

**Method of Treating Acute Myocardial Infarction
with Hirudin and Acetylsalicylic Acid in
Patients Not Undergoing Thrombolytic Treatment**

Background of the Invention

5 *Field of the Invention*

The invention relates to the therapeutic and pharmaceutical combination of a platelet inactivating drug, acetylsalicylic acid, and a direct thrombin inhibitor, hirudin, for the treatment of patients with acute myocardial infarction who are not undergoing thrombolysis.

10 *Related Art*

I. Acute Vascular Diseases and Clot Formation

Acute vascular diseases, such as myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis, peripheral arterial occlusion and other blood system thromboses constitute major health risks. In particular, acute myocardial infarction (AMI) occurs with a mortality rate of about 35 %, with about half of the deaths occurring before the patient reaches a hospital; an additional 15 to 20% of survivors die in the first year following myocardial infarction (Pasternak, R.C., *et al.*, Chapter 190 in *Harrison's Principles of Internal Medicine*, 11th ed., Braunwald *et al.*, Eds., McGraw-Hill Book Co., New York (1987), pages 982-993). Acute vascular diseases are caused or aggravated by either partial or total occlusion of a blood vessel by a blood clot, which typically consists of fibrin and/or aggregated platelets.

Blood platelets play an essential role in normal hemostasis. They have both a distinct hemostatic function and a thromboplastic function. Hemostasis is initiated within a few seconds following a trauma, when platelets begin to adhere to the edges of the lesion. This initial adherence of platelets may be

mediated by collagen exposed at the site of blood vessel wall trauma or by newly generated thrombin. Once in contact with collagen or thrombin, platelets undergo activation (release reaction) and release a variety of chemicals, including ADP and thromboxane A₂. The released ADP and thromboxane A₂ cause additional platelets from the issuing blood to aggregate to those already attached to the vessel wall. The newly attached platelets also undergo the release reaction and the process continues until a hemostatic platelet plug forms. In addition to releasing ADP and thromboxane A₂, platelets also expose platelet factor 3, which promotes the clotting cascade, ultimately resulting in thrombin generation and fibrin deposition at the site of injury. Thrombin also binds to receptors on the platelet membrane and causes further platelet aggregation and release. The ultimate result is a mixed clot composed of aggregated platelets and polymerized fibrin (Handin, R.I., Chapter 154 in *Harrison's Principles of Internal Medicine*, 11th ed., Braunwald *et al.*, Eds., McGraw-Hill Book Co., New York (1987), pages 266-272).

Although necessary for normal hemostasis, clot formation resulting from platelet aggregation and release reaction is now recognized as being responsible for, or contributing to, a variety of life-threatening vascular diseases, such as myocardial infarction, stroke, peripheral arterial occlusion and other blood system thromboses. In patients suffering from such diseases, clot formation is an undesirable event, and inhibiting or controlling clot formation is prophylactic or therapeutic for such patients.

2. *Acetylsalicylic Acid*

Commonly known as aspirin, acetylsalicylic acid is commonly described as an analgesic (see, e.g., p. 16 of *The Merck Index*, 11th Ed., Budavari *et al.*, eds., Merck & Co., Rahway, NJ (1989)). Recently, however, ASA has been investigated for use as a therapeutic anticoagulant, although the degree of therapeutic value of ASA treatment for specific

cardiovascular conditions remains a subject of controversy (Pasternak, R.C.,
et al., Chapter 190 in *Harrison's Principles of Internal Medicine*, 11th ed.,
Braunwald et al., Eds., McGraw-Hill Book Co., New York (1987), page 987;
Aldhous, P., *Science* 263:24 (1994)). ASA is synthesized by known methods
(e.g., German Patent No. 236,196 (1908)).

3. Heparin

Heparin is a complex linear polysaccharide of 60 to 100 kilodaltons
that, due to a series of modification reactions that do not go to completion, is
comprised of a variety of oligosaccharide structures. The use of heparin,
which is water-soluble, to prevent the *in vitro* clotting of blood removed from
the body, eventually led to its *in vivo* use to treat venous thromboembolism,
in which thrombin activity is responsible for the development or expansion of
a thrombus (Majerus, P.W., et al., Chapter 55 in *Goodman and Gilman's The
Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY
(1990)).

