methotrexate, prednisolone, and/or TNF-α inhibitors for more than one year. HBV reactivation was observed in two of five patients with HBsAg, compared with only in one of the remaining 45 patients without it. Therefore, HBV reactivation leading to de novo hepatitis B was observed in 2% (1%/year) of patients. It should be noted that the lone HBsAg-negative reactivation patient had been treated with methotrexate but not with TNF-α inhibitors. Mori[47] performed a cross-sectional analysis of 239 patients with RA who were treated with biological and/or non-biological agents, among whom 60 were found to have HBV markers indicating earlier HBV infection. Of these, two were signal-positive for serum HBV DNA but without ALT elevation or HBsAg positivity: one patient was treated with tacrolimus, prednisolone, and methotrexate, and the other was treated with adalimumab, prednisolone, and methotrexate. Whereas HBV DNA level in the former patient increased and HBsAg and HBeAg became weakly positive after 10 weeks, the latter patient became HBV DNA-negative without additional anti-viral therapy. The authors also concluded that biological and non-biological agents are relatively safe in RA patients with past HBV infection. Thus, these studies suggested that the occurrence of de novo hepatitis B was rare in RA patients who were treated with TNF-α inhibitors in addition to DMARDs over the medium term. A large-scale post-marketing surveillance study was carried out in Japan to determine the safety profile of infliximab in patients with RA.[18] All patients with RA who were treated with infliximab were prospectively monitored for any adverse events for a period of 6 months after the initiation of infliximab. No cases of de novo hepatitis B were found. Although the follow-up period was short, the number of patients enrolled was over 5000. This report indicated that de novo hepatitis B due to TNF-α inhibitors would be very rare over the short-term as well.

In contrast to the abovementioned reports, several studies have suggested a relatively high incidence of de novo hepatitis B due to TNF-α inhibitor therapy. Kim *et al.*[48] followed 266 patients with RA who were treated with TNF-α inhibitors and analyzed the occurrence of clinically significant (over two times higher than normal range) and persistent (two or more incidences) alanine aminotransferase (ALT) elevation in relation to HBV markers. Elevation of ALT was significantly more frequent in patients with HBcAb (HBsAg negative) than in those without (16% vs. 6%, $P = 0.009$). In multiple logistic regression analysis controlling for various potential confounding factors, such as methotrexate, nonsteroidal anti-inflammatory drugs, and type of TNF-α inhibitor, only potential occult HBV infection was identified as a significant risk factor for ALT elevation, suggesting a close association between HBcAb-positivity and ALT elevation during TNF-α inhibitor therapy in RA patients. However, it cannot be confirmed whether ALT elevations in that study were indeed caused by reactivation of occult HBV because HBV DNA was not measured along with ALT. Urata *et al.*[49] prospectively followed 135 patients with RA who had HBV markers suggesting past HBV infection for 12 months. The cohort was treated with biological and/or non-biological anti-rheumatic agents and followed for a total mean period of approximately 20 months, including the period before follow-up. Serum HBV DNA was measured every 3 months during the study period, and revealed that HBV reactivation occurred in seven patients (5%/year). HBV reactivation was significantly associated with use of TNF-α inhibitors with a hazard ratio of 10.9 ($P = 0.008$). This study suggested that careful monitoring of HBV DNA level is required in RA patients with resolved hepatitis B when receiving anti-rheumatic agents, especially biologic ones.

In Japan, HBV reactivation rates tend to differ regionally. A study from Aomori prefecture[49] in the northern part of Japan reported a relatively higher rate of de novo hepatitis stemming from TNF-α inhibitors than studies from Osaka[46] and Kumamoto[47] prefectures in the central and southern parts of Japan, respectively. It is speculated that these differences are attributed to variations in HBV genotype distribution; whereas genotype B is predominant in the former area, genotype C is more frequent in the latter areas.[50] Further studies are required to address this phenomenon.

In light of the above findings, it is evident that RA patients with past HBV infection who are treated with anti-rheumatic agents are at risk of developing HBV reactivation and ensuing de novo hepatitis B, especially those being treated with anti-rheumatic agents, such as TNF-α inhibitors, for an extended time. Spontaneous remission of HBV reactivation was observed in one of the two patients reported by Mori[47] and two of the seven patients reported by Urata *et al.*,[49] and so it should be noted that HBV reactivation does not necessarily result in the occurrence of de novo hepatitis B.

PROPHYLACTIC MEASURES FOR DE NOVO HEPATITIS B

THREE MEASURES ARE generally used to prevent de novo hepatitis B due to immunosuppressive therapy.[7] The first measure is to regularly check for

serum HBV DNA during immunosuppressive therapy and administer NAs should it be detected. The second measure is to administer NAs from the onset of immunosuppressive therapy. The third measure is to maintain circulating HBsAb titer using HB vaccines and/or HB immunoglobulins. Reports have suggested that regular evaluation of HBV DNA is effective in avoiding de novo hepatitis in patients treated with TNF-α inhibitors because HBV reactivation could be controlled by NAs when found at an early stage.[46,49] It is still unclear how often and for how long patients should be tested to detect HBV viremia. Prophylactic administration of NAs is also an option to preempt de novo hepatitis B due to TNF-α inhibitors because NAs are normally used to prevent reactivation in carrier patients. However, the issue of cost-efficiency versus relatively low incidence of de novo hepatitis B needs to be reconciled. Lastly, maintenance of circulating HBsAb titer using HB vaccines may be effective in responders since several studies[44,46] have shown that HBsAb titer decreases during TNF-α inhibitor therapy. As with HBV DNA monitoring and prophylactic NA administration, further studies are required to clarify the extent of HB vaccination effectiveness in preventing de novo hepatitis B due to TNF-α inhibitors.

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＜特別寄稿＞

核酸アナログ薬中止に伴うリスク回避のための指針2012
—厚生労働省「B型肝炎の核酸アナログ薬治療における治療中止基準の作成と治療中止を目指したインターフェロン治療の有用性に関する研究」の報告—

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索引用語：　核酸アナログ薬　治療中止　B型肝炎　肝炎再燃
HBV cccDNA

はじめに

近年B型肝炎の治療に導入された核酸アナログ薬はHBVの増殖を強力に抑制するため，多くの症例で血中HBV DNA量は速やかに低下しALT値の正常化がもたらされる[1)]．さらに，組織学的な改善が得られ肝発癌率が低下することや[2)3)]，経口薬で副作用も少ないことから臨床的に広く使用されている．しかし，核酸アナログ薬を使用してもウイルスを完全に排除することは困難であり，本治療薬には耐性株の出現や治療中止に伴う肝炎の再燃が問題点として残されている[4)]．この原因の一つとして，血中のHBV DNA量が低下しても，HBV複製の起源となる肝細胞核内のHBV cccDNA量はほとんど減らず，これが長期に残存することが挙げられている[5)]．

