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(54) Title: METHODS FOR THE TREATMENT OF THROMBOSIS

(57) Abstract

Patients having a predisposition for thrombus formation are effectively treated with compositions of acetylsalicylic acid and citric acid, and optionally a zinc salt. The combination of aspirin and citric acid has been found to be significantly more effective in inhibiting platelet aggregability than aspirin alone. Accordingly, new methods and compositions are disclosed for reducing the thrombotic potential of human or animal subjects, such as in the prophylaxis or treatment of atherosclerosis, vascular surgery patients or other cardiovascular diseases.

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METHODS FOR THE TREATMENT OF THROMBOSIS

Field of the Invention

This invention relates to methods and compositions for the treatment or prophylaxis of thrombosis. More particularly, this invention provides methods and compositions for reducing platelet aggregation in the circulating blood of a patient.

Background of the Invention

At least half of the victims of cardiovascular disease (CVD) are asymptomatic until the occurrence of a major vascular obstruction due to thrombus formation. Such an event frequently results in the loss of vital circulation, often endangering life and limb viability.

Thrombus formation involves a complex interaction of aggregated platelets and activated coagulation factors with the damaged vessel wall. Circulating non-activated platelets do not adhere to normal endothelium or to each other, but when the endothelial lining of a vessel is broken, such platelets adhere to the exposed subendothelial collagen. This is the first step in the formation of hemostatic plugs, and requires participation of a protein made by endothelial cells called the von Willebrand (vW) factor. The vW factor is found both in the vessel wall and in plasma, and binds during platelet adhesion to a receptor present on the platelet surface membrane. Next, platelets are activated in reactions initiated by collagen and by thrombin formed at the injury site. These stimuli activate phospholipase C, an enzyme that hydrolyzes the membrane phospholipid, phosphatidyl inositol triphosphate. Products of this reaction activate protein kinase C and also increase the calcium concentration of platelet cytosol. As a result, a series of progressive, overlapping events ensue. The platelets change shape and develop long pseudopods. A receptor

is assembled on the platelet surface membrane, and fibrinogen and other adhesive proteins bind to this receptor causing platelets to stick to each other. Arachidonic acid is liberated from membrane phospholipids and undergoes oxidation to products that include prostaglandin H_2 (PGH_2), which serves as an important cofactor for collagen-induced platelet activation, and thromboxane A_2 (TxA_2), which can act itself as an additional platelet activator. The contents of platelets are secreted, including adenosine diphosphate (ADP) which can also stimulate platelet activation and recruit new platelets into the growing hemostatic plug.

Subsequent to platelet aggregation, fibrinogen in the circulating blood is converted to fibrin to physically tie the hemostatic platelet plug in place. The platelet surface undergoes a reorganization that exposes procoagulant phospholipids needed for enzyme/cofactor complexes of blood coagulation to form on the platelet surface. Secretion of platelet factor V from platelet a-granules provides a key component for one of the enzyme/cofactor complexes. As a result, thrombin is generated in increasing amounts on the platelet surface, and converts fibrinogen into fibrin with the formation of fibrin strands that radiate outward from aggregated platelets helping to secure the platelet plug to the site of injury. Additionally, a mechanism within the platelets is activated which results in contraction of platelet actinomycin. This compresses and consolidates the platelet plug, further securing it to the site of injury.

In the in vivo regulation of thrombus formation, platelet aggregation is mediated by the PGH_2 derivative prostacyclin (PGI_2). Prostacyclin is also a vasodilator and is believed to render the vessel lining inert to platelet interactions. Thus, TxA_2 and PGI_2 have opposing effects on platelet aggregation, and the degree of the physiological effect of each in the cardiovascular system on the regulation of thrombus formation is determined mainly by their quantitative balance (Bush, H.L., Jr. et al., "Favorable Balance of Prostacyclin and Thromboxane A_2 Improves Early Patency of Human In Situ Vein Grafts," *J. Vasc. Surg.*, **1**:149-159 (1984); Coker, S.J. et al., "Thromboxane and Prostacyclin Release From Ischemic Myocardium in Relation to Arrhythmias," *Nature*, **291**:323-334 (1981); Hunter, G.C. et al., "Arterial Wall Thromboxane: Dominance After Surgery Predisposes to Thrombosis," *J. Vasc. Surg.*, **1**:314-319 (1984); and Zmuda, A., et al., "Experimental Atherosclerosis in Rabbits: Platelet Aggregation, Thromboxane A_2 Generation and Antiaggregatory Potency of Prostacyclin," *Prostaglandins*, **14**:1035 (1977)).

