This Sheet Has Been Replaced

METHOD FOR TREATING HEPATITIS B CARRIERS WITH MINIMAL DISEASE

Background of the Disclosure

Technical Field

This invention relates to treatment of disease resulting from infection with the hepatitis B virus ("HBV"). More particularly, this invention relates to treating minimal disease hepatitis B carriers using thymosin α-1, or using a biologically active fragment or analog of thymosin α-1.

Background Art

Various disease states are caused by infection with the hepatitis B virus, and HBV infection is the second most common infectious disease worldwide. The hepatitis B virus ("HBV") is a DNA virus. HBV is known to be transmissible through blood transfusions, contaminated hypodermic needles, and sexual contact, and from mother to child (vertical transmission). A large number of people are infected by unknown means.

A description of the hepatitis B virus and its modes of transmission, and of disease states associated with HBV infection, can be found in, *e.g.*, J.H. Hoofnagle (1990), *New England Jour. Med.*, Vol. 323, pp. 337-39; J.H. Hoofnagle (1990a), *Vaccine*, Vol. 8 Suppl.; H. Popper *et al.* (1987), *Hepatology*, Vol. 7.pp. 764-72; Zakim and Boyer, eds. (1990), *Hepatology, a Textbook of Liver Disease*, W.B. Sauders Co., 2d ed.; F.B. Hollinger *et al.*, eds. (1991), *Viral Hepatitis*, Raven Press, 2d ed.

About 5 % of humans who become infected with HBV as adults develop a long-term infection, that is, they become "carriers" of the virus.

Of humans who are infected perinatally, which accounts for most of the hepatitis B infections worldwide, the vast majority (possibly 90 %) become carriers. Approximately 5 % of the world's population are carriers of HBV, and the majority of carriers are contagious, that is, they can transmit HBV infection to others *via* sexual contact, through blood or blood products, or vertically (at birth).

Various clinically distinguishable disease states can be exhibited in persons infected with the hepatitis B virus, including chronic hepatitis and minimal disease hepatitis. About 50 % of persons infected with HBV show chronic inflammatory changes in the liver and, of those, about 50 % have histopathologic changes (termed "chronic active hepatitis") that can lead to fibrosis and ultimately to cirrhosis and progressive liver failure.

Carriers of HBV who do not have chronic inflammatory changes in the liver may develop chronic active hepatitis; and liver cancer develops in about 10 % to 30 % of persons infected with HBV. It has been estimated that approximately 4 million carriers of hepatitis B virus die each year from liver cancer or cirrhosis.

The persistent infection seen in individuals presenting with chronic hepatitis may owe to a defective or physiologically immature immunological response, resulting in an impaired ability to clear the virus. Although the mechanisms responsible for liver damage are poorly understood, it is thought that in most individuals such damage results from attack by the body's immune system on infected liver cells, rather than from liver destruction by HBV. Cytotoxic T cells appear to be responsible for immune-mediated hepatic damage. The balance between suppression of immune system activity against normal tissue and the immunological response mounted against the virus also appears to be impaired in chronic hepatitis B. A number of specific immune defects have been described in chronic hepatitis, including defective production of α-interferon by HBV-infected hepatocytes and inhibition of cytotoxic T cell responses (*reviewed in*

This Sheet Has Been Replaced

M. Peters *et al.* (May 1991), *Hepatology (United States)*, Vol. 13 no. 5, pp. 977-94).

Patients having severe chronic active hepatitis are only a small percentage of the total population whose liver biopsy specimen is interpreted as showing chronic active hepatitis.

Roughly 40 % of hepatitis B carriers manifest a "minimal disease" state, sometimes referred to as "asymptomatic" hepatitis. A minimal disease carrier is a person who has detectable hepatitis B antigens or viral DNA in the serum, and yet has no clinically evident signs or symptoms of liver damage, or has, at most, only relatively slight clinically evident signs or symptoms of liver damage. Minimal disease carriers may develop chronic active hepatitis, which can lead to cirrhosis with liver failure, as described above. Clinical progression is slower in asymptomatic hepatitis, and the factor or factors that contribute to development of active disease in patients initially presenting as minimal disease carriers are not at present known.

