HCV COMBINATION THERAPY

Field of the Invention

This invention relates to a HCV combination therapy.

Background to the Invention

Hepatitis C Virus (HCV) infection is a major global healthcare problem. The World Health Organisation has estimated that 170m people have been infected although few of them show symptoms yet because the disease has a long incubation period. Ten to twenty percent of these people are likely to suffer serious liver disease, such as cirrhosis or cancer. According to the US National Institutes of Health, HCV kills 10,000 Americans every year and is responsible for 17,000 patients waiting for liver transplants every year. There are still three to four million new HCV infections every year. Approximately 70% of those infected will develop liver disease and 1-5% cancer. There is no vaccine for HCV

Existing therapy for HCV is based on alpha2 interferon or alpha2 interferon coupled to polyethylene glycol which is known as pegylated interferon or peginterferon. Serious side-effects and poor therapeutic benefit are associated with current methods of treatment. The usual course of treatment includes injections of 3 million units of recombinant alpha-interferon, 3 times per week over 12 months. In some studies, less than half of patients on interferon treatment gained any therapeutic benefit.

The antiviral drug Ribavirin is used in combination with interferon therapy to improve efficacy. Combination therapy improves the proportion of patients benefiting from treatment, if they are infected with HCV genotype-2 or genotype-3. However, only 40% of patients infected with HCV-1B, the most common genotype in the USA, respond to combination therapy.

It is well known that existing interferon treatment causes severe influenza-like symptoms, and side-effects with interferon plus Ribavirin are generally worse that the side-effects of interferon alone. Prolonged treatment with alpha-interferon is accompanied by the development of additional side-effects which get worse over the course of treatment. The majority of patients present complaints of weakness, irritability, insomnia, head and muscular pains, and arthralgia (joint pain) in the course of treatment. Frequent complications of interferon therapy include anemia, neutropenia, thrombocytopenia and alopecia. More rarely, patients develop one or more of hypo-thyroidism, pathologic damage of the capillaries, lupus, sarcoidosis

and bullous injuries of the skin, depression and psychoses and also the serious polyorgan toxic effects.

US5773573A describes HEP1 (Gepon). GB2354241A describes regulatory/unfolding peptides of Ezrin. These peptides are immune amplifiers and can be used to treat viral disease.

Summary of the Invention

The present invention is based on the finding that either HEP1 (Gepon) or regulatory/unfolding peptides of Ezrin, when used in combination with interferons, for example alpha-interferon or peginterferon, will reduce the side-effects of interferon therapy and enhance interferon efficacy. An example is provided of the successful combination therapy of recombinant alpha-interferon with the immunomodulator HEP1 (Gepon) that reduced the side effects of interferon, and increased the effectiveness of the antiviral treatment.

According to one aspect of the invention, a product comprising a peptide and an interferon, wherein the peptide comprises a sequence having at least 80% identity to a fragment of Domain A or B of the Hepreceptor of ezrin, and wherein the peptide binds to the Hepreceptor with at least as great affinity as HEP1, is a combined preparation for simultaneous, separate or sequential use in the treatment of viral hepatitis.

According to another aspect of the invention, the peptide is used for the manufacture of a medicament for use in the treatment of a patient who has viral hepatitis and is undergoing treatment with interferon.

Description of the Invention

The peptide that is used in the invention preferably has 5 to 50 amino acids. It is or comprises a fragment of ezrin or closely related thereto. Thus, it may be, for example, TEKKRRETVEREKE (SEQ ID NO:1); see, for example, US5773573A (the contents of which are incorporated herein by reference). SEQ ID NO:1 corresponds to amino acids 324-337 of ezrin. Any peptide having at least 80%, preferably at least 90% identity (or 100% identity) to this sequence, or to any fragment of ezrin, and which has at least substantially the same activity, may be used. Suitable fragments of ezrin, from Domain A and B of the Hepreceptor, are described in GB2354241A and US Patent Application Serial No. 09/856070, filed May 17, 2001, the content of which is incorporated herein by reference (see in particular claims 6, 9 and 19).

The active agents used in the invention are known, or can be prepared by known methods. In particular, methods of synthesis of alpha-interferons and peptides such as HEP1, i.e. a peptide with the amino acid sequence TEKKRRETVEREKE, or regulatory/unfolding peptides of ezrin, are known by those skilled in the art.

The respective active agents may be administered in either order or simultaneously, e.g. in the same composition. They may be formulated together or independently, preferably in a form suitable for oral or parenteral administration.

Formulations, routes of combinations and dosages of the active components used in the invention are known, or can be determined, by those skilled in the art, based on the usual factors. By way of example, administration by mouth of 1 to 10 mg or by injection of 0.1 to 1mg of HEP1 or a regulatory/unfolding peptide of ezrin, one to five times daily or up to 35 times weekly, is suitable in combination with standard treatment protocols for interferon therapy.

The following Example illustrates the invention. "HEP1" is the peptide having SEQ ID NO:1.