Therapeutic treatments to alter thrombus formation have focused mainly on inhibition of the aggregation response. The most widely accepted agent for this

purpose is acetylsalicylic acid (ASA), or aspirin. In the arachidonic acid cascade, aspirin acts as a cyclooxygenase inhibitor, blocking the conversion of arachidonic acid to the PGH_2 precursor prostaglandin G_2 (PGG_2). Since PGG_2 is a precursor to both TxA_2 and PGI_2, aspirin blocks both the aggregation inducing and aggregation inhibiting effects of these factors, respectively. As an antithrombotic agent, aspirin has had varying degrees of success. Although minimal amounts of aspirin are required for platelet inhibition, most of the clinical experience relates to relatively large doses. The nonselective, potent inhibition of high-dose aspirin on both TxA_2 and PGI_2 has caused investigators to consider the theoretical advantage of the use of low-dose therapy. Preferred inhibition of proaggregatory TxA_2 in humans has been limited to single-dose aspirin administration or short-term cumulative effect. It has been further found that the degree and duration of aspirin's beneficial effect is highly dependent on each subject's inherent thrombotic potential (see Zammit, M. et al., "Aspirin Therapy in Small Caliber Arterial Prostheses; Long Term Experimental Observations," *J. Vasc. Surg.*, **1(6):**839-851 (1984)). For many individuals, aspirin has little or no discernable antiaggregatory activity, and is not effective in reducing a predisposition for thrombus formation.

To overcome some of the problems associated with aspirin therapy, it has been previously proposed to utilize certain thromboxane synthetase inhibitors (TSIs), which inhibit the formation of proaggregatory TxA_2 without interfering with the formation of antiaggregatory PGI_2. Various imidazole derivatives have been proposed for this purpose, for use either alone or in connection with low dose aspirin therapy. See, for example, Kaplan, S. et al., "A New Combination Therapy for Selective and Prolonged Antiplatelet Effect: Results in the Dog," *Stroke*, **17**:450-454 (1986). It has also been suggested that zinc ions exhibit an inhibitory activity toward collagen-induced platelet aggregation and serotonin release in vitro (Chapvil, M. et al., "Inhibitory Effect of Zinc Ions on Platelet Aggregation and Serotonin Release Reaction," *Life Sciences*, **(16):**561-572 (1975)) and toward platelet activating factor (PAF) in vitro (*Arch. Biochem. Biophys.*, **272(2):**466-475 (1989); *Arch. Biochem. Biophys.*, **260(2):**841-846 (1988)), and that hydroxyurea, citric acid and ascorbic acid inhibit plant lipoxygenase activity *in vitro* (Bekheet, I.A. et al., "The Effect of Some Inhibitors on the Activity of Lipoxygenase," *Alex. Sci. Exch.*, **7(3):**389-398 (1986)).

Summary of the Invention

It has now been found that patients having a predisposition for thrombus formation can be effectively treated with compositions comprising aspirin and citric acid, and optionally a pharmaceutically acceptable zinc salt. The combination of

aspirin and citric acid has been found to be significantly more effective in inhibiting platelet aggregability than aspirin alone. Accordingly, new methods are disclosed for reducing the platelet aggregability of human or animal subjects. The new methods are highly effective in the treatment of cardiovascular patients, such as in the prophylaxis or treatment of the thrombotic complications of atherosclerosis.

Brief Description of the Drawings

FIGURE 1 is a graph of a representative adenosine diphosphate (ADP)-induced platelet aggregation response showing the percent of light transmission through platelet rich plasma (PRP) as a function of time after induction of aggregation; and

FIGURE 2 is a graph of the ADP-induced *in vitro* platelet aggregation response in PRP with no treatment (baseline, represented by solid dots), and after exposure to sodium citrate (open squares), aspirin and sodium citrate (closed squares), aspirin alone (open circles), citric acid (closed triangles), aspirin and citric acid (open triangles), water alone (dotted line) or water and ethanol (dashed line).

Description of the Preferred Embodiments

In accordance with the present invention, it has been found that therapeutic compositions comprising aspirin and citric acid are significantly more effective for reducing platelet activity than aspirin alone. Accordingly, one aspect of the present invention provides a method for reducing a predisposition for thrombus formation in a patient comprising administering to the patient a therapeutically effective amount of a composition comprising aspirin and citric acid. In one particularly preferred embodiment, a patient is treated by administering to the patient amounts of aspirin, citric acid, and optionally a zinc salt, effective to reduce platelet aggregation, thereby decreasing the likelihood of thrombus formation by at least partially blocking the platelet aggregation pathway.

