



121

SEÑOR**SUPERINTENDENTE DE INDUSTRIA Y COMERCIO****E.****S.****D.****REF:****Expediente 06-106-506**

TÍTULO SOLICITADO: FORMAS POLIMÓRFICAS DE HIDROBROMURO
ACETATO DE METIL (+)-(S)-ALFA-(2-CLOROFENIL)-
6,7-DIHIDROTIENO [3,2-C] PIRIDINA-5-(4H),
HIDROBROMURO DE CLOPIDOGREL.

PUBLICACIÓN:**Gaceta 583 de 18/12/2007, N° de publicación 725****PRIORIDAD:****US 60/563,796 de 20/04/2004****SOLICITANTE:****SANOFI-AVENTIS.****APODERADO:****EMILIO FERRERO****TRÁMITE:****SUSTENTACION DE OPOSICIÓN**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LABORATORIO FRANCO COLOMBIANO S.A. – LAFRANCOL S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2° de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada en los siguientes términos:

I. COMPLEMENTO DE INFORMACIÓN.

La solicitud colombiana 06-106-506 en trámite, que se refiere a la obtención de

177
122

dichos polimorfos y un método de inhibición de la agregación plaquetaria útil en el tratamiento de episodios ateroscleróticos; carece de los requerimientos fundamentales para otorgársele el beneficio de patente de invención, tal y como se puede evidenciar a través de los siguientes argumentos:

- La solicitud colombiana en trámite, específicamente en sus reivindicaciones 1 a 18, se refiere a la formas polimórficas B, C y D de acetato de metil (+)-S- α -(2-clorofenil)-6,7-dihidrotieno[3,2-C]piridina-5(4H)hidrobromuro; como es de amplio reconocimiento por este despacho, el polimorfismo, en los términos de la Farmacia Práctica de Remington¹, es "la capacidad de cualquier elemento o compuesto de cristalizar en más de un sistema cristalino".

Así mismo, en la literatura científica está claramente definido que los sólidos pueden existir, de acuerdo con su estructura u ordenamiento interno, en formas cristalinas o amorfas; en las formas amorfas las partículas componentes no presentan una disposición interna ordenada, sino que se encuentran distribuidas al azar, mientras que en las formas cristalinas se presenta un arreglo interno ordenado y regular de las partículas, que implica la apreciación de formas geométricas definidas. De igual manera, es importante resaltar que dicha apariencia cristalina o amorfa depende de factores manipulables como son temperatura, presión, velocidad de cristalización, concentración de saturación, e inclusive, la influencia de sustancias extrañas y campos de fuerza exteriores al cristal².

Como el tema del polimorfismo es un tema ampliamente divulgado y discutido, es necesario recordar que, el descubrimiento del polimorfismo como propiedad de las entidades químicas se atribuye al farmacéutico Martín Enrique Klaproth en 1798, así mismo, la tesis según la cual los mismos elementos químicos pueden adoptar formas sólidas diversas – polimorfos - y mostrar propiedades físicas diferentes, se atribuye a Louis Jacques Thénard (1777-1857) y Jean Baptiste Biot (1774-1862)³, lo que indiscutiblemente demuestra que el encontrar o el atribuir a un compuesto "nuevas" formas polimórficas no es algo

723
123

que se conozca recientemente, es una propiedad inherente de la molécula y que está influenciada por el medio y las condiciones en el que se encuentre.

De igual manera, la caracterización de dicha forma cristalina (polimorfo), dada por los patrones de difracción de rayos X en polvo y como consta en algunas de las reivindicaciones de la solicitud colombiana 06-106-506, carece de nivel inventivo, pues es bien sabido que la difracción de rayos X corresponde a una técnica analítica útil y rutinaria en la caracterización de formas cristalinas. En otras palabras, la caracterización hecha de las formas cristalinas por el solicitante, en función de sus patrones de difracción de RX (método de identificación que data de 1912⁴) permite a cualquier persona versada en la materia identificar y caracterizar diversas variaciones en la estructura cristalina de una molécula. Los resultados de un procedimiento de caracterización por difracción de RX son una manifestación del comportamiento natural de una molécula, en función de las condiciones ambientales y demás variables a que esta sea sometida, incluyendo desde luego la orientación e intensidad de los rayos, sin que dichos resultados constituyan de manera alguna aporte inventivo de quien realiza el procedimiento de identificación⁵.

En conclusión, el reporte de las formas polimórficas B, C, y D de acetato de metil (+)-S- α -(2-clorofenil)-6,7-dihidrotieno[3,2-C]piridina-5(4H) hidrobromuro es un hallazgo previsible y no una invención, en el sentido que es una propiedad inherente a la materia en su estado sólido y dicho reporte es el simple encuentro de características fisicoquímicas que ya se sabía podían ser halladas y que hacen parte de la práctica rutinaria en el campo farmacéutico.

Ahora bien, es importante también destacar lo revelado en la patente americana US 4,847,265, titulada "Dextro-rotatory enantiomer of methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate and the pharmaceutical compositions containing it" y publicada el 11 de Julio de 1989; en este documento se revelan las formas enantioméricas dextrorrotatorias de

⁴ Ver "Remington - The Science and Practice of Pharmacy", 20th Edition, Philadelphia, PA, USA, 1955, p. 157-158.

724
1224

Clopidogrel y de sus sales, cuya actividad farmacológica es la inhibición de la agregación plaquetaria.

Más específicamente se refiere a las formas enantioméricas dextrorrotatorias de methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate hidrogenosulfato, taurocolato e bromhidrato, obtenidas de manera convencional mediante la reacción de un ácido sobre una base en solución en un solvente adecuado y posterior precipitación espontánea o luego de la adición de un no-solvente.

En la columna 6, línea 64 a la columna 7 línea 13, vemos que se describe claramente la forma de obtención del bromhidrato de Clopidogrel dextrorrotatorio que corresponde a:

$m.p. = 140^{\circ} C.$

empirical formula: $C_{16}H_{16}ClNO_2S.HBr$

$[\alpha]_D^{20} = +59.23 (c=2.09 g/100 ml; methanol).$

Si comparamos lo anterior con lo descrito en cualquiera de las reivindicaciones de la solicitud colombiana en trámite tenemos que la misma carece de novedad ya que se está frente a la misma sustancia, es decir, en los dos casos se revela la forma dextro-rotatoria de methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate hydrobromide (US 4,847,265) ó acetato de metil(+)-(S)-alfa-(2-clorofenil)-6,7-dihidrotieno [3,2-c] piridina-5-(4H) bromhidrato (CO 06-106-506).

Ahora, si la supuesta novedad está dada en que la solicitud colombiana se refiere a las formas B, C y D, es del caso hacer notar no sólo lo expuesto a través de todo el escrito sino que, la anterioridad mencionada tiene un punto de fusión para el Bromhidrato de Clopidogrel de $140^{\circ}C$, punto de fusión idéntico o que se encuentra dentro del rango propuesto en las reivindicaciones 5 a 12 de la solicitud en trámite ($140-143^{\circ}C$ Forma B y $138-148^{\circ}C$ Forma C), lo que llevaría a pensar que se trata de la misma sustancia, independientemente de la nomenclatura que se le haya dado a dicha forma polimórfica, o se

12/5

Así mismo, es importante resaltar que la solicitud en trámite no resalta el efecto inesperado o sobresaliente de tales polimorfos dentro de una formulación con respecto a las formas ya existentes en el estado del arte, y si así lo hiciera, es del caso hacer notar que, para cualquier persona medianamente versada en la materia y aquella que se dedica a la fabricación de medicamentos, la etapa de preformulación es un estadio crítico del desarrollo de un medicamento ya que en esta etapa se definen las propiedades fisicoquímicas del fármaco frente a los excipientes y al proceso de fabricación, por tanto, el control o el conocimiento de la naturaleza cristalina del fármaco es fundamental ya que como se sabe de manera amplia en la literatura científica, los polimorfos suelen presentar diferencias en sus parámetros fisicoquímicos y, evidentemente, la elección de la forma cristalina o polimórfica deberá estar dada por la biodisponibilidad y por aquella que termodinámicamente sea más estable para que ni durante las etapas del proceso ni durante el almacenamiento se den cambios no deseables.

- En adición a los argumentos anteriores, puede argumentarse además que la obtención de dicha sal de clopidogrel, tampoco representa de ninguna manera un motivo suficiente como para solicitar protección ante la ley de patentes, puesto que la formación de sales es un recurso que es ampliamente reconocido en la industria farmacéutica y que se aplica sobre diversos compuestos, ya sea para modificar sus características de estabilidad durante el proceso de producción, de estabilidad en la formulación, de solubilidad, de biodisponibilidad o de cualquier otro tipo de consideraciones biofarmacéuticas que deban ser tenidas en cuentas para asegurar la efectividad y seguridad del medicamento.

En otras palabras, la solicitud colombiana en trámite puede reducirse a la formulación de una sal de clopidogrel, para aplicarse en la prevención de eventos ateroscleróticos; argumento de la invención que también puede desvirtuarse si, como cualquier persona medianamente versada en la materia reconoce, debido a su actividad antiagregaria sobre las plaquetas (debido a su mecanismo de acción que corresponde a un bloqueo irreversible del

76
125

trastornos cardiovasculares relacionados. Prueba de esto es la amplia cantidad de información de carácter científico⁶ que demuestra la aplicación de clopidogrel en este tipo de trastorno, veamos una pequeña muestra:

❖ **Clopidogrel Antiplatelet Activity Is Independent Of Age And Presence Of Atherosclerosis⁷.**

Abstract: The pharmacodynamic and pharmacokinetic effects of clopidogrel 75 mg taken once daily in the morning before breakfast for 10 days were compared among three groups: 12 healthy young subjects, 10 healthy elderly subjects (>65 years) and 10 otherwise healthy elderly subjects with atherosclerosis, manifested by intermittent claudication. Platelet aggregation induced by 5 microM of ADP was measured in plasma samples taken at screening, 2 hours after dosing on day 1 to day 9; 2, 5, and 24 hours after dosing on day 10; and on alternate days until day 24. The inhibition of platelet aggregation was expressed as the percent reduction in maximum platelet aggregation with respect to baseline. The bleeding time was measured at screening, 5 hours after dosing on day 10, and on day 18. Plasma concentrations of SR26334, the main circulating metabolite of clopidogrel, were determined before dosing on day 1 to day 10 and at regular intervals over 72 hours after dosing on day 10. Inhibition of platelet aggregation appeared 2 hours after the first dose, became significant after the second dose, and progressed to a steady-state value of 55 to 57% by day 7 in the three groups, with no statistically significant difference between groups. A moderate, statistically significant prolongation of bleeding time of similar extent (prolongation factor of 1.5 to 1.6) was found on day 10 in the three groups. The pharmacodynamic parameters generally returned to baseline within 8 days after treatment. Based on AUC(0-24h) values, drug exposures were very similar for the two groups of elderly subjects but approximately twice that for the young group. The pharmacodynamic effects of clopidogrel were comparable in all three groups.

