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Open-label trial of therapeutic immunization with oral V-5 Immunitor (V5) vaccine in patients with chronic hepatitis C

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Summary We evaluated whether V-5 Immunitor (V5) — tableted therapeutic bivalent vaccine comprising heat-inactivated HCV antigens from pooled blood of HBV- and HCV-infected donors — may produce clinical benefit through induction of oral tolerance and reduction of immune-mediated liver injury. Once daily dose of V5 was administered *per os* to 10 patients with chronic hepatitis C in an open-label study that lasted 1 month. Every patient who entered the study had elevated liver enzyme levels, which at the end of study have decreased in 100% of analyzed patients. The reduction was highly significant, from 157.7 ± 73.4 to 49.9 ± 43.8 U/L ($P = 0.0013$) and 147.0 ± 79.2 to 58.7 ± 56.6 U/L ($P = 0.0132$), for ALT and AST, respectively. The AST/ALT ratio has improved from 0.93 to 1.18 ($P = 0.00058$) indicating the reversion of progression to cirrhosis. None of intent-to-treat patients who were anti-HCV antibody positive at study entry, became negative after 1 month on V5 ($P = 0.998$). All patients, except one, reported complete recuperation from hepatitis C-associated clinical symptoms present at baseline ($P = 0.0016$) with Mantel Haenszel's odds ratio 9.4 ($P = 0.0021$) at 95% confidence interval: $2.7 < \text{OR} < 476.3$. No adverse events were observed at any time. The favorable biochemical and clinical responses have been observed in a small number of individuals for a limited time period. Larger scale and longer studies are needed to confirm our preliminary observations suggesting that V5 is safe and effective means for immunotherapy of chronic hepatitis C.

Introduction

Hepatitis C virus (HCV) is a global public health problem, infecting estimated 120–180 million people [1,2]. Mongolia has the highest reported rate of HCV infection in Asia; anti-HCV antibodies are found in 10–48% of adult popula-

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tion [3—5]. Approximately 85% of acute infections progress to chronic persistence of HCV and about 25% of these chronically infected individuals develop fatal liver diseases. Currently, there is no prophylactic vaccine to prevent the disease and no specific antiviral drug controlling HCV replication [1,2].

The current standard of care are interferon alfa-2a (Roferon-A) and interferon alfa-2b (Intron-A) or pegylated interferons (PEG-Intron, Pegasys) alone or in combination with ribavirin (Copegus, Rebetol). However, they have shown limited success and are associated with undesirable side effects [1,2]. The high cost of medications is another barrier that prevents wider use of anti-HCV therapy. For example, in Mongolia, only about 100 patients were treated with interferon in 2001 [6]. Thus, the treatment of the chronic HCV infection represents an unmet medical need. New strategies are being developed including therapeutic vaccines. The development of a therapeutic vaccine has considerable potential to aid chronic HCV carriers. They might help to clear the infection or prevent and delay cirrhosis and primary liver cancer.

Natural immune responses, both cellular and humoral, are not capable of terminating HCV infection in most patients. Over the last several years, studies have revealed some of the causes for the failure of the immune system to eliminate HCV infection [2]. It is now believed that if a therapeutic vaccine is administered to already-infected individuals it may help to induce a proper immune response that can be clinically beneficial. Nevertheless, therapeutic vaccines tested so far had shown modest clinical benefit [1]. In previous study we have shown that oral therapeutic hepatitis vaccine V-5 Immunitor (V5) developed by us, is beneficial to patients with hepatitis B [7]. In the present study we describe results from a pilot, open-label trial of V5 in patients with chronic hepatitis C.

Materials and methods

Subjects

Four female and six male patients with chronic hepatitis C infection were enrolled into an open-label, 1-month study. Patients were recruited among NRCID outpatients who gave their consent. Individuals who had higher than normal baseline liver enzyme transaminase ALT and AST levels were enrolled into the trial. All patients were positive for hepatitis C antibodies at study entry. The median age of patients was 31.5 years, range 17—64 years, mean $\pm$ S.D. equal to 35.7 ± 17.6 years. The average and median duration of infection prior to study initiation was between 10 and 11 years. None of patients were treated with interferon and/or anti-hepatitis drugs before or during the trial.

Vaccine

The vaccine is made from pooled blood of hepatitis B and C carriers by employing proprietary technology developed by us. The hepatitis viruses were killed by heat- and chemical inactivation and then formulated into a tablet. The process of manufacturing is described in detail earlier for a similar vaccine, V1, which is derived from the blood of HIV-positive patients many of whom had concomitant hepatitis virus infections [8]. The principle for production of V5 is not much different from established principles with old-fashioned killed vaccines, e.g., Hepatitis B vaccine made from pooled plasma. V-5 Immunitor is presented as 850 mg coated pill, 10 of which are sealed in a ''blister'' pallet, with 30 pills per one package. The recommended dose is 1—2 pills per day. The preparation is stable at ambient temperature for 5 years.

Administration schedule and monitoring

Patients were instructed to self-administer one oral tablet of V5 once per day at least half-an-hour before or after the morning meal. Each patient received 30 pills of V5 and was asked to come back 30 days later. The baseline and outcome parameters were established at study entry and at the return visit. The ALT and AST values were measured by LiquiUV test (Human GmbH., Germany). ELISA test kit for anti-HCV antibodies was from the same manufacturer.

Statistical analysis

Primary endpoints for this study were changes in serum ALT and AST liver aminotransferases, effect on anti-HCV antibodies, and clinical response. Parametric paired values were assessed by Student t-test and qualitative changes were analyzed by Chi-square (χ^2) 2×2 contingency table using GraphPad software. The odds ratio (OR) was estimated by Mantel Haenszel test with Wald's 95% confidence interval (CI). The significance level was set at $P \leq 0.05$.

Results

The results of V5 immunotherapy which lasted 1 month are shown in Table 1. The statistical values are provided at the bottom of the Table. One male patient (#10) failed to test for ALT and AST and have not completed his HCV-antibody tests. The patient #6 instead of taking one daily pill, took 2 pills per day for 15 days. Nevertheless, the statistical analysis of available, per intent-to-treat data shows that V5 produced significant positive changes, including three-fold decrease in ALT and AST levels and remarkable amelioration of clinical symptoms. Every endpoint has shown highly significant statistical value compared to baseline, indicating that these parameters were correlated with each other. The status of anti-HCV antibodies has not changed in any of tested patients.

Serum alanine aminotransferase rather than AST, is considered to be more reliable parameter in assessment of hepatic damage. Table 1 provides results from unpaired t-test since one patient had not ALT measured at follow-up. The reduction was highly significant, from 157.7 ± 73.4 to 49.9 ± 43.8 U/L ($P = 0.0013$) and from 147.0 ± 79.2 to 58.7 ± 56.6 U/L ($P = 0.0132$), for ALT and AST respectively. In order to prevent the bias we have excluded this patient and conducted more robust paired Student t-test on remaining nine patients. However, the overall outcome was not affected, as baseline mean ALT level 148.6 ± 71.5 U/L still shows the decrease to 49.9 ± 43.8 U/L ($P = 0.0009$). When