Other aspects of the invention provide therapeutic compositions comprising an amount of aspirin and citric acid effective to reduce platelet aggregation when administered to a patient. In a particularly preferred embodiment, the compositions of the invention comprise aspirin, citric acid and a zinc salt.

The methods and compositions of the invention may be used prophylactically or therapeutically in the treatment of patients who are at high risk of thrombus formation, such as in the treatment of atherosclerosis, vascular surgery patients, and patients with other types of cardiovascular disease.

In accordance with one aspect of the present invention, amounts of acetylsalicylic acid and citric acid effective to reduce platelet aggregation are

administered to the patient. Effective but nontoxic amounts of acetylsalicylic acid and citric acid may be readily determined by the ordinary skilled physician. The precise amounts required for this purpose will depend upon the body weight of the patient, physiological condition of the patient, thrombotic potential of the patient, desired effect, and other factors. However, for most purposes, at least about 0.5 mg of acetylsalicylic acid per kg of body weight of the patient per day, more preferably from about 2 to about 4 mg of acetylsalicylic acid per kg of body weight of the patient per day, and at least about 0.5 mg of citric acid per kg of body weight of the patient per day, more preferably from about 2 to about 4 mg of citric acid per kg of body weight of the patient per day is administered to the patient.

In yet another aspect, a patient is treated in accordance with the foregoing and by additionally administering to the patient an amount of a pharmaceutically acceptable zinc salt effective to result in a reduction in platelet aggregability of the patient. In most cases, at least about 0.01 mg of the zinc salt per kg of body weight of the patient per day will be effective for this purpose, but significantly larger doses may be employed, if desired. Suitable zinc salts include, for example, the pharmaceutically acceptable acid addition salts of Zn^{2+}, such as the hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, gluconic, fumaric, succinic, ascorbic, maleic, methanesulfonic, arginine or similar pharmaceutically acceptable salts. Presently particularly preferred salts include zinc acetate and zinc sulfate.

In another aspect of the invention, compositions are provided which comprise amounts of acetylsalicylic acid and citric acid effective to reduce platelet aggregation and an amount of a pharmaceutically acceptable zinc salt effective to result in a reduction in platelet aggregability of the patient, as described above, as well as pharmaceutically acceptable carriers. Preferred compositions of the invention are designed for unit dosage administration, such as, for example, for routine one dose a day administration, and may contain representative amounts of the active compounds within the following ranges:

Component	Amount (mg/dose)
acetylsalicylic acid	50 - 650
citric acid	50 - 650
zinc salt	0 - 100

In a presently particularly preferred illustrative embodiment for oral administration, compositions of the invention comprise from about 100 to about 200 mg/dose of acetylsalicylic acid and from about 100 to about 200 mg/dose of citric

acid. The compositions may additionally comprise from about 0.5 to about 2 mg/dose of a pharmaceutically acceptable zinc salt.

The compositions of the invention can be administered in any effective pharmaceutically acceptable form to warm blooded animals, e.g., in oral, parenteral or infusible dosage forms, in transdermal formulations or as a buccal or nasal spray. Suitable parenteral routes of administration include, for example, intramuscular, intravenous, intraperitoneal or subcutaneous administration of the compounds. For most purposes, oral administration will be preferred.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compounds may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills may additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions of the may also comprise adjuvants such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

In addition to the active compounds, compositions according to the invention for parenteral injection may comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, suspensions or emulsions. Examples of suitable nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Such compositions may also contain adjuvants, such as preserving, wetting, emulsifying and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or other sterile injectable medium, immediately before use.

The foregoing may be better understood in connection with the following examples, which are presented for purposes of illustration and not by way of limitation.

EXAMPLES

As used in the following examples, platelet aggregations were performed on a Payton dual channel aggregometer with an Omniscribe B-5000 chart recorder with platelet rich plasma (PRP) prepared from venous blood anticoagulated with 3.8% sodium citrate according to the turbidometric method of Born, GVR, "Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal," *Nature*, **194**:927-929 (1962). Platelet aggregations were induced with adenosine diphosphate (ADP). It has been previously shown that the rate and persistence of platelet aggregation in response to adenosine diphosphate can be used as a measure of the thrombotic potential of blood (see, Kaplan, S., et al., "The effect of Predetermined Thrombotic Potential of the Recipient on Small-Caliber Graft Performance," *J. Vasc. Surg.*, **3**:311-321 (1986), the disclosure of which is incorporated herein by this reference). Using autologous platelet poor plasma (PPP), the platelet count in PRP was adjusted to 250,000 ± 25,000 platelets/ml. Following a three minute period at 37°C, ADP was added to the PRP to a final concentration of 2.5 μM, and the change in aggregation response was assessed by degree of inhibition as represented by alteration of the aggregation curve. Referring to FIGURE 1 (a typical adenosine diphosphate-induced platelet aggregation curve depicting percent transmission (%Tran) as a function of time), platelet aggregation (PA) was determined by the following Equation (1):

