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(54) Title: METHOD FOR TREATING CYTOKINE MEDIATED HEPATIC INJURY (57) Abstract A method of modulating cytokine mediated hepatic injury by administering deltorphins to a mammal. Deltorphin I SEQ ID NO:1, deltorphin II SEQ ID NO:2 or combinations of deltorphins I SEQ ID NO:1 and II SEQ ID NO:2 may be administered. A deltorphin concentration in the range of about 0.5 mg/kg to about 20 mg/kg in a physiologically acceptable formulation blocks a cytokine cascade in a murine model of septic shock. A therapeutic method of modulating cytokine mediated acute inflammatory, trauma induced and toxin induced hepatic injury, particularly via tumor necrosis factor modulation, is thus disclosed.		

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METHOD FOR TREATING CYTOKINE MEDIATED HEPATIC INJURY

Field of the Invention

The invention relates to the use of deltorphins to attenuate or prevent cytokine mediated hepatic injury.

Background

5 Hepatic injury can be caused by a number of different agents including viruses such as Hepatitis A, B, C, D and E, both gram positive and gram negative bacteria, chemical agents such as ethanol, carbon tetrachloride and lead, and by physical trauma resulting in ischemia (ischemic hepatitis) injuries as can occur in right-sided congestive heart failure. It is now believed
10 that all of these types of hepatic injury are caused at least in part by the liver's inflammatory or cytokine response to these agents. The inflammatory

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response of the liver results in the overexpression of a cascade of inflammatory/acute phase cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6, IL-8 and transforming growth factor beta (TGF β). It is now believed that it is the cascade of these cytokines which is the ultimate cause of much of the hepatic injury resulting from these agents. Thus, there is a need for a therapeutic agent which can be useful in alleviating or modulating the inflammatory response associated with liver disease or injury.

Summary of the Invention

The present invention fills this need by providing a method of treating or preventing a cytokine mediated hepatic injury in a mammal comprised of administering a pharmaceutically effective amount of a deltorphin to said mammal. The hepatic injury can be an acute inflammatory reaction, as a result of a viral or bacterial infection or a chemical agent such as ethanol, lead, carbon tetrachloride or acetaminophen, or from trauma resulting in ischemia or reperfusion injury in the liver.

The present invention is also directed to a method of treating a viral or bacterial infection-related hepatic damage in a mammal comprised of administering a pharmaceutically effective amount of a deltorphin to said mammal.

The present invention is also directed to a method of treating alcohol induced liver injury in a mammal comprised of administering a pharmaceutically effective amount of a deltorphin to said mammal.

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Preferably, the deltorphin is administered in a pharmaceutical composition at a dosage of from about 0.5 mg/kg to about 20 mg/kg, or alternatively lower doses from about 1 μ g/kg to about 1000 μ g/kg of deltorphin per body weight of the mammal.

5 Preferably, the mammal is a human.

Detailed Description

Deltorphins are endogenous linear heptapeptides isolated from skin extracts of the South American frog *Phyllomedusa bicolor*. These may be further divided into deltorphin I SEQ ID NO:1 and deltorphin II SEQ ID NO:2 depending on their amino acid sequence. Deltorphan I SEQ ID NO:1 has the amino acid sequence Tyr-Ala-Phe-Asp-Val-Val-Gly-NH₂ with alanine as either the D- or L- isomer. Deltorphan II SEQ ID NO:2 has the amino acid sequence Tyr-Ala-Phe-Glu-Val-Val-Gly-NH₂ with alanine as either the D- or L- isomer. Either deltorphin I SEQ ID NO:1, deltorphin II SEQ ID NO:2 or a combination of deltorphin I SEQ ID NO:1 and II SEQ ID NO:2 may be used in the invention. Deltorphins may be obtained from frog skin extracts, or they may be purchased from a vendor such as Peninsula Laboratories, Inc. (Belmont, CA) or may be synthesized using a peptide synthesizer such the type available from Applied Biosystems.

20 Deltorphins are administered to a mammal to modulate cytokine activation by blocking one or more steps in the cytokine cascade. Deltorphins may be formulated for administration in an aqueous based liquid such as phosphate buffered saline to form an emulsion, or they may be formulated in an organic liquid such as dimethylsulfoxide to form a solution. The solution or

emulsion may be administered by any route, but it is preferably administered parenterally such as by intravenous, intramuscular, intradermal or intraperitoneal injections. A preferred dose is in the range of about 0.5-20 mg, or alternatively lower doses of about 1-1000 μ g of deltorphin per kg of body weight of the mammal. The time of administration of the deltorphin is preferably prior to initiation of cytokine activation. However, the deltorphin may be administered concurrently with another agent that induces cytokine activation or even subsequent to an agent that induces cytokine activation and still produce a protective effect.