B型肝炎の核酸アナログ薬治療において，同薬の中止はしばしば肝炎の再燃を伴うため，安易な中止はすべきでないとされている．しかし，中止後，いつ頃どの様な形で肝炎が再燃するかは必ずしも明らかにされてはいない．また，中止後に肝炎が再燃しない症例や再燃しても軽度で最終的に安定化する症例も少なからず存在するが，この様な症例を効率よく見分ける方法も確立されていない．

我々は，厚生労働省の科学研究費により「B型肝炎の核酸アナログ薬治療における治療中止基準の作成と治療中止を目指したインターフェロン治療の有用性に関する研究」（平成21年度～23年度）を行い，治療中止後の経過の特徴や肝炎再燃の定義，さらには再燃率の予測を検討した．本稿では，この研究成果を元に「核酸アナログ薬中止に伴うリスク回避のための指針2012」をまとめたので報告する（Table 1）．本指針は必ずしも核酸アナログ薬の中止を推奨するものではなく，様々な理由により中止を検討する必要がある場合の参考になるよう定めた．

I. 本指針の目指すもの

本指針は，核酸アナログ薬の中止を検討する際に，中止成功の可能性が高い症例や逆に治療を継続すべき症例を明らかにすること，さらに，中止後の経過観察

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Table 1　核酸アナログ薬中止に伴うリスク回避のための指針　2012

I. 本指針の目指すもの

B型慢性肝炎の核酸アナログ薬治療において，同薬の中止によりdrug free を目指すことは重要な治療目標の一つである．しかし，同薬の中止によりしばしば肝炎が再燃し，時に重症化することがある．このため，中止に際してはその危険性に十分配慮する必要がある．

核酸アナログ薬治療はHBs抗原の陰性化を目標とするが，必ずしも容易ではない．このため，HBs抗原が陰性化しなくても治療の中止を考慮する場合がある．本指針は，この様な状況下で核酸アナログ薬を中止し，最終的に非活動性キャリアの状態（ALT＜30 IU/L かつ血中 HBV DNA＜4.0 log copies/m*l*）に落ち着くことを目標として作成した．

核酸アナログ薬の中止と継続のどちらが生命予後や肝発癌に対して有利かは現在のところ明らかではない．このため，本指針は様々な理由により中止を検討する必要がある場合の参考になるよう定めた．この際，中止成功の可能性が高い症例を見いだすことや逆に治療を継続すべき症例を明らかにすること，さらに，中止後の経過観察の指標を設定することにより，核酸アナログ薬中止に伴うリスクを極力回避することを目指した．

II. 肝炎再燃に伴う重症化のリスクを回避するための必要条件

重症化のリスクをあらかじめ想定し，これを回避するため，以下を中止の必要条件とした．

1. 核酸アナログ薬中止後には肝炎再燃が高頻度にみられ，時に重症化する危険性があることを主治医，患者共に十分理解している．
2. 中止後の経過観察が可能であり，再燃しても適切な対処が可能である．
（専門医が関与することが推奨される．）
3. 肝線維化が軽度で肝予備能が良好であり，肝炎が再燃した場合でも重症化しにくい症例である．
（肝硬変やこれに近い線維化の進行した慢性肝炎の症例では中止すべきでない．）

III. HBV 増殖能の評価と再燃のリスクを低下させるための条件

1. 核酸アナログ薬中止の必要条件

HBV増殖能が高い症例では中止後の再燃はほぼ必発である．この様な症例で中止を行わないことが肝要であり，このための必要条件を以下に示す．

中止の必要条件
◇中止時，血中 HBV DNA（リアルタイム PCR 法）が陰性．
◇中止時，血中 HBe 抗原が陰性．

2. 核酸アナログ薬治療期間の条件

核酸アナログ薬治療期間が短いと再燃しやすいため，以下の条件を満たすことが望ましい．

治療期間の条件
◇核酸アナログ薬投与開始後2年以上経過している．

3. ウイルス抗原量のスコア化による再燃の危険性の評価

中止の必要条件（中止時 HBV DNA 陰性かつ HBe 抗原陰性）を満たす症例について，中止時の HBs 抗原量と HB コア関連抗原量をスコア化し，合計スコアから再燃のリスクを以下の3群に分けて予測することが可能である．この予測リスクを参考に中止の可否を決定することにより再燃のリスクを低下させることを目指す．

中止時 HBs 抗原量	スコア	中止時 HB コア関連抗原量	スコア
1.9 log IU/m*l* 未満（80 IU/m*l* 未満）	0	3.0 log U/m*l* 未満	0
1.9 ～ 2.9 log IU/m*l*（80 ～ 800 IU/m*l*）	1	3.0 ～ 4.0 log U/m*l*	1
2.9 log IU/m*l* 以上（800 IU/m*l* 以上）	2	4.0 log U/m*l* 以上	2

再燃リスク	総スコア	予測成功率	評価
低リスク群	0	80-90%	中止を考慮しても良い群．ただし，低リスク群でも肝炎再燃症例が存在するため，再燃に対する注意は必須である．
中リスク群	1-2	約 50%	状況によって中止を考慮しても良い群．この群では，中止の条件や方法を今後さらに検討する必要がある．
高リスク群	3-4	10-20%	治療の継続が推奨される群．ただし，35歳未満では中止成功例が比較的高く 30-40% である．

Table 1　核酸アナログ薬中止に伴うリスク回避のための指針　2012

Ⅳ. 中止後の経過観察方法と再治療開始の条件

1. 核酸アナログ薬中止後は定期的にHBV DNA（リアルタイムPCR法）とALTを測定し，HBVの再増殖とこれに伴う肝炎再燃に注意を払う．
2. 中止後の再燃は，中止直後から1年以内が多く，その後徐々に減少し，3年目以降はまれになる．このため，特に中止直後は再燃に対する注意が必要である．具体的には，中止後16週までは2週毎，その後は4週毎の血液検査による経過観察が望ましい．
3. 中止が成功し，最終的に非活動性キャリア状態に落ち着く症例においても，約2/3では一過性のALTまたはHBV DNAの異常値が出現する．このため，中止後の経過観察でALTまたはHBV DNAの異常値が出現しても，軽度の上昇であれば再治療を行わずに経過をみることが可能である．ただし，以下の条件では，最終的に非活動性キャリア状態に落ち着く可能性は低く，核酸アナログ薬による再治療を考慮する．