Heparin is an indirect thrombin inhibitor which acts by increasing, by
at least 1,000-fold, the rate at the inhibition of thrombin by antithrombin III,
a member of the "serpin" family of serine protease inhibitors (Olson, S.T.,
J. Biol. Chem. 263:1698-1708 (1988); Tollefsen, D.M., pages 257-273 in
Heparin: Chemical and Biological Properties, Clinical Applications, Lane,
D.A., et al., eds., Edward Arnold Publishers, Ltd., London (1989)).
Recently, it has become clear that heparin has additional activities *in vivo*
including but not limited to (1) increasing the rate at which HCII (heparin
cofactor II), another member of the "serpin" family of serine protease
inhibitors, inhibits thrombin; (2) promoting the binding of thrombin to fibrin
polymers (Derechin, V.M., et al., *J. Biol. Chem.* 265:5623-5628 (1990);
Hogg, P.J., et al., *J. Biol. Chem.* 265:241-247 (1990)).

Unfractionated Heparin is widely used in AMI patients as adjunctive
antithrombotic therapy. Because of its mode of action, heparin is useful to

prevent only thrombin-induced platelet aggregation. Even in that application, however, the overall efficacy of heparin is questionable. Furthermore, therapy with heparin is limited by several factors, including but not limited to the following.

5 First, heparin produces many undesirable side effects, including hemorrhaging, heparin-induced thrombocytopenia, abnormalities in hepatic function, osteoporosis, hyperkalemia and the like (Majerus, P.W., *et al.*, Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)).

10 Second, many blood proteins, including platelet factor 4, secreted by activated platelets, and lactoferrin, secreted by activated neutrophils, inactivate heparin. Histidine-rich glycoprotein and S-protein (vitronectin) also competitively inhibit the binding of antithrombin to heparin (Majerus, P.W., *et al.*, Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)). Due to a variable
15 degree of protein binding and heparin inactivation, the dosing of heparin has to be individually adjusted for each patient, and the clotting time (i.e., the activated partial thromboplastin time (aPTT)) of the patient's blood must be closely monitored during the course of treatment (Majerus, P.W., *et al.*,
20 Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)).

 Third, immunological responses limit heparin use in some patients. In about 5 to 10% of patients treated with heparin for more than 5 days intravenously, antibodies are induced. In about 0.2 to 0.5% of patients
25 treated with unfractionated heparin, the heparin induced antibodies result in thrombocytopenia and severe thromboembolic complications. In patients suffering from heparin-induced thrombocytopenia, immune-mediated thrombocytopenia may have dire thrombotic consequences, as heparin actually accelerates platelet aggregation, often with fatal consequences.

Thus, prior to the present invention, a need existed for an adjunctive antithrombotic therapy for AMI that utilizes a thrombin inhibitor that does not have the undesirable characteristics of heparin.

4. *Hirudin and Variants Thereof*

5 Hirudin is a family of related polypeptides, each of about 65 amino acids in length, the first member of which was isolated from the leech *Hirudo medicinalis* (Markwardt, F., *Methods Enzymol.* 19:924-932 (1970)). At least two different isospecific forms of hirudin, HV-1 and HV-2, have been sequenced and have been shown to differ slightly in amino acid sequence
10 (Harvey, R.P., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83:1084-1088 (1986)). Both HV-1 and HV-2 comprise a single polypeptide chain protein containing 65 amino acids in which the amino terminus primarily comprises hydrophobic amino acids and the carboxyl terminus typically comprises polar amino acids. Sequence analysis of naturally occurring forms of hirudin revealed homologies
15 of about 80%, and six cysteine residues were assigned to three disulfide bonds connecting Cys⁶ to Cys¹⁴, Cys¹⁶ to Cys²⁸, and Cys²² to Cys³⁹ (Dodt, J., *et al.*, *Biol. Chem. Hoppe-Seyler* 366:379-385 (1985); Mao, S.J.T., *et al.*, *Anal. Biochem.* 161:514-518 (1987); Harvey, R.P., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83:1084-1088 (1986)). All forms of hirudin are characterized by an
20 N-terminal domain (residues 1-39) stabilized by the three disulfide bridges, and a highly acidic C-terminal segment (residues 40-65) characterized by the presence of a tyrosine residue at amino acid position 63 which is sulfated. The three-dimensional structure of hirudin in solution has been determined
25 (Clore, G.M., *et al.*, *EMBO J.* 6:529-537 (1987); Haruyama *et al.*, *Biochemistry* 28:4301-4312 (1989)).