10 Deltorphin administration should be continued on a daily basis until hepatic function returns to normal and is maintained at normal levels, preferably for at least one to two days. Hepatic injury can be determined by elevated levels of hepatic enzymes, as well as by depressed albumin levels (less than about 35 g/liter). Hepatic function is routinely monitored by
15 quantitating serum levels of hepatic enzymes such as alanine aminotransferase (ALT) (normal < 35 U/L), aspartate aminotransferase (AST) (normal < 30 U/L), alkaline phosphatase (ALP) (normal $\leq$ 100 U/L) and gamma glutamyltransferase (GGT) (normal $\leq$ 45 U/L for males, $\leq$ 30 U/L for females), as well as bilirubin, both conjugated (normal $\leq$ 0.2 mg/deciliter) and total
20 (normal $\leq$ 1.0 mg/deciliter) bilirubin. Deltorphin modulation of hepatocyte cytokine activation may be used therapeutically in a variety of hepatic injury processes. As used herein, the term hepatic injury broadly encompasses all types of injury such as hepatic trauma, physical and/or chemical insult, stress, inflammation, toxicity, disease and so on. For example, deltorphins can be

used in treating hepatic injury due to alcoholic liver disease, acetaminophen toxicity, cadmium toxicity, lead poisoning, bacteremia due to, for example, *Staphylococcus* species, *Streptococcus* species, *Neisseria* species, *Salmonella* species, *Shigella* species, *Escherichia coli*, *Clostridium perfringens*, *Klebsiella* species, *Proteus* species, *Enterobacter* species, *Bacteroides* species, *Brucella* species, *Francisella tularensis*, *Listeria monocytogenes*, *Acinetobacter* species, *Streptobacillus moniliformis*, *Vibrio* species, *Helicobacter pylori*, *Pseudomonas* species, *Haemophilus* species, *Bordetella pertussis*, viral infections due to, for example, influenza viruses, adenoviruses, paramyxoviruses, rubella viruses, polioviruses, hepatitis viruses, herpesviruses, rabies viruses, human immunodeficiency viruses and papilloma viruses, as well as trauma, ischemia reperfusion injury and metabolic liver disease.

While the specific mechanism of deltorphin action on the modulation of cytokine mediated hepatic injury such as acute inflammatory reactions, trauma and toxin induced biological responses is unknown, deltorphins exhibit a specific and reproducible effect on decreasing hepatotoxicity. This invention will be further appreciated in light of the following example.

EXAMPLE

Hepatic failure was induced in mice by intraperitoneal injections of a mixture of lipopolysaccharide (LPS) (100 μ g/kg) and D-galactosamine (GAL) (700 mg/kg) to form LPS/GAL. LPS is a bacterial endotoxin that is a major stimulus for production of many cytokines, particularly tumor necrosis factor (TNF), thought to be involved in liver injury. GAL is an amino sugar that

sensitizes hepatocytes by depleting the base uridine and thus preventing hepatic transcription, and sensitizes animals to normally innocuous amounts of endotoxin or TNF. The LPS/GAL treatment induces fulminate hepatic and multiorgan failure, and serves as a model for septic shock. The major effects
5 of LPS/GAL are elicited by the inflammatory cytokine TNF- α . Hepatocytes sensitized to TNF- α then undergo apoptosis or programmed cell death in response to inflammatory processes.

Mice were treated with deltorphin and were subsequently rendered hepatotoxic by LPS/GAL treatment. An emulsion of deltorphin I SEQ
10 ID NO:1 (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH₂) was prepared at a concentration of 80 μ g/200 μ l in sterile phosphate buffered saline (PBS). An emulsion of DADLE (Tyr-D-Ala-Gly-Phe-D-Leu-enkephalin) was also prepared at a concentration of 80 μ g/200 μ l in sterile PBS. Male BALB/c HSD mice were treated by intraperitoneal injections according to the following protocol.
15 Control mice (n = 7) received only LPS/GAL (280 μ l) at 6.5 h. Deltorphin I SEQ ID NO:1 treated mice (n = 15) received injections of 4 mg/kg deltorphin I SEQ ID NO:1 at 0 h, 2 h, 4 h, 6 h and 7.5 h and received an LPS/GAL injection at 6.5 h. DADLE treated mice (n = 15) received 4 mg/kg DADLE at 0 h, 2 h, 4 h, 6 h and 7.5 h and received an LPS/GAL injection at 6.5 h.

20 Both control and treated mice were monitored at 15 h, 20 h, 40 h and 65 h after the last injection. The results are shown in the following table.

