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Treatment of Hepatitis C Virus Infection with Human Ezrin Peptide One (HEP1) in HIV Infected Patients

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Abstract

This report shows the therapeutic benefit of HEP1 (human ezrin peptide 324–337; TEKKRRETVEREKE) monotherapy of hepatitis C virus (HCV) infection in HIV infected patients in two clinical studies. In the Pilot Study I, 16 of 18 patients responded well to the treatment with significant reductions of HCV viral load and a normalization of serum liver enzymes. In 8 of 18 patients, HCV RNA became undetectable, and 3 of 8 interferon/ribavirin treatment failure patients showed undetectable HCV load following HEP1 treatment. In the second study, 8 of 10 patients responded well to the treatment with a pronounced reduction of the HCV viral load and a normalization of serum liver enzymes. Three of 15 patients (20 %) showed an undetectable viral load 30 days after the end of a 30-day course of HEP1 treatment. In both studies, all genotypes of HCV were sensitive to HEP1 treatment. Analysis of the combined data from both studies showed the overall efficacy of HEP1 therapy: in 37 HCV+HIV patients, HEP1 therapy gave the following results: 10 of 37 (27 %) HCV+HIV patients showed a reduction of viral load between –7 log (–10,000,000x) and –3 log (–1,000x); 4 of 37 (11 %) a reduction of –3 log (–1,000x); 6 of 37 (16 %) a reduction of –2 log (–100x); 11 of 37 (30 %) a reduction of –1 log (–10x); 6 of 37 (16 %) a reduction of less than –1 log (–10x); 0 of 37 (0 %) had an increase in viral load, and the average reduction in viral load for all 37 patients was –2 log (–100x). No adverse reactions or side effects were detected and the improving CD4/CD8 ratio showed that the therapy had no negative impact on the immunological status. Thus, oral HEP1 therapy matches the efficacy results for injectable peginterferon/oral ribavirin therapy with the advantages of more rapid action and less side effects. HEP1 therapy should be used in patients where either peginterferon/ribavirin therapy fails or is contraindicated.

Key words

- **Ezrin**
- **Hepatitis C therapy**
- **Hepatitis C virus**
- **Human ezrin peptide one**
- **Human immunodeficiency virus**
- **Gepon®**

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1. Introduction

Ezrin is a member of the ERM (ezrin-radixin-moesin) family of closely related 80 kDa proteins constituting multifunctional structural and regulatory proteins located on the interior surface of cell membranes. Ezrin plays a central role in cell membrane movement and fusion, and interacts with cell surface adhesion molecules, cytoskeletal components and various kinases involved in cell activation cascades. Ezrin connects receptors on the cell surface to kinases such as PI-3-kinase, protein kinase C and cAMP activated protein kinase A. Ezrin is also a tyrosine kinase substrate and becomes tyrosine phosphorylated during cell-signalling processes [1]. Human ezrin peptide 324–337 (HEP1, TEKKRRETVEREKE, Gepon®[1)]) is a synthetic 14 amino acid peptide [2]. In vitro, HEP1 is relatively inactive in the absence of other immunological signals, but in the presence of antigens, mitogens and cytokines such as IL-2, it amplifies immune activation particularly in macrophages, monocytes, and granulocytes. *In vivo,* HEP1 enhances antibody formation and amplifies cellular immune responses to a wide range of infectious agents. Clinically, HEP1 is used as an immune amplifier for the treatment and prophylaxis of opportunistic infections in HIV patients. HEP1 is also used for the treatment of skin/mucus infections caused by Candida, and has been shown to have a broad spectrum of activity against different viral, bacterial and fungal infections. The therapeutic benefit of combining oral HEP1 therapy with standard alpha interferon injection therapy has been clinically investigated [3]. HEP1 has also been used as an oral monotherapy to treat acute HCV hepatitis in children [4, 5]. No toxicity or adverse reactions have been detected with HEP1 treatment. Here we demonstrate the therapeutic benefit of HEP1 monotherapy of HCV infection in HIV infected patients.

[1)] Manufacturer: Immapharma Ltd, Moscow (Russian Federation).

Abbreviations

ALT	alanine amino transferase
APRICOT	*A*IDS *P*egasys *R*ibavirin *I*nternational *CO*-infection *T*rial
AST	asparagine amino transferase
CMV	cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
EDTA	ethylene diamine tetraacetate
ELISA	enzyme linked immunosorbent assay
ERM	ezrin-radixin-moesin
EVR	early virological response
HBsAg	hepatitis B surface antigen
HEP1	human ezrin peptide 1
PCR	polymerase chain reaction
SVR	sustained virological response
TEKKRRETVEREKE	amino acid sequence Thr-Glu-Lys-Lys-Arg-Arg-Glu-Thr-Val-Glu-Arg-Glu-Lys-Glu