$$PA = Amp\ R \cdot S_{sum} \cdot Plt\ R \qquad (1)$$

where Amp R is equal to Amp1 divided by A_{max} (see FIGURE 1), Amp1 is the height of the initial aggregation wave amplitude (FIGURE 1), A_{max} is the maximum height of the aggregation curve (Amp1 + Amp2, FIGURE 1), S_{sum} is the total aggregation area (i.e., $S_1 + S_2$, FIGURE 1), S_1 is the primary aggregation area (see FIGURE 1), S_2 is the secondary aggregation area, and Plt R is a normalization parameter determined by the ratio of the actual platelet count divided by the adjusted platelet count.

Example 1

Sixteen human patients shown to have an inadequate platelet aggregation response to aspirin administration alone, were given daily doses of a composition having the following formulation:

aspirin	158 mg
citric acid	158 mg
thiamine hydrochloride	8 mg
zinc acetate	1 mg
Total per dose	325 mg

The PA score (expressed in % transmission · minutes) of each patient was determined prior to treatment with any medication (NM) and/or after aspirin treatment alone (ASA), and then again after varying periods of daily administration of a single dose of the composition set forth above (Post Treatment). The results are shown in the following Table 1:

TABLE 1

	PA		
ID	NM	ASA	PT
1	nd	17.24	9.34
2	nd	11.81	8.90
3	nd	14.44	8.44
4	nd	9.76	6.27
5	nd	20.18	12.30
6	nd	10.31	11.11
7	nd	5.37	3.33
8	16.67	16.60	8.3
9	nd	6.22	3.11
10	nd	7.33	6.04
11	nd	11.51	6.75
12	nd	11.38	6.50
13	24.17	11.20	8.96
14	17.87	11.92	11.42
15	nd	24.76	13.68
16	nd	3.90	3.25
MV	19.57±4.03	12.12±5.53	7.98±3.21

NM = no medication
ASA = aspirin alone
PT = post treatment
nd = not determined
MV = mean value

As can be seen from Table 1, the administration of aspirin (ASA) alone results in a partial reduction in platelet aggregation in humans, as is known in the art. However, the daily administration of a representative composition of the invention unexpectedly results in a further substantial reduction in platelet aggregation over that elicited by aspirin alone.

Example 2

To demonstrate the effect of various components on platelet aggregation *in vitro*, eight identical samples of platelet rich plasma (PRP) from dog blood were treated with the components described below, induced with a final concentration of 10 μM ADP and tested for platelet aggregation response, as described above. The

results are shown in platelet aggregation curves of FIGURE 2, in which the baseline (no medication control) is represented by solid dots, PRP treated with 2.5 mMol aspirin in ethanol is represented by open circles; PRP treated with 10 mMol of sodium citrate alone is represented by open squares, PRP treated with 2.7 mMol aspirin and 10 mMol sodium citrate in ethanol and water, respectively, is represented by closed squares; PRP treated with 10 mMol citric acid alone is represented by closed triangles, PRP treated with 2.7 mMol aspirin and 10 mMol citric acid in ethanol and water, respectively, is represented by open triangles; and controls with PRP treated by 10 μL of water or 10 μL water and 2.5 μL ethanol are represented by a dotted or dashed line, respectively. As can be seen in FIGURE 2, the combined inhibitory effect of aspirin and citric acid is substantially greater than that of each component separately, or that of aspirin combined with sodium citrate.

Example 3

Six human patients were selected for treatment after experiencing one or more occurrences of thrombotic vascular occlusion following vascular surgery. The thrombotic condition of each subject was clinically determined to be inadequately controlled by conventional aspirin therapy. Each of the six subjects was judged to be at high risk of subsequent vascular occlusions, and of significant potential for loss of life or limb. Each subject was placed on a daily treatment regimen of a single dose of the composition of Example 1. All of the subjects were routinely examined, and remained free from clinically observable thrombotic occlusions from beginning the treatment regimen for periods extending from two months to over 12 months. Each subject remained free from complications associated with their prior symptoms, and were judged to have avoided significant hospitalization and medical care which would have otherwise been required.

