E. 結論

Preliminary な解析から NA 治療後の血中 HBV DNA 量は肝組織 ccc DNA 量を反映する可能性が示唆された。HBV 感染指標の多寡により肝組織 ccc DNA 量が推測可能であれば、NA 投与を PegIFN 投与に切り換えるに際しての症例選択の参考になる他、治療反応性、治療終了後の HBV 動態と肝炎再燃とその後の沈静化、緩解の予測に応用できる可能性があるため、本厚労省班研究において更なる検討が求められる。

F. 研究発表

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研究論文

なし

G. 知的所有権の取得状況

1. 特許申請：なし
2. 実用新案登録：なし
3. その他：なし

厚生労働科学研究費補助金（肝炎等克服緊急対策研究事業）

分担研究報告書

Sequential 療法の HBs 抗原に対する効果

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研究要旨：核酸アナログの長期投与後に、Drug free と HBs 抗原の陰性化を目標として Peg-interferon による Sequential 療法を施行した。HBs 抗原陰性化率は全体では 10%だが、Sequential 療法開始時の HBs 抗原が 1000 未満の症例では 33%であった。HBs 抗原の 1.0Log 以上の減少は全体では 30%であったが、Sequential 開始時の HBs 抗原が 1000 未満の症例では 66%、Sequential 開始時 HBs 抗原が 1000～10000 では 20%であった。ALT 上昇は全体の 90%で見られたが、重症化する症例はなく、HB s 抗原が 1.0Log 以上減少した症例では全例で ALT が上昇した。以上の preliminary な結果から、HBs 抗原の陰性化を目指した Sequential 療法においては、核酸アナログの長期投与後に HBs 抗原量が 1000 未満に減少した症例が最も良い適応と推察された。Sequential 療法においては ALT の上昇が高率にみられるが、これはインターフェロンの免疫賦活作用による HBV 感染細胞の排除を反映する可能性がある。どのような症例を Sequential 治療の適応とし、その効果をいかに判定するかは今後の検討課題である。

A. 研究目的

核酸アナログの長期継続治療により、HBV DNA の持続陰性と ALT 値の正常を維持することが可能であるが、治療を中止すると高率に肝炎が再燃する。Sequential 療法は、核酸アナログ治療例に対してインターフェロンを投与することで、核酸アナログ治療から離脱し、Drug free を目指す治療であるが、近年は HBs 抗原の陰性化に促進的に働く作用が注目されている。本研究では、核酸アナログ長期継続治療により持続的に HBV DNA が陰性化している症例を対象として Peg-interferon を Sequential に投与することで、Drug free が得られる確率、および HBs 抗原の陰性化あるいは減少効果について検討を行うことを目的とした。

B. 研究方法

当院で核酸アナログ長期継続治療を行い、1 年以上 HBV DNA の陰性化が持続した 10 症例を対象として、Peg-interferon- alpha2a を 4 週間併用投与した後に核酸アナログを中止し、以後は Peg-interferon- alpha2a を合計 48 週間投与する Sequential 療法を施行した。経時的に生化学データ、HBV DNA、HBs 抗原量、HBe 抗原を測定し、治療効果をモニターした。

（倫理面への配慮）

治療導入に当たっては、Sequential 療法の目的、予想されるメリット、デメリットについて文書で説明し、十分なインフォームドコンセントのもとに書面による同意を取得した。本研究については、当院の臨床研究委員会により倫理的妥当性を検討し、承認を得ている。

C. 研究結果

対象症例の 90%は男性、平均年齢は 38.5 歳で、70%が HBe 抗原陰性であった。HBV DNA<2.1Log/ml が 50%、高感度 RTD-PCR 法で陰性が 50%であった。先行する核酸アナログ派 80%がエンテカビルで、20%がラミブジン・アデフォビル併用療法であった。核酸アナログの平均投与期間は 4 年であった。

Sequential 療法により、全例で HBs 抗原量が減少し、HBs 抗原の減衰は最終観察時点で-0.02～-3.47Log/ml であった。1 例において治療開始後 24 週時点で HBs 抗原が陰性化し、治療終了後も再燃することなく、治療終了後 24 週時点でも Drug free かつ HBV DNA 陰性化かつ HBs 抗原陰性が維持できている。他の 1 例において、治療終了時に HBs 抗原量が 0.96 まで減少し、治療終

了後も再燃することなく、HBV DNA 陰性かつ HBs 抗原低値を持続している。他の 8 症例は、現時点では継続治療中である。

HBs 抗原陰性化率は全体では 10％だが、Sequential 療法開始時の HBs 抗原が 1000 未満の症例では 33%であった。HBs 抗原の 1.0Log 以上の減少は全体では 30% であったが、Sequential 開始時の HBs 抗原が 1000 未満の症例では 66%、Sequential 開始時 HBs 抗原が 1000～10000 では 20%であった。ALT が 40 以上への上昇は全体の 90%、80 以上への上昇は 40%で見られたが、重症化する症例はなく、HB s 抗原が 1.0Log 以上減少した 3 症例では全例で ALT が上昇した。