Hirudin is a direct thrombin inhibitor with a very high affinity for human thrombin (Stone, S.R., *et al.*, *Biochemistry* 25:4622-4628 (1986)) as well as thrombin from other mammalian species (Dodt, J., *et al.*, *FEBS Lett.* 229:87-90 (1988)). One molecule of hirudin binds to one molecule of

thrombin, forming a tight, noncovalent complex and thereby inactivating thrombin essentially irreversibly, i.e., the conversion of fibrinogen to fibrin and the thrombin-mediated activation of platelets is blocked. In contrast to heparin, hirudin also inactivates clot-bound thrombin (Weitz *et al.*, *J. Clin. Invest.* 86:385-391 (1990)). No antithrombin III or any other cofactor is required for inactivation of thrombin. Hirudin is not inactivated by platelet factor 4, and does not induce thrombocytopenia or severe thromboembolic complications (Markwardt, F., *et al.*, *Thromb. Haemostasis* 52:160-163 (1984); Talbot, M., *Semin. Thromb. Hemost.* 15:293-301 (1989); Prager, N.A., *et al.*, *J. Am. Coll. Cardiol.* 22:296-301 (1993)). In animal studies, hirudin exhibits low toxicity, little or no antigenicity and a very short clearance time from circulation (Markwardt, F., *et al.*, *Thromb. Haemostasis* 47:226-229 (1982); Heras, M., *et al.*, *Circulation* 79:657-665 (1989)).

In comparison to heparin, plasma levels of hirudin are much more stable and reproducible, as protein binding is rather low; as a result, there is less interindividual variety of treatment for hirudin as contrasted to heparin (Badimon, L., *et al.*, *Trends Cardiovasc. Med.* 1:261-267 (1991)). In several pilot studies in AMI patients undergoing thrombolysis, treatment with recombinant hirudin in conjunction with ASA has shown some promising results (Neuhaus *et al.*, *J. Am. Coll. Cardiol.* 21:418A (1993); von Essen *et al.*, *Ann. Hematol.* 68 (Suppl. II):A43 (1994); Cannon *et al.*, *J. Am. Coll. Cardiol.* 23:993-1003 (1994)). Treatment of patients having unstable angina with recombinant hirudin in combination with ASA has also been examined (Topol, E.J., *et al.*, *Circulation* 89:1557-1566 (1994)).

Methods for the preparation and biological or biochemical evaluation of various natural forms of hirudin, as well as many variants of hirudin (a.k.a. "hirulogs"), have been described. In an effort to create a greater supply of hirudin, attempts have been made to produce the polypeptide through recombinant DNA techniques. The presence of an O-sulfated tyrosine residue on native hirudin and the inability of microorganisms to perform a similar protein modification made the prospect of recombinant production of

biologically active hirudin highly speculative. However, desulfato-hirudins are almost as active as their sulfated counterparts (U.S. Patent Nos. 4,654,302 (1987) and 4,801,576 (1989)), and recombinant (i.e., desulfato-) hirudin has been produced in *Escherichia coli* (European patent application Nos. 158,564 (1985), 168,342 (1986), 171,024 (1986), 448,093 (1991), and 506,883 (1994)) and yeast (European patent application Nos. 200,655 (1986) and 252,854 (1988)). Furthermore, methods are known in the art for effecting the O-sulfation, or comparable modifications, of recombinant hirudin *in vitro* (U.S. Patent Nos. 225,633 (1987), 4,745,177 (1988), and 5,256,559 (1993)).

Efforts have been made to identify variants of hirudin, including peptide fragments of hirudin, which are effective in inhibiting clotting. For example, hirudin-PA and its derivatives are structurally very similar to hirudin; however, at several important places on the protein chain, hirudin-PA and its derivatives differ in a characteristic manner from the sequence of hirudin. In hirudin-PA the strongly acid sulfate monoester group on the phenolic hydroxyl of the tyrosine group in position 64 (position 63 in hirudin) is conspicuous (U.S. Patent No. 4,767,742 (1988)). Oligopeptides and peptidomimetic analogs derived from hirudin, as well as mutant forms of hirudin, having anticoagulant and platelet inhibitory effects are known in the art (U.S. Patent Nos. 5,256,559 (1993), 4,654,302 (1987), and 4,668,662 (1987); European Patent Appl. No. 576,792 (1994); International Patent Appl. Nos. WO 86/03517, WO 92/08736 (1992), and WO 94/080034 (1994)). Furthermore, methods are known in the art for producing soluble, biocompatible compositions comprising hirudins (U.S. Patent No. 5,112,615 (1992)).

Incorporation by Reference

The entire text of all publications cited above and below are hereby incorporated by reference.

Summary of the Invention

5 We have discovered that the administration of the combination of
hirudin and ASA to patients not undergoing treatment with any thrombolytic
agent, inhibits and/or prevents myocardial or cardiovascular events in the
patient treated thereby. Myocardial or cardiovascular events include, but are
not limited to, myocardial infarctions of various degrees of severity, strokes,
coronary revascularization and cardiovascular death. In particular, we have
discovered that the administration of the combination of hirudin and ASA to
patients is effective in preventing the recurrence of acute myocardial
10 infarctions (AMI).