2. Patients and methods

2.1. Clinical pilot study I: treatment of HCV infection with HEP1 in 19 HIV infected patients

2.1.1. Patients

Treatment was performed in the Satellite HIV Clinic, Russian Medical Academy of Sciences, Moscow, between July and August 2004 with a follow-up until December 2004. No formal permission by the Ministry of Health was required since HEP1 (Gepon) is registered in Russia for the treatment of HIV infected patients (registration number 000015/04-2001, dated 08/09/2001; instruction number 6, dated 02/12/2001). Patients were recruited for the study in accordance with ethical standards and statutory regulations of the Russian Federation. Twenty HIV infected patients with symptoms of hepatitis volunteered to participate. Their sera were tested by ELISA for anti-HCV antibodies, and 19 of 20 tests were positive. One of the 20 HIV infected volunteers was identified as being co-infected with hepatitis B virus, and although he benefited from HEP1 treatment, he was not considered part of the HCV+HIV pilot study. Of the 19 patients who were infected with HCV+HIV, active HCV infection was confirmed in 18 patients by PCR. All patients were Russian residents: age: 23–29 years (m = 26.5); sex: 15 men and 3 women; average duration of hepatitis: 4.5 ± 1 years. Patients entering the trial had different treatment histories: 11 had received no therapy, and 8 had been on interferon (CAS 36791-04-5)/ribavirin (CAS 98530-12-2) therapy for 3 to 14 months but were considered to be 'treatment failures'. All patients in the pilot study received no other therapy for either HCV or HIV for three months prior to the start of treatment.

2.1.2. Laboratory assays

HCV RNA PCR, AmpliSens HCV-240/VCO-440 and genotyping of HCV, AmpliSens-HCV-genotype: Central Scientific Research Institute of Epidemiology, Ministry of Health, Moscow, Russia. HCV ELISA, Recombibest Anti-HCV: Vector-Best, Novosibirsk, Russia; Serum ALT, AST transaminases, Alsyon 300: Abbott Laboratories, Abbot Park, Illinois, USA; HIV-1 ELISA: Organon-Technica, Turnhout, Belgium; HIV-1 RNA PCR, Amplicor HIV-1 Monitor: Roche Diagnostic Systems, Hoffmann-la Roche, Nutley, NJ, USA; Immunology (CD4,CD8), Epics XL: Beckman Coulter, Fullerton, CA, USA.

2.1.3. Treatment protocol

Patients were treated with a 30-day course of an aqueous solution of 2 mg HEP1 in 2 ml water given orally twice a day for the first 10 days, and once a day for the following 20 days. The patients received no other drug therapy for either their HCV or HIV infection during the 30-day treatment period or during the subsequent 30-day follow-up period.

2.2. Clinical study II: Phase II, randomized, placebo controlled blind study (2005–2006) – treatment of HCV infection in 20 HIV infected patients with HEP1

2.2.1. Patients

Treatment was performed in the Satellite HIV Clinic, Russian Medical Academy of Sciences, Moscow. No formal permission by the ministry of health was required since HEP1 (Gepon) is registered in Russia for the treatment of HIV infected patients (registration number 000015/04-2001, dated 08/09/2001; instruction number 6, dated 02/12/2001). Patients as characterized in Table 1 were selected from a cohort of HIV infected patients suffering from HCV infection who volunteered for the

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Table 1: Results of Pilot Study I.

Patient code	Treatment history with Inf+ Ribavirin	HCV genotype	HCV RNA before	HCV RNA after	Log drop	ALT before	ALT change	AST before	AST change	CD4 cells/µl before	CD4 cells/µl change	CD4/CD8 index before	CD4/CD8 index change
03GIN	no	?	UD	UD	0.0	15	–3	20	–6	191	28	0.51	0.11
01ANV	no	1a	1100	UD	0.0	29	5	22	6	476	44	0.51	0.19
06ZNG	no	1a	3200	UD	–0.5	92	–67	43	–11	535	325	0.49	–0.04
14PAG	no	1a	17000	1300	–1.1	103	–32	84	–32	471	52	0.39	0.13
13NDS	3m in 2003	3a	78000	5200	–1.2	62	–19	32	0	1071	104	1.96	0.04
07KKA	no	3a	130000	UD	–2.1	64	–14	39	–6	545	–141	0.40	–0.03
10LSL	6m in 2001	1b	150000	130000	–0.1	213	–45	111	–41	505	26	0.54	0.02
09KOG	no	1a	270000	2100	–2.1	167	–83	78	–39	375	60	0.43	0.17
05GMA	12m in 2002	3a	1400000	2500	–2.7	98	0	56	0	412	75	0.70	0.23
15PAU	no	1b	2700000	1700	–3.2	154	–68	78	–31	480	219	0.33	0.3
08KVV	no	1a	3400000	UD	–3.5	47	–12	40	–12	830	80	0.93	0.33
17FDV	9m in 2003	1a	3600000	UD	–3.6	56	–37	52	–25	596	96	0.38	–0.01
02BVV	no	2a	5900000	34000	–2.2	84	–67	43	–19	496	42	0.73	0.11
11MOA	12m in 2001	2a	6000000	42000	–2.2	18	–1	22	0	403	13	0.49	0.1
19CAA	no	1b	6400000	230000	–1.4	60	–20	40	–9	228	106	0.37	0.18
04GLG	12m in 2003	3a	6800000	UD	–3.8	52	4	44	0	305	9	0.34	0.13
16RAA	3m in 2001	1b	8000000	3000000	–0.4	79	9	68	–13	495	137	0.83	0.04
18HSH	6m in 2002	1b 3a	35000000	16000	–3.3	197	–53	141	–48	258	44	0.66	–0.03
12MDA	no	1b 3a	68000000	480000	–2.2	44	20	29	17	247	17	0.51	–0.12