It is not at present understood why some persons infected with HBV do not have inflammation or liver damage, while others do. These appear to represent two different disease conditions. In the minimal disease state, carriers do not show overt signs of immunological imbalance, as evidenced by lack of readily observable destruction of hepatic tissue.

There is currently no effective treatment for minimal disease carriers, yet treatment of such carriers, before they manifest a more serious form of the disease or transmit it to others, would be highly desirable. In addition, because the carrier state can be diagnosed by measuring hepatitis B antigens or HBV DNA in the blood, minimal disease carriers could be qualified for treatment without resort to liver biopsy. Although a vaccine to prevent hepatitis B is available, it is not used on a massive basis and does not alleviate the disease in existing carriers. Thus, even if the existing vaccine were implemented on a massive scale, the need for an effective treatment of individuals with hepatitis B viral infection would persist for at least several

This Sheet Has Been Replaced



decades, owing to the large number of carriers and the highly contagious nature of the disease.

Thymosin α-1 is a 28 amino acid peptide. This peptide, originally isolated from calf thymus thymosin fraction 5, is one of several polypeptides present in thymosin fraction 5 which participate in the regulation, differentiation and function of thymic dependent lymphocytes (T-cells). The isolation, characterization and use of thymosin α-1 is described in, for example, U.S. Patent No. 4,079,127.

Although the mechanism(s) by which thymosin α-1 mediates its effects are unknown, evidence suggests that thymosin α-1 may function through modulation of the immune system. Thymosin α-1 has been shown to trigger maturational events in lymphocytes, to augment T-cell function, and to promote reconstitution of immune defects (T.L.K. Low *et al.* (1984), *Thymus*, Vol. 6, pp. 27-42). Thymosin has also been shown to cause increases in lymphocyte counts and enhance production of γ-interferon in individuals suffering from chronic active hepatitis B (M.G. Mutchnick *et al.* (1991), *Hepatology*, Vol. 14, pp. 409-15).

M.G. Mutchnick *et al.* (1991) also describes the use of thymosin α-1 in treatment of chronic active hepatitis B, that is, in patients with evidence of liver injury on biopsy. Patients administered the peptide cleared hepatitis B virus DNA from serum and tested persistently negative for serum HBV DNA after treatment was terminated. No asymptomatic carriers without histopathological evidence of liver injury were included in this study.

Summary of the Invention

We have discovered that thymosin α-1, and biologically active fragments and analogs of thymosin α-1, can be used to treat minimal disease hepatitis B infection. That is, administration of an appropriate amount of thymosin α-1 to a patient having minimal disease hepatitis B can result in a

This Sheet Has Been Replaced



decrease in the patient in a parameter characteristic of minimal disease hepatitis B infection.

Disclosure of the Invention

This invention provides a treatment of minimal disease hepatitis B infections. In particular, this invention provides for use of thymosin α-1 (and of biologically active analogs and fragments of thymosin α-1) in compositions for treatment of minimal disease hepatitis B, that is for use in treatment of persons who may test seropositive for HBsAg and/or may test positive for HBV DNA in the serum, but who are asymptomatic and show no biochemical or histopathologic evidence of liver disease (or show only mild biochemical or histopathologic evidence of liver disease). Treatment of such minimal disease carriers of hepatitis B virus can result in elimination of HBV from the subject, as evidenced by loss of HBV DNA from the serum, and can thereby render the subject no longer at risk of developing active hepatitis or liver cancer or of transmitting HBV to others.

Accordingly, in one embodiment, the invention features treating minimal disease hepatitis B infection in a subject by administering to the subject a therapeutically effective amount of a composition that includes at least one peptide selected from the group consisting of thymosin α-1, a biologically active fragment of thymosin α-1, and a biologically active analog of thymosin α-1, in a pharmaceutically acceptable vehicle.

In particularly preferred embodiments, the peptide is thymosin α-1 and the composition is administered subcutaneously.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Detailed Description

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of protein chemistry, molecular biology, microbiology and recombinant DNA technology, which are within the skill

This Sheet Has Been Replaced

of the art. Such techniques are explained fully in the literature. See, e.g., Scopes, R.K., Protein Purification Principles and Practice, 2nd ed. (Springer-Verlag 1987); Methods in Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press 1989); Oligonucleotide Synthesis (M.J. Gait ed. 1984); and Handbook of Experimental Immunology, Vols. I-IV (D.M. weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications).