❖ **Acute Antithrombotic Effect Of A Front-Loaded Regimen Of Clopidogrel In Patients With Atherosclerosis On Aspirin⁸.**

Abstract: There is a need for a rapid antithrombotic effect after the administration of antiplatelet drugs in the setting of acute coronary syndromes and percutaneous interventions. Clopidogrel, a new thienopyridine derivative, is an efficient antiplatelet agent. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant antithrombotic effects. Patients with stable arterial disease on chronic aspirin therapy (n=520) were treated with clopidogrel either with a front-loaded regimen, 300 mg the first day and 75 mg/d the next 7 days, or with a standard regimen, 75 mg/d for 8 days. Blood thrombogenicity was assessed by quantification of platelet-thrombus formation in an ex vivo perfusion chamber, by ADP-induced platelet aggregation, and by ADP-induced fibrinogen binding. At 2 hours, mean total thrombus area with the standard regimen was not significantly reduced. In contrast, at 2 hours, the mean total thrombus area with the front-loaded regimen was significantly decreased by 23.168.5%

⁶ <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer>

De
127

versus baseline ($P,0.05$). ADP-induced platelet aggregation (with 5 and 10 mmol/L) was also significantly ($P,0.05$) reduced with the front-loaded regimen at 2 hours, with the mean platelet aggregation being 82.264.4% and 81.864.5%, respectively, versus baseline. Similarly, flow cytometry demonstrated a significant decrease ($P,0.05$) in the ADP-induced fibrinogen binding (with 0.12 and 0.6 mmol/L) at 2 hours in this front-loaded regimen group (36.162.0% and 53.269.3%). With the standard regimen, platelet activity was not significantly reduced at 2 hours. Our data suggest that a front-loaded regimen of clopidogrel added to aspirin achieves a significant antithrombotic effect at 2 hours in patients with known atherosclerotic disease on chronic aspirin therapy. This provides a rationale for using front-loaded clopidogrel in combination with aspirin in percutaneous coronary interventions.

❖ Clopidogrel: Hemorheological Effects In Subjects With Subclinical Atherosclerosis⁹.

Abstract: The usefulness of Clopidogrel as inhibitor of platelet aggregation has been demonstrated, but its effect on hemoreological parameters, such as whole blood viscosity at low and high shear rate, red cell aggregation and deformability indexes, filterability rate and deformability of red blood cells has not been studied. This study revealed that, in subjects with impaired blood rheology and ultrasound evidence of atherosclerosis, 3-weeks treatment with clopidogrel (75 mg daily) improved blood viscosity at high shear rate and other hemorheological parameters, including red cell filterability rates and dynamic red cell deformability index ($p<0.01$ for all), without any unwanted side effect. Blood viscosity at low shear rate and red cell aggregation index improved after only 1-week treatment, and the reductions were maintained after two and three weeks ($p<0.01$). These results indicate that, similarly to another thienopyridine such as ticlopidine, Clopidogrel may have a positive influence on several hemorheological parameters, thus exerting its protection not only through inhibition of platelet function, but also through changes in the hemorheological profile.

Así mismo en el FDA Electronic Orange Book se puede apreciar que se han aprobado formulaciones farmacéuticas de clopidogrel en su forma de sal Bisulfato¹⁰, veamos:

Search results from the "OB_Rx" table for query on "020839."

Active Ingredient:	CLOPIDOGREL BISULFATE
Dosage Form/Route:	TABLET; ORAL
Proprietary Name:	PLAVIX
Applicant:	SANOFI AVENTIS US
Strength:	EQ 75MG BASE
Application Number:	020839
Product Number:	001
Approval Date:	Nov 17, 1997
Referente Listed Drug	Yes

n7
128

RX/OTC/DISCN: RX
 TE Code: AB
 Patent and Exclusivity Info for this product: [View](#)

Active Ingredient: CLOPIDOGREL BISULFATE
 Dosage Form/Route: TABLET; ORAL
 Proprietary Name: PLAVIX
 Applicant: SANOFI AVENTIS US
 Strength: EQ 300MG BASE
 Application Number: 020839
 Product Number: 002
 Approval Date: Sep 20, 2007
 Referente Listed Drug: No
 RX/OTC/DISCN: RX
 TE Code:
 Patent and Exclusivity Info for this product: [View](#)

- Para terminar, la solicitud colombiana en trámite reivindica, específicamente en sus reivindicaciones 20 y 21, un método para inhibir la agregación plaquetaria que comprende la administración a un paciente necesitado de esta de una cantidad eficaz de uno de los compuestos descritos en reivindicaciones anteriores -polimorfos B, C y D de acetato de metil (+)-S- α -(2-clorofenil)-6,7-dihidrotieno[3,2-C]piridina-5(4H)) hidrobromuro- para reducir los episodios ateroscleróticos; con lo cual, dicha solicitud, incurre directamente en una improcedencia de patentabilidad, ya que como lo expone claramente el literal d) del artículo 20 del capítulo 1 del título II *"No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales"*.

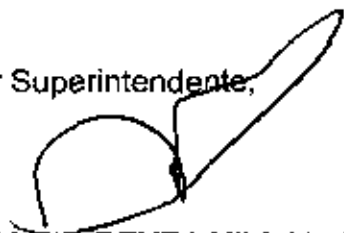
Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

129
129

III. ANEXOS

- Abstracts de los artículos científicos mencionados en el acápite de Nuevos Argumentos.
- La patente americana US 4,847,265, titulada "Dextro-rotatory enantiomer of methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate and the pharmaceutical compositions containing it" y publicada el 11 de Julio de 1989
- Copia de los resultados arrojados por el meta-buscador de <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer> relacionados a Clopidogrel y aterosclerosis.
- Copia de las paginas del "FDA Orange Electronic Book" con la información para medicamentos aprobados para Clopidogrel.

Señor Superintendente,


JOSE LUIS REYES VILLAMIZAR
 C.C. 79.152.473 de Usaquén
 T.P. 44655 del C. S de la J.

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Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

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Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

Gérard Heffl, Julio I. Osende, Stephen G. Worthley, Azfar G. Zaman, Oswaldo J. Rodriguez, Eli I. Lev, Michael E. Farkouh, Valentin Fuster, Juan J. Badimon, James H. Chesebro

Abstract—There is a need for a rapid antithrombotic effect after the administration of antiplatelet drugs in the setting of acute coronary syndromes and percutaneous interventions. Clopidogrel, a new thienopyridine derivative, is an efficient antiplatelet agent. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant antithrombotic effects. Patients with stable arterial disease on chronic aspirin therapy ($n=20$) were treated with clopidogrel either with a front-loaded regimen, 300 mg the first day and 75 mg/d the next 7 days, or with a standard regimen, 75 mg/d for 8 days. Blood thrombogenicity was assessed by quantification of platelet-thrombus formation in an ex vivo perfusion chamber, by ADP-induced platelet aggregation, and by ADP-induced fibrinogen binding. At 2 hours, mean total thrombus area with the standard regimen was not significantly reduced. In contrast, at 2 hours, the mean total thrombus area with the front-loaded regimen was significantly decreased by $23.1 \pm 8.5\%$ versus baseline ($P<0.05$). ADP-induced platelet aggregation (with 5 and $10 \mu\text{mol/L}$) was also significantly ($P<0.05$) reduced with the front-loaded regimen at 2 hours, with the mean platelet aggregation being $82.2 \pm 4.4\%$ and $81.8 \pm 4.5\%$, respectively, versus baseline. Similarly, flow cytometry demonstrated a significant decrease ($P<0.05$) in the ADP-induced fibrinogen binding (with 0.12 and $0.6 \mu\text{mol/L}$) at 2 hours in this front-loaded regimen group ($36.1 \pm 2.0\%$ and $53.2 \pm 9.3\%$). With the standard regimen, platelet activity was not significantly reduced at 2 hours. Our data suggest that a front-loaded regimen of clopidogrel added to aspirin achieves a significant antithrombotic effect at 2 hours in patients with known atherosclerotic disease on chronic aspirin therapy. This provides a rationale for using front-loaded clopidogrel in combination with aspirin in percutaneous coronary interventions. (*Arterioscler Thromb Vasc Biol*. 2000;20:2316-2321.)