The invention is also directed to a pharmaceutical composition
comprising the synergistic combination of hirudin and ASA, in the absence of
any thrombolytic agent. The pharmaceutical composition of the invention is
particularly useful in the therapeutic and prophylactic methods of the
15 invention.

Detailed Description of the Preferred Embodiments

A preferred method of the invention includes the administration of
ASA (aspirin) in combination with hirudin to a patient such that the
occurrence of one or more myocardial or cardiovascular events is thereby
20 inhibited and/or prevented, wherein the patient being treated is not undergoing
treatment with any thrombolytic agent. The term "myocardial or
cardiovascular events" is meant to include, but not be limited to, myocardial
infarctions of various degrees of severity, strokes, coronary revascularization
and cardiovascular death. By the term "inhibited and/or prevented" is
25 intended the prevention, amelioration, or both, of such events. Thus, the
invention includes both a therapeutic and a prophylactic modality.

Included as well in the present invention are pharmaceutical
compositions comprised of ASA and hirudin in combination with a

pharmaceutically acceptable carrier. Because the compositions of the invention are intended for treatment of patients who are not undergoing treatment with any thrombolytic agent, such agents are excluded from the composition of the invention. The term "thrombolytic agent" includes streptokinase, urokinase, tissue Plasminogen Activator (t-PA or, when produced via recombinant DNA technology, rt-PA), anisoylated-Plasminogen-Streptokinase activator complex (APSAC), and the like (Majerus, P.W., *et al.*, Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)).

By the term "ASA" is intended acetylsalicylic acid, also known as aspirin, 2-(acetyloxy)benzoic acid 2-carboxyphenyl ester, 2-hydroxybenzoic acid acetate 2-carboxyphenyl ester, salicylacetylic acid, and Disposal Acetate. Also included by the term "ASA" are derivatives thereof having the biological activity of ASA.

The dosage of ASA varies according to different embodiments of the invention, as well as the age, size and medical condition of the patient. Thus, in one embodiment, the dose of ASA does not vary over the period of treatment and is about 50 to about 500 mg/day, in particular about 80 to about 300 mg/day, and preferably about 80 to about 100 mg/day. In another embodiment, the dose of ASA is about 160 to about 325 mg/day for the first 1 to 5 days following an AMI, and is about 80 to about 160 mg/day for on subsequent days. In all embodiments, ASA is preferably administered orally, and, in therapeutic modalities, is preferably administered beginning as soon as possible after a diagnosis of AMI has been made and continuing for up to about 3 to 6 months after the event.

By the term "hirudin" is intended hirudin family members isolated from their natural sources; recombinant hirudin, produced by isolating a gene encoding a hirudin protein and introducing the gene into an appropriate vector/host system for production of hirudin; and synthetic oligopeptides and peptidomimetics derived from hirudin having the anticoagulant activity of hirudin. There are many *in vitro* and *in vivo* methods known in the art for

determining the anticoagulant activity of a particular hirudin. For example, *in vitro* tests include (1) determinations of inhibition of platelet aggregation, thrombin-induced or otherwise, by a hirudin variant (often as measured by the activated partial thromboplastin time or aPTT), (2) determinations of thrombin-induced serotonin release and/or thromboxane-A₂ generation, and/or (3) determinations of inhibition of thrombin's enzymatic activity on chromogenic substrates such as Chromozym TH (U.S. Patent Nos. 5,256,559 (1993), 5,246,715 (1993), 5,242,810 (1993), 5,227,469 (1993), 4,832,849 (1989), and 4,801,576 (1989)). The term "hirudin" includes, but is not limited to, hirudin HV1, desulfato hirudin, (Ile^{1,2})-desulfato hirudin HV1, (Gly¹)-desulfato hirudin HV1, (Gln^{57,58,61,62})-desulfato hirudin HV1, (Pro⁶⁶)-desulfato hirudin HV1, (Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Met⁰, Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Gln²⁷, Gln³⁶, Arg⁴⁷)-desulfato hirudin HV1, (des-Val¹, Thr²)-desulfato hirudin HV1, hirudin HV2, (Lys⁴⁷)-hirudin HV2, and hirudin PA.

