Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.6 No.1, Issue of April 15, 2003

Received November 15, 2002 / Accepted January 6, 2003
SHORT COMMUNICATION

Normalization of elevated liver enzymes due to V-1 Immunitor therapy

Vichai Jirathitikal
Immunitor Corporation Co., Ltd.
71 Moo 5, Bangpakong Industrial Park
Takarm, Chachoengsao 24130, Thailand
Tel: 66 09 2446398
Fax: 66 038 828524
E-mail:immunitor@aol.com

Orapun Metadilogkul
Occupational and Environmental Medicine Association of Thailand
Rajavithi General Hospital, The Ministry of Public Health
Rajavithi Road, Bangkok 10400, Thailand
Tel: 66 02 2456084
Fax: 66 02 2468270
E-mail: dr_orapun@yahoo.com

Aldar S. Bourinbaiar *
Immunitor Corporation Co., Ltd.
71 Moo 5, Bangpakong Industrial Park
Takarm, Chachoengsao 24130, Thailand
Tel: 66 09 2446398
Fax: 1 775 6406636
E-mail:immunitor@aol.com

Financial support: All work was supported by the Immunitor Corporation. Dr. Orapun Metadilogkul's salary is paid by the Thai Ministry of Public Health, she did not receive any compensation for participating in this study.

Keywords: AIDS, hepatitis, oral, therapeutic, transaminase, vaccine.

Abbreviations: AST/SGOT: aspartate aminotransferase; ALT/SGPT: alanine aminotransferases; V1: V-1 Immunitor; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome; CBC: complete blood count; FDA: Food and Dug Administration.

V-1 Immunitor (V1) is an oral AIDS vaccine currently being used as a therapeutic modality by HIV-positive patients. Upon interim analysis of phase I safety trial it has been discovered that patients who had elevated liver enzymes aspartate (AST/SGOT) and alanine (ALT/SGPT) aminotransferases have experienced the reduction of enzyme levels back to normal. Two other hepatitis markers alkaline phosphatase and bilirubin have also decreased. V1's effect may be hepatitis-specific since liver enzymes in normal patients treated with V1 have not changed and three patients who were Hepatitis B antigen positive at baseline became negative after therapy. The results suggest that V1 supplementation reduces hepatic damage caused by hepatitis viral infection.

Currently there are two major types of hepatitis viruses of particular concern, *i.e.*, B and C, which combined affect over 500 million individuals worldwide (Tangkijvanich et al. 1999). However, no effective and at the same time non-toxic therapy is available for chronic hepatitis – a serious and potentially life-threatening disease which may culminate in liver cancer. Diagnosis of virally induced chronic hepatitis is often made when a patient presents with elevated aspartate (AST/SGOT) and alanine (ALT/SGPT) aminotransferases. Many clinicians treat patients solely on the basis of clinical and biochemical abnormalities, *i.e.*, elevated liver enzymes. Other markers aiding the hepatitis diagnosis are abnormal levels of alkaline phosphatase and bilirubin.

V-1 Immunitor (V1) is an orally available polyvalent vaccine containing heat-inactivated viral antigens derived from the pooled blood of HIV infected donors and was originally developed as a therapeutic vaccine. We have published earlier that V1, tested in a small group of AIDS patients in Thailand increases T cell counts and body weight, decreases the viral load, (Jirathitikal and Bourinbaiar, 2002) and results in prolonged survival (Metadilogkul et al. 2002). This vaccine has been approved by the Thai FDA as a food supplement and has been used primarily by HIV-infected patients as a therapeutic modality. Our recently published data from placebo-controlled, phase II study suggests that V1 may be also effective as a prophylactic AIDS vaccine (Jirathitikal et al.

* Corresponding author

2003).

Materials and Methods

Clinical evaluation

The sample population consisted of 61 HIV-infected individuals who were randomly selected among HIV-seropositive outpatients at the Bangpakong Clinic in Chachoengsao province, Thailand. After obtaining standard written informed consent, the subjects were given a supply of V-1 Immunitor and were instructed to take one pill in the morning and one pill at bedtime. The seropositive status was confirmed by two rapid tests for HIV antibodies (Bioline HIV1/2, Immunochemical Lab., Thailand and Determine HIV1/2 Abbott/Dainabot, Japan). Hepatitis B surface antigen was determined by a similar rapid test (Abbott/Dainabot, Japan). Blood samples were taken by venipuncture and analyzed for liver and kidney function and for complete blood count (CBC) by a routine procedure (Tietz, 1995).

