中文摘要

丁型肝炎病毒和乙型肝炎病毒合并感染的分子病毒学特点研究

实验背景和目的：

丁型肝炎病毒（Hepatitis D virus，HDV）是乙型肝炎病毒（Hepatitis B virus，HBV）的卫星病毒，理论上所有乙型肝炎患者都应该接受丁型肝炎筛查。但是由于乙型肝炎患者基数庞大，实际丁型肝炎筛查率较低，不同地区丁型肝炎流行率差距大等原因，目前还无法准确估算丁型肝炎的流行率。HDV 入侵肝细胞后，产生的小丁型肝炎抗原（Small-hepatitis D antigen，S-HDAg）促进 HDV 的复制。复制进行一段时间后，HDV 利用宿主酶进行 RNA 编辑，成为编辑型丁型肝炎病毒（edited-HDV）。后者产生的大丁型肝炎抗原（Large-hepatitis D antigen，L-HDAg）抑制 HDV 的复制，促进 HDV 的组装和释放。S-HDAg 和 L-HDAg 均可以通过多种机制抑制辅助病毒 HBV 的复制。Edited-HDV 也可以释放并重新入侵细胞，不过单独感染肝细胞后，无法进行病毒复制和建立丁型肝炎感染，产生的 L-HDAg 有防止 HBV 或原始型 HDV 感染细胞的潜力。

本实验的目的是研究 HDV 在乙型肝炎患者中的流行率以及分子病毒学特点，寻找更加合理的筛查策略；以及研究 edited-HDV 对肝炎病毒的影响，探究其在病毒性肝炎治疗上的应用前景。

实验方法：

本实验在吉林省收集了 5594 份乙型肝炎血清样本，对乙型肝炎表面抗原（Hepatitis B surface antigen，HBsAg），HBV DNA，抗-HDV 和 HDV RNA 进行检测，利用统计学知识进行数据分析。通过基因测序的方法分析丁型肝炎患者血清中 edited-HDV 在总 HDV 中的所占比例。利用体外实验和分子生物学手段，通过病毒全基因组质粒使细胞感染对应病毒，研究 edited-HDV 对其他肝炎病毒是否有抑制作用。

实验结果：

吉林省乙型肝炎患者中抗-HDV 的阳性率约为 3.6%（3.2-4.2%），HDV RNA

的阳性率约为 1.2%（0.9-1.5%）。男性和女性乙型肝炎患者的 HDV RNA 筛检阳性率相似（1.2% vs 1.1%，P＞0.05）。51-80 岁的乙型肝炎患者的 HDV RNA 筛检阳性率高于 31-50 岁的患者（2.1% vs 0.2%，P＜0.05），87.7%的丁型肝炎患者年龄在 51-70 岁之间。HDV 清除患者（抗-HDV 为阳性，HDV RNA 为阴性）的 HBsAg 定量值小于丁型肝炎患者（65.83 IU/mL vs 892.90 IU/mL，P＜0.05）。丁型肝炎患者的 HBV DNA 定量结果较单独乙型肝炎患者低（18.6 IU/mL vs 338 IU/mL，P＜0.05），HBV DNA 水平较低的乙型肝炎患者，HDV 筛查结果为阳性的可能性较大，95.2%的丁型肝炎患者 HBV DNA 定量值小于 2000 IU/mL。丁型肝炎患者的抗-HDV 吸光度较 HDV 清除患者高（2.219 vs 1.397，P＜0.05），抗-HDV 水平较高的患者更有可能同时为 HDV RNA 阳性。在丁型肝炎患者中，HBsAg 和抗-HDV 之间存在较弱的相关性（r=0.256，P=0.043）。

83.3%的丁型肝炎患者血清中的 edited-HDV 占总 HDV 的 35%-43%。细胞实验中，edited-HDV 的初始占比在 50%以上时，HDV 的复制较转染原始型 HDV 时少（P＜0.05），HBV 的复制与不转染 HDV 时无显著性差异（P＞0.05）。HDV 单独感染时，HDV RNA 水平与 edited-HDV 的初始占比呈负相关（r=-0.857，P=0.029）；HBV-HDV 同时感染时，HDV RNA 水平与 edited-HDV 的初始占比没有相关性（r=-0.790，P=0.061），HBV DNA 水平与 edited-HDV 的初始占比也没有相关性（r=-0.547，P=0.262）。提前加入细胞的 edited-HDV 可以减少后续进入的 HBV 的复制（0.56 vs 1.00，P＜0.05），不过对后续进入的原始型 HDV 没有影响（0.99 vs 1.00，P＞0.05）。Edited-HDV 进入原本就感染 HBV 的细胞中后，增加细胞中 HBV 的复制（1.46 vs 1.00，P＜0.05）。