All patents, patent applications and publications mentioned herein, whether supra or infra, are hereby incorporated herein by reference in their entirety.

Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "minimal disease hepatitis B" is meant the disease state present in a carrier of hepatitis B, the carrier being identified as having hepatitis B serum antigens or as having HBV DNA in the serum, but having normal or slightly elevated serum enzyme levels, and no or only slight biochemical or histopathological evidence of liver disease. The minimal disease state is readily understood and identifiable by a skilled practitioner.

"Thymosin α-1", as used herein, refers to the 28 mer described below, with or without the N-acetyl group, and is meant to include biologically active fragments of this sequence and biologically active analogs of the sequence (*i.e.*, deletion, substitution and addition mutants), which are substantially homologous to the peptide sequence shown below.

A peptide that is "substantially homologous" to thymosin α-1 is one in which at least about 30%, preferably at least about 85% to 90%, and most preferably at least about 95%, of the amino acids match over a defined length of the molecule, with the sequence depicted below.

A "biologically active" fragment or analog of thymosin α-1, is a fragment or analog of thymosin α-1 which retains a significant amount of

This Sheet Has Been Replaced

the activity of the native molecule, *i.e.*, which is capable of decreasing serum HBV DNA and/or hepatitis B surface antigen, as described further below.

The term "treatment" as used herein refers to either (i) the prevention of infection or reinfection (prophylaxis), or (ii) the reduction or elimination of indicators of minimal disease hepatitis B (therapy).

A "therapeutically effective amount" of thymosin α-1 is an amount of the peptide which has the capability of changing the measurable parameters of hepatitis infection. Since the patients included in the minimal disease category have normal liver enzyme biochemistry, the parameters that will normally be monitored are serum hepatitis B envelope antigen (HBeAg) and serum viral DNA (HBV DNA). Response is defined as a significant decrease of either of these parameters. An amount of peptide which has the ability of eliciting a response is considered a "therapeutically effective amount." HBV DNA can be monitored using the spot hybridization assay described in M.G. Mutchnick *et al.* (1991), *Hepatology*, Vol. 14, pp. 409 15, and in H.M. Lieberman *et al.* (1983), *Hepatology*, Vol. 3, pp. 285-91. Alternatively, the presence of HBV DNA in the blood can be measured using standard PCR technology. See, *e.g.*, U.S. Patent Nos. 4,683,202 and 4,683,194, incorporated herein by reference in their entirety. Serum HBcAg levels can be monitored using, for example, standard RIAs, as described in M.G. Mutchnick, M.G. *et al.* (1991), *Hepatology*, Vol. 14, pp. 409-15, or by standard ELISAs.

General Methods

The present invention relates to the use of thymosin α-1 for the treatment of minimal disease hepatitis B infection. Thymosin α-1 is one of several peptides present in thymosin fraction 5 from the thymus gland. The native molecule is a 28-mer, having the following amino acid sequence:

Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-
Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-
Val-Val-Glu-Glu-Ala-Glu-Asn-OH.

This Sheet Has Been Replaced

Thymosin α-1, as well as fragments and analogs thereof, are easily synthesized using standard methods of peptide synthesis, known to those of skill in the art. U.S. Patent Nos. 4,148,788 and 4,844,407 described the solution phase and solid phase synthesis, respectively, of thymosin α-1, and are incorporated herein by reference in their entirety. See also, J.D. Young, *Solid Phase Peptide Synthesis*, 2d ed. (Pierce Chemical Company 1984); and G. Barany *et al.*, *The Peptides: Analysis, Synthesis, Biology*, Vol. 2 (E. Gross and J. Meienhofer, eds., Academic Press 1980), for a discussion of solid phase peptide synthesis; and M. Bodansky, *Principles of Peptide Synthesis* (Springer-Verlag 1984); and E. Gross, E. and J. Meienhofer, eds., *The Peptides: Analysis, Synthesis, Biology*, Vol. 1 (Academic Press 1980), for solution phase peptide synthesis.

