A retrospective study: The Influence of human immunodeficiency virus co-infection with hepatitis C virus or hepatitis B virus on the efficacy with HAART in China AIDS area

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Abstract: To evaluate the impact of human immunodeficiency virus (HIV) co-infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) on the efficacy of highly active anti-retroviral therapy (HARRT) and analysis on the variable pattern of resistant’s sites in HIV RNA. The patients were divided into three groups: HIV/HBV/HCV co-infection group (23 patients), HIV/HCV co-infection group (168 patients), and HIV-only group (178 patients). All patients in the 3 groups were given the same HAART, that was, AZT+DDI+NVP, but not given other antivirus treatment including HCV and HBV antivirus therapy. HIV RNA, HCV RNA or HBV DNA were detected by real time PCR every 90 days, meanwhile the counts of CD4+ T lymphocyte and liver function including ALT(alanine transaminase), AST (aspartate aminotransferase), and total bilirubin (T-Bil) were tested. According to the titer of HIV RNA (>10⁴ copies/ml) in sera during the one year HAART, polymerase genes of HIV RNA were sequenced and analyzed. During one-year HAART, HIV RNA of HIV-only group, HIV/HBV/HCV co-infection group and HIV/HCV co-infection group decreased significantly from 6.78±1.08, 6.23±1.34, 6.54±1.23 log copies/ml to 0.53±0.15, 0.67±0.16, 0.43±0.11 log copies/ml respectively (P-value < 0.001). And CD4+ T lymphocyte counts of the three groups elevated significantly from 197±127, 184±113, 213±143 cells/μl to 382±74, 383±70, 378±76 cells/μl respectively (P-value<0.001). However there were no differences among the three groups in HIV RNA and CD4+ T cell counts. There were no differences in liver functions including ALT, AST and T-Bil among the three groups. The detection of sites of drug resistance: the major mutant sites to AZT+DDI were at M41L, E44A, K70KR, D67N, L210W, T215Y or K219W which were highly resistant and to NVP were at A98G, V179H, Y181C, K103N or G190A which were highly resistant in the 3 groups. Meanwhile, the rates resistant of emergence were similar and there were no sites to 3TC and protease inhibitors (PIs) in the above HAART groups. HIV co-infected with HBV and/or HCV does not impact on the efficacy of HAART. What more, HAART does not impact HCV replication.


Key words: HIV; Co-infection; High active anti-retroviral therapy

1. INTRODUCTION

HIV, HBV and HCV share similar routes of transmission, with sexual, parenteral and perinatal transmission being the most frequent modes of acquiring these infections. In contrast, exposure to these viruses is followed by an immune response which differs markedly in its ability to clear the infection[1]. Highly active antiretroviral therapy (HAART) has improved the life expectancy of HIV infected patients, but, by extending survival, it permits the development of HCV cirrhosis. Relatively little is known regarding hepatitis viral co-infections among HIV infected patients, so this study was therefore carried out to estimate the effects of HBV and/or HCV seropositivity in a cohort of people living with HIV/AIDS in China and to investigate the effect to these viruses on CD4+ lymphocytes in the HAART.

2. MATERIAL AND METHODS

(1) HIV RNA, HBV DNA and HCV RNA ELISA kits were from Invitrogen Co., Netherlands. Extractive kits for PCR product were obtained from QIAGEN Company (Germany). Real-time PCR was from Roche Co. Sephadex G-100 and Sepharose CL-4B were from Pharmacia Ltd. FACS was from Beckman (USA).

(2) Blood was collected aseptically into 10 ml vacutainer tubes (BD, NJ USA) for biochemical, CD4+ count and viral serology tests. Biochemical and CD4+ T lymphocyte assays(FACS, Beckman, USA), were performed within three hours of collection, while serum for serological assays of hepatitis B and C markers were stored at -20°C until
(3) Extraction of HCV RNA, HIV RNA or HBV DNA was according to the extractive kit’s direction. Briefly, the extracted HIV RNA from the 100μl serum was amplified by PCR with reverse-transcriptase procedure: 42°C, 45min and 94°C, 5min, and so rounded to major procedure: 92°C 30 s, 55°C 30 s, 72°C 30 s for 45 cycles. Amplification products were resolved by agarose gel electrophoresis, stained with ethidium bromide. If HIV RNA was positive (>10⁴ copies/ml) from the patients who had been given 3 months’ treatment of HAART, The polymerase gene of HIV RNA was amplified and sequenced. The resistant sites were analyzed by resistant soft and resistant data provided from Stanford University. The serum amino-transferase determined was ALT, AST, and total T-Bil. The catalytic activity of ALT, AST and T-Bil was determined in serum using a COBAS MIRA chemistry analyzer (GMI, MI, USA) after it was calibrated.