HCV viral load PCR assay
UD: HCV RNA Not Detected, assay baseline <1000 copies per ml
After: 30 days after the end of treatment

study. Patients were recruited in accordance to ethical standards and statutory regulations of the Russian Federation. Twenty-seven HIV infected patients with symptoms of hepatitis volunteered to participate. Their sera were tested for anti-HCV antibodies by ELISA and for HCV viral load by polymerase chain reaction (PCR), and 20 patients were selected. The study group of clinically stable patients consisted of 19 men and one woman (average age 27.5 years). From the time of selection to the end of the study period, none of the patients received HAART (highly active antiretroviral therapy) anti-HIV therapy or any other therapy for HCV. For each patient entering the study, the following information was recorded: age of the patient, documentation of HIV-1 infection by ELISA confirmed by Western Blot, documentation of hepatitis C infection by detection of hepatitis C antibody and a HCV RNA level of 1000 U/ml or above. Patients agreed to participate in the trial after a verbal explanation of the trial and after having read the package insert for HEP1.

2.2.2. Patient eligibility

HIV entry criteria: HIV positive by ELISA; CD4 greater than 100/µL.

HCV entry criteria: HCV positive by ELISA; HCV positive by PCR (greater than 1000 copies per ml); HCV genotype identified; records of treatment history for HCV available.

2.2.3. Exclusion criteria

Preexisting pancreatitis; use of long-term systemic corticosteroids, immunosuppressives or cytotoxic agents within 60 days of enrolment into the trial; chronic viral hepatitis of etiology other than hepatitis C; alcohol or substance abuse that potentially could interfere with patient compliance; pregnant women; breastfeeding women; coexisting neoplastic disease except for Kaposi's Sarcoma; any nonmetastatic skin cancer that has been resected; nonmetastatic cervical or anal cancer that has been resected; severe psychiatric disorder that would interfere with the adherence to protocol requirements; preexisting autoimmune disorders including inflammatory bowel diseases, psoriasis, and optic neuritis; preexisting uncontrolled seizure disorder; severe retinopathy; active systemic infections other than hepatitis C and HIV.

All tests were performed in accordance with the clinic's routine medical screening practice: Quantitative HCV RNA measurement by PCR, HCV genotype, HIV determination by anti-HIV abs and HIV RNA measurement, total WBC, leucocytes, lymphocytes and platelet count, CD4+ and CD8+ lymphocytes, CD4/CD8 ratio, hemoglobin, hematocrit, serum glucose, creatinine, biochemical markers of liver injury and assessment of hepatic function: serum AST and ALT; serum albumin, serum total bilirubin, alkaline phosphatase, lactate dehydrogenase, alpha-amylase, HBsAg, toxoplasmosis, syphilis, and CMV.

2.2.3. Randomization of patients

Patients were assigned a three-letter initial code based on their full names, which was then converted to a number from 1 to 20. Patient medical records consisted of a blinded notebook and blinded laboratory results tables. The patients were then screened for HCV genotype, and split into two groups: genotype 1 and non-genotype 1. A computerized randomization process was then performed: patients were randomly selected from two genotype groups (first HCV genotype 1, then the re-

mainder) and allocated to each treatment group on the basis of their code number. If possible an equal proportion of the difficult to treat HCV genotype 1 was allocated to each treatment group: 10 patients were allocated to the normal HEP1 treatment group: "Treatment"; 5 patients were allocated to the 10% dose HEP1 treatment group: "10% Dose"; 5 patients were allocated to the placebo treatment group: "Placebo".

2.2.4. Blinding of treatment

All treatments were presented to patients as masked and coded vials containing either 2 mg or 0.2 mg lyophilized peptide or nothing. The investigator gave detailed verbal instructions for the preparation of the aqueous solution of HEP1 at their first visit. Patients were asked to dispense 2 ml of water into the vials for oral administration.

2.2.5. Treatment day 1 to day 30

Treatment group: 2 mg/2 ml aqueous solution of HEP1 orally twice a day (morning and evening) for ten days, then once a day for 20 days.

Treatment 10% dose group: 0.2 mg/2 ml aqueous solution of HEP1 orally twice a day (morning and evening) for ten days, then once a day for 20 days.

Placebo group: 2 ml water orally twice a day (morning and evening) for ten days, then once a day for 20 days. No other drug therapy for HCV was given for 30 days prior to HEP1 treatment, or during the 30 day treatment period, or 90 days after the end of the HEP1 treatment period.