Thymosin α-1 can also be isolated directly from appropriate tissue expressing thymosin α-1, using techniques readily known in the art. This is generally accomplished by first preparing a crude tissue extract which lacks cellular components and several extraneous proteins. The thymosin α-1 can then be further purified by, for example, column chromatography, HPLC, immunoadsorbent techniques or other conventional methods well known in the art. U.S. Patent No. 4,079,127 discloses a method for purifying thymosin α-1 from calf thymus and is incorporated herein by reference in its entirety.

Finally, thymosin α-1 and fragments or analogs thereof may be produced recombinantly using methods well known to those of skill in the art. See, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, 2d ed. (Cold Spring Harbor Laboratory Press 1989); and M.J. Gait, ed. (1984), *Oligonucleotide Synthesis*.

Thymosin α-1, or an active fragment thereof, or an analog thereof, can be administered to subjects diagnosed or suspected of having minimal disease hepatitis B infection. The thymosin α-1 may be administered alone or in mixed with pharmaceutically acceptable vehicle or excipient.

Typically, the thymosin α-1 compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in,

This Sheet Has Been Replaced

or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. As explained above, the active ingredient is often mixed with vehicles containing excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. *See, e.g.*, Remington's Pharmaceutical Sciences (Mack Publishing Company, Easton, Pennsylvania, 15th edition, 1975). The composition or formulation to be administered will, in any event, contain a quantity of the peptide adequate to reduce or eliminate HBV DNA and/or HBeAg from the serum of the subject being treated.

The injectable compositions are preferably administered by the subcutaneous route; intramuscular injection or intravenous injection can also be acceptable. Injectable formulations will contain an effective amount of the active ingredient in a vehicle, the exact amount being readily determined by one skilled in the art. The active ingredient may typically range from about 1 % to about 95 % (w/w) of the composition, or even higher or lower if appropriate. The quantity to be administered can depend on such factors as the age, weight and degree of health of the subject to be treated. With the present formulations, an amount between about 600 and about 3000 μg of thymosin α-1 per square meter of body area will be administered; amounts between about 400 and about 3200 μg or between about 600 and about 1500 μg or between about 900 and about 1200 μg per square meter of body area may be effective. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

This Sheet Has Been Replaced

Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, aerosol, intranasal, oral formulations, and sustained release formulations. For suppositories, the vehicle composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range from about 0.5 % to about 10 % (w/w), and preferably from about 1 % to about 2 %. Oral vehicles include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral compositions may be taken in the form of solutions, suspension, tablets, pills, capsules, sustained release formulations, or powders, and contain from about 10 % to about 95 % of the active ingredient, preferably about 25 % to about 70 %.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject protein by the nasal mucosa.

Controlled or sustained release formulations are made by incorporating the peptide into carriers or vehicles such as liposomes, nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures. The peptides can also be presented using implanted mini-pumps, well known in the art.

Furthermore, the peptides may be formulated into compositions in either neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active peptides)

This Sheet Has Been Replaced

and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The subject is treated by administration of the thymosin α-1 peptide, or fragment thereof, or analog thereof, in at least one dose. Preferably, thymosin α-1 is administered from one to several times weekly for several weeks to several months. More preferably, the compositions are given one to seven times weekly, most preferably twice weekly, for at least about three to about twelve months or longer, most preferably for at least about six months. The compositions can be given more frequently in earlier weeks and then less frequently in subsequent weeks (as, for example, daily for two weeks and then biweekly thereafter). The progress of treatment can be monitored by measuring the presence of HBV DNA and/or HBeAg, as described above.

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Example 1

This Example provides an illustrative protocol that can be used in a clinical trial of the efficacy of thymosin α-1 or any biologically active analog or fragment of thymosin α-1 in treatment of minimal disease hepatitis B. The trial employs 33 patients in each of three groups.

Criteria for selecting subjects for the trial

Candidates for inclusion in the trial are males and females between 18 and 64 years old at the time the trial is initiated; subjects are selected for inclusion using criteria designed to maximize safety and to ensure that a

This Sheet Has Been Replaced



relationship can be established between the subjects' disease states and the treatment regimes.

