Review

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Drug Resistance to HCV in Direct-Acting Antiviral Treatments

Doğrudan Etkili Antiviral Tedavilerde HCV İlaç Direnci

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ABSTRACT

Approved treatment protocols for hepatitis C virus (HCV) include direct-acting antiviral drugs (DAA), which consist of NS3 protease inhibitors (PI), NS5A replication complex inhibitors, and NS5B polymerase nucleoside and non-nucleoside inhibitors (NNI). First-generation DAAs are effective in specific genotypes (GT), such as NS5AIs (dasabuvir), PIs (asunaprevir, grazoprevir, paritaprevir/r, and simeprevir), and NNIs (ledipasvir, ombitasvir, and elbasvir). Second-generation DAAs are "pan-genotypic" and include NS5AIs (velpatasvir and pibrentasvir), PIs (voxilaprevir and glecaprevir), and an NSBNI (sofosbuvir). DAAs can potentially cure people with hepatitis C. However, although generally well tolerated, DAAs have not been observed to produce sustained virological response in some patients. Resistance-associated amino acid changes (RAS), which can lead to treatment failure, may be the reason for this. The RASs can occur naturally or during treatment and are influenced by various factors such as the treatment plan, HCV-GT/subtype, and endemic characteristics. While there are no standardized tests for investigating HCV-RAS, Sanger dideoxynucleotide sequencing-based approaches are generally reliable, even though their sensitivities range from 10% to 25%. However, new generation sequencing techniques are more sensitive and can detect variants with a prevalence of 1%, despite some ongoing debate about the clinical significance of variants at this level.

Keywords: Hepatitis C, HCV, direct-acting antiviral, drug resistance

ÖZ

Hepatit C virüs (HCV) için onaylanmış tedavi protokolleri, NS3 proteaz inhibitörleri (PI), NS5A replikasyon kompleksi inhibitörleri ve NS5B polimeraz nükleozid ve non-nükleozid inhibitörlerden (NII) oluşan direkt etkili antiviral ilaçları (DAA) içerir. Birinci nesil DAA'lar, NS5AI'lar (dasabuvir), PI'lar (asunaprevir, grazoprevir, paritaprevir/r ve simeprevir) ve NNI'lar (ledipasvir, ombitasvir ve elbasvir) gibi spesifik genotiplerde (GT) etkilidir. İkinci nesil DAA'lar "pan-genotipiktir" ve NS5AI'ları (velpatasvir ve pibrentasvir), PI'ları (voksilaprevir ve glekaprevir) ve bir NSBNI'yı (sofosbuvir) içerir. DAA'lar, hepatit C'li hastaları potansiyel olarak tedavi edebilir. Ancak, genellikle iyi tolere edilmelerine rağmen, DAA'ların bazı hastalarda kalıcı virolojik yanıt sağlamadığı gözlemlenmiştir. Tedavi başarısızlığına yol açabilen dirençle ilişkili aminoasit değişiklikleri (RAS), bunun nedeni olabilir. RAS'ler doğal olarak veya tedavi sırasında ortaya çıkabilir ve tedavi planı, HCV-GT/alt tipleri ve endemik özellikler gibi çeşitli faktörlerden etkilenir. HCV-RAS'leri araştırmak için standartlaştırılmış testler bulunmamakla birlikte, duyarlılıkları %10 ila %25 arasında değişse de Sanger dideoksinükleotid sekanslama genellikle güvenilirdir. Bununla birlikte, yeni nesil sıralama teknikleri daha hassastır ve klinik önemi hakkında devam eden bazı tartışmalara rağmen, %1'lik prevalansa sahip varyantları saptayabilir.

Anahtar Kelimeler: Hepatit C, HCV, direkt-etkili antiviral, ilaç direnci

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Introduction

According to the World Health Organization (WHO), approximately 58 million individuals across the globe are currently living with hepatitis C infection. Even children and adolescents are not spared, as an estimated 3.2 million people are affected by this infection. Shockingly, approximately 79% of these cases have not been diagnosed, and only 13% have received treatment. Although hepatitis C virus (HCV) is still prevalent among specific populations, such as intravenous drug users (IVDUs) and homosexual men, there is good news. Direct-acting antiviral drugs (DAAs) can potentially cure people with hepatitis C. However, limited access to diagnosis and treatment worldwide is still a concern (1,2).