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TABLE

TREATMENT	SURVIVAL			
	15 Hours	20 Hours	40 Hours	65 Hours
LPS/GAL	0	---	---	---
DADLE + LPS/GAL	3 (20%)	3 (20%)	3 (20%)	3 (20%)
5 Deltorphin I SEQ I NO:1 + LPS/GAL	12 (80%)	11 (73%)	11 (73%)	11 (73%)

Of the seven mice receiving only LPS/GAL (control group), none survived more than 15 h post-LPS/GAL injection and some died as early as 6 to 8 h post injection. Of the 15 mice treated with DADLE, 20% (3 out of 15) survived for 20 h, 20% (3 out of 15) survived for 40 h and 20% (3 out of 15) survived for 65 h after the last injection. In contrast, of the 15 mice treated with deltorphin I SEQ ID NO:1, 80% (12 out of 15) survived for 15 h, 73% (11 out of 15) survived for 20 h, 73% (11 out of 15) survived for 40 h and 73% (11 out of 15) survived for 65 h after the last injection. The markedly increased survival time for deltorphin I SEQ ID NO:1 treated mice that had been made septic by LPS/GAL treatment was significant compared to mice receiving LPS/GAL only. In contrast, the survival time for DADLE treated mice was not as striking at any time point. These results indicate that treatment with deltorphin I SEQ ID NO:1 prevents acute inflammatory hepatic injury and can reduce lethality in a murine model.

A treatment for attenuating and/or preventing cytokine mediated acute inflammatory, trauma induced and toxin induced hepatic injury is thus disclosed. Deltorphins, administered at a concentration of about 0.5 mg/kg to about 20 mg/kg, or alternatively at lower concentrations of about 1-

1000 $\mu\text{g/kg}$, inhibit hepatic injury and result in decreased lethality of the animal.

It should be understood that the embodiments of the present invention shown and described in the specification are only preferred
5 embodiments of the inventors who are skilled in the art and thus are not limiting in any way. Therefore various changes, modifications or alterations to these embodiments may be made or resorted to without departing from the spirit of the invention and the scope of the following claims.

What is claimed is:

1. A method of modulating a cytokine mediated hepatic injury response in a mammal comprising administering a deltorphin to the mammal in a pharmaceutically acceptable formulation.
2. The method of claim 1 wherein said deltorphin is selected from the group consisting of deltorphin I SEQ ID NO:1, deltorphin II SEQ ID NO:2 and combinations thereof.
3. The method of claim 1 wherein said deltorphin is administered prior to said response.
4. The method of claim 1 wherein said deltorphin is administered subsequent to said response.
5. The method of claim 1 wherein said deltorphin is administered substantially concurrently with said response.
6. The method of claim 1 wherein said deltorphin is administered in the formulation selected from the group consisting of a solution, an emulsion and a suspension.
7. The method of claim 1 wherein said deltorphin is administered parenterally.

8. The method of claim 1 wherein said deltorphin is administered at a concentration in the range of about 0.5 mg/kg to about 20 mg/kg.
9. The method of claim 1 wherein said deltorphin is administered at a concentration in the range of about 1 μ g/kg to about 1000 μ g/kg.

10. A method for treating hepatic injury in a mammal by a chemical toxin comprising administering a pharmaceutically effective concentration of a deltorphin.

11. The method of claim 10 wherein the chemical toxin is selected from the group consisting of ethanol, lead, cadmium, carbon tetrachloride, and acetaminophen.

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12. A method for treating a bacterial or viral infection related hepatic injury in a mammal comprising administering a pharmaceutically effective concentration of a deltorphin.

13. The method of claim 12 wherein the bacterial or viral infection is caused by an organism selected from the group consisting of *Staphylococcus* species, *Streptococcus* species, *Neisseria* species, *Salmonella* species, *Shigella* species, *Escherichia coli*, *Clostridium perfringens*, *Klebsiella* species, *Proteus* species, *Enterobacter* species, *Bacteroides* species, *Brucella* species, *Francisella tularensis*, *Listeria monocytogenes*, *Acinetobacter* species, *Streptobacillus moniliformis*, *Vibrio* species, *Helicobacter pylori*, *Pseudomonas* species, *Haemophilus* species, *Bordetella pertussis*, influenza viruses, adenoviruses, paramyxoviruses, rubella viruses, polioviruses, hepatitis viruses, herpesviruses, rabies viruses, human immunodeficiency viruses and papilloma viruses.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- (ii) TITLE OF INVENTION: METHOD FOR TREATING CYTOKINE
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- (iii) NUMBER OF SEQUENCES: 2
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- (v) COMPUTER READABLE FORM:
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 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: Windows 95
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0b
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- (viii) ATTORNEY/AGENT INFORMATION:
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(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Ala Phe Asp Val Val Gly
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Ala Phe Glu Val Val Gly
1 5