The therapeutic effects of the practice of the invention in most human and animal subjects have included a dramatic inhibition of platelet aggregation, together with a resulting substantial decrease or elimination of thrombotic occlusion events. The inhibitory action of the compositions of the invention on platelet activity has been shown to be much more effective than that elicited by aspirin alone. In addition, the subjective clinical experience in patients treated according to the invention has been highly favorable as judged by lack of symptoms, a relative freedom from complications and an overall higher quality of life, which is in marked contrast to their pretreatment state. The methods and compositions of the invention therefore provide a safe, effective means for the long-term, on-going prophylactic or therapeutic

treatment of patients having a predisposition for life threatening thrombus formation, where adequate chronic treatment has heretofore been unavailable.

Example 4

A single center, double blind study was designed to test the hypothesis that daily administration of a combination of aspirin (acetylsalicylic acid, ASA) and citric acid provides greater inhibition of platelet aggregation than ASA therapy alone.

To evaluate this hypothesis 268 apparently healthy volunteers were screened to ascertain their platelet aggregation status and sensitivity to ASA therapy. All participants were instructed to fast after 10:00 p.m. the evening before their screening evaluation and to avoid ingesting ASA-containing products ten days prior to their appointment. Blood samples were collected between 8:00 and 10:00 a.m. using the standard venipuncture technique. Platelet aggregation responses were measured using the turbidimetric procedure of Born, "Aggregation of Blood Platelets by Adenosine Diphosphate and its Reversal," *Nature* **4832:**927-929 (1962), and quantified by the platelet aggregation scoring method of Saad et al., "Platelet Aggregometry Can Accurately Predict Failure of Externally Supported Knitted Dacron Femoropopliteal Bypass Grafts," *J. Vascular Surgery* **18(4):**587-594 (1993). Those individuals having a base line platelet aggregation (PA) score above 30 were invited back to be retested after ingesting 325 mg of ASA daily for a minimum of three days. Volunteers with ASA medicated PA scores remaining above 30 were asked to continue participating in the study. Twenty-four volunteers, 15 men and 9 post-menopausal women, were enrolled in the next phase of the study.

Each person enrolled in the study was instructed to not take ASA-containing products throughout their participation in the study. Each person in the study was randomly placed in one of four groups, with each group being designated to receive daily administration of one of the following four medications:

1. placebo (no medication);
2. 325 mg ASA;
3. 162.5 mg of ASA and 162.5 mg of citric acid (CA); and
4. 325 mg of citric acid.

The foregoing medication was prescribed to be taken daily for a period of two weeks, and platelet aggregation analyses for each participant were performed at the beginning and end of the two-week treatment period, as described below. A two-week non-medicated rest period was prescribed following the treatment period, and then each patient was randomly placed into another one of the four groups identified above. The foregoing procedure was repeated until each study participant had participated in

each of the four study groups. During the course of this study, all four medications were placed in identical looking gelatin capsules, and participants were unaware of the capsule contents. The order and sequence of medication distribution were unknown to the study participants. Individuals were assigned a medication sequence in the order they were recruited. Medications were prepared and distributed by a research chemist not involved in sample collection and platelet aggregation analysis. Blood drawing and scheduling appointments was handled by a phlebotomist who was not involved in distribution of the capsules or platelet aggregation analysis. Platelet aggregation tests were performed by a research associate who was not involved in capsule distribution or blood sample collection, on coded samples which did not reveal the participant group or medication taken. At the completion of data collection for all 24 participants, the data collected was analyzed.

During the study described above, the platelet aggregation of each participant was determined as follows. Using the standard venipuncture technique fasted blood was collected in a 5 mL vacutainer tube (Becton Dickinson Co., Rutherford, NJ) with EDTA Na_2 for the actual platelet count determination. Two 4.5 mL vacutainer tubes with 3.8% buffered sodium citrate were drawn for aggregation studies.