核酸アナログ薬の再投与を考慮する条件
◇中止後 ALT≧80 IU/L または HBV DNA≧5.8 log copies/m*l* となる場合

Ⅴ. 注意点と今後の課題

1. 患者の状況は個々に異なる．また，中止の目的や意義も個々に異なるため，実際に中止するか否かの判断は，これらの条件を考慮し主治医が行う．また，中止を考慮する場合は肝臓専門医に相談することが推奨される．
2. 核酸アナログ薬中止後に肝炎が再燃し再投与した場合，中止しなかった場合と比較し核酸アナログ薬耐性株の出現頻度が増加するか否かについては不明である．
3. HBVキャリアでは非活動性キャリア期（HBV DNAが4.0 log copy/m*l*未満かつALTが30 IU/L未満）となってもまれに肝炎の再燃がみられるので，中止に成功してもキャリアとしての経過観察は継続する必要がある．また，肝発癌に関しても同様に経過観察が必要である．
4. 今後の検討課題としては，核酸アナログ薬中止基準の精度をさらに高めること，本指針で用いた基準を前向き検討で検証すること，インターフェロン併用によるシークエンシャル療法で核酸アナログ薬を積極的に中止しようとする方法の検討などが挙げられる．

の指標を設定することにより，核酸アナログ薬中止に伴うリスクを極力回避することを目指して作成した（Table 1-Ⅰ）．ここでの中止成功は，最終的に非活動性キャリアの状態，すなわちALTが30 IU/L未満かつ血中HBV DNAが4.0 log copies/m*l*未満に落ち着くこととした．この基準は日本のB型慢性肝炎治療ガイドラインに準拠して設定したが，このような非活動性キャリア状態になると肝病変の進行はなく発癌率も低下することが知られており[6][7]，適切なものと考えられる．

Ⅱ. 肝炎再燃に伴う重症化のリスクを回避するための必要条件

現状では，核酸アナログ薬中止後の肝炎再燃を十分高い確率で予測することはできない．このため，重症化の危険性[8]が存在することを想定し，重症化防止のための必要条件を設定した（Table 1-Ⅱ）．肝炎再燃や重症化の危険性を主治医と患者が共に理解していること，さらに，中止後の経過観察体制があり，再燃しても適切な対処が可能であることは当然の条件と考えられる．また，肝硬変やこれに近い線維化の進行した慢性肝炎症例では重症化しやすいこと，さらには将来的に発癌の危険性が高いことを考慮すると，現状では安易に中止すべきでないと判断した．

Ⅲ. HBV増殖能の評価と再燃のリスクを低下させるための条件

これまでも，核酸アナログ薬中止時にHBV DNAが十分低下しない症例またはHBe抗原陽性の症例では中止後に肝炎が高率再燃することが経験されていたが，本研究班の検討でもこれが科学的に確認された[9]．肝炎再燃を予測するHBV DNA量のcut-off値はROC解析で3.0 log copies/m*l*であり，これ以上の症例ではほとんど全例が1年以内に再燃したのに対し，3.0 log copies/m*l*未満の症例では長期に安定化する症例が30%近く存在した．さらに，HBV DNA量が3.0 log copies/m*l*未満の症例に限った場合，HBe抗原陽性例は1年以内に90%以上が再燃したのに対し，HBe抗原陰性例では長期に安定化する症例が少なからず存在した．この結果から，HBV DNA量の十分な低下とHBe抗原の陰性化は中止の必要条件として設定した．ここで，HBV DNA量の十分な低下の基準値については，実際の指針では3.0 log copies/m*l*未満ではなく，安全を考慮してリアルタイム

PCR法で陰性であることとした．

明らかに中止後の肝炎再燃が予測される症例，すなわち，核酸アナログ薬中止時にHBV DNA量が3.0 log copies/m*l*以上またはHBe抗原陽性の症例を除いて中止後の肝炎再燃と関連する因子をさらに解析すると，核酸アナログ薬治療期間，中止時HBs抗原量，中止時HBコア関連抗原量が有意な因子として算出された[9]．治療期間のcut-off値は16カ月と算出されたため，本指針では余裕をもって2年以上経過していることが望ましいとの条件を設定した．

中止時のHBs抗原量とHBコア関連抗原量については，ROC解析の結果からそれぞれ2つのcut-off値の存在が示唆され，HBs抗原量は1.9と2.9 log IU/m*l*，HBコア関連抗原量は3.0と4.0 log U/m*l*であった[9]．この事から，Table 1-IIIに示す如くHBs抗原量とHBコア関連抗原量をスコア化し，総スコアから低リスク群，中リスク群，高リスク群の3群を設定した．それぞれの予測成功率は低リスク群が80～90%，中リスク群が約50%，高リスク群が10～20%であった．各群の中で肝炎再燃と関連する因子をさらに検討すると，低リスク群と中リスク群では新しい因子はなかったが，高リスク群では年齢が有意な因子であった．すなわち，予測成功率が10～20%と低い高リスク群であっても，年齢が35歳未満ではこの成功率がやや高く30～40%であった．

以上の如く，治療期間やウイルスマーカーの結果から核酸アナログ薬中止後の経過を予測することが可能であり，治療中止を計画する際の指標となると考えられた．近年，HBs抗原量の測定は新しいマーカーとして注目されており，インターフェロン治療効果の予測などに有用なことが報告されている[10][11]．一方，HBコア関連抗原量は核酸アナログ薬使用下においても肝細胞核内のHBV cccDNA量を反映することが報告され[12]～[14]，この量が中止後の肝炎再燃と関連することはこれまでも報告されていた[15][16]．今回，これらの抗原量の組み合わせが中止の指針作成に有用であった点は興味深い[9]．

IV．中止後の経過観察方法と再治療開始の条件

核酸アナログ薬中止後の経過観察は，定期的にHBV DNA量（リアルタイムPCR法）とALT値を測定することにより行う．中止後の再燃は，中止直後から1年以内が多く，その後徐々に減少し，3年目以降はまれになることが今回の研究で明らかになった[9]．このため，特に中止直後は再燃に対する注意が必要であると判断した．具体的には，中止後16週までは2週毎，その後は4週毎の血液検査による経過観察が望ましいとした．