There are several qualifications for inclusion of a candidate in the trial.

Included subjects have been hepatitis B surface antigen ("HBsAg") seropositive for at least 6 months prior to initiation of the trial; they have serum alanine aminotransferase ("ALT") levels no greater than 2.0 times the upper limit of normal, determined as described further below; they are "HBcAg" seropositive, and they have HBV DNA in the serum, determined as described further below.

Included subjects have a hematocrit $\geq$ 30 %, platelet count $\geq$ 80 x 10^9/L, white blood cell count $\geq$ 3 x 10^9/L, and polymorphonuclear white cell count $\geq$ 1.5 x 10^9/L. Renal function is at least reasonably good as demonstrated by serum creatinine $\leq$ 180 μmol/L. Prothrombin time is less than 5 seconds over control, serum albumin $\geq$ 30 g/L, and bilirubin $\leq$ 68 μmol/L; included subjects have no history of hepatic encephalopathy or bleeding esophageal varices. Liver biopsy of included subjects shows either "minimal change disease" or "chronic persistent hepatitis".

Candidates are excluded from the trial if they have any of several indications.

A candidate is excluded if he or she has any progressive decrease in serum HBV DNA level prior to initiation of the study, as indicated by a most recently determined serum HBV DNA level less than 50 % of the level from the preceding determination. Such a candidate may be included if, upon a further determination at least one month later, the serum HBV DNA level has stabilized.

A candidate is excluded if he or she has been treated using interferon or any other type of immunotherapy within one year prior to initiation of the study, or has been treated using adrenocorticoid steroids within six months prior to initiation of the study. A candidate is excluded if there is evidence suggesting toxic hepatitis, alcoholic liver disease, Wilson's disease, primary

This Sheet Has Been Replaced



biliary cirrhosis, hepatitis C, or presence of antibody to either of hepatitis C virus ("HCV"), hepatitis delta, hemochromatosis, or autoimmune hepatitis; or if he or she has HIV infection diagnosed by HIV seropositivity and confirmed by Western blot; or if she or he has concomitant or prior history of malignancy other than curatively treated skin cancer or surgically cured *in situ* carcinoma of the cervix; or if he or she has any active infectious process other than HBV that is not of a self-limiting nature, such as for example tuberculosis "TB" or acquired immune deficiency syndrome "AIDS"; or if she or he has rheumatoid arthritis or other autoimmune disease, or serum ANA > 1:40. A candidate is excluded if he or she has any history of intravenous drug abuse within the 5 years prior to initiation of the trial; or if he or she is concomitantly using any drug known to be hepatotoxic. A candidate is excluded if she is pregnant as documented by urine pregnancy test, and included female subjects must agree to practice birth control for the duration of the study.

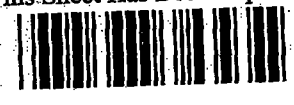
A candidate is excluded if she of he is a poor medical or psychiatric risk, or has any non-malignant systemic disease that, in the opinion of the investigator, would make it unlikely that the candidate could complete the protocol or if, in the opinion of the investigator, there is any indication that the candidate would not comply with the conditions of the protocol.

Screening phase

Subjects preferably are recruited for the trial by screening a large population of candidates according to a scheme such as the following.

Generally, candidates are identified as suitable for treatment according to the invention by a pattern of criteria for minimal disease hepatitis B. Particularly, a preferred screening protocol includes first and second screening visits between four weeks and two months apart and, for cases where the criteria for inclusion are not clearly met in the first two visits, a third screening visit between four weeks and two months after the second visit. A candidate for treatment meets the criteria set out above.

This Sheet Has Been Replaced



With respect to serum ALT for qualification of subjects, preferably the level of serum ALT was no greater than 2.0 times the upper limit of normal at each of the first and second screening visits; or, alternatively, the mean of the serum ALT at the three screening visits was no greater than 2.0 times the upper limit of normal.

With respect to HBeAg and HBV DNA for qualification of subjects, the determination must be positive on each of the first two screening visits; if the second visit HBV DNA level is less than 50 % the first visit level, then the level upon a third determination must be at least 50 % of the first in order for the candidate to be included.