The dosage of hirudin, and means of administration thereof, vary according to different embodiments of the invention, as well as the age, size and medical condition of the patient. Thus, in one embodiment, the dose of hirudin does not vary over time and is 0.05 to 0.2 mg/kg body weight/day, preferably 0.1 to 0.15 mg/kg body weight/day, and is administered intravenously. In a related embodiment, the dose of hirudin does not vary over time and is 0.25 to 1.0 mg/kg body weight/12 hours, preferably about 0.5 mg/kg body weight/12 hours, and is administered subcutaneously. In another embodiment, an initial dose of hirudin is 0.2 to 0.4 mg/kg body weight administered intravenously in a bolus followed by a current infusion of 0.05 to 0.2 mg/kg body weight/day of hirudin, preferably 0.1 to 0.15 mg/kg body weight/day hirudin. In all embodiments, hirudin is preferably administered orally, and, in therapeutic modalities, is preferably administered beginning as soon as possible after a diagnosis of AMI has been made and continuing for up to about 3 to 6 months after the event.

By the term "patients not undergoing thrombolytic treatment" is intended those patients suffering from AMI for whom thrombolytic therapy is not available due to the presence of one or more medical contraindications or for any other reason. Medical contraindications to thrombolytic therapy include, but are not limited to, (1) surgery (e.g., organ biopsy, puncture of noncompressible vessels, serious trauma or cardiopulmonary resuscitation) within 10 days, (2) serious gastrointestinal bleeding within three months, (3) history of hypertension, (4) active bleeding or hemorrhagic disorder and (5) previous cerebrovascular accident or active intracranial process) (Majerus, P.W., *et al.*, Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)). Additionally, patients may elect to not pursue thrombolytic therapy for religious or other personal reasons. By the terms "thrombolytic therapy" and "thrombolytic treatment" is meant the administration of one or more thrombolytic agents to a patient in need thereof. Thrombolytic agents include, but are not limited to, streptokinase, urokinase, tissue Plasminogen Activator (t-PA or, when produced via recombinant DNA technology, rt-PA), anisoylated-Plasminogen-Streptokinase activator complex (APSAC), and the like (Majerus, P.W., *et al.*, Chapter 55 in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Pergamon Press, NY (1990)).

Acetylsalicylic Acid in Patients with Acute Myocardial Infarction

As shown in a large, double-blind, randomized and placebo-controlled trial, the use of acetylsalicylic acid (ASA) reduces mortality, reinfarctions and strokes in patients with acute myocardial infarction (AMI). Specifically, in the Second International Study of Infarct Survival (ISIS-2), performed on 17,187 patients with AMI treated within 24 hours of the onset of symptoms, daily treatment with 162.5 mg of ASA reduced the 35-day mortality by 23 % (ISIS-2 Collaborative Group, *Lancet* ii:349-360 (1988)). In addition, the incidence of nonfatal reinfarctions decreased by 49 % and of nonfatal stroke

by 46%. There was no increase in major bleeds (defined as bleeds requiring transfusions) (i.e., 31% with ASA versus 33 % with placebo), and only a modest increase (0.6%) of minor bleeds (ISIS-2 Collaborative Group, *Lancet* ii:349-360 (1988)). The beneficial effects of ASA were observed in AMI patients undergoing thrombolysis, as well as in those not receiving thrombolysis.

Heparin and ASA for Treatment of AMI Patients

Despite the fact that heparin is widely used in AMI, evidence for its net benefits in patients receiving an adequate dose of ASA is sparse. In the ISIS-2 trial, the effects of the thrombolytic agent, streptokinase, alone or in combination with ASA, and ASA alone were compared against placebo in a two-by-two factorial design. The trial provided conclusive evidence of the beneficial effects of thrombolytic therapy with streptokinase, and of antithrombotic therapy with ASA, with the benefits being largely additive (ISIS-2 Collaborative Group, *Lancet* ii:349-360 (1988)). Furthermore, in subgroup analyses, patients receiving heparin in addition to ASA were exploratively compared with patients receiving only ASA. No increased clinical benefit due to the additional use of heparin was demonstrable.

Other studies have suggested that heparin treatment might have negative effects when used in combination with ASA. In ISIS-3 (ISIS-3 Collaborative Study Group, *Lancet* 339:753-770 (1992)), 41,299 patients were treated with ASA, thrombolytic drugs (i.e., streptokinase, recombinant tissue Plasminogen Activator (rt-PA), or anisoylated-plasminogen-streptokinase activator complex (APSAC)), and either (1) subcutaneous heparin started four hours after randomization or (2) no heparin. The daily dose of ASA was 162.5 mg. The 35-day mortality in the group treated with heparin (10.3%) was similar to that of the group without heparin (10.6%). However, the incidence of probable cerebral hemorrhages or cerebral hemorrhages was significantly higher in the group treated with heparin. The GISSI-2 study

(Gruppo Italiano per lo Studio della Sopravvivenza Nell'Infarto Miocardico, *Lancet* 83:65-71 (1990)) showed similar results. Overall, there was no significant benefit with regard to mortality, but a 2 per 1,000 increase in risk of cerebral hemorrhage.