Key Words: platelet aggregation inhibitors ■ thrombus ■ atherosclerosis

The central role of platelets in arterial thrombosis is well established.¹ Platelet activity and thrombin and thromboxane generation are markedly increased after myocardial infarction, thrombolysis, unstable angina, and percutaneous coronary interventions.^{1,2} Numerous studies have shown that antiplatelet agents like aspirin or ticlopidine are effective for secondary prevention of coronary and cerebrovascular events.³ Combined antiplatelet therapy with aspirin and ticlopidine improves clinical outcome after stent implantation compared with aspirin alone or aspirin plus full anticoagulation.⁴⁻⁷ New antiplatelet drugs, including glycoprotein IIb/IIIa antagonists and clopidogrel, a new ADP-receptor antagonist, also reduce coronary events.^{8,9}

Aspirin inhibits cyclooxygenase, whereas clopidogrel inhibits the binding of ADP to its platelet receptor, which leads to reduced binding of fibrinogen to glycoprotein IIb/IIIa receptors.^{10,11} Given the different mechanisms of action and proven safety of these combined drugs, combined therapy appears logical and has already given encouraging results in nonrandomized comparisons in patients undergoing coronary stenting.¹²⁻¹⁷

There is a need for rapid efficacy after the administration of antiplatelet drugs in the setting of percutaneous interventions and acute coronary syndromes. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant effects occur in platelet aggregation.¹⁸

The major objective of this study was to investigate whether a front-loaded regimen of clopidogrel added to aspirin could achieve a significant antithrombotic activity 2 hours after its administration. Antithrombotic activity was assessed by evaluating (1) the quantitative growth of thrombus in a well-validated ex vivo perfusion chamber, (2) ADP-induced platelet aggregation, and (3) ADP-induced fibrinogen binding by flow cytometry in patients with atherosclerosis receiving chronic aspirin therapy.¹⁹

No clinical or experimental study has assessed, in patients with atherosclerosis, the acute antithrombotic effect of the combination of clopidogrel and aspirin (load and no-load) compared with aspirin alone. Therefore, the secondary objective was to compare the antithrombotic effect at day 8 of the standard regimen of clopidogrel versus the antithrombotic effect of

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baseline aspirin therapy alone in atherosclerotic patients on chronic aspirin therapy.

Methods

Patient Population

The study population included patients with stable coronary or peripheral arterial disease ($n=20$). All patients were receiving chronic aspirin therapy (325 mg/d) for ≥ 3 months. The presence of coronary artery disease was documented by coronary angiography, history of previous myocardial infarction, or typical angina pectoris with a positive stress test. The existence of peripheral disease was documented by lower-extremity claudication, previous peripheral angioplasty, bypass grafting, or $\geq 50\%$ stenosis in any artery distal to the aortic bifurcation by angiography or ultrasound. Exclusion criteria included hemoglobin < 12 g/L, history suggestive of recent blood loss, or poorly controlled hypertension ($> 180/100$ mm Hg). The study was approved by the Institutional Review Board, and all patients signed informed consent to participate.

The selected patients were randomized to 1 of 2 treatment groups in a double-blind, placebo-controlled design. The front-loaded regimen group received a loading dose of 300 mg (four 75-mg capsules) of clopidogrel on the first day, followed by 75 mg/d for the next 7 days. The standard regimen group received a loading dose of 3 identical placebo capsules plus a 75-mg clopidogrel capsule on the first day followed by 75 mg/d of clopidogrel for the next 7 days. All patients continued taking 325 mg/d of aspirin for the duration of the study. The CAPRIE Trial used a dose of 75 mg/d clopidogrel, which is approved by the FDA.⁸ The effects of the 2 treatments were studied at serial time points throughout the study and were compared with their respective pretreatment values. This design allowed each patient to serve as his or her own control. The investigators performing the perfusion and platelet studies, described further below, were blinded as to treatment assignment.

Quantification of Platelet-Thrombus Formation

Quantification of platelet-thrombus formation was assessed at baseline, after 2 hours, and on day 8. The effect of clopidogrel on ex vivo arterial thrombus formation was assessed by measuring the area of thrombus formation in a perfusion chamber. The description, validity, and reliability of the Badman perfusion system to study thrombus formation on defined thrombogenic substrates under stable rheological conditions were reported previously.¹⁰⁻¹² In brief, a 19-gauge needle was carefully inserted into an antecubital vein. After the first 5 mL of blood had been discarded, the needle was connected to the perfusion chamber. Native, nonanticoagulated blood was directly perfused from the antecubital vein to the perfusion chambers at a constant flow rate with a peristaltic pump (Masterflex, Cole-Parmer Instruments) distal to the chambers. The Plexiglas perfusion chamber consists of a cylindrical flow channel (11-mm diameter, 2.5-cm length) that allows the blood to flow over the thrombogenic substrate. All perfusions were performed for a period of 5 minutes at a flow rate of 10 mL/min (calculated shear rate of 1490/s; Reynolds number of 60; average blood velocity 21.2 cm/s). The selected dynamic conditions model the local rheology associated with mildly stenosed coronary arteries. Previous work has demonstrated that these rheological conditions result in consistent levels of platelet deposition.¹⁴

To mimic the in vivo situation of severe arterial injury, porcine aortic tunica media was used as the substrate to trigger thrombus formation. Segments of porcine aorta were cut into segments (2.8 × 0.8 cm) and surgically prepared to expose the deeper components of the arterial wall as previously described.¹⁷

The histological assessment of thrombus formation was performed by histomorphometry as previously described.¹⁸⁻¹⁹ In brief, after blood perfusion, the segments were removed from the chambers, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (5 μ m) were cut and stained with a combined Masson's trichrome-elastin to visualize the total thrombus formed on the exposed substrates. Morphometric analysis of thrombus was conducted at 400-fold magnification. Images were digitized with a Sony DSC-F5000 camera using Adobe Photoshop 4.0 software on a Power Macintosh 8500 computer. The thrombus area was measured

measured by computerized planimetry using Image Pro-plus software (Media Cybernetics). Results are given as the average of the analyzed sections ($n=6$) per chamber and expressed as the percentage of thrombus area compared with baseline. The measurements were performed by a single investigator blinded to the assigned therapy.

Platelet Aggregation

Platelet aggregation was performed at baseline, 2 hours, day 2, and day 8. Blood (16 mL) was drawn from an antecubital vein through a 19-gauge needle into 2 mL of 110 mmol/L trisodium citrate solution. Platelet-rich plasma and platelet-poor plasma were prepared by differential centrifugation. Platelet concentration in platelet-rich plasma was adjusted to $2.5 \times 10^8/L$ by the addition of platelet-poor plasma. ADP (Chronolog) at concentrations of 5 and 10 μ mol/L served as an agonist to trigger platelet aggregation. The aggregation response was recorded for 5 minutes after addition of the agonist in an aggregometer (Chronolog model 440, Chronolog Corp). Platelet aggregation was expressed as the mean percentage of maximum platelet aggregation compared with baseline.

Flow Cytometry

Platelet activation was assessed at baseline, 2 hours, day 2, and day 8. Samples were prepared within 1 hour of collection. FITC-conjugated chicken anti-human antibody (10 μ L; Wako-Chemie Medical GmbH) was added to 3 tubes containing 250 μ L WB-PV8 buffer or HEPES-EDTA buffer for the negative control.²⁰ Thereafter, 20 μ L platelet-rich plasma was added and incubated for 10 minutes at room temperature. Twenty microliters of a solution of ADP in HEPES buffer (0.012 and 0.6 μ mol/L) was added to each sample tube, and 20 μ L of HEPES-EDTA buffer was added to the negative control. After exactly 10 minutes of incubation, 2 mL of ice-cold HEPES buffer was added to each tube. Samples were analyzed within the next 3 hours in a Becton-Dickinson flow cytometric analyzer. Activated platelets are expressed as a percentage, and the results are expressed as the relative fibrinogen binding compared with baseline.

Blood Samples

Blood was collected at baseline and day 8 for complete blood examination. Blood (4.5 mL) was also collected in SCAT tubes (Hematologic Technologies Inc) containing PPACK, EDTA, and aprotinin. Repeated blood samples for prothrombin fragment 1 + 2, a marker of thrombin generation, were obtained from the 20 patients at baseline, 2 hours, and day 8. Aliquots of plasma were stored at -70°C until analysis with ELISA (Enzygnost, Dade Behring).

Statistical Analysis

As already mentioned, the study design allowed each patient to serve as his or her own control (pretreatment versus posttreatment). Continuous variables were expressed as percentage of baseline $\pm$ SEM and compared by a paired Student's *t* test within group comparisons. Statistical significance was considered as a 2-tailed probability < 0.05 .

Results

Study Population and Hematological Parameters

Twenty patients with stable coronary or peripheral arterial disease were studied; all of them completed the platelet reactivity data and 16 of them the perfusion chamber data. Baseline characteristics of these patients are summarized in the Table. No statistically significant differences were observed between the groups. In particular, use of antianginal and hypolipidemic drugs did not differ between groups.

There were no adverse events related to the medication. All patients received aspirin (325 mg/d) with the clopidogrel. In particular, no bleeding, gastrointestinal upset or rash was observed. Hematological parameters, including hemoglobin, hematocrit, platelet count, and platelet count were

Baseline Characteristics in Patients Randomized to Standard (n=10) Versus Front-Loaded (n=10) Regimen of Clopidogrel

	Standard Regimen	Front-Loaded Regimen
Age, y (mean $\pm$ SEM)	56.5 $\pm$ 2.4	57.1 $\pm$ 2.5
Male, n	9	8
Coronary artery disease, n	8	8
Diabetes, n	1	3
Hypertension, n	3	4
Current smoking, n	0	1
Hypolipemic drugs, n	6	5
β -blocker	5	4
Ca-channel blocker	1	2
Long-acting nitrate	1	1

measured in EDTA-blood at baseline and at day 8 and did not change significantly.

Effects of Clopidogrel Treatments on Thrombus Area

The effects of the 2 regimens on thrombus formation were assessed at 2 time points (2 hours and day 8) and compared with their respective baseline (aspirin-treated) values (Figure 1). No significant difference was observed in the mean thrombus formation between the 2 groups at baseline, indicating the homogeneity of the study groups. At 2 hours, mean thrombus area with the standard regimen was decreased by 9.0 $\pm$ 7.9%, but was not significantly different from baseline (aspirin alone, P =NS). In contrast, at 2 hours, the mean thrombus area in patients receiving the front-loaded regimen was 23.1 $\pm$ 8.5% less than the mean pretreatment value (P <0.05). As expected, the mean thrombus areas were significantly (P <0.05) reduced at day 8 in both groups compared with the baseline (22.2 $\pm$ 6.9% with the standard regimen and 29.1 $\pm$ 9.1% with the front-loaded regimen).