实验结论：

吉林省乙型肝炎患者中抗-HDV 和 HDV RNA 的阳性率分别约为 3.6%和 1.2%。年龄 50 岁以上，HBV DNA 定量小于 2000 IU/mL 的乙肝患者应作为丁型肝炎的重点筛查对象。抗-HDV 水平较高的患者更有可能同时为 HDV RNA 阳性。大部分丁型肝炎患者血清中的 edited-HDV 占总 HDV 的 35%-43%。在细胞实验中，当 edited-HDV 的初始占比在 50%以上时，edited-HDV 可以抑制同时进入细胞的原始型 HDV 的复制，但对同时进入细胞的 HBV 的复制没有抑制作用。细

胞中的 edited-HDV 可以预防 HBV 感染，但是无法预防原始型 HDV 感染。

关键词：

丁型肝炎病毒，乙型肝炎病毒，流行病学，分子机制，筛查策略。

Abstract

Molecular Virological Characteristics of Hepatitis D Virus and Hepatitis B Virus Dual-Infection

Background and objective:

Hepatitis D virus (HDV) is a satellite virus of hepatitis B virus (HBV). Theoretically, all hepatitis B patients should be screened for hepatitis D infection. However, due to high burden of hepatitis B infection, insufficient screening of HDV, and HDV prevalence varies according to geography, it is difficult to estimate the certain prevalence rate of hepatitis D infection. After HDV entries hepatocytes, small hepatitis D antigen (S-HDAg) is translated to promote HDV replication. After a period of replication, HDV uses host enzyme to edit RNA, and is converted into an edited-HDV. The latter produces Large Hepatitis D antigen (L-HDAg), which inhibits HDV replication and promotes HDV assembly and release. Both S-HDAg and L-HDAg can inhibit the replication of HBV through various mechanisms. Edited-HDV can release from and entry into hepatocytes, but when edited-HDV momo infects hepatocytes, it can't replicate and establish hepatitis D infection. The L-HDAg producted by edited-HDV may prevent HBV or original-HDV infection.

One of the objectives was to study the prevalence and the characteristics of molecular virology of HDV in hepatitis B patients. We used these results, to find a more reasonable screening strategy. The other objectives was to find the effects of edited-HDV infection on other hepatitis viruses, exploring its application prospects in the treatment of viral hepatitis.

Methods:

5594 serum samples of hepatitis B patients were collected in Jilin Province, hepatitis B surface antigen (HBsAg), HBV DNA, anti-HDV, and HDV RNA were detected, and study data were performed statistical analysis. The proportion of edited-HDV to total HDV in the serums of patients with hepatitis D were analyzed by gene

sequencing. Using in vitro experiments and molecular biology methods, hepatocytes were infected with virus through the corresponding virus whole genome plasmid, to investigate the effects of edited-HDV infection on other hepatitis viruses.

Results:

In the hepatitis B patients of Jilin Province, the positive rate of anti-HDV was about 3.6% (3.2-4.2%), and the positive rate of HDV RNA was about 1.2% (0.9-1.5%). The screening positive rates of HDV RNA in male and female hepatitis B patients were similar (1.2% vs 1.1%, $P>0.05$). The screening positive rate of HDV RNA of 51-80 years old hepatitis B patients was higher than that of 31-50 years old patients (2.1% vs 0.2%, $P<0.05$), 87.7% of hepatitis D patients were between 51 to 70 years old. The HBsAg quantity of HDV-resolved patients (anti-HDV positive and HDV RNA negtive) was lower than that of hepatitis D patients (65.83 IU/mL vs 892.90 IU/mL, $P<0.05$). The HBV DNA quantity of hepatitis D patients was lower than HBV mono-infected patients (18.6 IU/mL vs 338 IU/mL, $P<0.05$), screening for HDV infection is more likely to yield positive results in hepatitis B patients with lower HBV DNA level, 95.2% of hepatitis D patients had HBV DNA quantity below 2000 IU/mL. The anti-HDV absorbance of hepatitis D patients was higher than that of HDV-resolved patients (2.219 vs 1.397, $P<0.05$), those with higher anti-HDV levels were more likely to test positive for HDV RNA. A weak correlation was observed between HBsAg and anti-HDV in hepatitis D patients (r=0.256, P=0.043).