5 The Global Utilization of Streptokinase and Tissue Plasminogen Activator to Treat Occluded Arteries (GUSTO-1) trial (The GUSTO Investigators, *N. Engl. J. Med.* 329:673-682 (1993)) compared different thrombolytic agents (i.e., streptokinase and rt-PA) and the adjunctive therapy of immediate intravenous or delayed subcutaneous therapy with heparin in
10 those who received streptokinase. All patients received a sufficient dose of ASA. There was no difference in benefit of heparin between the two modes of administration, i.e., intravenously (I.V.) and subcutaneously (S.C.), as adjunctive therapy to streptokinase. The 30-day mortality in the group receiving heparin I.V. (7.4%) was comparable to that in the group receiving
15 heparin S.C. (7.2%). Furthermore, there was no evidence for less reinfarction, stroke, or bleeding with immediate I.V. heparin.

Overall, the available data are inconclusive with regard to a net clinical benefit of heparin in AMI patients treated with ASA (Ridker *et al.*, *Lancet* 341:1574-1577 (1993); Fibrinolytic Therapies Trialists Collaborative Group, *Lancet* 343:311-322 (1994)), whether or not the patients received thrombolytic
20 therapy.

Use of Hirudin in AMI Patients Not Undergoing Thrombolysis

This invention relates to the use of hirudin in combination with ASA in AMI patients not undergoing thrombolysis. Currently, more than 50% of
25 all AMI patients in the U.S.A. and a variable proportion of AMI patients in Europe (from 30% in UK to about 60 to 70% in France) are not treated by thrombolysis. The reasons for this can be, e.g., a long delay from onset of symptoms to treatment, an uncharacteristic ECG, old age, uncontrolled hypertension, recent stroke, or gastroduodenal ulcer. For this patient group

it has been shown that mortality rates and the incidence of reinfarction and of other clinical complications are higher than in patients undergoing thrombolysis.

5 At present it is established that ASA confers clinical benefit to these patients via the partial inactivation of platelets. The additional use of hirudin with the inactivation of thrombin is expected to result in additional net clinical benefit resulting in a reduction of mortality or reinfarctions, as well as other clinical complications.

10 The results of several trials raise the possibility that the combination of recombinant hirudin and ASA with differential antithrombotic mode of action seems to be superior to the combination of heparin and ASA or to ASA alone. Recently it has been demonstrated that adjunctive antithrombotic therapy with recombinant hirudin in addition to thrombolysis with rt-PA or streptokinase resulted in a higher incidence of early, complete and sustained
15 patency of the infarct-related coronary artery. In other clinical studies it was demonstrated that a higher incidence of early and complete patency correlates with a reduced mortality (Neuhaus *et al.*, *J. Am. Coll. Cardiol.* 21:418A (1993); von Essen *et al.*, *Ann. Hematol.* 68 (Suppl. II):A43 (1994); Cannon *et al.*, *J. Am. Coll. Cardiol.* 23:993-1003 (1994)). In addition it has been
20 demonstrated that open vessels are important also for AMI patients not undergoing thrombolysis (GUSTO Angiographic Investigators, *N. Engl. J. Med.* 329:1615-1622 (1993); Vogt *et al.*, *J. Am. Coll. Cardiol.* 21:1391-1395 (1993)).

25 Currently, the need of improved therapy of AMI patients who cannot be treated by thrombolysis has not been specifically addressed in preclinical models, which only focused e.g., on reocclusions. No clinical study addresses the question of how to improve treatment of MI patients with Q-wave infarctions not treated by thrombolysis. The ongoing GUSTO-2b study assesses the treatment of patients with non-Q wave myocardial infarction in
30 a subgroup of patients with acute coronary syndromes. This study will not definitely answer the question of optimal treatment of patients with AMI not

undergoing thrombolysis, as only a specific subgroup of these patients are assessed.

5 From preliminary studies we deduce that the combined use of hirudin and ASA in AMI patients where treatment with thrombolysis is not advisable will result in a higher incidence of open coronary vessels. In addition, spontaneous reperfusion via the endogenous thrombolytic system will be increased by the use of hirudin. This innovative and original combination of platelet inhibition and direct thrombin inhibition results in net clinical benefit in AMI patients not undergoing thrombolytic treatment.



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What Is Claimed Is:

1. A method of treating or preventing acute myocardial infarction in a patient comprising the administration of ASA and hirudin.