2.2.6. Treatment day 60 to day 90

On day 60 all patients were decoded, and after they had provided their day 60 blood sample, the "Placebo" and "10% Dose" groups of patients were started on a 30-day course of normal HEP1 treatment.

2.2.7. Diagnostic procedures

The trial protocol required blood analysis of all patients on days 0, 60, and 120 of the trial; one clot and 4 EDTA tubes were collected for each patient. All blood samples were sent to the laboratories on the same day. The clot tube was sent to The Epidemiology Research Institute Laboratory, Moscow, Russia, for analysis of AST and ALT transaminase enzymes in blood and all other biochemistry evaluations. The EDTA tubes were spun down at 2750 rpm/min for 5 min, and the plasma aliquoted to 4 Eppendorf tubes. One sample was sent to The Russian Federal Research AIDS Centre (AIDS Centre) Immunology Laboratory, Moscow, for analysis of CD4+ and CD8+ lymphocytes, leucocytes and other immunology tests. This laboratory is also the AIDS Reference Laboratory of the Russian Federation and was the location for the storage of the study back-up plasma samples. Another plasma sample was sent to The Institute of Virology, Reference Laboratory for the determination of HCV and HIV viral loads using Roche PCR assays (Hoffmann-la Roche, Nutley, NJ, USA). Laboratory Assays were performed as indicated for study I.

3. Results

3.1. Clinical pilot study I

3.1.1. Reduction in HCV viral load

Eighteen out of 19 patients had detectable HCV RNA at the start of treatment and all of these 18 patients showed a decrease in HCV viral load, with a log reduction of HCV RNA down to – log 7 (–10,000,000x). The average drop in HCV viral load for the group was –3 log (–1,000x). All genotypes of HCV responded to treatment with an average reduction of: -l000x for HCV-1a (6 patients), –10x for HCV-1b (4 patients), –100x for HCV-2a (2 patients), –1000x for HCV-3a and –100x for a mixed infection of HCV-1b and HCV-3a. In 8 out of 18 patients (44%) HCV RNA became undetectable thirty days after the end of the 30 day treatment period.

3.1.2. Normalization of liver enzymes

There was a –25% average decrease in the pathologically elevated levels of ALT in serum ($p < 0.01$) with a > –10% fall in 13 of 19 patients (68%). There was also a –14% average decrease in AST in serum ($p < 0.01$) with a > –10% fall in13 of 19 patients (68%). In 5 of the 8 patients in which HCV became undetectable, the ALT levels normalized.

3.1.3. Effect on CD4, CD8 and HIV RNA

There was an average increase of 70 cells/μL in the pathologically decreased CD4 levels ($p < 0.01$) with an increase in 18 of 19 patients (95%), and an average increase of 0.1 in the CD4/CD8 immunoregulatory index ($p < 0.01$) with an increase in 14 of 19 (74%) patients. HIV RNA was measured in 4 patients and an average ten fold decrease in HIV viral load was detected with a > –1 log fall in 3 of 4 patients.

3.1.4. Conclusion

The pilot clinical study involved treatment of HCV disease in 19 HIV infected patients. 18 of 19 patients had detectable HCV RNA by PCR and all 18 patients (100%) responded to treatment with a reduction of viral load. The average viral load reduction in the group was –3 logs (–1000x) and in 8 of 18 patients the HCV RNA became undetectable at the end of the 30-day treatment period. The 8 out of 18 patients who had failed to respond to earlier interferon/ribavirin treatment all responded with a drop in HCV viral load. There was a reduction of ALT liver transaminase in the serum of 13 out of 19 patients, and a normalization of enzyme levels was achieved in 5 of the 8 patients in which HCV RNA became undetectable. All HCV genotypes characterized in the study responded to treatment including HCV-1a and HCV-1b. No side effects or adverse events were observed.

3.2. Clinical study II

3.2.1. Objective of the study

This Phase II, randomized, placebo controlled, blind study was performed to investigate the safety and effi-

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Table 2: Log decrease in HCV viral load in HCV-HIV patients due to HEP1 treat ment or 10 % dose or placebo.