Example

Eleven patients with chronic HCV infection (measured by HCV serum RNA PCR) and liver disease (measure by elevated levels of Asp and Ala transaminases), were treated with injections of recombinant alpha2 interferon plus 2mg HEP1 (Gepon)/5ml water solution by mouth twice a day (Treatment Group). Ten patients with chronic HCV infection were treated with recombinant alpha2 interferon alone (Control Group).

The result was that in the Treatment Group there was at least a 20-fold enhancement in the suppression of HCV viral load compared to the Control Group and a reduction of transaminase levels to healthy levels (the Control Group still had significantly elevated level of transaminases in the blood). Further there was an unexpected and remarkable reduction in interferon side-effects in the Treatment Group, particularly in the areas of general pain, aching joints, bad taste in the mouth and weakness.

The data are shown in the following Table (Group Mean for each data point).

	Treatment group: HEP1 (Gepon) + Interferon 11 patients				Control Group: Interferon only 10 patients			
	Before	Month 1	Month 2	Month 3	Before	Month 1	Month 2	Month 3
Symptoms								
% of patient group								
Weakness	55	27	18	27	50	50	70	80
Pain	82	36	18	18	70	80	70	70
Bitter taste	64	27	9	9	80	70	80	80
aching joints	27	36	18	18	0	0	20	90
Virology+biochemistry								
HCV_RNA Log load	3.55			1.36	3.5			2.2
Ala Trans	133.36	84.55	68.55	53.09	194.7	90.7	62.4	71.5
Asp Trans	108.82	62.09	50.27	43.36	97.1	72.2	53.7	63
Immunology								
Leucocytes	7.13	6.15	5.55	4.73	6.09	4.7	4.11	4.51
Lymphocytes	27.73	33.36	33	32.82	26.3	29.1	31	37.5
Monocytes	8.27	6.82	7.09	6.45	5.4	5.1	6.4	4.7

Study details

21 patients (12 women, 9 men) aged from 18 to 55 years, sick with chronic hepatitis C participated in the study at The Gastroenterological Center in the Clinical Infectious Hospital № 1, Moscow. The diagnosis of chronic hepatitis C was based on the presence of specific antibodies to HCV in the blood of the patients during at least 6 months. The presence of HCV RNA in the peripheral blood was determined by PCR (polymerase chain reaction), and in all patients in the trial were in the replication phase of HCV infection. According to clinical data, and to the results of a biochemical study of the blood, in the majority of patients a moderate activity of chronic hepatitis C disease was well established.

All patients who participated in this investigation were treated with a recombinant alpha interferon (reaferon) at a dose of 3 millions units, 3 times a week. In addition to the background interferon therapy, 11 patients were treated with Gepon, 2mg orally (the preparation was dissolved in 5 ml of water, held 2-3 min in the mouth, then swallowed) once a day, daily during 3 months. Patients were divided into 2 groups, i.e. the Treatment Group (11 patients who obtained the combination of

interferon antiviral therapy with Gepon) and the Control Group (10 people patients who obtained interferon antiviral therapy only).

Treatment within the framework of the investigation continued for 3 months. Subsequently, the treatment with interferon was continued up to 1 year, even in the cases where Gepon treatment had resulted in the disappearance HCV RNA from the blood. For the duration of this study, all patients attended the designated doctor not less than 4 times, directly before beginning treatment, and 1, 2 and 3 months after the beginning of treatment. Within the same period, in addition to the clinical inspection, clinical and biochemical analyses of the blood were conducted, and viral load was assessed by HCV RNA in a semi-quantitative PCR assay. The final estimation of efficacy of the treatment was conducted 3 months after the beginning of treatment. The criteria for efficacy were prevalence of symptoms; frequency of an improvement (or normalization) in the activity of hepatic enzymes Al-AT, As-AT, gamma GT; frequency of improvement in the clinical indices of the blood; and results of PCR analysis of HCV RNA (viral replication). Statistical processing of the results of treatment was conducted with the use of a computer program called 'Statgraphics'.

Initial clinical status of the patients (before treatment)

Patients in both treatment and control groups prior to the beginning of treatment were similar in the presence and manifestation of the symptoms characteristic of the hepatitis disease of the hepato-biliary system. Complaints of pain in the right subcostal area were presented in 16 out of 21 (76%) patients. Bitterness in the mouth was perceived by 15 patients (71%). Express weakness, a sign of general asthenovegetative syndrome, appeared distinctly in 11 (52%) patients.

On inspection, only one of the subjects had established jaundice of the skin and sclera. Hepatomegalia and splenomegalia occurred in 20 (95%) and 17 (81%) respectively of the patients. Diffuse changes in parenchyma of the liver were registered with UZI in 20 (95%) of 21 patients. One patient had reactive pancreatitis. In certain cases, pathology of the gall bladder was observed, including thickening of the walls of the gall bladder in 9 patients, distortion of the bile duct in 6 patients, and gall stone formation in 1 patient.