Effects of Clopidogrel Treatments on Platelet Aggregation

Mean maximum percentages of ADP-induced platelet aggregation at baseline were comparable in both groups (Figure 2A

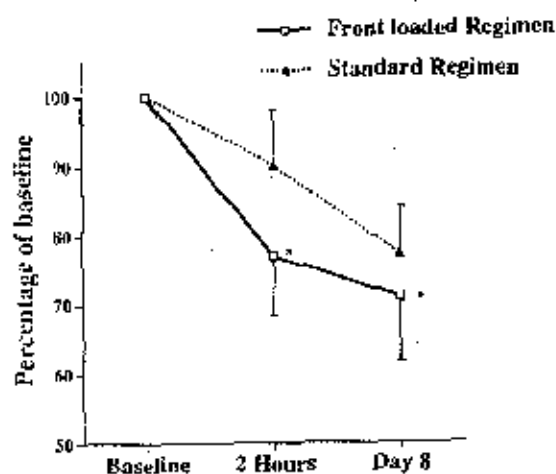


Figure 1. Effects of clopidogrel treatments on thrombus area. Results on thrombus formation at baseline, 2 hours, and day 8 with the respective treatments. Data are expressed as mean thrombus area $\pm$ SEM (μ m²). * P <0.05 vs baseline.

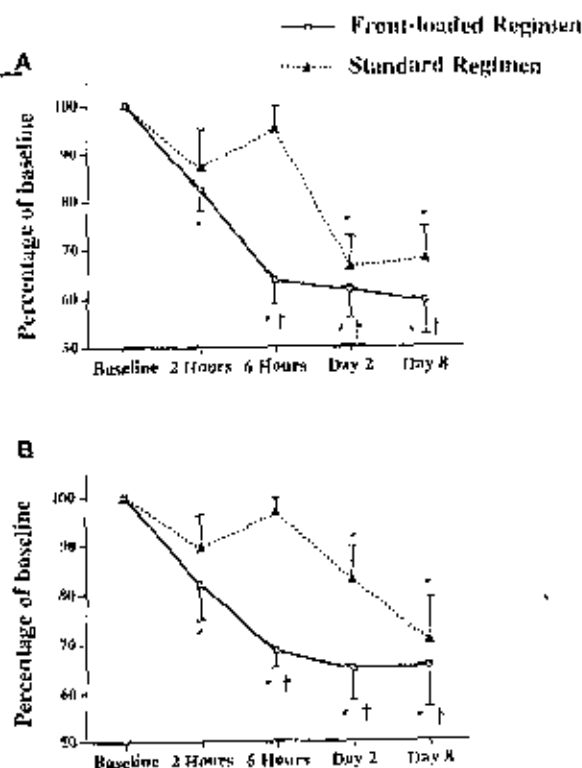


Figure 2. Effects of clopidogrel treatments on platelet aggregation to ADP 5 μ mol/L (A) and 10 μ mol/L (B). Results on maximal aggregation at baseline, 2 hours, 6 hours, day 2, and day 8 with the respective treatments. Data are expressed as mean percentage $\pm$ SEM. * P <0.05 vs baseline. † P <0.05 vs 2 hours.

and 2B). No significant inhibition of ADP-induced platelet aggregation was noted at 2 and 6 hours with ADP 5 and 10 μ mol/L with the standard regimen of clopidogrel (mean platelet aggregation: 86.9 $\pm$ 8.0%, 95.0 $\pm$ 4.7%, and 89.3 $\pm$ 6.8%, 96.5 $\pm$ 3.2%, respectively). At day 2, significant inhibition was achieved, the mean platelet aggregation with ADP 5 and 10 μ mol/L being 66.3 $\pm$ 6.2% and 82.6 $\pm$ 6.7%, respectively.

In contrast, a significant inhibition of ADP-induced platelet aggregation was observed at 2 hours with the front-loaded regimen of clopidogrel. At this time, the mean platelet aggregation for ADP 5 and 10 μ mol/L was 82.2 $\pm$ 4.4% and 81.8 $\pm$ 4.5%, respectively, compared with baseline (aspirin alone). Further significant inhibition (P <0.05) was observed at 6 hours, with a mean platelet aggregation of 63.5 $\pm$ 4.9% and 68.4 $\pm$ 5.2% for both concentrations of ADP used. At day 2 and day 8, no further significant inhibition of platelet aggregation was noted.

Effects of Clopidogrel Treatments on Fibrinogen Binding to ADP-Activated Platelets

There was no significant difference at baseline between the 2 groups (Figure 3A and 3B). With the front-loaded regimen, platelet activation, as determined by ADP-induced platelet-fibrinogen binding by flow cytometry, was significantly reduced (P <0.05) at 2 hours for ADP concentrations of 0.12 and 0.6 μ mol/L, the fibrinogen binding being 36.1 $\pm$ 2.0% and 53.2 $\pm$ 9.3%, respectively, compared with baseline. In contrast, with the standard regimen, platelet activation was not significantly reduced at 2

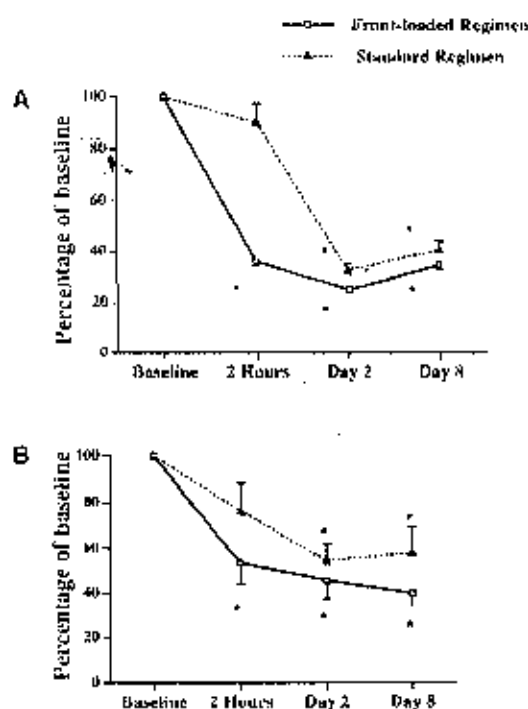


Figure 3. Effects of clopidogrel treatments on fibrinogen binding by flow cytometry induced by ADP 0.12 $\mu\text{mol/L}$ (A) and 0.6 $\mu\text{mol/L}$ (B). Results on activated platelets at baseline, 2 hours, day 2, and day 8 with the respective treatments. Data are expressed as the relative fibrinogen binding $\pm$ SEM compared with baseline. * $P < 0.05$ vs baseline.

hours in the standard regimen, the fibrinogen binding being $93.4 \pm 7.1\%$ and $74.6 \pm 11.8\%$, respectively.

Effect of Clopidogrel Treatments on Thrombin Generation

Baseline values of prothrombin fragment F1+2 plasma levels were similar in both groups at baseline on aspirin (0.79 ± 0.08 nmol/L with the front-loaded regimen and 0.91 ± 0.09 nmol/L with the standard regimen). These values remained unchanged when the patients were treated, at 2 hours and day 8 (0.89 ± 0.11 and 0.86 ± 0.06 nmol/L with the front-loaded regimen and 0.84 ± 0.09 and 0.86 ± 0.12 nmol/L with the standard regimen, respectively).

Discussion

We have shown that a front-loaded regimen of clopidogrel (300 mg) added to aspirin achieved a significant antithrombotic activity as early as 2 hours after its administration, as assessed by reduction in thrombus formation and platelet reactivity. We have also demonstrated that the antithrombotic effect at day 8 of a standard regimen of clopidogrel (75 mg/d) in patients with atherosclerosis receiving chronic aspirin therapy is greater than the antithrombotic effect of chronic aspirin therapy alone.

Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel

With a front-loaded regimen of clopidogrel (300 mg), it is possible to achieve a significant antithrombotic effect at 2 hours, an effect not obtained with the standard regimen. In patients with known atherosclerotic disease receiving chronic aspirin therapy, the antithrombotic effect of clopidogrel

aspirin. To the best of our knowledge, this is the first report of quantitative reduction in thrombus formation in a patient population with documented atherosclerotic disease receiving aspirin. Previous data in normal subjects have indicated that a dose of 300 to 400 mg produced a rapid onset of the pharmacodynamic activity of clopidogrel, with levels of platelet inhibition close to steady state observed within the first hours after dosing.¹¹ Our findings confirm and extend these results in patients with atherosclerotic disease receiving chronic aspirin therapy. But more importantly, we have shown that the antithrombotic effect, assessed in a perfusion chamber mimicking an arterial injury atop a mild stenosis in a coronary artery, is achieved as early as 2 hours after front-loaded regimen administration. This provides evidence for the rapid onset of an antithrombotic effect with a loading dose (300 mg) of clopidogrel in the treatment of acute coronary syndromes and in coronary stent implantation. This is important because the median time from stent implantation to stent thrombosis in patients treated with ticlopidine and aspirin is ≈ 12 hours.¹² To date, it appears that a standard regimen of ticlopidine or clopidogrel does not achieve a significant early antithrombotic effect.

We have also demonstrated that although a significant reduction in ADP-platelet aggregation is obtained with 300 mg of clopidogrel after 2 hours, a further significant reduction is achieved at 6 hours. This confirms recent findings showing that a loading regimen of clopidogrel induces maximal inhibition of platelet aggregation at 5 hours.¹³

The early antithrombotic effect on platelet activation we observed with a loading dose of clopidogrel, as assessed by flow cytometry, is consistent with the reduction in thrombus formation. The reduction in the fibrinogen binding obtained with a front-loaded regimen of clopidogrel is already significant at 2 hours, whereas with the standard regimen, a significant reduction was observed only at day 2. Similarly, an acceleration of platelet inhibition has been shown with a loading dose of a similar agent, ticlopidine (500 mg), in patients undergoing coronary angiography at 24 hours, but no data at 2 hours were provided.¹⁴

Prothrombin fragment F1+2 plasma levels were similar throughout the study and between the groups. This is consistent with other published data^{15,16} showing that thrombin generation is not directly reduced with thienopyridines.