Phase II trial groups results					Data sorted by HCV genotype		
Patient	Genotype	Day 0–Day 60 log drop	Day 60–Day 120 log drop		Genotype 1b		Day 0-Day 60 log drop
Treatment		Treatment	None				Treatment or 10 % dose
SAN	3a	–1	0		SLY	1b	–1
AACh	3a	–1	0		AAI	1b	0
SLY	1b	–1	0		IMR	1b	–5
AAI	1b	0	–1	relapse	AIL	1b	–1
IMR	1b	–5		6	SGT	1b	0
AIL	1b	–1	0		LGK	1b	–1
LGN	3a	–6	0		AVB	1b	0
SGT	1b	0	–1		AIM	1b	–1
MNT	3a	–1	0		ShAH	1b + 3a	–1
LGK	1b	–1	0				
	Average	**–2**	**0**			**Average**	**–1**
10 % dose		10 % dose	Treatment		Genotype 3a		Treatment or 10 % dose
GTS	3a	–4	0		SAN	3a	–1
ShAH	1b + 3a	–1	–1		AACh	3a	–1
AVB	1b	0	0		LGN	3a	–6
SAP	3a	–3	1		MNT	3a	–1
AIM	1b	–1	0		GTS	3a	–4
					SAP	3a	–3
	Average	**–2**	**0**			**Average**	**–3**
Placebo		Placebo Treatment			Placebo		Placebo
YAV	3a	0	–2		YAV	3a	0
KSB	1b	0	–7		KSB	1b	0
LSL	1b	0	–1		LSL	1b	0
ANM	3a	0	–1		ANM	3a	0
AYP	3a	n.a.	n.a.		AYP	3a	n.a.
	Average	**0**	**–3**			**Average**	**0**

n.a.: not available.

cacy of HEP1 treatment of HCV in HIV infected patients, and to confirm the therapeutic benefit observed in the pilot clinical study I. A low dose (10 %) treatment group was included to determine if there was a dose dependency of the therapeutic benefit of HEP1.

3.2.2. Reduction in HCV viral load

The reduction in HCV viral load in all patients is shown in Table 2: all 10 patients in the Treatment Group and all 5 patients in the 10 % Dose Group responded with a decrease in HCV viral load, with a log reduction of HCV RNA down to –6 log (–1,000,000x). The efficacy of treatment was similar for the Treatment Group and for the 10 % Dose Group with an average –2 log (–100x) fall in HCV viral load. The treatment group consisted of six HCV-1b infected patients and four HCV-3a infected patients, and the ten percent dose group consisted of two HCV-1b infected patients, one HCV-1b+HCV-3a and two HCV-3a infected patients. Both genotypes responded to treatment with an average reduction of –10x for HCV-1b (9 patients) and an average reduction of –1000x for HCV-3a (6 patients). In 2 out of 10 patients in the Treatment Group (20 %) and in one out of 5 in the 10 % Dose Group (20 %), HCV RNA became undetectable 30 days after the end of the 30-day treatment period. In contrast, there was no significant drop in viral load in the placebo group by day 60. On day 60, the blinding of the patient groups was decoded, and the 10 % Dose Group and the Placebo Group were started on a 30 day course of normal HEP1 treatment (placebo patient AYP left the study). The Treatment Group received no further treatment. On day 120, 30 days after the end of the 30-day course of normal HEP1 treatment, the HCV viral loads were assessed again. There was no significant change in viral load in the Treatment Group apart from one patient who relapsed. There was also no significant change in the 10 % Dose Group, suggesting the lower dose of HEP1 had been sufficient to induce the anti-HCV effect. In contrast, the response of the previous Placebo group to normal HEP1 treatment was an average –3 log (–1000x) drop in viral load (Fig. 1).

3.2.3. Normalization of liver enzymes

In the Treatment Group, there was a –49 % average decrease in the pathologically elevated levels of AST in serum ($p < 0.01$) with a decrease in 9 of 10 patients (90 %) (Fig. 2). There was also a 60 % average decrease in the pathologically elevated levels of ALT in serum ($p < 0.01$) with a decrease in 9 of 10 patients (90 %). In the 2 of 10 patients (20 %) in which HCV became undetectable, the ALT and AST levels normalized. In the 10 % Dose Group,

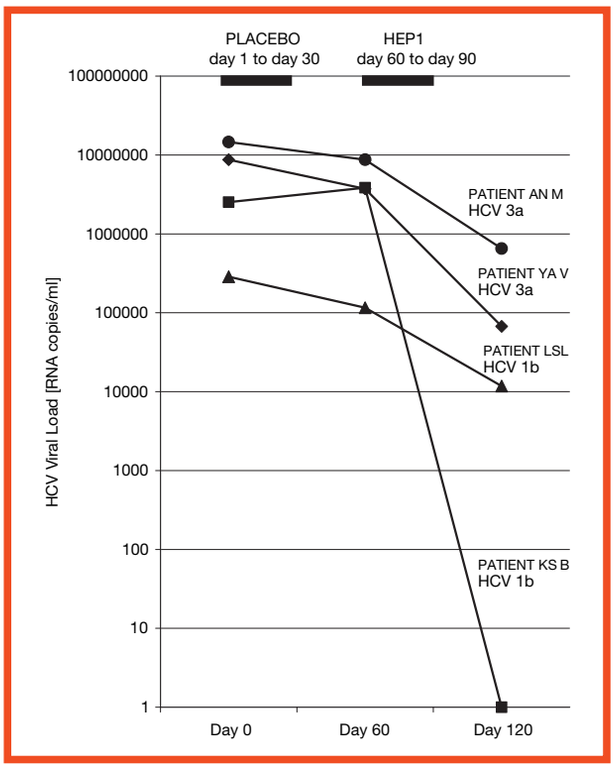

Fig 1: Effect of placebo followed by HEP1 treatment on hepatitis C virus (HCV) viral load. Viral load was measured as copy number per ml using an HCV RNA PCR assay and expressed as HCV viral load. Four randomly selected HCV+HIV patients were assessed for viral load (trial day 0), then treated with placebo (water) for 30 days. The viral load was assessed again 30 days after the end of placebo treatment (trial day 60). Subsequently, patients were treated with HEP1 for 30 days, and viral load was assessed again 30 days after the end of treatment (trial day 120).