肝炎再燃をどのように定義し，中止後の経過観察をどのように行うかは本指針の要点の一つである．最終的に非活動性キャリア状態に落ち着く症例においても，約2/3では一過性のALTまたはHBV DNAの異常値が出現する．このため，中止後の経過観察でALTまたはHBV DNAの異常値が出現しても，軽度の上昇であれば再治療を行わずに経過をみることが可能である．しかし，どこまでなら経過をみて良いのかの基準はこれまで明らかにされていない．この点を明らかにするため我々は，核酸アナログ薬中止後のALT値とHBV DNA量の推移を平均値と最高値で評価した．この結果，両者とも平均値と最高値の間にきわめて強い相関があることが明らかになった[9]．ROC解析の結果より，平均ALT値の30 IU/Lは最高ALT値の79 IU/Lに，一方，平均HBV DNA量の4.0 log copies/m*l*は最高HBV DNA量の5.7 log copies/m*l*に相当することが明らかになった．すなわち，中止後にALT値が80 IU/L以上になる場合は平均値が30 IU/Lを超える可能性が高く，最終的に中止成功の基準を満足しないことが予測される．同様に，中止後のHBV DNA量が5.8 log copies/m*l*以上となる場合は平均値が4.0 log copies/m*l*を超える可能性が高く，中止成功の基準を満足しないことが予測される．これらの結果より，中止後にALT値が80 IU/L以上，またはHBV DNA量が5.8 log copies/m*l*以上となる場合は最終的に非活動性キャリア状態に落ち着く可能性は低く，核酸アナログ薬による再治療を考慮するとする条件を設けた．この条件設定により，より効率的で具体的な中止が可能になると考えられる．安全を考慮し，主治医の判断でこの基準をより厳しく設定することは可能である．逆に，この基準を緩く設定することも可能であるが，その場合は漫然と経過観察は行わず，何らかの方針を立てて対処することが望ましい．

V．注意点と今後の課題

核酸アナログ薬中止の指針についてはこれまで本格的なものはなく，その意味で本指針は初めてのものとも言える．しかし，多くは後向きの検討データを基に作成したものであり，まだ不明な点も多く残されている．そのため，注意点や今後の課題を一つの項目としてまとめた（Table 1-V）．本指針では核酸アナログ薬の中止に関する判断材料を提供したが，実際に中止す

るかは否かの判断は主治医が行うべきと考える．それは，核酸アナログ薬を継続した場合と中止した場合の長期の予後が必ずしも明らかになっていないことが大きな要因であり，患者の希望と主治医の判断が優先されると考えられる．

中止が成功しなかった場合，核酸アナログ薬を再投与することが選択肢の一つとなる．しかし，この場合，継続投与した場合と比較して耐性株出現率が高くなるか否かについては明らかになっておらず，この点は患者に同意を得ておく必要があると考えられる．

今後の検討課題としては，中止後の肝炎再燃の予測精度を向上させることが一つである．このためには新しい因子の検討が必要であり，候補としては，より高感度なHBV DNA量の測定，HBV RNA量の測定[17)18)]，HBV遺伝子型，HBV遺伝子変異の検出などが挙げられている．また，今回の指針は後ろ向き研究で作成された基準であり，今後，前向き検討でこれを検証する必要がある．さらに，インターフェロン併用によるシークエンシャル治療で核酸アナログ薬を積極的に中止しようとする方法も今後の重要な検討課題と考えられる．

おわりに

核酸アナログ薬が日本で使用可能になって12年目になるが，本格的な中止の指針作成は今回が最初である．難しい課題であるが，第一歩を踏み出さないことにはその次はない．すなわち，本指針はまだ十分とは言えないが，少なくとも，今後より良いものを作るための出発点になることが期待される．

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Guidelines for avoiding risks resulting from discontinuation of nucleos(t)ide analogues in patients with chronic hepatitis B (2012)

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Key words: nucleos(t)ide analogue　discontinuation of treatment　hepatitis B　hepatitis relapse　HBV cccDNA

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Review Article

Reactivation of hepatitis viruses following immunomodulating systemic chemotherapy

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Reactivation of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection following anticancer chemotherapy and immunosuppressive therapy is a well-known complication. HBV reactivation has been reported to be associated with anti-CD20 monoclonal antibody rituximab-containing chemotherapy and tumor necrosis factor-α inhibitor-containing immunosuppressive therapy in HBV resolved patients (hepatitis B surface antigen negative and antibodies against hepatitis B core antigen positive and/or antibodies against surface antigen positive). On the other hand, HCV reactivation has been reported to be associated with liver damage or hepatic dysfunction, but fulminant hepatitis due to HCV reactivation is a rare complication. In this review, we describe the pathophysiology of the reactivation of HBV and HCV infection, as well as the clinical evidence and management of HCV reactivation.

Key words: chemotherapy, hepatitis B virus, hepatitis C virus, immunosuppressive, occult infection, reactivation

INTRODUCTION

REACTIVATION OF HEPATITIS B virus (HBV) or hepatitis C virus (HCV) infection following anticancer chemotherapy and immunosuppressive therapy is a well-known complication. In particular, HBV reactivation is a potentially fatal complication that needs to be followed up carefully. Most HBV reactivation occurs in hepatitis B surface antigen (HBsAg) positive patients prior to treatment; however, HBV reactivation has been observed increasingly in HBV resolved patients without HBsAg, but with antibodies against hepatitis B core antigen (anti-HBc) and/or HBsAg (anti-HBs). Moreover, HBV reactivation has been reported to be associated with anti-CD20 monoclonal antibody rituximab-containing chemotherapy and tumor necrosis factor (TNF)-α inhibitor-containing immunosuppressive therapy in patients with prior resolved HBV infection. On the other hand, HCV reactivation has been reported to be associated with liver damage or hepatic dysfunction, but fulminant hepatitis due to HCV reactivation is a rare complication.

Hematopoietic stem cell transplantation (HSCT) is often the chosen treatment for hematological malignancies and it has been suggested that the incidence and clinical characteristics of reactivation of HBV or HCV infection may depend on immune reconstitution, which may be associated with graft-versus-host disease (GVHD) and the combined immunosuppressant, especially in the allogeneic HSCT setting.

As several review papers about HBV reactivation had been already reported, we described here the pathophysiology of the reactivation of HBV and HCV infection, as well as the clinical evidence and management of HCV reactivation.

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PATHOPHYSIOLOGY OF REACTIVATION OF HBV AND HCV INFECTION

Immunity to HBV and HCV

BECAUSE HBV AND HCV are not cytopathogenic, it is widely accepted that both viral control and liver pathology are mediated by the host immune system (Table 1). Many studies of host genetics and

Table 1 Putative host immune system of HBV and HCV infection

	HBV	HCV
Innate immunity		
Hepatocytes	Stealth response	Type I and/or III IFN production
Main components	NK and NKT cells	DC
Critical cytokines	IFN-γ, TNF-α	Type I and/or III IFN
Adaptive immunity		
Components	T cells and B cells	T cell and B cells

DC, dendritic cells; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon; NK, natural killer; NKT, natural killer T cells.

immunology demonstrate an important role for T lymphocytes in protective immunity against HBV and HCV.