In the serum of 83.3% of hepatitis D patients, edited-HDV accounts for 35%-43% of the total HDV. In vitro experiments, when the initial proportion of edited-HDV was more than 50%, HDV replication was lesser compared to all original-HDV group ($P<0.05$); but HBV replication remained unchange compared to the group that didn't transfect HDV($P>0.05$). In HDV mono-infection, there was a negative correlation between the initial proportion of edited-HDV and HDV RNA level (r=-0.857, P=0.029); but in HBV-HDV co-infection, there was no correlation between the initial proportion of edited-HDV and HDV RNA level (r=-0.790, P=0.061), and no

correlation between the initial proportion of edited-HDV and HBV DNA level too (r=-0.547, *P*=0.262). Edited-HDV added to hepatocytes in advance can reduce the replication of HBV entered subsequently (0.56 vs 1.00, $P<0.05$), but can't affect the replication of original-HDV entered subsequently (0.99 vs 1.00, $P>0.05$). After entering hepatocytes that were already infected with HBV, edited-HDV increased HBV replication (1.46 vs 1.00, $P<0.05$).

Conclutions:

Among the hepatitis B patients of Jilin Province, the positive rates of anti-HDV and HDV RNA were about 3.6% and 1.2%, respectively. Hepatitis B patients over 50 years old, or HBV DNA quantity less than 2000 IU/mL, should be screened for hepatitis D infection. Patients with higher anti-HDV levels are more likely to test positive for HDV RNA. In the serum of most hepatitis D patients, edited-HDV accounted for 35%-43% of the total HDV. In vitro experiments, when the initial proportion of edited-HDV was more than 50%, edited-HDV could inhibit the replication of original-HDV that entered simultaneously, but had no effect on the replication of HBV that entered simultaneously. Edited-HDV in hepatocytes might prevent HBV infection, but couldn't prevent primitive original-HDV infection.

Key words

Hepatitis D virus, hepatitis B virus, epidemiology, molecular mechanisms, screening strategies.

目录

英文缩写词表

缩写	全称	中文
aa	amino acid	氨基酸
ADAR1	Adenosine deaminases acting on RNA 1	1 型作用于 RNA 的腺苷脱氨酶
AGL	Antigenic loop	抗原环
agRNA	antigenome RNA	反基因组 RNA
cccDNA	covalently closed circular DNA	共价闭合环状 DNA
CCS	Coiled-coil sequence	螺旋线圈序列
CHD	Chronic hepatitis D	慢性丁型肝炎
CI	Confidence intervals	置信区间
CYL-I	Cytosolic loop I	胞浆环 I
ELISA	Enzyme linked immunosorbent assay	酶联免疫吸附试验
ESCRT/MVB	Endosomal sorting complex required for transport-dependently multivesicular body	需要内体分选复合物的运输依赖性多泡体
gRNA	genome RNA	基因组 RNA
HBsAg	Hepatitis B surface antigen	乙型肝炎表面抗原
HBV	Hepatitis B virus	乙型肝炎病毒
HCV	Hepatitis C virus	丙型肝炎病毒
HDAg	Hepatitis D antigen	丁型肝炎抗原
HDV	Hepatitis D virus	丁型肝炎病毒
hNTCP	human Sodium taurocholate coreceptor peptide	人钠离子/牛磺胆酸共转运受体肽
HSPG	Heparan sulfate proteoglycans	硫酸乙酰肝素蛋白聚糖
IQR	Interquartile distance	四分位间距
L-HBsAg	large-HBsAg	大乙型肝炎表面抗原
L-HDAg	large-HDAg	大丁型肝炎抗原

续表

缩写	全称	中文
M-HBsAg	middle-HBsAg	中乙型肝炎表面抗原
NES	Nuclear export signal	核输出信号
NLS	Nuclear localization signal	核定位信号
ORF	Open reading frame	开放阅读框
Pol-I	Polymerase I	RNA 聚合酶 I
Pol-II	Polymerase II	RNA 聚合酶 II
Pol-III	Polymerase III	RNA 聚合酶 III
quasi-ds	quasi-double-stranded	准双链
RBD	RNA-binding domain	RNA 结合域
RBS	Receptor-binding site	受体结合位点
RIA	Radioimmunoassay	放射免疫分析
RNP	Ribonucleoprotein	核糖核蛋白
RT-qPCR	quantitative Reverse transcription Polymerase chain reaction	定量逆转录聚合酶链反应
SD	Standard deviation	标准差
S-HBsAg	small-HBsAg	小乙型肝炎表面抗原
S-HDAg	small-HDAg	小丁型肝炎抗原
SVP	Subviral particle	亚病毒颗粒
TLM	Translocation motif	转移基序