D. 考察

HBs 抗原の陰性化を目指した Sequential 療法においては、核酸アナログの長期投与後に HBs 抗原量が 1000 未満に減少した症例が最も良い適応と推察された。Sequential 療法においては ALT の上昇が高率にみられるが、これはインターフェロンの免疫賦活作用による HBV 感染細胞の排除を反映する可能性がある。

E. 結論

核酸アナログの長期継続治療後に Peg-interferon を Sequential に投与することで、Drug free あるいは HBs 抗原の陰性化が得られる症例が存在する。今後は治療効果を予測する因子を明らかにすることで、治療適応症例の基準を明確にする必要がある。

F. 健康危険情報

特になし

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2. 学会発表

なし

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

特になし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅳ．研究成果の刊行物・別刷

Title page

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

Short running title: Guidelines to discontinue NUCs

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Figure legends	1	page
Figures	5	pages

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

Abstract

Since nucleos(t)ide analogues (NUC) can lead to rapid reduction in HBV DNA levels in blood and normalization of alanine aminotransferase levels in many patients. They also provide histological improvement which results in a reduction in liver carcinogenesis. However, it is difficult to completely remove viruses even by NUCs and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment. One of the reasons is that NUCs reduce the HBV DNA level in blood but have almost no effects on the HBV cccDNA level in hepatocyte nuclei which are the origins of HBV replication and HBV cccDNA remains for a long period.

For treatment with NUCs in patients with hepatitis B, it is considered that NUCs should not be easily discontinued since discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses after discontinuation. Although there are not a few patients without hepatitis relapse after discontinuation or with mild relapse and finally in a stable condition, it has not been established how to identify such patients efficiently.

We performed research to investigate characteristics of the course after discontinuation of treatment and definition of hepatitis relapse and estimate the relapse rate. "Guidelines for avoiding risks resulting from discontinuation of NUCs 2012," is summarized based on the study results. Because the guidelines are written in Japanese, we explain those in English as a review article.

Introduction

Since nucleos(t)ide analogues (NUC) recently introduced to treatment of hepatitis B strongly inhibit proliferation of HBV, they can lead to rapid reduction in HBV DNA levels in blood and normalization of alanine aminotransferase (ALT) levels in many patients.[1] They also provide histological improvement which results in a reduction in liver carcinogenesis[2, 3] and can be orally administered with few side effects, so they are widely used in clinical practice. However, it is difficult to completely remove viruses even by NUCs and there are some problems such as emergence of resistant strains and hepatitis relapse resulting from discontinuation of treatment.[4] One of the reasons is that NUCs reduce the HBV DNA level in blood but have almost no effects on the HBV cccDNA level in hepatocyte nuclei which are the origins of HBV replication and HBV cccDNA remains for a long period.[5]

For treatment with NUCs in patients with hepatitis B, it is considered that NUCs should not be easily discontinued since discontinuation often results in hepatitis relapse. However, it has not been clearly revealed when and how hepatitis relapses after discontinuation. Although there are not a few patients without hepatitis relapse after discontinuation or with mild relapse and finally in a stable condition, it has not been established how to identify such patients efficiently.

We performed research funded by the Health and Labour Sciences Research Grant to investigate characteristics of the course after discontinuation of treatment and definition of hepatitis relapse and estimate the relapse rate.[6] "Guidelines for avoiding risks resulting from discontinuation of NUCs 2012," is summarized based on the study results (Kanzo 2012; 53: 237-242, The Japan Society of Hepatology). The guidelines don't always recommend discontinuation of NUCs. We determined them to be referred

if it is necessary to consider discontinuation due to various reasons.

Serum markers reflecting amount of HBV cccDNA in hepatocytes

The replication process of HBV in hepatocytes is shown in Fig. 1. HBV is an enveloped DNA virus containing a relaxed circular DNA genome converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells. [7-10] These cccDNA molecules serve as transcriptional templates for production of viral RNAs that encode both viral structural and non-structural proteins. Hepatitis B surface antigen (HBsAg) is transcript from 2.1 kb and 2.4 kb mRNAs. On the other hand, hepatitis B core antigen (HBcAg), p22cr antigen (p22crAg)[11], and hepatitis B e antigen (HBeAg) are transcript from 3.5 kb mRNA which also serves as pregenome RNA. HBeAg is secreted into blood stream as a secretion protein, and p22crAg forms genome-negative core particles. HBcAg forms nucleocapsid particles by incorporating pregenome RNA. Once the pregenome RNA is reverse transcripted to DNA, the particles are enveloped with lipid layer containing HBsAg and then secreted into blood stream as virions.[8, 9] When the reverse transcriptation is inhibited by NUCs, virus particles with RNA genome are secreted instead of those with DNA genome.[12, 13]

HBV cccDNA is a stable molecule like chromosomal DNA which hardly be destroyed by DNases in natural conditions. Because NUCs are inhibitors of reverse transcriptase, they have no direct effect on reducing intrahepatic cccDNA levels. Therefore, reactivation of HBV replication which originates from HBV cccDNA and incidental hepatitis relapse occurs when NUCs are discontinued.