Antithrombotic Effect of Clopidogrel Administration in Atherosclerotic Patients Receiving Chronic Aspirin Therapy

We showed that the antithrombotic effect of treatment with clopidogrel for 8 days (75 mg/d) in patients with atherosclerosis receiving chronic aspirin therapy is greater than the antithrombotic effect of chronic aspirin therapy alone. In animal models, clopidogrel has antithrombotic effects in both arterial and venous thrombosis.¹⁷⁻²⁰ In a porcine *ex vivo* model of high shear induced stent thrombosis, intravenous clopidogrel produced a dose-dependent inhibition of stent thrombosis.²⁰ In nonhuman primates, clopidogrel reduced thrombosis in a baboon model of arterial thrombosis.²¹ To the best of our knowledge, only 1 study focused on the inhibition of thrombogenesis by clopidogrel in humans.²² In this study, a standard regimen of clopidogrel significantly inhibited thrombus formation in healthy young subjects after 2 weeks of

a standard regimen of clopidogrel. We found that the inhibition was significant at day 5 in patients with atherosclerosis receiving chronic aspirin therapy when a standard regimen of clopidogrel was used.

Large-scale, randomized, placebo-controlled trials have clearly established that oral aspirin therapy decreases the risk of thrombotic events in patients with symptomatic atherosclerotic disease by 20% to 25%. Because aspirin is only a cyclooxygenase inhibitor,² its effectiveness is limited, because it fails to block platelet activation by other important agonists such as shear stress, thrombin, collagen, and ADP. Clopidogrel is an ADP-receptor antagonist that is free of the hematological side effects of ticlopidine therapy.⁸ Clopidogrel acts by irreversibly inactivating platelet ADP receptor-initiated signaling in a dose-dependent manner.¹⁵ In a large-scale, randomized clinical trial, clopidogrel was shown to be significantly more effective than and at least as safe as aspirin in decreasing arterial thrombo-occlusive episodes in patients with symptomatic atherosclerotic disease.³ However, aspirin and clopidogrel interfere with different pathways of platelet activation. Therefore, to improve the antithrombotic effect, the combination of these 2 drugs has been suggested. Additive antithrombotic effects of clopidogrel and aspirin have been demonstrated in experimental models in the rabbit.¹⁶ Interestingly, clopidogrel and aspirin have produced additive antithrombotic effects as assessed by measurements of ¹¹¹I-labeled platelets and ¹²⁵I-labeled fibrin deposition in a porcine ex vivo model of high shear-induced stent thrombosis.²⁵ In our study, the combination of aspirin and clopidogrel significantly improved the antithrombotic effect of aspirin alone in patients receiving chronic aspirin therapy. The rheological conditions (1600/s) we used mimicked those typical of a mildly stenosed coronary artery. These rheological conditions were selected because >70% of the plaques responsible for an acute myocardial infarction have <50% stenosis.¹³

Limitations

Our data support the added benefit of combining clopidogrel with aspirin on thrombus formation without any bleeding complications. However, this is a pilot study, and large-scale clinical trials are needed to further clarify the efficacy and safety of the combination of aspirin and clopidogrel.

Conclusions

Our data suggest that a front-loaded regimen of clopidogrel 300 mg added to aspirin achieves a significant antithrombotic effect at 2 hours, an effect not achieved with the standard regimen. In patients with known atherosclerotic disease on chronic aspirin therapy, this supports the efficacy of a loading dose of clopidogrel in acute coronary syndromes and percutaneous coronary interventions.

Acknowledgments

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References

1. Fuster V, Badier C, Badier J, Chesebrough J. The pathogenesis of coronary artery disease and the acute coronary syndromes. *N Engl J Med*. 1992;326:248-259.
2. Anti K, Lerman C, Bittencourt L, McLellan L, Tracy RP, Newson AJ, Remington JP, Braunholtz H. Thrombolysis in Myocardial Infarction 2 trial: outcomes in patients after acute coronary syndromes treated with the TPA-2 trial. *J Am Coll Cardiol*. 1998;31:634-637.
3. Antiplatelet Trialists' C. Collaborative overview of the cumulative risk of stroke in patients with atherosclerotic disease: a meta-analysis of individual and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*. 1998;316:1105-1109.
4. Schomig A, Neumann F, Kastrup A, Nebelunz H, Dierckx R, Thiele A, M. Walter H, Ziegler-Ruck P, Richardt G, Al F, Schmitt C, Thiele A. A randomized comparison of antiplatelet and anticoagulant therapy to the placement of coronary artery stents. *N Engl J Med*. 1996;344:1014-1018.
5. Bertrand M, Legrand V, Boland J, Cluck E, Benoit J, Emmanuel H, Viole M, Kisch J, Chierchia S, Casaccia M, Nesch C, Ott A, R. Lee C, Wiest-Pleple M, Van Belle P, Mc Fisher EP. Randomized trial comparing conventional anticoagulation versus aspirin plus clopidogrel in acute coronary stenting: the Full Antithrombotic Versus Aspirin Plus Ticlopidine (FANTASTIC) study. *Circulation*. 1998;98:1507-1513.
6. Urban P, Muegge C, Ruppel M, Karmali P, Rommel H, Fontana A, Pieper M, Wesseling T, Siegmund L. Randomized evaluation of antiplatelet versus antiplatelet therapy after coronary stent implantation in high-risk patients: the multicenter aspirin and ticlopidine trial after coronary stenting (MASTIS). *Circulation*. 1998;98:1126-1132.
7. Leon M, Baim D, Poppat J, Garwood P, Cutlip DE, Ho KK, Gosselin M, Diver D, Lerman D, Williams D, Pocock S, Kim P. Stent Anticoagulation Restenosis Study Investigators. A clinical trial comparing three antithrombotic-drug regimens after coronary stent stenting. *N Engl J Med*. 1998;338:1665-1671.
8. CAPRIE Steering Committee. A randomized, blinded trial of clopidogrel versus aspirin in patients at risk of ischemic events of AHA/ACC. *Circulation*. 1996;94:323-329.
9. Voronchikhin D, Badier C, Fuster V. Platelet glycoprotein IIb/IIIa receptor antagonists in cardiovascular disease. *Circulation*. 1999;100:1407-1414.
10. Patena C, Collet B, Zeller J, Fuster V, Gent M, Flucker L, Hsu J, Roth G. Platelet-activating agents, the relationship among dose, effectiveness, and side effects. *Chest*. 1998;114(suppl):3470-3480.
11. Miller D, Puri R, Hu C, Ahmed C, Arora G, Friedman MG, Collins RF, Cohen RW. Clopidogrel inhibits the binding of ADP analogs to the receptor mediating inhibition of platelet adenosine cyclase. *Arterioscler Thromb*. 1992;12:430-436.
12. Meuwé J, Gengen M, Rothman J, Chakraborty A, Wang X, Byr S, Meuwé R, Collins M, Kreps E, Moses JW. Effectiveness of clopidogrel and aspirin versus ticlopidine and aspirin in preventing stent thrombosis after coronary stent implantation. *Circulation*. 1999;99:2264-2269.
13. Badier C, Bergeron G, Savary S, Demich E, Shalunovich A, Pankin M, Sanborn TA. Effectiveness of aspirin and clopidogrel combination therapy in coronary stenting. *Int J Cardiol*. 1999;64:725-728.
14. Bergeron G, Ball MR, Rehal CS, Ting H, Bousquet G, Caron R, Bouché V, Mathias V, Kelly S, Gosselin J, Chabot D, Holmes DR, Jr. Clopidogrel versus ticlopidine after myocardial stent placement. *J Am Coll Cardiol*. 1999;34:1891-1894.
15. Mikkel L, Aguirre LV, Ligon RW, Rocha-Singh KJ, Ligon CL. Clopidogrel as adjunctive antiplatelet therapy during coronary stenting. *J Am Coll Cardiol*. 1999;34:1894-1899.
16. Shatz R, Cannon C, Janssen L. The antiplatelet effects of clopidogrel and aspirin. *Am J Med*. 1998;29:394-398.
17. Badier C, Fuster V, Bousquet J, Bousquet J, George V. A new mechanism of a tubular flow chamber for studying platelet interaction with biological and prosthetic materials: deposition of human ¹¹¹I-labeled platelets on collagen, subendothelium, and expanded polytetrafluoroethylene. *J Lab Clin Med*. 1987;109:704-718.
18. Mathias A, Badier C, Fuster V, Bousquet J, Bousquet J, George V, Fuster V, Badier C, Fuster V, Bousquet J. A new mechanism of a tubular flow chamber for studying platelet interaction with biological and prosthetic materials: deposition of human ¹¹¹I-labeled platelets on collagen, subendothelium, and expanded polytetrafluoroethylene. *J Lab Clin Med*. 1987;109:704-718.
19. Darius G, Badier C, Collet B, Fuster V, Sharma SK, Hayes RM, Altaba P, Aguirre JA, Mounier JD. Administration of abciximab during percutaneous coronary intervention reduces both ex vivo platelet reactivity