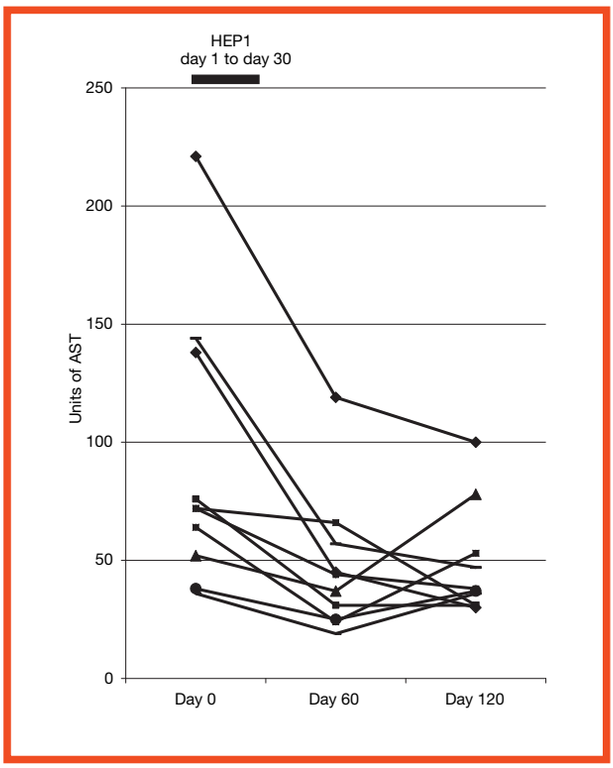

Fig 2: Normalization of asparagin amino transferase (AST) levels in the Treatment Group. AST was assessed by an AST transaminase assay and expressed as international units of AST in ten randomly selected HCV+HIV patients prior to treatment (trial day 0). Patients were treated with HEP1 for 30 days, and AST levels were assessed again 30 days (trial day 60) and 90 days (trial day 120) after the end of HEP1 treatment.

there was a –68 % average decrease in the pathologically elevated levels of AST in serum with a decrease in 4 of 5 patients (80 %). There was also a –72 % average decrease in the pathologically elevated levels of ALT in serum with a decrease in 4 of 5 patients (80 %). In the 1 of 5 patients (20 %) in which HCV became undetectable, the ALT and AST levels normalized. In the Placebo Group, there was an insignificant –21 % average decrease in the pathologically elevated levels of AST in serum. There was also an insignificant –24 % average decrease in the pathologically elevated levels of ALT in serum.

3.2.4. Effect on CD4, CD8 and HIV RNA

In the Treatment Group, there was a +100 cells/μL average increase in the pathologically decreased CD4 levels ($p < 0.01$) with an increase in 9 of 10 patients (90 %) and a +0.25 average increase in CD4/CD8 immunoregulatory index ($p < 0.01$) with an increase in 8 of 10 (80 %) patients. An average 10 % decrease in HIV viral load was detected with a decrease in 9 of 10 patients. In the Ten Percent Dose Group, we observed a +71 cells/μL average increase in the pathologically decreased CD4 levels with an increase in 3 of 5 patients (60 %) and an non-significant +0.04 average increase in the CD4/CD8 immunoregulatory index with an increase in 4 of 5 (80 %) patients. An average 33 % decrease in HIV viral load was detected with a decrease in 5 of 5 patients. In the Placebo Group, there was a +106 cells/μL average increase in the decreased CD4 levels with an increase in 3 of 4 patients and an insignificant +0.09 average increase in CD4/CD8 immunoregulatory index with an increase in 3 of 4 patients. An average 26 % decrease in HIV viral load was detected with a decrease in 3 of 4 patients.

3.2.5. Effect on opportunistic infections

HEP1 therapy reduced the incidence of opportunistic infections. Between day 0 and day 120, after all the patients in the study had received normal HEP1 therapy, the following differences were observed: the incidence of Herpes Zoster (HZV) dropped from 4 of 20 to 1 of 20, the incidence of vaginal candidasis dropped from 1 of 20 to 0 of 20, the incidence of oral candidasis dropped from 2 of 20 to 1 of 20, and the incidence of acne vulgaris dropped from 7 of 20 to 3 of 20.

