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Short communication

Comparison of human cytomegalovirus (HCMV) protease sequences among laboratory strains and seven clinical isolates

Tom K. Huffaker*, Susan Binford, Amy K. Patick, Christopher Pinko, Chen-Chen Kan, Leora S. Zalman

Pharmacology and Molecular Biology Groups, Agouron Pharmaceuticals, 3565 General Atomics Court, CA 92121, USA

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Abstract

The nucleotide sequence of the human cytomegalovirus (HCMV) protease gene from two laboratory strains and seven clinical isolates, both ganciclovir-sensitive and -resistant, was examined to determine the genetic variability of the HCMV protease catalytic domain and to identify changes that may alter the efficacy of designed protease inhibitors. The Towne strain varied from AD169 at 12 nucleotides and led to one amino acid change at position 12 (Ala to Thr). The clinical isolates had amino acid substitutions relative to the laboratory strains, with a Ser to Pro change at position 8, a His to Tyr change at position 44 and a Gly to Ser change at position 47. None of these changes occurred in any of the conserved domains of the protease, nor do they appear necessary to confer ganciclovir resistance in the isolates. These findings suggest that no changes exist in the protease of the clinical isolates examined that may diminish the effectiveness of a drug targeting the HCMV protease.

Keywords: Human cytomegalovirus; Protease inhibitors; Drug resistance

Human cytomegalovirus (HCMV) belongs to the family Herpesviridae, together with herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), and varicella-zoster virus (VZV). HCMV is a serious pathogen in immunocompromised individuals, especially those patients with AIDS, those receiving organ or bone marrow transplants, or those undergoing cancer chemotherapy or steroid therapy. Ganciclovir, foscarnet and cidofovir, inhibitors of viral DNA polymerase, are currently the only therapeutics approved for use in the treatment of HCMV infections. However, drug resistance by the virus to each of these has been observed (Erice

* Corresponding author.

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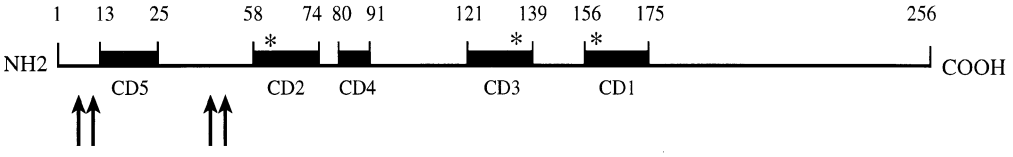
et al., 1989; Stanat et al., 1991; Tatarowicz et al., 1992) and the drugs have the undesired side effects of nucleoside analogs (Darby, 1995). The incidence of resistance to ganciclovir can be quite significant, with studies ranging from 8 to 66% of treated patients (Drew et al., 1991; Chou et al., 1995). In addition, it has been noted that patients who have been treated with ganciclovir can develop cross-resistance to other DNA polymerase inhibitors (Tatarowicz et al., 1992; Dunn et al., 1995). As an alternative treatment strategy, inhibitors of the viral enzyme HCMV protease have been considered. Mutations in its HSV-1 homolog, the UL26 open reading frame (ORF), have been shown to render virions non-infectious (Gao et al., 1994). Together, these data make HCMV protease an attractive alternative target for antiviral chemotherapy. It is the purpose of this paper to determine the genetic variability of the HCMV protease catalytic domains in both laboratory strains and ganciclovir-sensitive and -resistant clinical isolates, and to identify changes that may alter the efficacy of designed protease inhibitors.