Whole blood platelet counts were performed by a Technicon H*1 hematology analyzer (Technicon Co., New York, NY). Platelet aggregation studies were performed according to the turbidimetric method of Born. Platelet-rich plasma was prepared from the citrated blood by centrifugation sufficient to sediment the red and white cells. After reserving an aliquot of the platelet-rich plasma supernatant, the blood was centrifuged again, at higher speed, to clear platelets into the pellet, yielding a platelet-poor plasma supernatant. A test plasma sample for aggregometry was prepared by diluting the aliquot of platelet-rich plasma with platelet-poor plasma, until the sample had a platelet count of $250{,}000 \pm 25{,}000/mm^3$. To quantify the platelet aggregation response, a standard transmission span of 10% and 90% was used for the platelet-poor plasma and platelet-rich plasma, respectively, and the test time was fixed at eight minutes. Adenosine diphosphate (ADP) reagent (Dade Laboratories, Miami, FL) was prepared daily by reconstitution with distilled water according to the manufacturer's instructions. After 450 µL of the standardized plasma sample had been incubated in a Chrono-log 560 VS aggregometer with a 707 recorder (Chrono-log Corp., Havertown, PA) driven by a Fluke 1752A Data Acquisition System (John Fluke Mfg. Co., Everett, WA) at 37°C for three minutes, 50 µL of ADP in a final concentration of 2.5 µM was added and the ensuing aggregation was monitored with

dedicated analytic software (Cybermed Technology Co., Seattle, WA). All aggregation studies were completed within three hours of sample collection.

The platelet aggregation (PA) score of each sample was obtained by the following formula:

$$\text{PA score} = \text{Amp R} \cdot S_{sum} \cdot \text{Plt R}$$

where:

Amp R = Amp1/Amax (see FIGURE 1, A_{max}=Amp1 + Amp2)

S_{sum} = Area under the curve (see FIGURE 1, $S_1 + S_2$)

Plt R = Actual platelet count/adjusted platelet count

The data described above are summarized in Tables 2 and 3, below. Table 2 shows the mean change from baseline in platelet aggregation score.

Table 2
Mean Change from Baseline ± Standard Deviation

Treatment	Adjusting for First Baseline Measurement	Adjusting for Baseline Before Receiving the Dose
Placebo (P)	0.08 ± 8.69	1.66 ± 1.77
ASA	-8.48 ± 7.17	-10.61 ± 8.58
ASA/CA	-19.07 ± 13.79	-18.95 ± 13.34
CA	0.003 ± 6.47	-0.81 ± 7.83

Table 3 shows the p-values for a comparison of the treatments.

Table 3
p-Values for Comparisons Between Treatment Groups; Unadjusted for Multiple Comparisons

	Adjusting for the Initial Baseline Value		Adjusting for the Baseline Just Before the Dose	
Groups Being Compared	t test	Wilcoxon two-sample test	t test	Wilcoxon two-sample test
p vs. ASA	0.00015	0.00028	<0.0001	0.00001
p vs. ASA/CA	<0.0001	<0.0001	<0.0001	<0.0001
p vs. CA	0.9720	0.7051	0.2625	0.6208
ASA vs. ASA/CA	0.00066	0.00119	0.0088	0.01231
ASA vs. CA	<0.0001	0.00017	<0.0001	0.00018
ASA/CA vs. CA	<0.0001	<0.0001	<0.0001	<0.0001

From the foregoing it is apparent that the ASA/CA therapy resulted in the greatest reduction in platelet aggregation, an average decrease of about 19. The next greatest change, a decrease of 8 to 10, was obtained by ASA alone. The placebo and citric acid (CA) treatments did not differ statistically; the average changes cannot be shown

to differ from zero. These data clearly indicate that treatment with both aspirin and citric acid results in a statistically significant reduction in platelet aggregation over treatment by aspirin alone, and that citric acid treatment alone has no *in vivo* effect on platelet aggregation.

Various modifications and applications of the methods and compositions of the invention will be apparent from the foregoing to those skilled in the art. Any such modifications and applications are intended to be within the scope of the appended claims except insofar as precluded by the prior art.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of inhibiting platelet aggregation in a patient comprising administering to the patient a composition comprising at least about 0.5 mg of acetylsalicylic acid per kg of body weight of the patient per day, and at least about 0.5 mg of citric acid per kg of body weight of the patient per day.

2. The method of Claim 1 wherein from about 2 to about 4 mg of acetylsalicylic acid per kg of body weight of the patient per day is administered to the patient.

3. The method of Claim 1 wherein from about 2 to about 4 mg of citric acid per kg of body weight of the patient per day is administered to the patient.

4. The method of Claim 1 which further comprises administering to the patient an amount of a pharmaceutically acceptable zinc salt effective to reduce the platelet aggregation activity of the patient.

5. The method of Claim 4 wherein at least about 0.01 mg of a pharmaceutically acceptable zinc salt per kg of body weight per day is administered to the patient.