The occurrence of HBV reactivation in patients with signs of resolved infection, particularly anti-HBc positive patients, relies on the existence of occult HBV infection. Patients with occult HBV infection are supposed to harbor HBV covalently closed circular DNA in the nuclei of their hepatocytes after the resolution of acute infection.[1] Most occult HBV infection individuals are infected with replicable viruses, whose replication and gene expression are strongly inhibited by the host immune system.[2] The exact mechanisms of inhibition have not yet been determined, but long-lasting specific host T-cell immune surveillance against HBV epitopes and epigenetic factors are presumably the major causes of long-term viral suppression.[3]

In contrast, although HCV reactivation following immunosuppressive therapy is rare,[4–8] fibrosing cholestatic hepatitis C (FCH) occurs in HCV positive liver transplant recipients with immunosuppressive therapy.[9–11] Whether immunosuppressive therapy leads to HCV reactivation in patients with cancer in whom the infection has cleared either spontaneously or secondary to therapy is uncertain. When HCV RNA clearance is achieved either spontaneously or in response to antiviral therapy in recipients of solid organ transplants, no relapse is observed in plasma, liver or peripheral blood mononuclear cells during chronic immunosuppressive treatment with agents such as calcineurin inhibitors, corticosteroids, antimetabolites, anti-thymocyte globulins, or anti-interleukin-2-receptor blockers.[12] This finding suggests the complete and permanent cure of HCV infection resulting from the elimination of HCV before transplantation.

Immunosuppression and viral replication in HBV reactivation

In general, there are three periods of HBV reactivation in patients with signs of resolved infection (Fig. 1).

The initial stage of HBV reactivation caused by chemotherapy-induced immune suppression is characterized by enhanced viral replication, as reflected by increases in the serum levels of HBV DNA, hepatitis B e-antigen (HBeAg) and HBsAg, indicating that suppression of a normal immunological response to HBV leads to enhanced viral replication and widespread infection of hepatocytes.[13] In particular, in cases of positive anti-HBs antibody, reactivation of HBV typically starts with a decrease of anti-HBs antibody titers. This may be related to the use of biologic therapy, such as anti-CD20 monoclonal antibody rituximab and anti-CD52 antibody alemtuzumab, which cause profound and long-lasting immunosuppression; however, a decrease of anti-HBs antibody titers is seen in all cases, including those on biologic drug-free chemotherapy, namely, tumor necrosis factor-α inhibitors.

There are at least two mechanisms by which immunosuppressive agents may increase HBV replication and expression. As the host immune response to the virus plays a crucial role in controlling HBV infection,[14] suppression of such immune responses should increase viral replication. Meanwhile, immunosuppressive agents may have a more direct stimulatory effect on viral replication. In fact, corticosteroid increases HBV DNA and RNA production *in vitro* by stimulating HBV transcription, by binding to the glucocorticoid responsive element and augmenting the HBV enhancer I;[15,16] however, it is controversial whether corticosteroid increases the secretion of HBsAg and HBeAg.[15–17] Although combinations of immunosuppressive agents may cause an increase in levels of intracellular HBV DNA, lower concentrations of prednisolone were presumably unable to stimulate HBV replication, so the doses of these compounds should be kept as low as practically possible when used clinically.

In the second stage of reactivation, functionality of the immune system is restored after chemotherapy is discontinued. Infected hepatocytes with recognizable viral antigens on their surface may then be exposed and would be cleared by T lymphocytes, leading to hepatic injury and necrosis. Clinically, this can lead to hepatitis with an increase in alanine aminotransferase (ALT) levels, hepatic failure and even death. Concurrently, HBV DNA levels may decrease

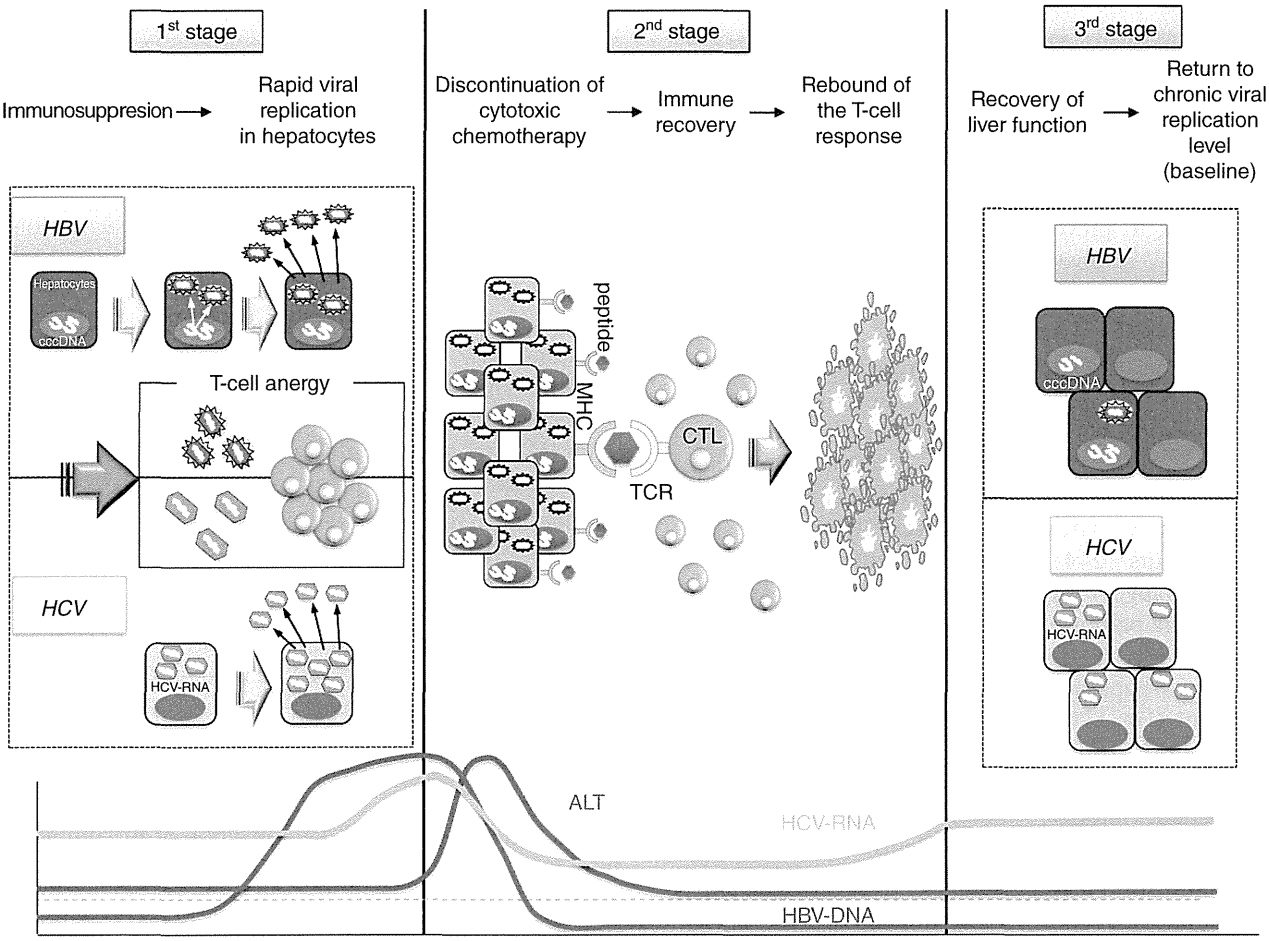

Figure 1 Pathophysiology of the reactivation of HBV and HCV infection. Reactivation of HBV or HCV as a result of chemotherapy can generally be divided into three stages. The first stage is characterized by enhanced viral replication, the second stage is the functional restoration of the immune system, and 3rd stage is the recovery stage. ALT, alanine aminotransferase; CTL, cytotoxic T lymphocyte; HBV, hepatitis B virus; HCV, hepatitis C virus; cccDNA, covalently closed circular DNA; MHC, major histocompatibility complex.