The viral genome of herpesviruses is replicated inside the nucleus of infected cells and is then packaged into an intermediate capsid. Following acquisition of a nuclear membrane envelope, the virion is released from the infected cell. Several reports have indicated that herpes protease plays a critical role in HSV-1 viral particle maturation by cleavage of the assembly protein precursor (ICP35), a major component of the intermediate capsid. This activity has been mapped to the HSV-1 UL26 ORF. The UL26 ORF overlaps with the ICP35-producing UL26.5 ORF and these two ORFs are 3′ co-terminal. Liu and Roizman (1991) reported that the product of the UL26 ORF is an 80-kDa protease. The HCMV UL80 ORF was reported to share homology with HSV-1 UL26 ORF (Chee et al., 1990) as well as with the assembly protein nested gene (APNG) ORF of the simian cytomegalovirus (SCMV) (Robson and Gibson, 1989; Welch et al., 1991). Gibson and coworkers more recently demonstrated that the C-terminal region of the HCMV UL80 encodes a protein which is proteolytically processed in a fashion similar to the HSV-1 ICP35 (Gibson et al., 1990; Schenk et al., 1991). The HCMV protease catalytic domain has been localized to its amino half comprising amino acid residues 1–256 (Baum et al., 1993). This region also contains five conserved sequence motifs which are highly homologous to proteases of other herpes viruses (Gibson et al., 1994).

The design of inhibitors for the HCMV protease requires a detailed understanding of its structure and catalytic mechanism. Any changes in primary sequence which exist among clinical isolates, especially in highly conserved sequence motifs, may alter the binding and effectiveness of any designed inhibitor. In addition, prior studies have shown ganciclovir resistance to be associated with mutations in the UL97 phosphotransferase coding region of HCMV (Chou et al., 1995; Wolf et al., 1995). While it is unlikely that changes would also be seen in the protease region as a result of acquired resistance to ganciclovir in these clinical isolates, it is important that any changes be ruled out.

We have compared the protease sequences of the HCMV laboratory strains AD169 and Towne, as well as seven clinical isolates, both ganciclovir-sensitive and -resistant. AD169 and Towne strains were obtained from American Type Culture Collection (Rockville, MD). All clinical isolates were obtained from Dr S. Stanat at Burroughs Wellcome (Research Triangle Park, NC) and passaged in MRC-5 cells. Cell-associated viral stocks were harvested from infected cells after three freeze–thaw cycles, followed by centrifugation and polyethylene glycol precipitation of supernatant. Pelleted virions were digested by proteinase K in the presence of sodium dodecyl sulfate, and the DNA was phenol extracted and ethanol precipitated. After polymerase chain reaction amplification, gel-purified products were ligated into pMGH4 (Kan et al., 1992) and transformed into *Escherichia coli* strain XL1-B (Stratagene, Cambridge, UK). Plasmid DNA was isolated (Qiagen-tip 100 kit, Qiagen) G-25 column purified, sequenced using Applied Biosystems Model 373A automated sequencer (Scripps Research Sequencing Facility, La Jolla, CA) and analyzed using Sequencher 3.0 software (Gene Codes).



AMINO ACID POSITION				STRAIN OR ISOLATE	GCV TOX
8	12	44	47		
S	A	H	G	AD169	SEN
S	T	H	G	TOWNE	SEN
P	A	H	G	8803	SEN
P	A	H	G	8912	SEN
P	A	Y	S	8915	RES
P	A	Y	S	8916	RES
P	A	Y	S	8918	RES
S	A	H	G	9208	SEN
S	A	H	G	9209	RES

Fig. 1. Amino acid sequence variations in laboratory strains and clinical isolates. HCMV protease catalytic domain (amino acids 1–256) is shown with the five conserved domains (CD1–5). Asterisks depict amino acids H63, S132 and H157 of the catalytic triad. Bars correspond to amino acid positions 8, 12, 44 and 47 as shown in the table. S, serine; P, proline; A, alanine; T, threonine; H, histidine; Y, tyrosine; G, glycine; GCV, ganciclovir; Sen, sensitive; Res, resistant.