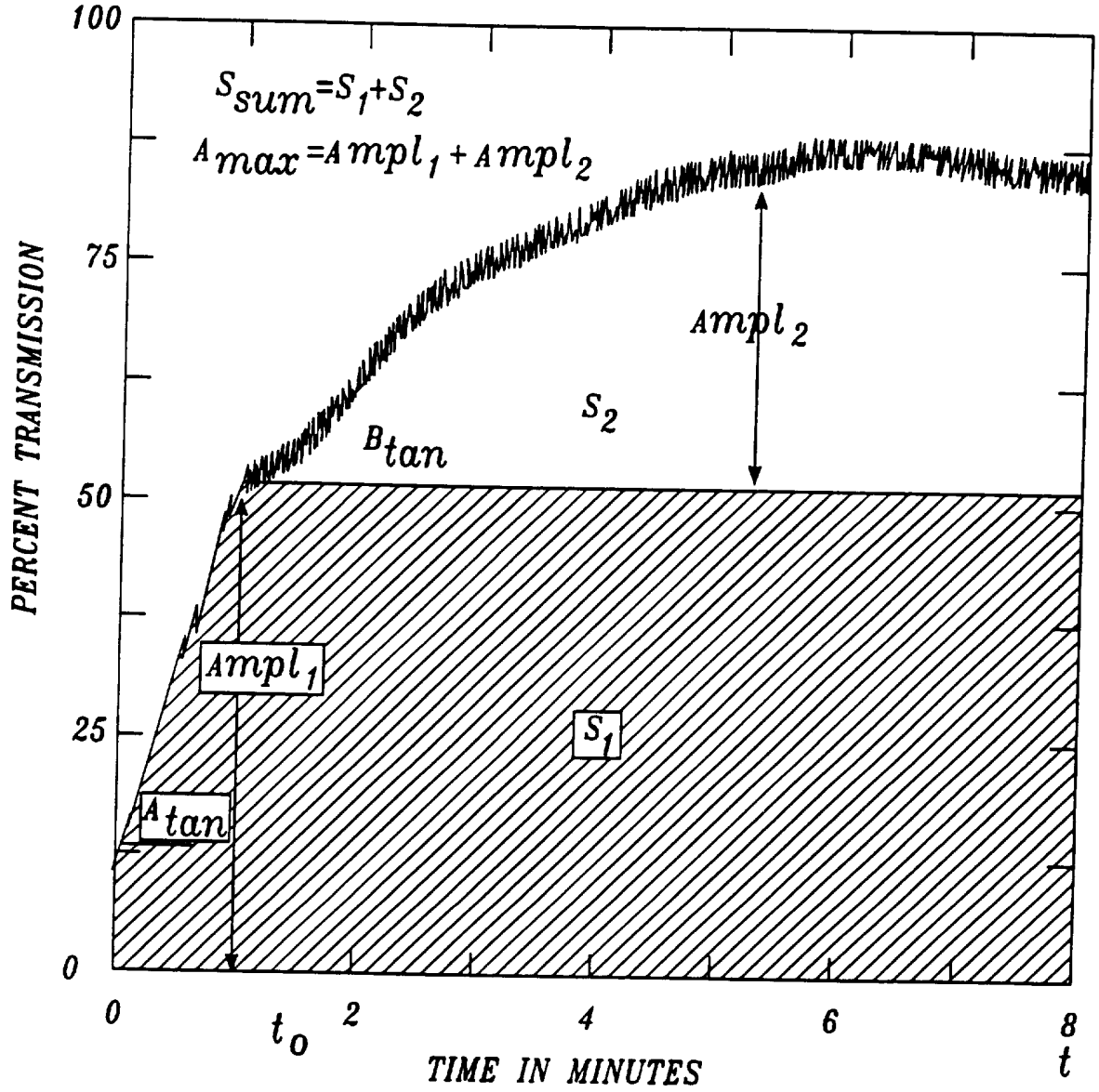
100
75
50
25
0
PERCENT TRANSMISSION
$S_{sum}=S_1+S_2$
$A_{max}=A_{ampl_1}+A_{ampl_2}$
$ampl_2$
S_2
B_{tan}
$ampl_1$
S_1
A_{tan}
0
t_0
2
4
6
8
t
TIME IN MINUTES

Fig. 1.