- formation and fibrin deposition: implications for a potential anti-coagulant effect of fibrinolytic. *Int J Vasc Med Ther*. 1998;10:1442-1449.
20. Lindahl T, Lonn P, Carlsson A. Studies of fibrinogen binding to platelets by flow cytometry: an improved method for analysis of platelet activation. *Thromb Haemostasis*. 1993;68:223-229.
21. Sasse M, Hanger J, Bachmann C, Wykl P, Gremelin B, Carlier B. Clopidogrel blocks platelet glycoprotein IIb/IIIa receptor-mediated response to platelet activators. *Stroke Thromb Haemostasis*. 1999;79(suppl 2):117-19.
22. Wilton G, Bond C, Bell M, Coleman B, Holmes DR Jr, Geiger PB. Timing of coronary stent thrombosis in patients treated with ticlopidine and aspirin. *Am J Cardiol*. 1998;81:1006-1011.
23. Bengtsson U, Lindahl T. Potential onset of platelet inhibition with a loading dose of ticlopidine in ASA-treated stable coronary patients. *Am J Cardiol*. 1998;84:715-717.
24. Boole F, Bessac B, Helft P, Stijnen C, Dickinson JP, Motter G, Sackmann EL. A novel way to platelet inhibition which inhibits thrombin-induced platelet aggregation in human blood independently of the blood flow condition. *Thromb Haemostasis*. 1998;71:655-662.
25. Geiger PB, Tashiro A, Sagami I, Barber A, Sakuragawa K, Warner B, Endler J. A double-blind randomized comparison of aspirin alone and aspirin plus ticlopidine versus aspirin or ticlopidine alone on experimental arterial thrombogenesis in humans. *Blood*. 1998;92:1518-1525.
26. Benoit A, Maffrand JP, Lefebvre G, Gordon J. The role of platelets and ADP in experimental thrombosis induced by various stress in the rat. *Thromb Haemostasis*. 1988;52:65-70.
27. Maffrand J, Benoit A, Bachmann G, Lefebvre G, Capreau JP, Gordon J. ADP plays a key role in thrombogenesis in rats. *Thromb Haemostasis*. 1993;69:225-230.
28. Sagami I, Benoit A, Hoffman M, Sagami G, Maffrand JP, Lefebvre G, Maffrand JP, Vigne P, Chen JP, Dinet JG. Comparative antiplatelet and antithrombotic activity of clopidogrel and acetyl salicylic acid in rats. *Thromb Haemostasis*. 1997;68:300-305.
29. Benoit A, Mares A, Lefebvre G, Maffrand J, Helft P. Effect of various antiplatelet agents on acute arterial thrombosis in the rat. *Thromb Haemostasis*. 1993;70:812-816.
30. Maffrand JP, Bigler N, Kott M, Lefebvre A, Nakamura M, Roth GJ, Capreau JP, Helft JM, Lefebvre G. Effects of clopidogrel in combination with aspirin in vivo model of high-dose aspirin-induced thrombosis. *Eur Heart J*. 1998;19:1538-1546.
31. Barker J, Marzari G, Kelly A, Channon NB, Sundell JD, Dawson GK, Helft JM. Clopidogrel inhibition of stent, graft, and vascular thrombogenesis with antithrombotic enhancement by aspirin in nonhuman primates. *Circulation*. 1998;98:2461-2469.
32. Parante O. Aspirin as an antiplatelet drug. *N Engl J Med*. 1993;329:1287-1294.
33. Gough PJ, Birch J, Kamigaito P, Lichtenhan GL, Schenckhoff CB, Helft JM, Walker C. Specific regulation of human platelet P2Y₁ ADP receptor-mediated signaling by the antiplatelet drug clopidogrel. *Arterioscler Thromb Vasc Biol*. 1999;19:2007-2011.
34. Helft J, Del F, Benoit A, Delange S, Lefebvre G, Sackmann EL. The vasoregulating and antithrombotic activity of clopidogrel is potentiated by aspirin in several experimental models in the rabbit. *Thromb Haemostasis*. 1998;76:512-518.
35. Falk E, Lindmark K, Gustav V. Coronary plaque disruption. *Circ Res*. 1995;77:657-671.

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Abstract

The usefulness of Clopidogrel as inhibitor of platelet aggregation has been demonstrated, but its effect on hemorheological parameters, such as whole blood viscosity at low and high shear rate, red cell aggregation and deformability indexes, filterability rate and deformability of red blood cells has not been studied. This study revealed that, in subjects with impaired blood rheology and ultrasound evidence of atherosclerosis, 3-weeks treatment with clopidogrel (75 mg daily) improved blood viscosity at high shear rate and other hemorheological parameters, including red cell filterability rates and dynamic red cell deformability index ($p < 0.01$ for all), without any unwanted side effect. Blood viscosity at low shear rate and red cell aggregation index improved after only 1-week treatment, and the reductions were maintained after two and three weeks ($p < 0.01$). These results indicate that, similarly to another thienopyridine such as ticlopidine, Clopidogrel may have a positive influence on several hemorheological parameters, thus exerting its protection not only through inhibition of platelet function, but also through changes in the hemorheological profile.

References

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United States Patent [19]

Badore et al.

[11] Patent Number: 4,847,265

[45] Date of Patent: Jul. 11, 1989

[54] DEXTRO-ROTATORY ENANTIOMER OF METHYL ALPHA-S (4,5,6,7-TETRAHYDRO (3,2-C) THIENO PYRIDYL) (2-CHLOROPHENYL)-ACETATE AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING IT

[75] Inventors: Alain Badore, Roquettes; Daniel Fréhel, Toulouse, both of France

[73] Assignee: Sanofi, France

[21] Appl. No.: 155,550

[22] Filed: Feb. 12, 1988

[30] Foreign Application Priority Data

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[51] Int. Cl.⁴ A61K 31/44; C07D 495/04

[52] U.S. Cl. 514/301; 546/114

[58] Field of Search 546/114; 514/301

[56] References Cited

U.S. PATENT DOCUMENTS

4,329,596 7/1985 Aubert et al. 546/115

FOREIGN PATENT DOCUMENTS

0099801 7/1983 European Pat. Off. .

OTHER PUBLICATIONS

Fieser et al., *Advanced Org. Chem.*—Reinhold Publishing Co., N.Y., (1961), pp. 85-88.

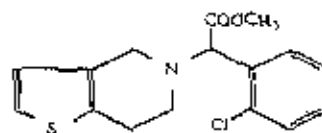
Primary Examiner—Mary C. Lee

Assistant Examiner—Bernard I. Deniz

Attorney, Agent, or Firm—Wegner & Bretschneider

[57] ABSTRACT

The present invention relates to the dextro-rotatory enantiomer of Formula



and its pharmaceutically acceptable salts with platelet aggregation inhibiting activity.

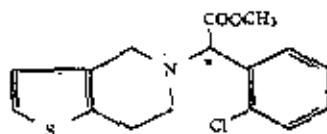
The invention also relates to a process for the preparation of this compound starting from the racemate and the pharmaceutical compositions containing it.

7 Claims, No Drawings

**DEXTRO-ROTATORY ENANTIOMER OF
METHYL ALPHA-5(4,5,6,7-TETRAHYDRO(3,2-C)
THIENO PYRIDYL)
(2-CHLOROPHENYL)-ACETATE AND THE
PHARMACEUTICAL COMPOSITIONS
CONTAINING IT**

The present invention relates to the dextro-rotatory enantiomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate, a process for its preparation and pharmaceutical compositions containing it.

The compound of the invention corresponds to the following formula (I):



in which the C* is an asymmetric carbon atom. In fact, this formula represents both the dextro-rotatory molecule claimed as well as its levo-rotatory enantiomer. The racemic mixture corresponding to this formula was described in the French patent application published under the No. 2 530 247. Hereafter the dextro-rotatory enantiomer claimed according to the invention will be designated by I_d and the levo-rotatory enantiomer by I_l.

It is known that the optical rotatory power of a compound depends on the solvent in which it is measured and on its concentration in this solvent. The optical rotatory power of the dextro-rotatory isomer according to the invention is positive in methanolic solution.

In an unexpected manner only the dextro-rotatory enantiomer I_d exhibits a platelet aggregation inhibiting activity, the levo-rotatory enantiomer I_l being inactive. Moreover, the inactive levo-rotatory enantiomer I_l is the less well tolerated of the two enantiomers.

The invention also relates to the addition salts of the compounds of formula (I_d) with pharmaceutically acceptable mineral or organic acids.

The compound (I_d) is an oil whereas its hydrochloride exists as a white powder. The oily products are usually difficult to purify and it is preferable to use for the preparation of pharmaceutical compositions crystalline products which can usually be purified by recrystallization.

However, it has been observed in the present case that some of the salts of compound (I_d) usually precipitate in an amorphous form and/or that they are hygroscopic, a property which makes them difficult to handle on an industrial scale. Thus, the salts of carboxylic acid and sulfonic acids classically used in pharmacy have been prepared, acids such as acetic, benzoic, fumaric, maleic, citric, tartaric, gentisic, methane-sulfonic, ethanesulfonic, benzenesulfonic and laurylsulfonic acids as well as the salts of dobesilic acid (m.p. = 70° C.) and para-toluenesulfonic acid (m.p. = 51° C.), the purification of which proved to be difficult.

Among the mineral and organic acid salts of the dextro-rotatory isomer of the compound of Formula (I_d) salts have been found which crystallize easily, are not hygroscopic and are sufficiently water-soluble as to make their use as active medicinal principles particularly advantageous.

The present invention thus relates more particularly to the hydrogen sulfate, the taurocholate and the hydrobromide of the dextro-rotatory enantiomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate.

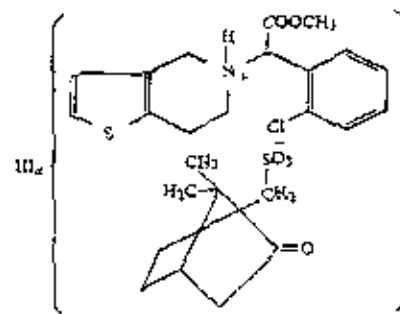
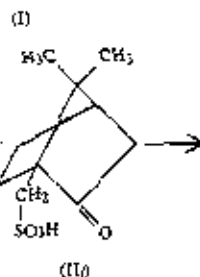
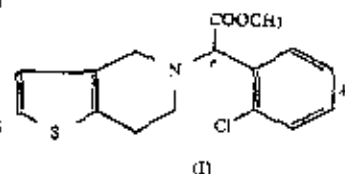
These salts are prepared in a standard manner by the action of the corresponding acid on the base in solution in a solvent from which they precipitate spontaneously or after addition of a non-solvent of the salt.

The dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate can be prepared by forming the salt of the racemic compound with an optically active acid in a solvent, repeated recrystallizations of the salt until a product of constant optical rotatory power is obtained, followed by the liberation of the dextro-rotatory isomer from its salts by a base; if required, a salt is formed between the dextro-rotatory isomer and a pharmaceutically acceptable acid.

The optically active acid may advantageously be levo-rotatory camphor-10-sulfonic acid.

One and the same solvent may be used for salt formation and recrystallization: acetone is ideally suited in this case.

