in incomplete synthesis of functional enzymes (non-functional). Two long homopolymer 7G and 6S are

the most frequent sites that experience mutation. Clinical report on U_L23 gene isolates TK negative identified strains that include the deletion of S in 3-S string or the elimination of G from 3-G stretch, or the deletion of one A (arginine) in 4A string that is a component of ATP-binding sites. Excepting G and S deletion from the active sites, cysteine modification is generated by tyrosine at 336 position. Mutation will result in TK low-producer mutant phenotype which is also known as TK partial mutant or TK altered isolate. C336Y mutation affects ATP-binding active sites and instigates a change in nucleoside analog binding affinity, which disrupts three-dimensional structures from all active sites by shifting LID domain and result in more open active catalytic conformation. Mutation may also be generated outside the conserved regions, and it may foster the resistance against acyclovir.[11,13]

There are three different phenotypes of TK mutation identified from varying clinical isolates, that are TK-negative mutant with an absolute TK insufficiency, low TK producing mutants with a decline in enzymatic activity, and a substrate specific mutant (TK-altered specificity isolate) which phosphorylates thymidine yet unable to be used for the phosphorylation of acyclovir. Around 95% of HSV clinical isolates that are resistant towards ACV are TK negative and TK low producer phenotypes, while the minority is comprised of TK-altered mutants. A mixture of viral mutants is discovered as well in the study.[7,11-13,24] Although TK has no association with viral replication, it is essential for viral pathogenesis especially viral activation and latency in trigeminal ganglion.[6,11]

Dental practitioners are advised to select a proper drug for the management of HSV-1 infection, specifically in immunocompromised patients, since drug resistance may result in chronic diseases and life-threatening conditions.

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