Liver biopsy preferably is evaluated according to the so-called Knodell scale, described in Knodell *et al.* (1981), *Hepatology*, Vol. 4, no. 5, pp. 434 *et seq.* The total Knodell score ("HAI" or "histology activity index") for the majority of trial subjects having minimal disease hepatitis B as defined above will be < 4, more probably ≤ 3 or ≤ 2. Candidates having HAI 4 or greater may not be properly classified as having minimal disease hepatitis B, as that expression is meant herein.

Treatment

For the trial, each subject will be assigned to one of three groups. Group 1 subjects receive Thymosin α-1 over a six months' treatment period, followed by six months of observation. Group 2 subjects receive Thymosin α 1 over a twelve months' treatment period, followed by six months of observation. Group 3 subjects are untreated, and will be observed for twelve months.

Following the observation period, the HBV DNA status of Group 3 subjects is determined, and subjects that are still HBV DNA positive are randomly assigned to a six months' or twelve months' treatment period. That is, after the first twelve months' observation period Group 3 subjects are reassigned to one of three groups. Group 3A subjects (HBV DNA negative at twelve months) receive no treatment. Group 3B subjects receive Thymosin α-1 over a six months' treatment period, followed by six months

This Sheet Has Been Replaced

of observation. Group 3B subjects receive Thymosin α-1 over a twelve months' treatment period, followed by six months of observation.

The subjects' liver function and hepatitis B markers are measured monthly during treatment periods, and each three months during observation periods. Preferably, each subject will have a liver biopsy at the end of the treatment period.

Dosage regimen

For the trial, Thymosin α-1 will be administered by subcutaneous injection twice weekly at 1.6 mg per injection for all treated patients. A range of 1.45 mg to 1.75 mg per injection can be expected to be safe and effective.

Efficacy

Virological response. The principal measure of efficacy in the preferred trial is the subject's virologic response. Three indicia of virologic response may be used, namely:

1. HBV becoming undetectable (negative) using a standard assay such as the Abbott HBV DNA kit;

2. Loss of HBcAg from serum (as detected by EIA);

3. Loss of HBsAg from serum.

The subjects' responses may be classified as:

1. Complete: Serum HBV DNA becoming negative and remaining negative at the end of the study, with loss of HBeAg; HBsAg may or may not be present at the end of the trial;

2. Partial: Loss of serum HBeAg and reduction of serum HBV DNA by 50 % or more, but not negative;

3. Non-responder: Reduction of serum HBV DNA of less than 50 % at the end of the trial;

4. Reactivation: Disappearance followed by recurrence of serum HBV DNA and HBeAg by the end of the trial;

This Sheet Has Been Replaced

5. Inconclusive: Persistence of HBeAg from initiation to the end of the trial, with reduction of serum HBV DNA of at least 50 % a the end of the study.

Clinical response. Because minimal disease HBV carriers generally have no symptoms or only slight symptoms specific to their carrier state, clinical response is not a significant measure of response to treatment. However, subjects may show improvement or no improvement in clinical symptomatology (malaise, fatigue, anorexia, daily activity).

Histological response. Where determined, the subject is classified according to histological response as a responder if there is improvement of at least 25 %, according to the method of and using the HAI index of Knodell *et al.*, in the histological grading of liver biopsy specimen, and loss of replicative HBV DNA forms or loss of hepatitis B core antigen in liver tissue on final biopsy; or as a non-responder if there is no significant improvement or worsening of the histological score.

Example 2

This example illustrates an alternative form of trial.

A randomized, open trial with a total sample size of 40 patients, each at least 18 years of age at the beginning of treatment. Candidates for inclusion in the trial have been hepatitis B surface antigen ("HBsAg") seropositive for at least 6 months prior to treatment, and have HBV DNA (as measured by PCR or any other standard DNA detection method) in the serum for at least 3 months prior to treatment. Only patients with minimal disease are included in the trial; these patients would have normal plasma levels of hepatic enzymes, and liver biopsy if performed shows minimal or no inflammatory changes, particularly showing no evidence or scarring or cirrhosis.