Viral Fitness

HCV is a type of RNA virus with a single strand that falls under the hepacivirus genus of the Flaviviridae family (3). The genomic RNA of HCV is ~9.6 kb, and the RNA-coding polyprotein precursor is ~3000 amino acids in size. The genome organization of HCV is shown in Figure 1. HCV has a remarkably high daily replication rate of 10^{12} virions. The virus's NS5B polymerase activity, which is responsible for RNA-dependent RNA polymerase, has a significant error rate of 10^{-3} to 10^{-5} per base pair copied and lacks error correction capabilities (3,4). This error rate generates mutant variants during viral replication, resulting in the replicative HCV population's significantly increased genetic diversity. Research suggests that this genetic diversity may lead to speciation associated with resistance to therapies (5).

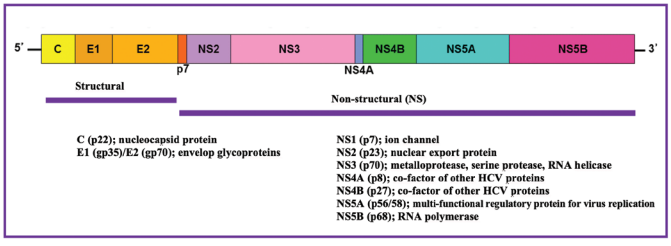

Figure 1. Genome organization of HCV. Genomic RNA is ~9.6 kb, and RNA encoding polyprotein precursor is ~3000 amino acids (6-8)
HCV: Hepatitis C virus

AASLD/IDSA 2023 Guideline

Guidelines for diagnosing, treating, and managing HCV infection were released for the first time in 2013 by the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) (www.aasld.org). These guidelines aimed to provide clinicians with unbiased and evidence-based recommendations. Second-generation DAAs have proven to be highly effective in eliminating hepatitis C, which is critical for achieving the WHO-HCV elimination targets. Although generally well tolerated, some patients may not achieve sustained virologic response (SVR). This can occur because of resistance-associated substitutions (RAS), which can cause treatment failure (9,10). Approved DAAs for hepatitis C treatment according to first- and second-generation specifications by the European Medicines Agency and the U.S. The Food and Drug Administration are shown in Table 1. However, the preferred treatment/retreatment regimens for HCV-infected patients according to the AASLD-IDSA 2023 update guide are shown in Table 2.

Table 1. Direct-acting antivirals approved for hepatitis C treatment according to first- and second-generation specifications (5,11,12)

HCV drug class target	Direct-acting antiviral	
	First generation	Second generation
NS3PIs	Simeprevir, grazoprevir, asunaprevir, and paritaprevir/r	Glecaprevir, voxilaprevir
NS5AIs	Daclatasvir, ledipasvir, elbasvir, and ombitasvir	Pibrentasvir, velpatasvir
NS5BNI/NNI	Dasabuvir	Sofosbuvir

HCV: Hepatitis C virus, NS3PIs: NS3 protease inhibitors, NS5AIs: NS5A replication complex inhibitors, NS5B NI/NNI: NS5B polymerase nucleoside and non-nucleoside inhibitors

Table 2. Preferred treatment/retreatment regimens for HCV-infected patients in the AASLD-IDSA guide 2023 update (13)

HCV-DAA target	DAA treatment regimen	Brand name	Manufacturer	Country
NS3PIs + NS5AIs containing	Grazoprevir/elbasvir	Zepatier	MSD	Rahway, NJ, USA
	Glecaprevir/pibrentasvir	Maviret	abbVie	North Chicago, IL, USA
NS5BNIs containing	Ledipasvir/sofosbuvir	Harvoni	Gilead	San Dimas, CA, USA
	Velpatasvir/sofosbuvir	Epclusa	Gilead	San Dimas, CA, USA
	Voxilaprevir/velpatasvir/sofosbuvir	Vosevi	Gilead	San Dimas, CA, USA

HCV: Hepatitis C virus, AASLD: American Association for the Study of Liver Diseases, IDSA: Infectious Diseases Society of America, DAA: Direct-acting antiviral, NS3PIs: NS3 protease inhibitors, NS5AIs: NS5A replication complex inhibitors, NS5BNIs: NS5B polymerase nucleoside inhibitors

Direct-Acting Antivirals

The treatment of hepatitis C has progressed significantly because of the development and authorization of DAA medications. More than 95% of cases are cured when a patient achieves SVR. Approved treatment plans for HCV now include DAAs, which consist of NS3 protease inhibitors (PIs), NS5A replication complex inhibitors (NS5AIs), and NS5B polymerase nucleoside (NS5BNIs) and non-nucleoside (NS5BNNIs) inhibitors. First-generation DAAs are effective in specific genotypes (GT), such as NS5AIs (dasabuvir), PIs (asunaprevir, grazoprevir, paritaprevir/r, and simeprevir), and NNIs (ledipasvir, ombitasvir, and elbasvir). Second-generation DAAs are "pan-genotypic" and include NS5AIs (velpatasvir and pibrentasvir), PIs (voxilaprevir and glecaprevir), and an NSBNI (sofosbuvir) (Table 1) (https://www.hcvguidelines.org/).