3.3. Results of both clinical studies I and II

3.3.1. Safety

HEP1 was safe in HCV+HIV double infected patients. No adverse reactions or side effects were detected (by blood,

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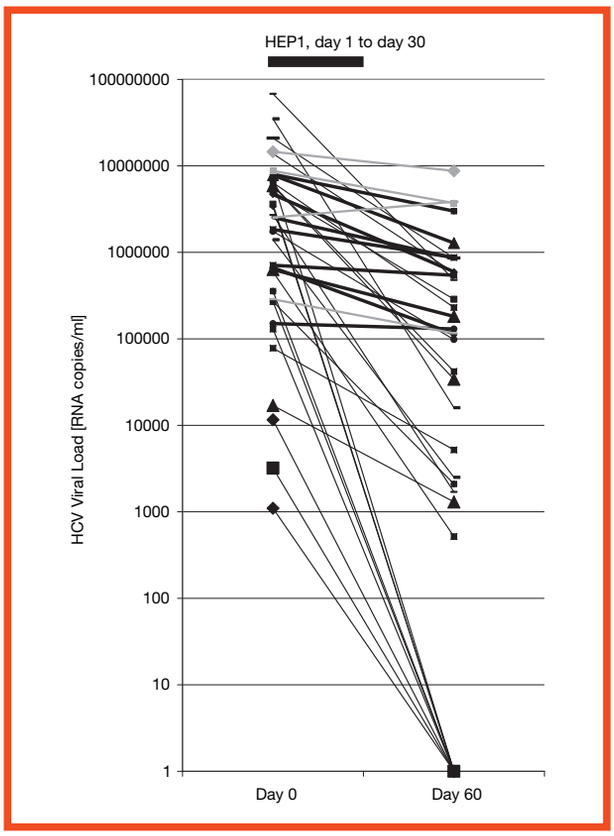

Fig 3: Combined hepatitis C virus (HCV) PCR data from Pilot and Phase II studies. The figure shows data of 37 HCV+HIV patients. HCV viral load was measured as copy number per ml using an HCV RNA PCR and expressed as HCV viral load. Treatment: 30 days placebo (4/37; grey, bold); 30 days HEP1, combined data of patients treated with the normal or the 10 % dose (weak responders 9/37, black, bold; strong responders 24/37, black).

urine and liver enzyme analyses, pancreatic tests, immunology and allergy tests) and the improving CD4/CD8 ratio showed that the therapy had no negative impact on the immunological status.

3.3.2. Efficacy

The analysis of the combined data from the Pilot and Phase II study is demonstrated in Fig. 3. In the 37 patients, HEP1 therapy followed by a 30-day treatment-free period showed the following results: 10 of 37 (27 %) HCV+HIV patients a reduction of viral load between –7 log (–10,000,000x) and –3 log (–1,000x), 4 of 37 (11 %) had a reduction of the viral load of –3 log (–1,000x), 6 of 37 (16 %) had a reduction of the viral load of -2 log (–100x), 11 of 37 (30 %) had a reduction of the viral load of -1 log (–10x), 6 of 37 (16 %) had a reduction of the viral load of less than –1 log (–10x), 0 of 37 (0 %) had an increase in viral load, and the average reduction in viral load for all 37 patients was –2 log (–100x).

4. Discussion

HCV infection of HIV patients is very common due the similar routes of transmission of HCV and HIV. About 25–30 % of all HIV patients in the USA and Europe are co-infected with HCV. About 50–90 % of HIV infected i.v. drug users are HCV infected and so are almost 100 % of HIV-infected hemophiliacs. There are approximately one million HCV+HIV patients in Europe and USA. HCV infection in HIV patients is a severe type of hepatitis that is difficult to treat with the existing therapy and may develop rapidly to liver cirrhosis, cancer and death. HIV seroconversion in HCV infected patients can lead to a ten fold increase in HCV viral load. In HCV+HIV patients, the frequency of liver cirrhosis ten years after HCV infection is 15 %, five times the frequency of liver cirrhosis in HCV mono-infected patients. HCV disease in HIV infected patients leads to a rapid development of hepatocellular carcinoma in less than 10 years after HCV infection, and HIV+HCV patients are six times more likely to die from liver disease than monoinfected HCV patients [6, 7]. Current therapy involves weekly peginterferon injections and oral ribavirin in combination for 48 weeks. A HCV+HIV patient is considered to be responding to this therapy if an early virological response (EVR) is achieved, defined as a 2 log reduction in HCV RNA by PCR assays after a 12-week course of treatment. The final objective of therapy for HCV infection is a sustained virological response (SVR) defined as undetectable HCV RNA six months after the discontinuation of a 24–48 week course of therapy. Patients who fail to achieve EVR almost always fail to achieve SVR and are defined as treatment failures. There is currently no alternative treatment for these patients. Sustained anti-HCV responses or SVR are achieved in no more than 40 % of HCV+HIV patients. In the AIDS Clinical Trials Group (ACTG) 5071 Study [8], of 66 patients who received peginterferon alfa-2 plus ribavirin, only 41 % achieved ETR (only 29 % of genotype 1) and only 27 % achieved SVR (only 14 % of genotype 1). In the 100 fold higher dose peginterferon study, the AIDS PEGASYS Ribavirin International Co-infection Trial (APRICOT) [9] of 289 patients who received peginterferon alfa-2 plus ribavirin, only 49 % achieved ETR (only 38 % of genotype 1) and only 40 % achieved SVR (only 14 % of genotype 1).