Vaccine

V-1 Immunitor is a polyvalent oral vaccine containing heat-inactivated, pooled HIV antigens which represent diverse clinical isolates derived from the blood of HIV-infected donors. Immunitor company manufactures the vaccine in Thailand according to a proprietary process developed by Vichai Jirathitikal (Jirathitikal and Bourinbaiar, 2002). V1 was first registered as a food on October 15, 1999 with health authorities of Chachoengsao province under License No. 1/2542. V1 was then licensed as a food supplement by the FDA on July 13, 2001 (License No. 152/44). Separately, in September of 2000 V1 received R & D Permit No. 1A1874/43 from the FDA for producing drug samples for R & D purposes. Thus, V1 has also a status as an experimental medicine, which will eventually lead to licensure of V1 as an immunomodulating drug or vaccine. V-1 Immunitor is provided as 850 mg coated pill, ten of which are sealed in a "blister" package. The recommended dose is one-to-four pills per day. The preparation is stable at ambient temperature for three years.

Statistical analysis

Obtained data were analyzed using StatMost version 2.5 program (DataMost Corp., Salk Lake City, UT). The significance threshold for all tests was set at $p<0.05$.

Results

Upon analysis of results of twice-a-day open label study of oral, therapeutic AIDS vaccine (V-1 Immunitor), we were surprised to discover that HIV-positive patients who initially had higher-than-normal AST and ALT levels have experienced the reduction of enzyme levels back to normal (Jirathitikal et al. 2002). All tests were carried out in the same lab and cut-off normal levels of AST and ALT were defined as being lower than 40 IU/L. Our study involved 61 antiretroviral drug naïve patients among whom 19 (31.1%) patients had elevated AST and ALT numbers at study entry (Table 1). Statistical analysis by both parametric and non-parametric tests revealed that hepatic enzyme levels were cut by about half and reduction was highly significant. While by the end of second month the enzyme levels seemed to rebound slightly this change was not significant. In contrast, patients who had normal baseline levels have not experienced changes in ALT levels (mean 26.8 vs. 25.3; $p=0.34$) or had diminished albeit normal AST levels (28.0 vs. 19.9; $p=3.34E-006$). At conclusion of study biochemical profiles of patients who entered this study with abnormal enzyme levels became indistinguishable from baseline figures of normal patients as assessed by both ALT (25.2 vs. 26.8; $p=0.2$) and AST (25.6 vs. 28.0; $p=0.06$) markers. Similar trend toward normalization was observed with bilirubin and alkaline phosphatase levels.

Discussion

According to AST or ALT profiles 18 out of 19 patients (94.7%) have responded to V-1 Immunitor therapy. This compares favorably with reported biochemical response of hepatitis C to interferon monotherapy (10-20%) or interferon and ribavirin combination (30-40%) (Bacosi et al. 2001). New hepatitis drugs like GlaxoSmithKline's Lamivudine and Gilead's Adefovir appear to have better response and fewer side-effects than interferon but they are still not ideal (Villamil, 2002). The virus generally returns once treatment is stopped and keeping a patient on a life-long treatment is not an option since drug resistance and toxicity are major concerns.

Unfortunately, since we did not anticipate such an outcome the diagnosis of hepatitis virus was not carried out in these patients and it is thus unclear whether V1 had a direct curative effect or brought down enzyme levels non-specifically. The later possibility, however, is not corroborated by lack of effect in normal patients. The normalization of two other markers of hepatitis, bilirubin and alkaline phosphatase also seem to lend support to the specificity of V1 activity. Finally, the identification of three separate patients with positive Hepatitis B antigen test at baseline who became negative after V1 therapy also suggests that V1 action may be specific.

Thus, it is possible that V-1 Immunitor might be useful for treating hepatitis, especially when one considers the fact that immunogenic constituents of our polyvalent vaccine are derived from HIV-infected donors at least half of whom are co-infected with hepatitis B and C viruses (Dodig and Tavill, 2001; Sud et al. 2001). In this aspect V1 is similar to the first generation of heat-inactivated prophylactic

hepatitis vaccine, which contained pooled viral antigens derived from the blood of hepatitis B carriers (Prince et al. 1984). This interesting observation needs to be further verified in controlled studies by recruiting patients with confirmed hepatitis diagnosis.