Resistance-Associated Substitutions

It is crucial to consider that RASs may hinder the success of DAA treatments for hepatitis C. Depending on the current DAA and HCV-GTs, Table 3 lists the most common RASs found in the NS3, NS5A, and NS5B drug target areas. These RASs can occur naturally or during treatment and are influenced by various factors such as the treatment plan, HCV-GT/subtype, and endemic characteristics (14).

Conducting HCV resistance analysis has become increasingly crucial for improving the effectiveness of treatment and avoiding the reemergence of HCV-resistant variants for DAAs. Although there are no standardized tests for investigating HCV-RAS, Sanger dideoxynucleotide sequencing-based approaches are generally reliable, even though their sensitivities range from 10% to 25.0%. However, new generation sequencing (NGS) techniques are more sensitive and can detect variants with a prevalence of 1%, despite some ongoing debate about the clinical significance of variants at

Table 3. Resistance-associated substitutions are natural or acquired after a failure to a DAA regimen in the HCV-NS3, NS5A, and NS5B drug class targets according to the latest-generation DAA and HCV genotype (5,11,12,15-17)

HCV drug class target	Direct-acting antiviral	HCV-RAS patterns associated with treatment failure considering HCV genotypes					
		GT1a/b	GT2	GT3	GT4	GT5	GT6
NS3	Simeprevir	V36M, Q80K/R, S122R/T, R155K/G/T, D168A/E/F/H/N/V/T	NA	NA	V36M, Q80K/R, S122R/T, R155K/G/T, D168A/E/F/H/N/V/T	NA	NA
	Grazoprevir	V36L/M, Y56F/H, Q80K/L, R155G/I/K/L/Q/S/T, A156G/M/V/T, V158A, D168A/C/E/G/H/K/N/V/Y	NA	NA	A156S/T, D168A/C/E/G/K/N/V/Y	NA	D168A/C/E/G/K/N/V/Y
	Asunaprevir	Q80K/L, D168A/E/H/Q/T/V/Y	NA	NA	NA	NA	NA
	Paritaprevir/r	Y56H, R155K, D168A/C/E/G/K/N/V/Y	NA	NA	Y56H, D168A/C/E/G/K/N/V/Y	NA	NA
	Glecaprevir	V36M, Y56H/N, Q80K/R, S122G, R155T, A156G/T/V, and Q168A/K/L/R/V	No RASs	V36M, Y56H/N, Q80K/R, R155T, A156G/T/V, and Q168A/K/L/R	ND	ND	ND
	Voxilaprevir	Q80K, A156L/T/V	No RASs	No RASs	A156S	No RASs	ND
NS5A	Daclatasvir	M28A/T, Q30E/H/K/R, L31IF/M/V, R30H, H58D, and Y93C/H/I/N/R	ND	A30K, L31I, Y93H	L28M/V, L30H/R/S, Y93C/H	ND	ND
	Ledipasvir	K24R, L28M, M28A/T/V, Q30E/H/K/R/Y, L31F/I/M/V, S38F, H58D, A92T, and Y93C/F/H/N	NA	No RASs	L28M, L30H/R, M31L/V, T/P58L, and Y93C/H/S	NA	NA
	Elbasvir	M28A/G/S/T, Q30D/E/G/H/K/R/Y, R30H, L31F/I/M/V, H58D, and Y93C/H/N/S	NA	NA	L28M/S, L30H/R, M31L/V, P58D, and Y93C	NA	NA
	Ombitasvir	L28M, M28T/V, Q30E/H/K/L/R/Y, R30Q, L31F/M/V, H58D, Y93C/F/H/L/N/S	NA	NA	L28S/V, L30R, M31L/V, Y93H	NA	NA
	Pibrentasvir	K24R, M28A/G, Q30K/R, L31F/M, P32del, H58D, and Y93H/N	F28C, L31M	S24F, M28G/K, A30G/K, L31F/I/M, P58T, and Y93H	ND	ND	ND
	Velpatasvir	M28T/V, Q30E/H/K/L/R, L31I/M/V, Y93C/H/L/N/R/S/W/T	L31I/M/V, Y93H	A30K/V, L31M/P/V, E92K, Y93H/N/R	ND	ND	ND
NS5B	Dasabuvir	C316H//N/Y, M414I/T/V, Y448C/H, A553T/V, G554S, S556G/N, G558R, D559N/G	NA	NA	NA	NA	NA
	Sofosbuvir	L159F ± C316N or L320F, L159F + V321A, S282G/R/T	S282T	L159F ± C316N or L320F, L159F + V321A, and S282R/T	S282C/T	S282T	S282T

DAA: Direct-acting antiviral, HCV: Hepatitis C virus, NS: Non-structural, RAS: Resistance-associated amino acid substitutions, GT: Genotype, NA: Not applicable, ND: No data

this level. There is still some controversy regarding the therapeutic importance of mutations below 15% in patient samples (18).