by improved cytopathic and non-cytopathic immune mechanisms.[18,19]

The third stage of reactivation is the recovery phase, during which clinical hepatitis resolves and HBV markers return to baseline levels.[20,21]

The retrospective and prospective studies of HBV reactivation in HBsAg negative patients with hematological malignancies were summarized in previous reviews.[22–24] As for the reason for considerable variation (1.0–23.3%) in the incidence of HBV reactivation in lymphoma patients with HBV resolved infection following rituximab-containing chemotherapy, there may be differences among institutions both in the study subjects (HBV serological status including baseline anti-HBs titer, steroid-containing chemotherapy, and salvage therapy including transplantation) and the assays used for HBV-related markers (cut-off values, sensitivity). Several guidelines for the management of HBV reactivation have been published by Asian, American and European societies (American Association for the Study of Liver Diseases, Asian Pacific Association for the Study of the Liver, and European Association for the Study of the Liver). In January 2009, the Japanese guideline was announced for HBV reactivation following immunosuppressive therapy and systemic chemotherapy.[25] Although the details of this guideline have been omitted from this review, in principle, antiviral prophylaxis is recommended for HBsAg

positive patients before treatment. For HBV resolved patients, monthly monitoring of HBV DNA levels is recommended during and for at least 1 year after the end of immunosuppressive therapy or chemotherapy. Preemptive antiviral therapy should be started as soon as possible if HBV DNA is detected during this monitoring; however, there is little evidence of HBV DNA monitoring to prevent hepatitis due to HBV reactivation in HBV resolved patients.

Reactivation of HCV infection

Although HCV reactivation is rare, hepatic toxicity related to chemotherapy is higher among patients with chronic HCV infection than in HCV uninfected patients,[26] suggesting that HCV reactivation occurred and can cause clinically relevant complications.

Hepatitis C virus-related liver dysfunction generally occurs 2–4 weeks after the cessation of chemotherapy.[27–30] A widely accepted hypothesis considering the pathogenesis indicates enhanced viral replication with a consequent increase in the number of infected hepatocytes following immunosuppressive treatment (Fig. 1). Withdrawal of immunosuppressive therapy leads to restoration of the host immune function, resulting in the rapid destruction of infected cells and hepatic injury.[27,31] Severe liver dysfunction was found to occur at a lower incidence in HCV positive patients than HBV positive patients.[5] The reason for this phenomenon is unknown; however, if severe hepatitis secondary to viral reactivation develops, mortality rates of HBV infected and HCV infected patients seem to be similar.[32–34]

CLINICAL EVIDENCE AND MANAGEMENT OF HCV REACTIVATION

Diagnosis for HCV reactivation

CHRONICALLY INFECTED PATIENTS have stable HCV RNA levels that may vary by approximately 0.5 $\log_{10}$ IU/mL;[35] therefore, an increase of the HCV viral load of more than 1 $\log_{10}$ IU/mL may be a sign of HCV reactivation. It was also reported that HCV reactivation showed an at least threefold increase in serum ALT in a patient in whom the tumor had not infiltrated the liver, who had not received hepatotoxic drugs and who had had no recent blood transfusions or other systemic infections besides HCV.[6,24] Changes in liver enzyme levels can be accompanied by the reappearance of HCV RNA or a sudden increase in the serum HCV RNA level.[6]

HCV reactivation after specific treatments

Patients with HCV infection who undergo HSCT or systematic chemotherapy including corticosteroids can experience severe hepatic dysfunction and fulminant hepatic failure (summarized in Table 2).

Corticosteroids have traditionally been associated with cases of HCV reactivation.[27,36] HCV reactivation has been associated with several immunosuppressive and chemotherapeutic agents, including rituximab, alemtuzumab, bleomycin, busulfan, cisplatin, cyclophosphamide, cyclosporin, cytarabine, dacarbazine, doxorubicin, etoposide, gemcitabine, methotrexate, vinblastine and vincristine;[27,37–44] however, many patients with HCV reactivation during treatment with one of these drugs were simultaneously treated with corticosteroids.[38,41,42,44,45] In a study by Zuckerman *et al.*,[46] 18 of 33 (54%) patients had mild to moderate increases of ALT, which occurred 2–3 weeks after the withdrawal of chemotherapy. HCV positive patients did not demonstrate a higher incidence of severe hepatic dysfunction during chemotherapy for malignancies than HCV negative patients; however, liver test abnormalities during therapy are very common and are seen in 54% HCV positive patients and in 36% HCV negative patients.

Whether corticosteroid therapy alone or in combination with other agents leads to reactivation of HCV infection and acute exacerbation of chronic HCV infection remains to be determined. A possible relationship between rituximab and HCV reactivation in patients with cancer has been reported.[41,44,45] Only the administration of rituximab-containing chemotherapy was associated with both acute exacerbation and reactivation of chronic HCV infection.[24]

Ennishi *et al.* also showed that the incidence of severe hepatic toxicity in HCV positive patients was significantly higher than in HCV negative patients, and HCV infection was determined to be a strong risk factor for this adverse effect in patients with diffuse large B-cell lymphoma (DLBCL) in the rituximab era.[44] These hepatic toxicities led to modification and discontinuation of immunochemotherapy, resulting in lymphoma progression. The study described that careful monitoring of hepatic function should be recommended for HCV positive patients, particularly those with high levels of pretreatment transaminase. More importantly, monitoring of HCV viral load demonstrated a marked enhancement of HCV replication, and it is suggested that increased HCV results in severe hepatic toxicity. Thus, HCV viral load should be

Table 2 Hepatic toxicity by HCV reactivation in HCV infected patients with hematological malignancies