The chiral, levo-rotatory camphor-10-sulfonic acid of Formula (II_l) is allowed to react in an inert solvent with the racemic mixture of Formula (I) according to the following reaction scheme:

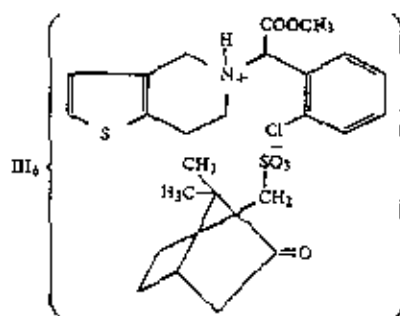
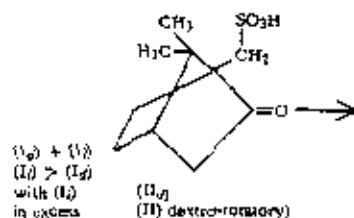


Salt formation may be carried out in solvents such as alcohols, ketones, dimethylformamide. The salt precipitates spontaneously or is isolated by salting out or evaporation of the solvent. A mixture of two diastereoisomers of Formula (IIIa) is formed. By repeated recrystallizations from a solvent such as acetone the precipitate

20
48
140

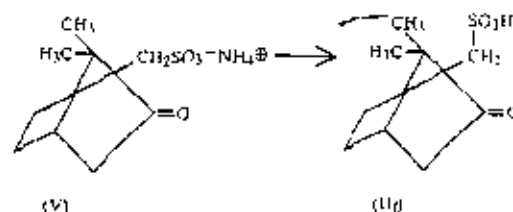
is enriched in the salt of the dextro-rotatory isomer of compound (I). After each recrystallization the optical rotatory power $[\alpha]_D^{20}$ of the precipitate is measured at 20° C. in methanol at a concentration varying between 1.5 and 2 g./100 ml. When the $[\alpha]_D^{20}$ stops increasing the base of Formula (I_D) is liberated from the salt (III_a) by the action of a base such as sodium or potassium hydrogen carbonate in aqueous media at temperatures varying between 5° C. and 20° C. Evaporation of the filtrate of the first recrystallization IV after the crystals of the precipitated salt (III_a) have been filtered off, gives a mixture enriched in the salt of (I₁) enantiomer. The basification of this mixture of diastereoisomeric salts with a weak base such as sodium or potassium hydrogen carbonate in aqueous solution at temperatures varying between 5° C. and 20° C. leads to a mixture of the two enantiomers (I_d) plus (I₁) enriched in the levo-rotatory enantiomer (I₁).

This mixture (I_d) + (I₁) enriched in enantiomer (I₁) is allowed to react with dextro-rotatory camphor-10-sulfonic acid which will be designated as (II_d) in a solvent according to the following reaction scheme:



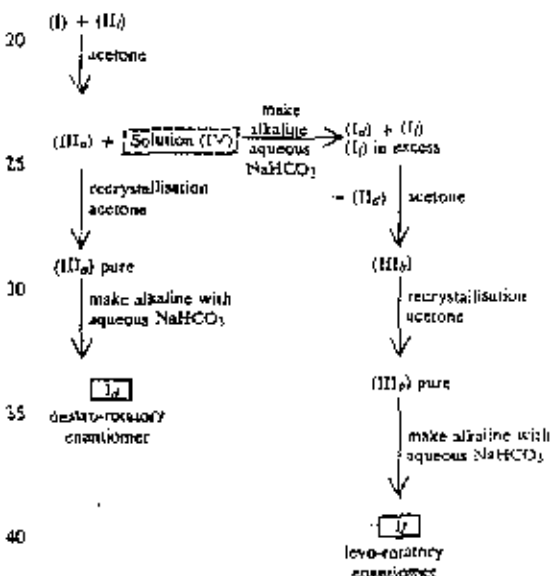
The crystalline mixture of diastereoisomeric salts (III_b) obtained is recrystallized from acetone until the optical rotatory power $[\alpha]_D^{20}$ of the precipitate remains constant. As previously mentioned the optical rotatory power $[\alpha]_D^{20}$ of the diastereoisomeric salt (III_b) is determined after each recrystallization.

The liberation of the stereoisomeric (I₁) from its salt is carried out in a standard manner, like that for compound (I_d). Levo-rotatory camphor-10-sulfonic acid (II_l) may be obtained from commercially available ammonium camphor-10-sulfonate of Formula (V) according to the reaction scheme:



An aqueous solution of the ammonium salt (V) is chromatographed on an Amberlite IRN-77 resin. After lyophilization of the eluate camphor-10-sulfonic acid of Formula (II_l) is recovered.

The entire sequence of the process is shown schematically below:



Each of the pure enantiomers (I_d) and (I₁) may be converted into a salt by means of the standard methods; for example, the hydrochlorides are prepared by the addition of a solution of hydrogen chloride gas in diethyl ether to a solution of (I_d) or (I₁) in diethyl ether.

DETERMINATION OF THE ENANTIOMERIC PURITY OF THE DEXTRO-ROTATORY (I_d) AND LEVO-ROTATORY (I₁) ENANTIOMERS

Two methods have been used:

proton NMR spectroscopy with the addition of a chiral rare earth
high pressure liquid chromatography using a chiral stationary phase.

(a) Proton NMR spectroscopy with the addition of a chiral rare earth. The enantiomeric purity (optical purity) was determined by ¹H 60 MHz NMR spectroscopy in the presence of a rare earth chiral complex according to the method described by G. M. WHITE-SIDES et al. (J. Am. Chem. Soc. 1974, 96, 1038).

In the racemic product (I), the hydrogen attached to the asymmetric centre in the α position to the ester function appears as a singlet (chemical shift δ=4.87 ppm in CDCl₃ as solvent. The addition of the rare earth complex Eu(tfc)₃ (tris 3-(trifluoromethyl) hydroxyme-

thylene)-3-camphorato europium (III) to the probe containing a solution of the racemate (I) in CDCl_3 leads to the resolution of the initial singlet into two, well-separated singlets corresponding to the proton of each of the two enantiomers (I_d) and (I_l). When the molar ratio of rare earth complex/compound (I)=0.4, the separation between the two singlets is about 6 Hz.

With each of the two enantiomers prepared, (I_d) and (I_l), the same procedure was used as for the racemate (I). The smaller chemical shift corresponds to the proton of the dextro-rotatory enantiomer (I_d) and the larger chemical shift to the levo-rotatory enantiomer (I_l).

The precision of the method was determined by comparing the ^1H (60 MHz) NMR spectra obtained with and without addition of the rare earth complex for each of the two enantiomers (I_d) and (I_l) in the pure state or as mixtures containing increasing quantities of one of the enantiomers. It was observed that it was possible to detect easily more than 5% by weight of one enantiomer in the presence of the other.

(b) High pressure liquid chromatography using a chiral stationary phase. The study was conducted with a liquid chromatograph HP-1084 using a UV detector at 215 nm. The chiral stationary phase was DEAE silica (10 microns) onto which was grafted alpha-1 acid glycoprotein (0.4x100 mm) (ENANTIO-PAC R-LXB). The mobile phase consisted of an aqueous phosphate buffer mixture 8 mM ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) containing 0.1M of NaCl, adjusted to pH=7.4, and 15% of isopropanol (v/v). The flow rate was fixed at 0.3 ml/minute and the temperature of the column was maintained at 18°-20° C. Under these conditions, the dextro-rotatory enantiomer (I_d) has a retention time of 45 minutes and the levo-rotatory enantiomer (I_l) has a retention time of 35 minutes.

The precision of the determination of the optical purity of the two enantiomers was estimated by chromatographing each of the two enantiomers (I_d) and (I_l) prepared either alone or as a mixture containing increasing amounts of one of the enantiomers. It was observed that it was easy to detect:

2% (weight/weight) of enantiomer (I_d) in enantiomer (I_l)

4% (weight/weight) of enantiomer (I_l) in enantiomer (I_d).

Under these conditions it may be concluded that the optical purity of the two enantiomers (I_d) and (I_l) obtained according to the examples is at least equal to 96% for the dextro-rotatory enantiomer (I_d) and at least equal to 98% for the levo-rotatory enantiomer (I_l).

The following examples are non-restrictive and are presented to illustrate the present invention.

EXAMPLE 1

Salts of dextro-rotary methyl-alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate

(a) levo-rotatory camphor-10-sulfonic acid

A column of Amberlite IRN-77 resin is prepared and tested by passing 1N hydrochloric acid through it and then by washing this column of acidified resin abundantly with water. Levo-rotatory ammonium camphor-10-sulfonate is dissolved in a minimum of water and applied to the column of resin previously prepared. Elution is carried out with water. The eluted fractions containing the acid are lyophilized.

White crystals, m.p.=198° C., $[\alpha]_D^{20} = -20.33$ (c=2.075 g/100 ml water).

(b) Camphor-10-sulfonic acid salt of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990 B).

32 g (0.0994 mole) of racemic methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate are dissolved in 150 ml of acetone; 9.95 g (0.0307 mole) of levo-rotary camphor-10-sulfonic acid monohydrate are added. The clear solution is left to stand at room temperature. After 48 hours some crystals have formed. The reaction mixture is concentrated to 50 ml and left to stand at room temperature for 24 hours. The crystals obtained are filtered off, washed with acetone and dried (yield: 55% on the basis of the starting racemate).

White crystals, m.p.=165° C., $[\alpha]_D^{20} = +14.67$ (c=1.58 g/100 ml; methanol).

The crystals obtained above are redissolved in the minimum of boiling acetone (50 ml). The crystals obtained after cooling are filtered off, washed with acetone and dried (yield: 88%).

White crystals, m.p.=165° C., $[\alpha]_D^{20} = +24.75$ (c=1.68 g/100 ml; methanol).

(c) Dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate.

12 g (0.032 mole) of the pure product obtained in (b) are dissolved in a minimum of water. After cooling to 5° C., the aqueous solution obtained is made alkaline with a saturated aqueous solution of sodium hydrogen carbonate. The alkaline aqueous phase is extracted with dichloromethane. The organic extracts are dried over anhydrous sodium sulfate. On evaporation of the solvent a colorless oil is obtained (quantitative yield). Oil, $[\alpha]_D^{20} = +51.52$ (c=1.61 g/100 ml; methanol).