Subjects who have had therapy with interferon within the previous year, who have HIV infection with HIV seropositivity confirmed by Western blot, who are hepatitis C virus ("HCV") antibody seropositive, or who have

This Sheet Has Been Replaced

engaged in intravenous drug abuse within the previous 5 years, are excluded from the trial. Subjects who are pregnant are excluded from the trial.

Patients undergo a pretreatment exam monthly for at least 3 months prior to the treatment protocol. Examination includes:

1. Blood studies including FBC with differentiated count, prothrombin time ["PT"], glucose, creatinine and electrolytes, liver function tests ["LFT"], α-fetoprotein ["AFP"], HIV antibody, HCV antibody, hepatitis-Δ antibody, HB markers (HBeAg and HBV DNA) and thymosin α-1 levels.
2. Liver biopsy and liver HBV DNA analysis.
3. Routine urinalysis.
4. A pregnancy test for women of child bearing age.

Selected patients are randomized into two groups. Group 1 patients will receive thymosin α-1 treatment, and group 2 patients will receive a placebo. Group 1 patients are given thymosin α-1 at a dosage of 1600 μg/injection by the subcutaneous route twice weekly for 6 months. Group 2 patients receive placebo injections under the same regimen. All patients are seen at 2 and at 4 weeks from the start of the treatment, and thereafter at monthly intervals during the 6 month treatment period. The patients are monitored monthly for 6 months to two years after completion of treatment.

Response to treatment is defined by loss of serum HBV DNA or serum HbeAg. A responder is a patient is one in whom a decreased level of serum HBV DNA or of HBeAg is achieved and sustained during the 12 month study. A nonresponder is one in whom no changes in either serum HBV DNA or HBsAg are shown at the conclusion of the study (12 months). Relapse status is given to patients who initially lose HBV DNA in their serum but regain the DNA by the conclusion of the study.

Group means are compared by Student's two-tailed t test. Changes in measurement between the inclusion values and subsequent time points are compared by Student's two-tailed paired t test or the Wilcoxon paired sample test.

This Sheet Has Been Replaced

Patients are monitored for any significant side effects or allergic manifestations resulting from the treatment.

Example 3

For treatment of minimal disease hepatitis B, the sera of members of the population to be treated will be tested for presence of HBsAg and/or HBV DNA. Those who are seropositive for HBV infection may be further examined as appropriate, to determine both that treatment will not pose an unacceptably high risk of iatrogenic harm and that they are in fact minimal disease hepatitis B carriers. Liver biopsy may or may not constitute a part of the further examination; and further blood chemistry (such as analysis for ALT or other enzymes) may or may not constitute a part of the further examination. Diagnosed minimal disease hepatitis B carriers will then be treated according to the invention, using a dosage amount and dosage regime appropriate for the particular patient and for the particular Thymosin α-1 or analog or fragment that is being used.

Other Embodiments

Preferred embodiments of the subject invention have been described in detail; it is understood that obvious variations can be made without departing from the spirit and scope of the invention as defined by the appended claims.

Other embodiments are within the following claims.

This Sheet Has Been Replaced



Claims

1. A method for treating minimal disease hepatitis B infection in a subject, comprising administering to the subject a therapeutically effective amount of at least one peptide selected from the group consisting of thymosin α-1, a biologically active fragment of thymosin α-1, and a biologically active analog of thymosin α-1, in a pharmaceutically acceptable vehicle.

2. The method of claim 1, wherein said peptide is thymosin α-1.

3. The method of claim 1 wherein said peptide is a biologically active fragment of thymosin α-1.

4. The method of claim 1 wherein said peptide is a biologically active analog of thymosin α-1.

5. The method of claim 1, said administering being by a subcutaneous route.

6. The method of claim 1 wherein from about 400 to about 3200 μg of said peptide per square meter of body area is administered.

7. The method of claim 1 wherein from about 600 to about 3000 μg of said peptide per square meter of body area is administered.

8. The method of claim 1 wherein from about 600 to about 1500 μg of said peptide per square meter of body area is administered.

9. The method of claim 1 wherein from about 900 to about 1200 μg of said peptide per square meter of body area is administered.

10. The method of any of claims 6 - 9 wherein said peptide is administered twice weekly.

This Sheet Has Been Replaced