2. The method of claim 1, wherein said ASA and said hirudin are separate unit dosage forms.

3. The method of claim 1, wherein said ASA and said hirudin are together in a single unit dosage form.

4. The method of claim 1, wherein said patient is not being treated with a thrombolytic agent.

5. The method of claim 1, wherein said hirudin is selected from the group consisting of

(a) hirudin variant HV1 having the formula

Val Val Tyr Thr Asp Cys Thr Glu Ser Gly
Gln Asn Leu Cys Leu Cys Glu Gly Ser Asn
Val Cys Gly Gln Gly Asn Lys Cys Ile Leu
Gly Ser Asp Gly Glu Lys Asn Gln Cys Val
Thr Gly Glu Gly Thr Pro Lys Pro Gln Ser
His Asn Asp Gly Asp Phe Glu Glu Ile Pro

W
|
Glu Glu Tyr Leu Gln,

wherein W represents the phenolic hydroxy group of Tyr (desulfato hirudin HV1) or a group of the formula $-O-SO_3H$ (hirudin HV1),

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(b) hirudin variant HV2 having the formula

5

Ile Thr Tyr Thr Asp Cys Thr Glu Ser Gly Gln Asn Leu Cys
Leu Cys Glu Gly Ser Asn Val Cys Gly Lys Gly Asn Lys Cys
Ile Leu Gly Ser Asn Gly Lys Gly Asn Gln Cys Val Thr Gly
Glu Gly Thr Pro Asn Pro Glu Ser His Asn Asn Gly Asp Phe
Glu Glu Ile Pro Glu Glu Tyr Leu Gln,

and

(c) hirudin variant PA having the formula

10

Ile Thr Tyr Thr Asp Cys Thr Glu Ser Gly Gln Asn Leu Cys
Leu Cys Glu Gly Ser Asn Val Cys Gly Lys Gly Asn Lys Cys
Ile Leu Gly Ser Gln Gly Lys Asp Asn Gln Cys Val Thr Gly
Glu Gly Thr Pro Lys Pro Gln Ser His Asn Gln Gly Asp Phe
Glu Pro Ile Pro Glu Asp Ala Tyr Asp Glu.

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6. The method of claim 1, wherein said hirudin is selected from the group consisting of (Ile^{1,2})-desulfato hirudin HV1, (Gly¹)-desulfato hirudin HV1, (Gln^{57,58,61,62})-desulfato hirudin HV1, (Pro⁶⁶)-desulfato hirudin HV1, (Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Met⁰, Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Gln²⁷, Gln³⁶, Arg⁴⁷)-desulfato hirudin HV1, (des-Val¹, Thr²)-desulfato hirudin HV1 and (Lys⁴⁷)-hirudin HV2.

20

7. The method of claim 1, wherein a human or animal patient is treated.

8. The method of claim 1, wherein said ASA is given orally.

9. The method of claim 1, wherein said hirudin is administered intravenously or subcutaneously.



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10. A pharmaceutical composition comprising ASA and hirudin.

11. The pharmaceutical composition of claim 10 additionally comprising one or more pharmaceutically acceptable carriers.

5 12. The pharmaceutical composition of claim 10, wherein said pharmaceutical composition does not contain any thrombolytic agents.

10 13. The composition of claim 10, wherein said hirudin is selected from the group consisting of hirudin HV1, desulfato hirudin, (Ile^{1,2})-desulfato hirudin HV1, (Gly¹)-desulfato hirudin HV1, (Gln^{57,58,61,62})-desulfato hirudin HV1, (Pro⁶⁶)-desulfato hirudin HV1, (Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Met⁰, Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Gln²⁷, Gln³⁶, Arg⁴⁷)-desulfato hirudin HV1, (des-Val¹, Thr²)-desulfato hirudin HV1, hirudin HV2, (Lys⁴⁷)-hirudin HV2, and hirudin PA.



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**Method of Treating Acute Myocardial Infarction
with Hirudin and Acetylsalicylic Acid in
Patients Not Undergoing Thrombolytic Treatment**

Abstract

5 The invention relates to the discovery that the administration of the
combination of hirudin and Acetylsalicylic Acid (ASA) to patients not
undergoing treatment with any thrombolytic agent, inhibits and/or prevents
myocardial or cardiovascular events in the patient. The invention further
relates to the pharmaceutical combination of a platelet inactivating drug,
10 acetylsalicylic acid, and a direct thrombin inhibitor, hirudin, for the treatment
of patients with acute myocardial infarction who are not undergoing
thrombolysis. A pharmaceutical composition comprising the synergistic
combination of hirudin and ASA, in the absence of any thrombolytic agent,
is particularly useful in the therapeutic and prophylactic methods of the
15 invention.