(4) Continuous variables were expressed as mean±standard deviation and were compared using the Mann-Whitney test or the student-t test. Categorical variables were expressed as proportions, compared using the chi-square test or Fisher exact test to evaluate differences between proportions. Datas were analyzed with SPSS (Statistical Package for Social Sciences) program version 10.0; p<0.05 were considered statistically significant.

(5) Selection of patients: One hundred and ninety-one naïve HIV/AIDS patients were selected from confirmed HIV-1 positive from China AIDS area. HIV, HBV and HCV infection would be testified by ELISA and western-Blot. Of the 191 patients, the sero-prevalence of HIV/hepatitis viruses were as follows: HIV only was 178 patients; HBV/HIV only 2(That type of patients was too rare and was not statistical analysis), HCV/HIV 166, and HIV/HBV/HCV were only 23 patients.

(6) Regimen of HAART: HIV-1 infected patients on failing HAART were prospectively submitted for consultation all patients were given the same treatment, that was AZT+DDI+NVP (AZT, zidovudine, 600mg/day; DDI, dideoxyinosine, 400mg/day; NVP, nevirapine, 400mg/day, for one year, but not given anti-HBV or HCV therapy corresponding.

(7) Periods of assay: HIV RNA, HCV RNA and HBV DNA in sera were assayed by real-time PCR. Resistance tests, antiretroviral history, adherence, CD₄⁺ counts, HIV-RNA levels and HCV/HBV co-infection were scheduled every 90 days during the HAART.

3. RESULTS

(1) Counts of CD₄⁺ T lymphocyte: After giving HAART for 1 year, there were obviously significant in the comparison of CD₄⁺ T lymphocyte counts and those were all elevated in every group, that is HIV/HCV/HBV, HIV/HCV and HIV group (P<0.001) (see Table 1). But there were no differences in the CD₄⁺ counts among the above 3 groups (P>0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Pre- HAART</th>
<th>Post-HAART</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/HCV/HBV</td>
<td>23</td>
<td>184±113</td>
<td>378±76</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV/HCV</td>
<td>168</td>
<td>213±143</td>
<td>383±70</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV</td>
<td>178</td>
<td>197±127</td>
<td>382±74</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(2) Real-time PCR

HIV RNA: In the AIDS serum of all the 3 groups, levels of HIV RNA were continuously declined during HAART and there were statistically significant in the copies of HIV RNA between the pre and post HAART in each group (see table 2). On the other, the ranges of decreasing of HIV RNA were the similar and there were no differences among the 3 groups during the HAART (P >0.05, see figure 1).

<table>
<thead>
<tr>
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<th>Pre-HAART</th>
<th>post-HAART</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/HCV/HBV</td>
<td>23</td>
<td>6.23±1.34</td>
<td>0.67±0.16</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV/HCV</td>
<td>168</td>
<td>6.54±1.23</td>
<td>0.43±0.11</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV</td>
<td>178</td>
<td>6.78±1.08</td>
<td>0.53±0.15</td>
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</tr>
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</table>
Figure 1 HIV RNA in the sera during the HAART by real-time PCR assay (x±s)

θ HCV RNA: Of the 23 patients with HIV/HCV/ HBV co-infection, HCV RNA position is 21 and 163 cases with HCV RNA(+) in all 168 HIV/HCV patients. From HIV/HCV/ HBV or HIV/HCV co-infection groups, level of HCV RNA had no overt alternation and was often in the range about 10^7 ~10^8 copies/ml during the HAART(see table 3). But to HIV RNA, it was obviously declined in the HIV/HCV co-infection group. Statistic data showed that it was no significant in HCV RNA between pre and post HAART (see figure 2).

Table 3. HCV RNA in sera at pre or post HAART (log copies/ml, x±s)

<table>
<thead>
<tr>
<th>Group</th>
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<th>Pre-HAART</th>
<th>Post-HAART</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/HCV/ HBV</td>
<td>21</td>
<td>7.23±1.54</td>
<td>6.86±1.36</td>
<td>0.67</td>
</tr>
<tr>
<td>HIV/HCV</td>
<td>163</td>
<td>6.76±1.47</td>
<td>7.15±1.41</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Figure 2. HIV RNA or HCV RNA in the sera during the HAART (x±s)

ρ HBV DNA: During the HAART, the change of HBV DNA was the similar as the trend of HCV RNA, but it was in low level about 10^3~10^5 copies/ml. Statistic showed that it was no significant in the level of HBV DNA between pre and post- HAART.