Dynamics of clinical manifestations in the course of treatment

During the course of treatment, the presence and the manifestation of dyspeptic phenomena were evaluated (pain in the right subcostal area, bitterness in the mouth), asthenovegetative symptoms (weakness, irritability) and arthralgia. In the Control Group, the frequency of complaints of expressed general weakness grew from 50% to 80%, irritability from 0 to 100%, and arthralgia from 0 to 90%. Related deterioration in the health of patients during the course of interferon therapy can be explained by the widely known side-effects caused by all preparations of interferon.

In the Treatment Group, the frequency of complaints of general weakness was reduced from 54% to 18% ($p<0.05$), and arthralgia from 27% to 18% ($p<0.1$). Irritability in the first month of treatment was noted in 54% of patients, and in the subsequent months of treatment this index was reduced to 18% ($p<0.05$). These results demonstrated the positive influence of Gepon on the side-effects and adverse reactions to interferon therapy in HCV patients.

In the majority of patients of both groups prior to the beginning of treatment, symptoms associated with liver disease were present. 70-80% of the patients noted pains in the right subcostal area and perceived bitterness in the mouth. In the Control Group, during the course of 3 months of treatment with interferon only, the frequency of complaints of pain in the right subcostal area and the bitterness in the mouth did not change. In the Treatment Group, the frequency of complaints of pain in the right subcostal area was reduced from 80% to 18% ($p<0.05$). After 2-3 months of treatment, bitterness in the mouth was perceived in only 9% of the Treatment Group, as against 64% in the Control ($p<0.05$) in comparison to the number of patients before treatment.

There were characteristic features of chronic hepatitis: hepatomegalia was noted by the doctor in attendance in 90-100% of patients, and splenomegalia in 70-90% of patients before treatment. In both groups of patients, treatment was equally effective in reducing hepatomegalia and splenomegalia. After the 3-month course of treatment, the frequency of hepatomegaly and splenomegalia was reduced to 30-40%.

Dynamics of the level of the hepatitis C virus in the blood

In all 21 patients investigated, a semi-quantitative PCR estimation of the level of HCV RNA in the blood was performed and the titre of HCV RNA varied from 10^3

to 10^4 at the start of the study. In the Control Group, after 3 months of treatment, in 7 (70%) of 10 people the titres of HCV RNA were reliably lower than the pretreatment levels. HCV RNA ceased to be detected in 2 (20%) of 10 patients. On average, the titre of HCV RNA was reduced by 1.3 log in the Control Group. Three patients in the Control Group (30%) failed to respond to interferon treatment and in one of these patients the level of virus increased 10 times during the course of interferon treatment.

In the Treatment Group, suppression of virus replication was far more effective than in the Control Group. All patients responded positively to the combination treatment and 10 out of 11 (91%) patients had a reliable reduction in the concentration of HCV RNA in the blood. The average titre of HCV RNA in the Treatment Group was reduced by 2,2 log, or 158 times, that is a reduction of viral load almost twenty times greater than in the Control Group. In 4 (36%) out of 11 patients in the Treatment Group, HCV RNA ceased to be detected. Not one patient of the Treatment Group registered an increase in the viral load.

Biochemical indices of the blood

The patients who participated in the study were sick with chronic hepatitis C, and suffered from a moderate increase in the level of Al-AT and As-AT in the blood, an indication of the destructive inflammation of the hepatocytes. In both groups, after only 1 month of treatment, a significant decrease in the level of Al-AT and As-AT in the blood occurred, which continued during the next month. In the Control Group, the levels tended to increase again in the third month. In the Treatment Group, there was a clear tendency toward a progressive and deeper reduction in Al-AT and As-AT. The average values of general bilirubin in both groups of patients were above the upper boundary of normal levels. In the course of treatment, the level of general bilirubin of the blood decreased by 15-20% to normal values.

Hematological indices

The side-effects of prolonged therapy with recombinant interferon appear in changes in a number of indices of the blood. In the Control Group, a significant reduction in the maintenance of hemoglobin levels, the number of thrombocytes and especially in the quantity of mature neutrophilic granulocytes was observed, and simultaneously there was an increase of the content of lymphocytes, eosinophiles and macrophages/monocytes. In the Treatment Group, the combination of Gepon with interferon prevented the majority of the pathological changes occurring, and the

levels of neutrophils, granulocytes, eosinophils, lymphocytes and macrophages/monocytes remained at normal values during the 3 months of treatment.

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CLAIMS

1. A product comprising a peptide and an interferon, wherein the peptide comprises a sequence having at least 80% identity to a fragment Domain A or B of the Hepreceptor of ezrin, and wherein the peptide binds to the Hepreceptor with at least as great affinity as HEP1, as a combined preparation for simultaneous, separate or sequential use in the treatment of viral hepatitis.

2. A product according to claim 1, wherein the peptide comprises TEKKRRETVEREKE or an active fragment thereof.

3. A product according to claim 1 or claim 2, wherein the viral hepatitis is hepatitis C.

4. Use of a peptide as defined in claim 1 or claim 2, for the manufacture of a medicament for use in the treatment of a patient who has viral hepatitis and is undergoing treatment with interferon.