Aspects for Clinics

For pan-genotypic DAA regimens, baseline RAS resistance testing is generally not recommended for velpatasvir/sofosbuvir and glecaprevir/pibrentasvir. It is worth mentioning that NS3 RASs can result in Q80K in up to 40% of patients with HCV-genotype 1a (GT1a), whereas NS5A RASs may be present in 5% to 15% of patients. For some cases, conducting a baseline RAS resistance analysis could be advantageous in identifying the best DAA treatment for patients with HCV-GT3 infections who have not been treated before. In hepatitis C, drug resistance is typically linked to RASs emerging due to DAA suppression. Notably, RASs in HCV can revert to their wild type, which can take several months for the NS3 gene region and years for the NS5A gene region (17,19,20).

The guidelines for treating GT1a infection have been updated in the IDSA 2023 guidelines, and the recommended treatment regimen has been revised. The previous suggestion of using elbasvir/grazoprevir has been substituted with a different treatment plan because it is necessary to conduct a baseline RAS analysis. The updated guidelines now suggest NS5A RAS analysis for treating HCV-GT3 infection with compensated cirrhosis and for initial treatment in adult patients infected with HCV-GT1-6. It is recommended to add ribavirin by weight or follow another recommended course of therapy if the natural prevalence of RAS is greater than 5% and baseline NS5A RAS Y93H is present (13).

Research has demonstrated that certain RASs can have an impact on SVR rates in hepatitis C, which can make it challenging to identify treatment options for patients who have already undergone treatment (17,21). Although newer and more effective DAA regimens are available for first- or second-line treatments, obtaining access to them can pose a challenge (22). Studies of in vivo infectious cDNA clones of HCV-GT3 in human hepatoma cells (Huh7.5) have shown that adaptation to NS5A drug resistance is also possible in pan-genotypic HCV-DAA regimens (23,24). A recently published real-life-based study focused on the NS3 and NS5B resistance of Asian HCV-GT3. Phylogenetic analyses indicate that GT3a is of Asian origin. GT3a has been observed to be similar to Asia in IVDUs in Europe (25). The rates of SVR with DAAs are very high; early treatment can reduce chronic hepatitis C complications and disease transmission. Considering this information, a more comprehensive understanding of RASs is essential to tailor first-line therapy and determine the most appropriate course of action for second-line therapy (26,27).

SHARED Initiative

The treatment of hepatitis C involves a range of genetic types and subtypes, making it challenging to obtain information about treatment resistance from local, regional, and short-term clinical trials. However, these studies have limited potential for generalized findings and are not commonly found in real-life cohorts. Although second-generation potent DAA treatment regimens can counter the adverse effects of RASs, the causes of virologic failure remain unclear. Studies addressing DAA resistance in hepatitis C lack standardization of analytical techniques and no clear definition of RASs. Despite the International Hepatitis C Treatment Guidelines recommending resistance testing for specific regimens or patient groups, the evaluation of drug resistance test results is not standardized. In response to this challenge, the Surveillance of Hepatitis C Antiviral Resistance, Epidemiology, and Methodologies (SHARED) is a global initiative that aims to develop, apply, and share HCV genomic data, methods, software, and technologies to better understand and prevent HCV drug resistance (28). The SHARED international collaboration is a formidable entity comprising physicians, virologists, and researchers from 22 countries and over 110 medical facilities and laboratories. This powerhouse possesses a vast database of HCV sequences, comprehensive patient information, disease characteristics, treatment histories, and clinical outcomes. The thorough and diverse nature of the data allows for unparalleled analysis. It provides unparalleled insights into HCV-DAA resistance on a global scale - insight that individual studies cannot match (5).

Conclusion

In conclusion, drug resistance caused by RAS to DAA medications can lead to an inadequate response to antiviral therapy and relapse in HCV-infected patients. We should be aware that HCV has high genetic variability, and RASs may lead to future failure of currently available DAA treatments. Therefore, monitoring drug resistance may be essential for pan-genotypic HCV-DAA regimens. RAS can occur naturally or be selected during therapy. To determine the most appropriate DAA treatment, it is crucial to identify the HCV-GT/subtype and detect pre-existing RAS. The analysis can be performed using either Sanger sequencing or NGS sequencing.

Ethics

Peer-review: Externally peer-reviewed.

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