Sequencing of the protease gene regions revealed the following changes relative to the AD169 strain. The Ala at residue 12 was replaced by a Thr in the Towne strain (see Fig. 1). Eleven other base changes were found in the Towne strain at base numbers 66, 252, 255, 258, 291, 513, 522, 540, 543, 576 and 756. These did not result in any amino acid substitutions. The sequences of the ganciclovir-sensitive clinical isolates 8803 and 8912 revealed a common Ser to Pro substitution (S8P) and two of the silent changes common to the Towne laboratory strain. Three ganciclovir-resistant isolates 8915, 8916 and 8918 were obtained from patients with AIDS at UCSF's Mt. Zion Hospital (Stanat et al., 1991). All exhibited the amino acid substitutions S8P, His to Tyr at residue 44 (H44Y) and Gly to Ser at residue 47 (G47S), as well as three silent mutations common to the Towne strain. The last two clinical isolates examined, 9208 and 9209, were obtained from the same patient before and after ganciclovir treatment and represent ganciclovir-sensitive and -resistant isolates, respectively. These samples showed no amino acid substitutions relative to AD169, three silent changes also seen in the Towne strain and three unique silent changes.

The HCMV protease catalytic domain of the Towne strain differs from the previously sequenced AD169 laboratory strain in at least one amino acid position, also exhibiting numerous silent changes predominantly at the C-terminal end. The three ganciclovir-resistant isolates 8915, 8916 and 8918 show sequence similarity, all containing the two substitutions, H44Y and G47S. It is intriguing to speculate that similar or identical viral strains may be isolated from patients in the same geographical area, although it is unknown when and where these patients acquired CMV. These changes in the protease region do not appear necessary for ganciclovir resistance, however, as evidenced by the absence of substitutions in the sensitive and the resistant isolates, 9208 and 9209, respectively, from the same patient. This is an important finding, given that an ideal HCMV protease inhibitor be effective against both ganciclovir-sensitive and -resistant viruses. Only substitution H44Y lies toward the end of the first α-helix. The other three substitutions lie either toward the N-terminus or in a flexible loop region. In addition, none of the clinical isolates shows substitutions in any of the five conserved domains common to the herpes protease family or

near the putative active site residues, His63, Ser132 and His157 (Chen et al., 1996; Shieh et al., 1996). These findings are particularly noteworthy from a drug design standpoint, supporting the notion that no changes exist in the protease region of the clinical isolates examined that may diminish the effectiveness of a drug that targets the CMV protease.

References

Baum, E.Z., Beebernitz, G.A., Hulmes, J.D., Muzithras, V.P., Jones, T.R. and Gluzman, Y. (1993) Expression and analysis of the human cytomegalovirus UL80-encoded protease: identification of autoproteolytic sites. J. Virol. 67, 497–506.

Chee, M.S., Bankier, A.T., Beck, S., Bohni, R., Brown, C.M., Cerny, R., Horsnell, T., Hutchinson, C.A., Kouzarides, T., Martignetti, J.A., Preddie, E., Satchwell, S.C., Tomlinson, P., Weston, K. and Barrell, B.G. (1990) Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169, Curr. Top. Microbiol. Immunol. 154, 125–169.

Chen, P., Tsuge, H., Almassy, R.J., Gribskov, C.L., Katoh, S., Vanderpool, D.L., Margosiak, S.A., Pinko, C., Matthews, D.A. and Kan, C.-C. (1996) Structure of the human cytomegalovirus protease catalytic domain reveals a novel serine protease fold and catalytic triad. Cell 86, 835–843.

Chou, S., Guentzel, S., Michels, K.R., Miner, R.C. and Drew, W.L. (1995) Frequency of UL97 phosphotransferase mutations related to ganciclovir resistance in clinical cytomegalovirus isolates, J. Infect. Dis. 172, 239–242.

Darby, G. (1995) In search of the perfect antiviral. Antiviral Chem. Chemother. 6, 54–63.