Serious side effects of treatment with interferons or peginterferons in combination with ribavirin are common and are more severe in HCV+HIV double infected patients compared to monoinfected HCV patients. Peginterferon alfa-2 plus ribavirin treatment is associated with serious injection site reactions, flu-like symptoms, birth defects, serious headaches, breathlessness, bone marrow suppression, anemia, drop of CD4 and CD8 levels, aggravation of immune disorders, retinopathy, interstitial pulmonary fibrosis, neuropsychiatric symptoms, serious depression and suicide attempts, seizures, acute cardiac and renal failure, and death. Up to 90 % of HCV+HIV patients never receive current therapy because they are excluded due to the risk of adverse reactions to peginterferon alpha-2 plus ribavirin combinations. In HCV+HIV patients infected with HCV genotype

1, some data suggest that more seriously ill patients with higher viral loads (more than 800,000 IU/ml) respond very poorly to treatment. There is a very high risk of retinal damage and other adverse events with peginterferon/ribavirin therapy for HIV patients with CD4 levels lower than 100 cells/µl. Current therapy is generally not recommended for patients with CD4 levels below 200 cells/µl. There are also high rates of withdrawal of HCV+HIV patients from existing therapy due to adverse events (drop-out rates due to adverse events can be as high as 29%). In the APRICOT study of 868 HCV+HIV patients, 273 patients suffered adverse events, of which 67 were very serious. There were 12 deaths, and two of the deaths were linked to adverse reactions to peginterferon-ribavirin treatment.

The therapeutic benefit of combining oral HEP1 therapy with standard alpha interferon injection therapy was clinically investigated in a previous study in 21 adult patients chronically infected with HCV (12 women and 9 men) [3]. All of the patients who participated in the study received recombinant alpha interferon as an injection of 3 million units 3 times per week. Eleven patients received additional oral HEP1 therapy (2 mg/5 ml aqueous solution of HEP1) once a day, and ten control patients received interferon therapy only. Patients were observed over a three month period. All the patients at the start of the study reported symptoms of liver disease such as pain in the right subcostal area of the liver and bitterness in the mouth. In the control group receiving interferon only, there was no change in the symptoms. In contrast, in the patients who received HEP1 in combination with interferon, there was a significant decrease in symptoms of HCV disease. The percentage of patients reporting liver pain decreased from 80% to 18%, and the percentage of patients reporting bitter taste decreased from 64% to 9%. On average in the control group treated with interferon only, the HCV viral load was reduced 20 fold. In the treatment group who received HEP1 plus interferon, on average the HCV viral load dropped 160 fold. In the HEP1-interferon group, HCV RNA could no longer be detected in 4 out of 11 patients (compared to 2 out of 10 in the control group). HEP1 in combination with interferon also improved the biochemical indices. In active HCV hepatitis, ALT and AST in the blood indicates the destructive inflammation of liver hepatocytes. Although interferon therapy improved ALT and AST levels, there was a more pronounced normalization in the group receiving the combination of HEP1 plus interferon. HEP1 also prevented the majority of adverse reactions and side effects normally induced by interferon therapy. The main conclusion of the trial was the clear therapeutic benefit of HEP1 given in combination with interferon therapy, the reduction of interferon related side effects, the reduction in HCV viral load, the increased proportion of patients responding to therapy, and the general improvement of HCV disease in the patients.

In a separate previous study, HEP1 was used as an oral monotherapy to treat acute HCV hepatitis in recently infected children. Seven children were given an oral dose of 1 mg twice a day for 28 days and compared to nine children who were untreated for HCV infection for the one month period of the study. HEP1 therapy led to a reduction of the pathologically elevated levels of ALT and AST whereas the patients in the control group showed increases in ALT and AST levels. In the same study, the disbacteriosis of the bowel was analyzed and HEP1 was shown to correct the microfloral homeostasis. The concentration of HCV virus in the treatment group dropped at least 10 fold using a local HCV RNA PCR titration assay. No side effects or adverse reactions were found [3, 4].

In summary, this report demonstrates the high therapeutic benefit of HEP1 therapy of HCV in HIV infected patients. In the pilot study I, 16 of 18 patients responded well to treatment with significant reductions of HCV viral load and a normalization of serum liver enzymes. In the second study, 8 of 10 patients responded to the treatment with a reduction of the HCV viral load and a normalization of serum liver enzymes; all genotypes of HCV were sensitive to treatment. No adverse reactions or side effects were detected. One patient relapsed to a high HCV viral load 90 days after the end of treatment suggesting that HEP1 therapy should be continued for a longer period. In summary, oral HEP1 therapy matches the efficacy of the peginterferon/ribavirin therapy with the advantage of more rapid action and easier use. HEP1 therapy could be used in patients where either peginterfron/ribavirin treatment fails or is contraindicated.

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