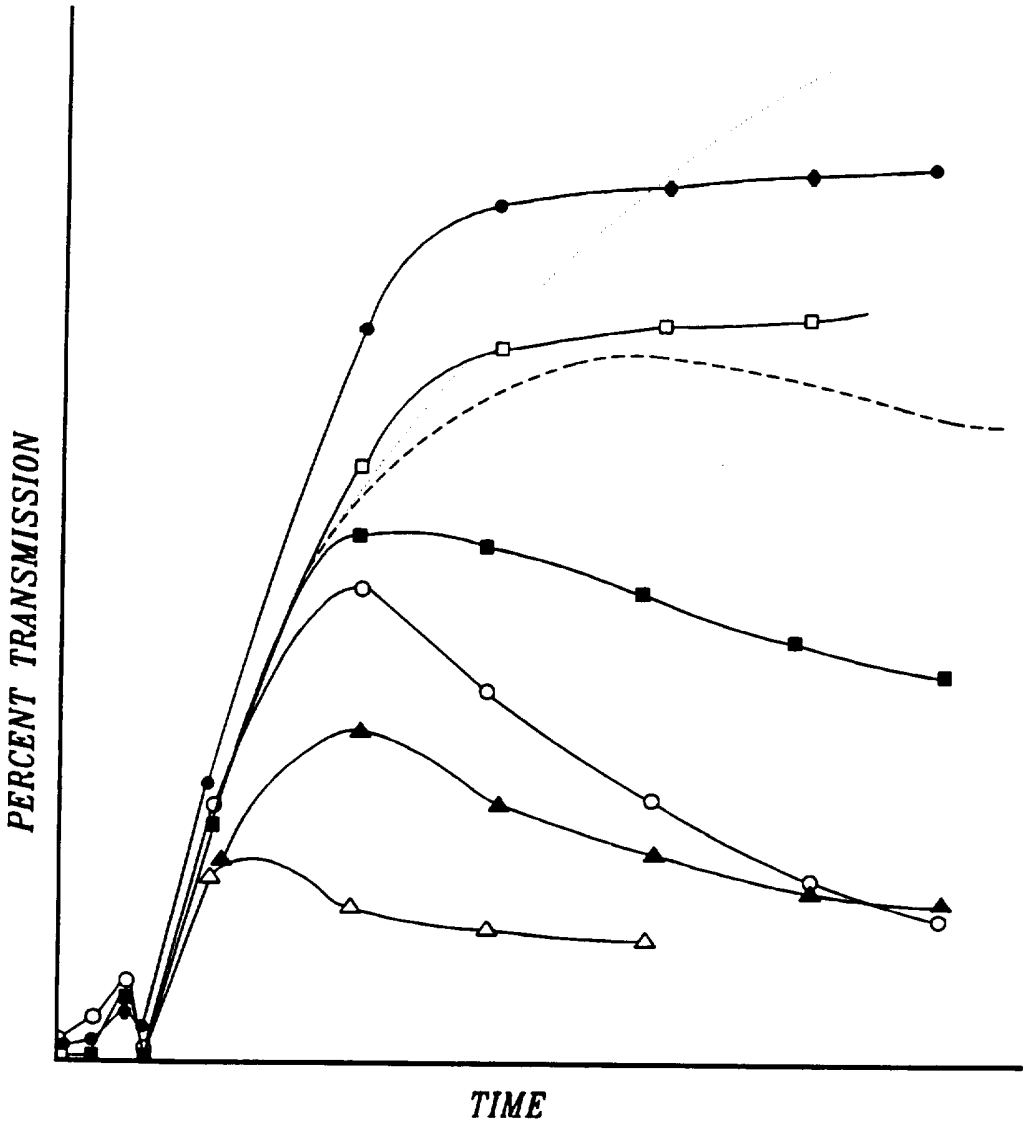

Fig. 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03429

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/61, 31/60, 31/19, 33/30
US CL :514/163, 165, 574; 424/643
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/163, 165, 574; 424/643

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN(CHEMICAL ABSTRACTS, MEDLINE, BIOSIS)
search terms: aspririn, acetylsalicylic acid, citric acid, citrate, zinc, platelet aggregation, thrombosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,904,761 (NOVICK, JR. ET AL) 09 SEPTEMBER 1975, column 2, lines 49-51.	1-5
Y	US, A, 4,193,813 (CHVAPIL) 18 MARCH 1980, column 1, lines 30 and 31.	4 and 5
Y	US, A, 4,845,129 (ANDERSON ET AL) 04 JULY 1989, column 29, line 41.	1-5
Y	Biological Abstracts, Volume 91, No. 7, issued 01 April 1991, Lin et al, "Study on the effect of various conditions and age to blood platelet aggregation test," see page 80, column 2, abstract no. 69072, Kaohsiung J. Med Sci. 6(12): 636-642.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 APRIL 1995

Date of mailing of the international search report
08 JUN 1995

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