Drew, W.L., Miner, R.C., Busch, D.F., Follansbee, S.E., Gullett, J., Mehalko, S.G., Gordon, S.M., Owen, W.F., Matthews, T.R., Buhles, W.C. and DeArmond, B. (1991) Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection, J. Infect. Dis. 163, 716–719.

Dunn, J.P., MacCumber, M.W., Forman, M.S., Charache, P., Apuzzo, L. and Jabs, D.A. (1995) Viral sensitivity testing in patients with cytomegalovirus retinitis clinically resistant to foscarnet or ganciclovir, Am. J. Ophthamol. 119, 587–596.

Erice, A., Chou, S., Biron, K.K., Stanat, S., Balfour, H.H. and Jordan, M.C. (1989) Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N. Engl. J. Med. 320, 289–293.

Gao, M., Matusick-Kumar, L., Hurlburt, W., DiTusa, S.F., Newcomb, W.W., Brown, J.C., McCann, P.J., Deckman, I. and Colonno, R.J. (1994) The protease of herpes simplex virus type 1 is essential for functional capsid formation and viral growth. J. Virol. 68, 3702–3712.

Gibson, W., Marcy, A.I., Comolli, J.C. and Lee, J. (1990) Identification of precursor to cytomegalovirus capsid assembly protein and evidence that processing results in loss of its carboxy-terminal end. J. Virol. 64, 1241–1249.

Gibson, W., Welch, A.R. and Hall, M.R.T. (1994) Assemblin, a herpes virus serine maturational proteinase and new molecular target for antivirals. Persp. Drug Discov. Des. 2, 413–426.

Kan, C.C., Gehring, M.R., Nodes, B.R., Janson, C.A., Almassy, B.J. and Hostomska, Z. (1992) Heterologous expression and purification of active human phosphoribosylglycinamide formyltransferase as a single domain. J. Prot. Chem. 11, 467– 473.

Liu, F. and Roizman, B. (1991) The herpes simplex virus 1 gene encoding a protease also contains within its coding domain the gene encoding the more abundant substrate. J. Virol. 65, 5149–5156.

Robson, L. and Gibson, W. (1989) Primate cytomegalovirus assembly protein: genome location and nucleotide sequence. J. Virol. 63, 669–676.

Schenk, P., Woods, A.S. and Gibson, W. (1991) The 45-kilodalton protein of cytomegalovirus (Colburn) B-capsids is an amino-terminal extension form of the assembly protein. J. Virol. 65, 1525–1529.

Shieh, H.-S. Kurumbail, R.G., Stevens, A.M., Stegeman, R.A., Sturman, E.J., Pak, J.Y., Wittwer, A.J., Palmier, M.O., Wiegand, R.C., Holwerda B.C. and Stallings, W.C. (1996) Three-dimensional structure of human cytomegalovirus protease. Nature 383, 279–282.

Stanat, S.C., Reardon, J.E., Erice, A., Jordan, M.C., Drew, W.L. and Biron, K.K. (1991) Ganciclovir-resistant cytomegalovirus clinical isolates: mode of resistance to ganciclovir, Antimicrob. Agents Chemother. 35, 2191–2197.

Tatarowicz, W.A., Lurian, N.S. and Thompson, K.D. (1992) A ganciclovir-resistant clinical isolate of human cytomegalovirus exhibiting cross-resistance to other DNA polymerase inhibitors. J. Infect. Dis. 166, 904–907.

Welch, A.R., McNally, L.M. and Gibson, W. (1991) Cytomegalovirus assembly protein nested gene family: four 3′ coterminal transcripts encode four in-frame, overlapping proteins. J. Virol. 65, 4091–4100.

Wolf, D.G., Smith, I.L., Lee D.J., Freeman, W.R., Flores-Aguilar, M. and Spector, S.A. (1995) Mutations in human cytomegalovirus UL97 gene confer clinical resistance to ganciclovir and can be detected directly in patient plasma. J. Clin. Invest. 95, 257–263.