Author	Year	Disease	Treatment	No. of cases with hepatic toxicity	Death from liver toxicity
Kanamori *et al.*	1992	AML	Allo-HSCT	2 patients	2
Maruta *et al.*	1994	AML, AA	Allo-HSCT	9 patients	2
Nakamura *et al.*	1996	Hematological malignancies	Various regimens	11 patients	5
Vento *et al.*	1996	B-cell NHL and HL	ABVD or CHOP- like regimen	2 patients	1
Luppi *et al.*	1998	B-cell NHL	Various regimens	20/35 patients	2
Zuckerman *et al.*	1998	Hematological malignancies	Various regimens	18/33 patients (55%)	0
Kawatani *et al.*	2001	Hematological malignancies	Various regimens	4/22 patients	1
Hamaguchi *et al.*	2002	Hematological malignancies	Allo-HSCT	40/58	9
Locasciulli *et al.*	2003	Hematological and solid malignancies	Allo-HSCT (21)/auto-HSCT (36)	21 6	2 1
Takai *et al.*	2005	Hematological malignancies	Various regimens	4/37 patients	0
Aksoy *et al.*	2006	DLBCL	Rituximab	0/1 patients	0
Besson *et al.*	2006	DLBCL	Various regimens	15/23 patients	3
Visco *et al.*	2006	DLBCL	CHOP-like, rituximab (35)	5/132 patients	1
Ennishi *et al.*	2008	DLBCL, MALT NHL	R-CHOP-like	1/5 patients	0
Hsieh *et al.*	2008	DLBCL	R-CNOP	1/1 patients	0
Ennishi *et al.*	2010	DLBCL	R-chemo	36/131 patients	6
Arcaini *et al.*	2010	NHL	R-chemo (28)	24/160 patients	3

AA, aplastic anemia; ABVD, doxorubicin hydrochloride (adriamycin), bleomycin, vinblastine and dacarbazine; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; auto-HSCT, autologous hematopoietic stem cell transplantation; CHOP, cyclophosphamide, vincristine and prednisolone; DLBCL, diffuse large B-cell lymphoma; MALT, extranodal-marginal zone lymphoma of the mucosa-associated lymphoid tissue; NHL, non-Hodgkin's lymphoma; R-chemo, rituximab plus steroids combined chemotherapy; R-CHOP, rituximab, cyclophosphamide, vincristine and prednisolone; R-CNOP, rituximab, cyclophosphamide, mitoxantrone, vincristine and prednisolone.

carefully monitored in HCV positive patients who receive immunochemotherapy.

Severity of HCV reactivation versus HBV reactivation

The health consensus regarding HCV reactivation seems to be less severe than that of HBV reactivation (summarized in Table 3). Previous reports described that the incidence of post-chemotherapy liver injury in HBV carriers was significantly higher than that in HCV carriers,[5,31,32] namely, the incidence of post-chemotherapy liver injury in 25 HBV carriers (36%) was significantly higher than that in 37 HCV carriers (10.8%, $P = 0.026$),[31] and 44 (51.8%) of the 85 patients reported to have severe hepatitis along with hematological malignancies were HBV carriers, while only 11 (12.9%) were HCV carriers;[32] however, the mortality rates did not differ between HBV and HCV carriers (40.9% vs 45.5%) once severe hepatitis developed.

In a large Italian study of 57 HCV infected patients who underwent HSCT, patients undergoing autologous HSCT had a significantly lower risk of reactivation post-transplant than the allogeneic group (16% vs 100%, $P = 0.004$). In the allogeneic HSCT group, HCV reactivation occurred mainly within 6 months after HSCT, whereas in the autologous group, reactivation occurred within the first 3 months post-transplant. In this cohort, one HBsAg positive and three anti-HCV positive patients before HSCT died of liver failure. The risk of death from liver failure was not significantly different between HBsAg and anti-HCV positive patients, being 3% and 8% at 24 months, respectively ($P = 0.6$), or between recipients of autologous (5%) and allogeneic HSCT (7%) ($P = 0.34$).[33]

In a Japanese multicenter study of 135 patients with HBV or HCV infection who received allogeneic transplants, transient hepatitis was more common in HBV infected patients than in HCV infected patients, but the rates of fulminant hepatitis and death due to hepatic failure were similar in both groups.[34]

Table 3 Clinical state by HCV reactivation versus HBV reactivation

Severity or prognosis	Year	Survey period	No. of patients	Country	% of HBV	% of HCV	*P*	Comments	Reference
Liver injury	2005	1996–2002	601	Japan	36% (9/25)	10.8% (4/37)	0.026	Patients with hematological malignancies	31
Severe hepatitis	1996	1987–1991	Surveillance in 250 hospitals	Japan	51.8% (44/85)	12.9% (11/85)	ND	In 85 patients having severe hepatitis along with hematological malignancies	32
Death from liver failure	2003	1996–2000	90	Italy	3% (1/33)	8% (3/38*)	0.6	Patients with HBV or HCV receiving HSCT (during 24 months)	33
Hepatic failure	2002	1986–1998	135	Japan	10% (8/77)	12% (7/58)	ND	Patients with HBV or HCV receiving HSCT	34

HBV, hepatitis B virus; HCV, hepatitis C virus; HSCT, hematopoietic stem cell transplant; ND, not done.
*Fifty-seven were anti-HCV positive; of these, 38 were also tested for HCV RNA.

Outcome of HCV infected hematological patients

As previously highlighted, there is no significant short-term impact of HCV on the outcome after HSCT. Nevertheless, the long-term impact of chronic HCV infection can be deleterious in the liver, causing significant fibrosis progression, liver failure and increased risk of hepatocellular carcinoma (HCC). One study reported the rapid progression of hepatitis C in patients with humoral immunodeficiency disorders.[47] Another group has recently reported a more rapid rate of fibrosis progression after HSCT, with median time to cirrhosis of 18 years, as compared to 40 years seen in the control group. HCV disease progression ranked third, behind infections and GVHD, as a cause of late death after HSCT.[48] Long-term survivors after HSCT thus appear to be at higher risk for HCV-related complications and treatment of HCV becomes critical. A possible explanation for the genesis of cirrhosis could be an immune imbalance or impaired regulation of B and T cells.[47,48]

In various regimens for hematological malignancies, Ennishi *et al.* reported that hepatic disease progressed in four patients, and HCC was found to increase the risk of death from hepatic failure significantly in lymphoma patients receiving conventional chemotherapy, even during short-term observation.[44] Cox multivariate analysis showed that older age and advanced stage had significant adverse effects on overall survival (OS); however, HCV infection was not associated with poor progression-free survival (PFS) or OS. Besson *et al.* described that the overall proportion of subjects undergoing hepatic toxicity was 65% (15/23 patients). Outcome of HCV positive patients was poorer for OS ($P = 0.02$), but not for event-free survival ($P = 0.13$).[49] Visco *et al.* also described that only five of 132 patients (4%) had to discontinue chemotherapy due to severe liver function impairment.[50] Although previous papers mentioned that rituximab induced HCV reactivation after spontaneous remission in DLBCL,[45,51] the addition of rituximab did not seem to affect patients' tolerance to treatment. Five-year overall survival of the entire cohort was 72%, while 5-year PFS of the 132 patients treated with intent to cure was 51%. The prognosis of HCV infected patients with DLBCL is still controversial.