It is generally considered that HBV cccDNA levels in hepatocytes is well correlated with proliferative potential of HBV,[5] serum markers reflecting the cccDNA

level are suggested to be useful as clinical indicators. Serum level of HBV DNA correlates well with intrahepatic level of HBV cccDNA in natural course but not under NUC treatment. NUCs reduce serum level of HBV DNA rapidly by inhibiting the reverse transcription, but this inhibition does not reduce the cccDNA level.[5] On the other hand, serum levels of HBsAg and HB core-related antigen (HBcrAg) have been reported as markers reflecting cccDNA levels in hepatocytes even under NUC treatment.[14-17] HBcrAg assay measures all antigens coded by pre-core/core genome simultaneously which include HBcAg, HBeAg, and p22crAg, and has been reported to be useful for predicting clinical outcomes of patients who were treated with NUCs.[6, 17-22] HBsAg level is focused recently as a new marker and has been reported to be efficient in prediction of treatment effects by interferon and others.[14, 15]

Aims of these guidelines

These guidelines aim to identify patients with a higher possibility of successful discontinuation or patients who should continue treatments and avoid risks resulting from discontinuation of NUCs as much as possible by establishing indicators for follow-up after discontinuation (Table 1-I). Successful discontinuation in the guidelines is defined to finally achieve the inactive carrier state with the ALT level of less than 30 IU/L and the HBV DNA level in blood of less than 4.0 log copies/ml. These criteria were defined in compliance with the guidelines for treatment of chronic hepatitis B in Japan.[23] It is known that patients in the inactive carrier state show no progression of hepatic diseases and a reduction in the carcinogenic rate[24, 25] and the criteria are considered to be appropriate.

Requirements to avoid risk of developing severe hepatitis resulting from relapse

It is currently unable to predict hepatitis relapse after discontinuation of NUCs with a sufficient high accuracy. Therefore, we reviewed the risk of developing severe hepatitis and established requirements to prevent severe hepatitis (Table 1-II).[26] The presence of understanding about the risks of hepatitis relapse and severe hepatitis by both doctors and patients as well as the availability of a follow-up system after discontinuation and appropriate treatment for relapse are the basic essential requirements. Given patients with hepatic cirrhosis or chronic hepatitis with progressed fibrosis similar to cirrhosis can easily develop severe hepatitis and have higher risks of carcinogenesis in the future, we determined that those patients should not easily discontinue NUCs.

Assessment of proliferative potential of HBV and conditions to reduce the relapse risk

It has been experienced that patients with insufficient reduction of HBV DNA level or with HBeAg positive at the time of discontinuation of NUCs can develop hepatitis relapse in higher rate after discontinuation. The tendency was also confirmed scientifically in our study.[6] The cut-off value of HBV DNA level to predict hepatitis relapse was 3.0 log copies/mL by the ROC analysis. Almost all patients with higher HBV DNA levels or with HBeAg positive relapsed within a year while nearly 30% of patients with the HBV DNA levels less than 3.0 log copies/mL and without HBeAg were in the stable condition for a long period. (Fig. 2) Based on these results, we included sufficient reduction in HBV DNA levels and HBeAg negative in requirements for discontinuation. We determined the reference range of sufficient reduction in HBV

DNA levels in the actual guidelines not to be less than 3.0 log copies /ml but to be negative by real-time PCR in consideration of safety.

Factors relating to hepatitis relapse after discontinuation were analyzed in the population except for patients who were obviously predicted to relapse after discontinuation, or those with the HBV DNA levels of not less than 3.0 log copies/mL or HBeAg positive. The following factors were calculated to be significant; duration of treatment period of NUCs, HBsAg levels at the time of discontinuation, and HBcrAg levels at the time of discontinuation. Since the cut-off value in duration of treatment period was calculated as 16 months, we overestimated and established that NUCs should be discontinued more than 2 years after the initial administration in the guidelines.[6]

Two cut-off values were suggested from the results of the ROC analysis for the HBsAg and the HBcrAg levels at the time of discontinuation (Fig. 3); 1.9 and 2.9 log IU/m*l* for the HBsAg level and 3.0 and 4.0 log U/m*l* for the HBcrAg level. Based on this, HBsAg and HBcrAg levels were scored as shown in Table 1-III and three groups of the low risk group, the medium risk group and the high-risk group were determined. The percentage of prediction success was 80% to 90% in the low risk group, approximately 50% in the medium risk group and 10-20% in the high-risk group (Fig. 4). In further investigation of factors relating to hepatitis relapse in each group, no factors were newly found in the low and medium risk groups but age was a significant factor in the high-risk group. Although the percentage of prediction success rate is low in the high-risk group (10% to 20%), it resulted slightly higher which was from 30% to 40% with those patients younger than 35 years old.[6] It was interesting to find that the combination of HBsAg and HBcrAg levels were useful in preparing these guidelines for