WHAT IS CLAIMED IS:

1. A use of a composition containing ASA and hirudin for preparing a pharmaceutical composition for treating or preventing acute myocardial infarction in a subject in need of such treatment, wherein said hirudin is selected from the group consisting of (Ile^{1,2})-desulfato hirudin HV1, (Gly¹)-desulfato hirudin HV1, (Gln^{57,58,61,62})-desulfato hirudin HV1, (Pro⁶⁶)-desulfato hirudin HV1, (Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Met⁰, Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Gln²⁷, Gln³⁶, Arg⁴⁷)-desulfato hirudin HV1, (des-Val¹, Thr²)-desulfato hirudin HV1 and (Lys⁴⁷)-hirudin HV2.

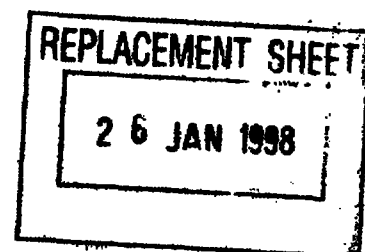
2. A use of a composition containing ASA and hirudin for preparing a pharmaceutical composition for treating or preventing acute myocardial infarction in a subject in need of such treatment, wherein said hirudin is selected from the group consisting of

(a) hirudin variant HV1 having the formula

Val Val Tyr Thr Asp Cys Thr Glu Ser Gly
 Gln Asn Leu Cys Leu Cys Glu Gly Ser Asn
 Val Cys Gly Gln Gly Asn Lys Cys Ile Leu
 Gly Ser Asp Gly Glu Lys Asn Gln Cys Val
 Thr Gly Glu Gly Thr Pro Lys Pro Gln Ser
 His Asn Asp Gly Asp Phe Glu Glu Ile Pro
 W
 |
 Glu Glu Tyr Leu Gln,

wherein W represents the phenolic hydroxy group of Tyr (desulfato hirudin HV1) or a group of the formula -O-SO₃H (hirudin HV1),

(b) hirudin variant HV2 having the formula



Ile Thr Tyr Thr Asp Cys Thr Glu Ser Gly Gln Asn Leu Cys
Leu Cys Glu Gly Ser Asn Val Cys Gly Lys Gly Asn Lys Cys
Ile Leu Gly Ser Asn Gly Lys Gly Asn Gln Cys Val Thr Gly
Glu Gly Thr Pro Asn Pro Glu Ser His Asn Asn Gly Asp Phe
Glu Glu Ile Pro Glu Glu Tyr Leu Gln,

and

(c) hirudin variant PA having the formula

Ile Thr Tyr Thr Asp Cys Thr Glu Ser Gly Gln Asn Leu Cys
Leu Cys Glu Gly Ser Asn Val Cys Gly Lys Gly Asn Lys Cys
Ile Leu Gly Ser Gln Gly Lys Asp Asn Gln Cys Val Thr Gly
Glu Gly Thr Pro Lys Pro Gln Ser His Asn Gln Gly Asp Phe
Glu Pro Ile Pro Glu Asp Ala Tyr Asp Glu.

3. The use of claim 1 or 2, wherein said ASA and said hirudin are separate unit dosage forms.

4. The use of claim 1 or 2, wherein said ASA and said hirudin are together in a single unit dosage form.

5. The use of claim 1 or 2, wherein a thrombolytic agent is not used in the subject in need of such treatment.

6. The use of claim 1 or 2, wherein the subject in need of such treatment is a human or animal.

7. The use of claim 1 or 2, wherein said ASA is given orally.



REPLACEMENT SHEET

26 JAN 1998

8. The use of claim 1 or 2, wherein said hirudin is administered intravenously or subcutaneously.

9. A pharmaceutical composition comprising ASA and hirudin, wherein said hirudin is selected from the group consisting of hirudin HV1, desulfato hirudin, (Ile^{1,2})-desulfato hirudin HV1, (Gly¹)-desulfato hirudin HV1, (Gln^{57,58,61,62})-desulfato hirudin HV1, (Pro⁶⁶)-desulfato hirudin HV1, (Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Met⁰, Gln²⁷, Arg⁴⁷)-desulfato hirudin HV1, (Gln²⁷, Gln³⁶, Arg⁴⁷)-desulfato hirudin HV1, (des-Val¹, Thr²)-desulfato hirudin HV1, Hirudin HV2, (Lys⁴⁷)-hirudin HV2, and hirudin PA.

10. The pharmaceutical composition of claim 9 additionally comprising one or more pharmaceutically acceptable carriers.

11. The pharmaceutical composition of claim 9, wherein said pharmaceutical composition does not contain any thrombolytic agents.