References

BACOSI, M.; PATSOURI, M.; MIGLIORESI, L.; PATRIZI, F.; RUSSO, F. and RICCI, G.L. Combined treatment of HCV infection: is there a need for meta-analysis? *Hepatology Research,* July 2001, vol. 20, no. 3, p. 359-371.

DODIG, M. and TAVILL, A.S. Hepatitis C and human immunodeficiency virus coinfections. *Journal of Clinical Gastroenterology*, November/December 2001, vol. 33, no. 5, p. 367-374.

JIRATHITIKAL, V.; SOOKSATHAN, P.; METADILOGKUL, O. and BOURINBAIAR, A.S. V-1 Immunitor: Oral AIDS vaccine with therapeutic and prophylactic potential. *Vaccine 2003*, vol. 21, no. 7-8, p. 624-628.

JIRATHITIKAL, V. and BOURINBAIAR, A.S. Safety and efficacy of an oral HIV vaccine (V-1 Immunitor) in AIDS patients at various stages of the disease. *HIV Clinical Trials*, January/February 2002, vol. 3, no. 1, p. 21-26.

JIRATHITIKAL, V.; BOURINBAIAR, A.S. and METADILOGKUL, O. Normalization of elevated liver enzymes due to V-1 Immunitor therapy. In: *Proceedings of the Symposium: Therapies for Viral Hepatitis.* (29th – 31st October, 2002, Boston, USA). Abstract Nº 2, 2002, Antiviral Therapy vol. 7, p. L115-116.

METADILOGKUL, O.; JIRATHITIKAL, V. and BOURINBAIAR, A.S. Survival of end-stage patients receiving V-1 Immunitor. *HIV Clinical Trials*, May/June 2002, vol. 3, no. 3, p. 258-259.

PRINCE, A.M. VNEK, J. and BROTMAN, B. An affordable multideterminant plasma-derived hepatitis B virus vaccine. *International Agency for Research on Cancer Scientific Publications 1984*, 63,Vol 6., p. 355-72.

SUD, A.; SINGH, J.; DHIMAN, R.K.; WANCHU, A., SINGH, S. and CHAWLA, Y. Hepatitis B virus co-infection in HIV infected patients. *Tropical Gastroenterology*, April/June 2001, vol. 22, no. 2, p. 90-92.

TANGKIJVANICH P, THEAMBOONLERS A, HIRSCH P, THONGNGAM D, KULLAVANIJAYA P, POOVORAWAN Y. Hepatitis viruses and chronic liver disease. *Southeast Asian Journal of Tropical and Medical Public Health September 1999*, vol. 30, no. 3. p. 489-495.

TIETZ, Norbert W. *Clinical Guide to Laboratory Tests*. 2nd ed. Philadelphia, PA., WB Saunders Company, 1995. 1096 p.

VILLAMIL, F.G. Hepatitis B: progress in the last 15 years. *Liver Transplantation*, October 2002, vol. 8, no. 10, p. 59-66.

APPENDIX

Table

Table 1. Safety evaluation of 61 patients including 19 patients with hepatitis.

Marker	Entry level	Month 2	P value (Kolmogorov-Smirnov test)	P value (Student t test)
BUN 13.	83	12.75	0.42	0.25
Creatinine 0.	98	0.97	0.32	0.75
Total protein	7.58	7.62	0.67	0.74
Albumin 4.	34	4.30	0.31	0.68
Globulin 3.	22	4.13	0.18	0.09
Hemoglobin 12.	77	12.07	0.72	0.15
Hematocrit 35.	90	36.83	0.82	0.46
Total bilirubin	0.89	0.65	5.24E-007	5.66E-006
High bilirubin	1.11	0.70	0.0005	0.0005
Normal bilirubin	0.79	0.62	8.15E- 005	0.0000 07
Direct bilirubin	0.18	0.09	2.37E-007	0.001
High AST	47.8	25.6	0.00018	6.44E-006
Normal AST	25.6	19.9	3.34E-006	0.06
High ALT	50.1	25.2	0.0002	4.9E-005
Normal ALT	26.8	25.3	0.34	0.34
Alkaline phosphatase	95.02	63.33	0.0001	0.0005
Platelets 217,	538.5	205,708.3	0.71	0.59
WBC 5,	592.5	5,127.5	0.45	0.23
Neutrophils (%)	51.86	52.33	0.97	0.86
Eosinophils (%)	5.71	6.08	0.63	0.88
Monocytes (%)	4.49	5.83	0.003	0.03
Basophiles (%)	1.43	0.92	0.85	0.05