Recently, Arcaini *et al.*[43] studied 160 HCV positive patients with NHL (59 indolent NHL, 101 aggressive). Among 28 patients treated with rituximab-containing chemotherapy, five (18%) developed liver toxicity, and among 132 independent patients who received chemotherapy, only nine (7%) had hepatotoxicity, suggesting

that rituximab was related to a slightly higher occurrence of toxicity. Median PFS for patients who experienced liver toxicity was significantly shorter than median PFS of patients without toxicity (2 and 3.7 years, respectively, $P = 0.03$). HCV infected patients with NHL developed liver toxicity significantly, often leading to interruption of treatment.

Based on these findings, the impact of HCV infection on the outcome after HSCT or rituximab-containing chemotherapy seems to be deleterious for OS but not for event-free survival. Further studies are required in prospective multicenter cohorts.

Treatment of HCV infected patients with hematological malignancies

The long-term impact of chronic HCV infection can be deleterious to the liver, causing significant fibrosis progression, liver failure and increased risk of HCC. Interestingly, a more rapid rate of fibrosis progression was reported after HSCT.[48] Therapy for HCV infection in patients with hematological malignancy can be considered once a patient's immunity and bone marrow have recovered, immunosuppressive drugs have been stopped, and there is no evidence of GVHD, because the hematological adverse effects of anti-HCV drugs can exacerbate the toxicity of chemotherapy, which can involve complications such as severe cytopenias and potentially life-threatening infections.[52] Overall, antiviral therapy for HCV in patients (e.g. HIV, transplant) is often associated with poor response rates, even though patients with chronic HCV infection were treated with the combination of pegylated interferon-α and ribavirin.[53–55] The use of direct-acting antiviral drugs (such as recently approved inhibitors of nonstructural protein 3/4A [NS3/4A] protease [boceprevir or telaprevir], or NS5B polymerase inhibitors) has not been evaluated in patients with cancer. Boceprevir and telaprevir can inhibit hepatic drug-metabolizing enzymes such as cytochrome P450 (CYP)2C, CYP3A4 or CYP1A;[56] therefore, these agents potentially interact with various drugs that are co-administrated in patients with cancer. These new antiviral drugs should be used with caution in patients with cancer.

Large-scale studies are needed to better define which patients with cancer are most likely to benefit from simultaneous antiviral therapy and cytotoxic chemotherapy. Notably, antiviral treatment with pegylated interferon-α and ribavirin should not be used early in the post-transplant period (<2 years after transplantation) in patients who have undergone allogeneic HSCT as interferon-α therapy may precipitate or induce the development of GVDH.[57]

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2′-Fluoro-6′-methylene-carbocyclic adenosine phosphoramidate (FMCAP) prodrug: In vitro anti-HBV activity against the lamivudine–entecavir resistant triple mutant and its mechanism of action

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Drug-resistant mutants

ABSTRACT

Novel 2′-fluoro-6′-methylene-carbocyclic adenosine (FMCA) monophosphate prodrug (FMCAP) was synthesized and evaluated for its in vitro anti-HBV potency against a lamivudine–entecavir resistant clone (L180M + M204V + S202G). FMCA demonstrated significant antiviral activity against wild-type as well as lamivudine–entecavir resistant triple mutant (L180M + M204V + S202G). The monophosphate prodrug (FMCAP) demonstrated greater than 12-fold (12×) increase in anti-HBV activity without increased cellular toxicity. Mitochondrial and cellular toxicity studies of FMCA indicated that there is no significant toxicity up to 100 μM. Mode of action studies by molecular modeling indicate that the 2′-fluoro moiety by hydrogen bond as well as the Van der Waals interaction of the carbocyclic ring with the phenylalanine moiety of the polymerase promote the positive binding, even in the drug-resistant mutants.

The chronic HBV infection is strongly associated with liver diseases like chronic hepatic insufficiency, cirrhosis and hepatocellular carcinoma (HCC).[1] According to the World Health Organization (WHO), currently about 2 billion people world-wide have been infected with HBV and more than 350 million live with chronic infection. Acute or chronic outcomes of HBV infection are estimated to cause the deaths of 600,000 people worldwide every year.[2]

Currently, there are several nucleos(t)ide analogues available to treat chronic hepatitis B virus infection.[3–6] The major target of these drugs is to inhibit the viral reverse transcriptase (RT)/DNA polymerase, which is responsible for the synthesis of the minus-strand DNA. Although the currently used agents are well tolerated and effective in suppressing the viral replication for extended periods, the significant rate of virological relapse caused by drug resistance remains a critical issue.

Lamivudine (LVD) was first introduced as the orally active anti-HBV agent in 1998. Lamivudine profoundly suppresses HBV replication in patients with chronic hepatitis B infection; however, lamivudine-resistant HBV (LVDr) was isolated from a significant numbers of patients during the treatment with lamivudine. Currently, there are several antiviral options exist for these patients viz., to use adefovir or high dose (1.0 mg/day) of entecavir, or more recently tenofovir. However, this resulted in also the development of resistance mutants during the long term therapy. At present, entecavir is the most prescribed drug, and is recommended for patients with the wild-type as well as for those harboring adefovir and lamivudine-resistant strains. However, recent clinical studies by Tanaka and his co-workers suggested that the entecavir mutant in the lamivudine-resistant patients (L180M + M204V + S202G) causes a viral breakthrough: 4.9% of patients at baseline increases to 14.6%, 24% and 44.8% at weeks 48, 96 and 144, respectively.[7] Therefore, it is of great interest to discover novel anti-HBV agent, which is effective against lamivudine- and entecavir-resistant triple mutants (L180M + M204V + S202G).

The potency of a nucleos(t)ide analogue is determined by its ability to serve as a competitive inhibitor of the HBV polymerase relative to that of the natural substrate, the nucleotide triphosphate.[8] However, host cellular kinases limit the pharmacological potency of nucleoside analogues by phosphorylation to their corresponding triphosphates. Particularly, the initial kinase action on the nucleoside to the monophosphate is the rate-limiting step. However, many synthetic nucleosides are not phosphorylated or the rate of phosphorylation is very slow due to the structural requirement of the kinases, resulting in only generating a low quantity of the triphosphate. To overcome this phosphorylation issue, nucleoside phosphoramidate prodrugs have been introduced,[8,9] which

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