σ Liver function: When clinical features and biochemical markers were considered together, ALT in only 1 patient with any of the markers for hepatitis had elevated (by history or examination). But in the following treatment, it became normal after 1 month. Importantly, only 5.1% of the patients co-infected with hepatitis B or C virus co-infection had abnormal serum ALT or AST.

τ Assay of resistance: The resistant strains were emergence since the third month with HAART, but only 7.8% of the patients co-infected with hepatitis C virus co-infection had abnormal serum HIV RNA in all groups. The
rates and positions of variant sites were the similar as the other 2 group patients. The sites for NRTI (AZT and DDI) were major in M41L, E44A, K70KR, D67N, L210W, T215Y, K219W A98G, V179H, Y181C, K103N, G190A, K20R, V35L, K43E, W88C, K122E, I135V, S162C, G196E, T200A, E203D, H221Y etc. And for NNRTIs(NVP), there was only in Y181C (see figure 3). There were no resistant sites to 3TC and protease inhibitors (PIs) in the above HAART groups.

Figure 3. Rate and position in the resistant strain of polymerase gene in the patients co-infected with hepatitis virus C.

4. DISCUSSION

HIV, HBV, and HCV are devastating disease agents that share common modes of transmission (Vincent Soriano, Pablo Barreiro and Marina Nuñez), therefore HIV positive individuals are at risk of co-infection with HBV and HCV infections[2]. With the increased lifespan of HIV-1 infected patients, HCV and HBV have recently emerged as important pathogens in these patients[3]. With the advent of highly active antiretroviral therapy (HAART) regimens capable of dramatically prolonging the survival of HIV-infected patients, the impact of co-morbid infections such as HBV and HCV has come into focus. Co-infection with HBV or HCV increases the risk for hepatotoxicity of HAART and likelihood of onset of an AIDS-defining illness, compared with infection with HIV-1 alone. Although the HIV co-infection with HBV and/or HCV has been recognized worldwide, limited data are available on the extent of co-infection. Few studies have been done on HIV, HBV, and HCV separately in developing country[5].

We collected 369 patients in China AIDS area and give them HAART for 1 year. We found: during the HAART, the declined trend of HIV RNA was almost similar in 3 groups (see graph 1), and there were obvious significant in HIV RNA between pre and post-HAART (P<0.05, see table 1). At the same time during the HAART, there were no differences in HIV RNA level among the 3 groups (P>0.05). Meanwhile, CD4+ T lymphocyte counts elevated following HIV RNA declined, there were obvious difference between pre and post HAART(see table 1). Notably, we observed no statistically significant association even in rates and positions between the occurrence of the either HBsAg or HCV (see graph 3), that was, it was no effect on the immune reconstruction and anti-virus to AIDS. The above results were the similar as Cooper and Milles’s reports[6]. But to the liver function, there were only 5.1% cases whose liver function was abnormal and rapidly recovered by themselves in a short time[9].

Otherwise, the knowledge about the interrelationship between these viruses and their effect on the immune system remains unclear[10]. Triple co-infected individuals are more likely to present with lower CD4 counts and therefore reduced host immunity[4]. In our study, with the immune reconstruction and declination of HIV RNA, the reproduction of HCV and HBV was not effected (see table 3 and graph 2) and the level of those was almost in stable. This results were liked as others[7, 11], so there was no kinetic interactions in reproduction between HIV and HCV( or HBV)[12, 13].

In other study, it has demonstrated that co-infection of HIV and hepatitis viruses (HBV and/or HCV) is on the increased and appears to decrease the CD4 counts of patients who are coinfected especially with triple coinfection of HIV, HBV, and HCV[8]. Treatment of either hepatitis virus
is complex because of pharmacokinetic interactions with components of HAART regimens. In our results, the reproduction of HIV can be inhibited and the immune system can be reconstructed after HAART (AZT+DDI+NVP) to AIDS with HBV and (or) HCV co-infection. The HAART have no effect on liver function and even there were no resistant sites to 3TC and protease inhibitors (PIs) in China AIDS area (see graph 3). So the regimen of AZT+DDI+NVP is a suitable therapy for developing country and the other regimens containing 3TC or PIs, perhaps, also are good choices in China.

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REFERENCES