(d) The hydrochloride of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (dextro-rotatory) (SR 25990 A).

7 g (0.0228 mole) of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate are dissolved in 100 ml of diethyl ether. 30 ml of a solution of 1N HCl in diethyl ether are added and the crystals obtained are filtered off. The crystals are washed with diethyl ether and dried (yield: 94%).

White crystals, m.p.=117° C., $[\alpha]_D^{20} = +62.23$ (c=1.82 g/100 ml; methanol).

(e) The hydrogen sulfate of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990 C).

800 ml of a saturated aqueous solution of sodium bicarbonate are added to a suspension of 200 g of SR 25990 B in 800 ml of dichloromethane. After vigorous shaking, the organic phase is separated, dried over sodium sulfate and the solvent is removed under reduced pressure. The residue is dissolved in 500 ml of ice-cold acetone and 20.7 ml of concentrated sulfuric acid (93.64%, d=1.83) are added drop-wise. The precipitate formed is isolated by filtration and washed with 1,000 ml of acetone, then dried in a vacuum oven at 50° C.

139 grams of analytically pure white crystals are thus obtained with a melting point of 134° C. Empirical formula: $\text{C}_{16}\text{H}_{16}\text{ClNO}_2\text{S}\cdot\text{H}_2\text{SO}_4$ $[\alpha]_D^{20} = +55.10$ (c=1.391 g/100 ml; methanol).

(f) The hydrobromide of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990 D).

150 ml of an aqueous solution of sodium bicarbonate are added to a suspension of 20 g of SR 25990 B in 200

ml of dichloromethane. The residue obtained after separation of the organic phase, drying and evaporation of the solvent is dissolved in 150 ml of diethyl or isopropyl ether, and 4.4 ml of a 48% (wt/v) aqueous solution of hydrobromic acid are added drop-wise. The precipitate formed is isolated. After drying, 14.4 g of crystals are obtained with a melting point of 111° C. (yield 99%).

13.4 g of these crystals are recrystallized from a mixture of isopropyl ether (100 ml) and isopropanol (150 ml) to give 10.2 g of analytically pure hydrobromide: m.p. = 140° C.; empirical formula: $C_{16}H_{16}ClNO_2S.HBr$; $[\alpha]_D^{20} = +59.23$ ($c = 2.09$ g/100 ml; methanol).

(g) The taurocholate of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990E).

The sodium salt of taurocholic acid is chromatographed on Amberlite IRN-77 resin by eluting with water. The fractions obtained are lyophilized.

3 g (0.0054 mole) of SR 25990B are treated with a saturated aqueous solution of sodium bicarbonate and the mixture is extracted with dichloromethane. The organic phase is separated, dried over sodium sulfate and evaporated to dryness. The free base obtained is taken up in 30 ml of isopropanol; 2.8 g (0.0054 mole) of taurocholic acid dissolved in 100 ml of isopropanol are added to this solution. The mixture is stirred overnight at room temperature, then evaporated to dryness. The residue solidified on being triturated with ether. 3.5 g of beige crystals are obtained. m.p. = 120° C. $[\alpha]_D^{20} = +39.53$ ($c = 1.79$ g/100 ml of methanol). $C_{16}H_{16}ClNO_2S.C_{24}H_{43}NO_7$; C, H, N analyses in agreement with theory.

EXAMPLE 2

Salts of levo-rotatory methyl
alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl)
(2-chlorophenyl)-acetate

(a) Salt of d-camphor-10-sulfonic acid (SR 25989 B)

After separation of SR 25990 B in Example 1-b the solvent is evaporated from the acetone filtrate obtained.

The residue is taken up in water and diethyl ether. The ethereal phase is decanted. The aqueous phase is cooled to 5° C. and made alkaline with a saturated aqueous solution of sodium bicarbonate. The aqueous alkaline phase is extracted with diethyl ether. The ethereal extracts are pooled and dried over anhydrous sodium sulfate.

On evaporation of the solvent an oil is obtained which is purified by filtration through a bed of silica (eluant: diethyl ether). A colorless oil is recovered consisting of a mixture of about 65% of the levo-rotatory enantiomer and 35% of the dextro-rotatory enantiomer, proportions determined by means of ^1H (60 MHz) NMR spectroscopy after the addition of chiral, rare earth complex.

16.66 g (0.0317 mole) of the mixture thus obtained are dissolved in 70 ml of acetone. 7.77 g (0.0310 mole) of dextro-rotatory camphor-10-sulfonic acid monohydrate are added. The homogeneous mixture is left to stand overnight at room temperature. The crystals obtained are filtered off, washed with acetone and dried (yield: 44% based on the mixture).

The crystals obtained are dissolved in a minimum of refluxing acetone (60 ml). The precipitate obtained on cooling to room temperature is filtered off, washed with

acetone and dried. White crystals, m.p. = 167° C. $[\alpha]_D^{20} = -24.35$ ($c = 1.79$ g/100 ml; methanol).

(b) Levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate.

11.3 g (0.0204 mole) of the camphor-10-sulfonate obtained in (a) are dissolved in a minimum of water. The aqueous solution obtained is cooled to 5° C. and made alkaline with a saturated aqueous solution of sodium hydrogen carbonate. The alkaline aqueous phase is extracted with dichloromethane. The organic solution is dried and the solvent is evaporated. A colorless oil is isolated (quantitative yield).

Oil $[\alpha]_D^{20} = -50.74$ ($c = 1.58$ g/100 ml; methanol).

(c) The hydrochloride of levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25989 A).

Prepared according to the method described in Example 1(d). Yield: 94%.

White crystals, m.p. = 117° C. $[\alpha]_D^{20} = -62.56$ ($c = 1.80$ g/100 ml; methanol).

(d) The hydrogen sulfate of levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25989 C).

70 g (0.126 mole) of the camphor sulfonate SR 25989 B obtained are treated as described in (a) above with a saturated aqueous solution of sodium bicarbonate in the presence of dichloromethane. The organic phase is separated, dried over sodium sulfate and evaporated to dryness. The residue is taken up in 300 ml of acetone and 7.2 ml (0.126 mole) of concentrated sulfuric acid are added drop-wise. After being stirred the crystals formed are filtered off and washed with acetone. 47.8 g of white crystals are obtained. m.p. = 182° C. $[\alpha]_D^{20} = -51.61$ ($c = 2.044$ g/100 ml; methanol). The analysis (C,H,N) are in agreement with theory.

PHARMACOLOGICAL STUDY

The platelet aggregation inhibiting activity and the toxicity of these new compounds were compared to those of the racemic mixture described in the French Pat. No. 82.12599 (Publication No. 2 520 247).

A description will now be given of the results of this study which demonstrates another advantage of the invention, namely that the salts of the dextro-rotatory isomer have a better therapeutic index than the salt of the racemic mixture; in fact, the levo-rotatory isomer exhibits almost no platelet aggregation inhibiting activity and its toxicity is markedly higher than that of its dextro-rotatory homologue.

The platelet aggregation inhibiting activities and the antithrombotic activities of the compounds were studied in the rat by standard methods.

The activity on the aggregation of plates induced by ADP or collagen was determined ex-vivo.

The products dissolved in ethanol (200 mg/ml) and diluted in water containing gum arabic (5%-wt/v) were administered by the oral route to groups of five female rats of the CD-COBS strain, weighing 250-300 g, in amounts of 10 ml of suspension per kilogram two hours before blood samples were taken.

The blood samples were taken from animals anesthetized with diethyl ether by puncture of the abdominal aorta and placed over a 3.8% aqueous solution of sodium citrate (1 vol/9 volumes of blood). The platelet-rich plasma was then isolated by centrifugation at 200 g for 10 minutes.

Aggregation is induced by the addition of 2 μ l of aggregating solution to 400 μ l of platelet-rich plasma.

The aggregating solutions used were: a 500 μ M aqueous solution of ADP marketed by Boehringer Mannheim (final concentration 2.5 μ M), and a solution of collagen marketed by Sigma (type 1) at a concentration of 0.25 g/100 ml in 3% acetic acid (v/v) (final concentration 12.5 μ g/ml).

The aggregation of the platelets was monitored as described in the method by G. V. R. Born in Nature 194, p. 927 (1967) using a Coultronics® aggregometer at a temperature of 37° C. and agitation of 900 rpm.

For aggregation with ADP, the aggregometer generates a curve representing a platelet aggregation as measured by a change in optical density. The height of this curve is defined as the height of aggregation. The percentage of aggregation is the relation between the aggregation height measured and the height corresponding to 100% aggregation $\times 100$. The percentage of inhibition is determined by the relation:

$$\frac{\text{Control aggregation height} - \text{produced aggregation height}}{\text{Control aggregation height}} \times 100$$

The results obtained for the aggregation with ADP for the hydrochloride of the racemic mixture (PCR 4099), the hydrogen sulfates of the dextro-rotatory (SR 25990 C) and levo-rotatory (SR 25989 C) isomers on the one hand, and for PCR 4099 and the hydrochlorides of the dextro-rotatory (SR 25990 A) and levo-rotatory (SR 25989 A) on the other, are shown in Table I; they demonstrate that the levo-rotatory isomer is inactive and that the dextro-rotatory isomer is at least as active as the racemate.

TABLE I

PRO- DUCT	DOSE mg/Kg P.O.	QUAN- TITY of base ad- ministered	% IN- HIBI- TION P**
% AGGREGATION			
Controls			42.4 $\pm$ 1.5
PCR 4099	4.48	3.84	29.8 $\pm$ 2.4
(racemate)	8.97	7.69	17.3 $\pm$ 2.2
	17.9	15.38	11.1 $\pm$ 2.1
SR 25989C	20	15.38	41.0 $\pm$ 1.5
	40	30.76	11.1 $\pm$ 1.7
SR 25990C	1.25	0.96	39.4 $\pm$ 1.3
	2.5	1.92	28.4 $\pm$ 1.3
	5	3.84	14.0 $\pm$ 1.6
	10	7.69	8.5 $\pm$ 1.6
Controls			13.8 $\pm$ 2.3
SR 25990E	10	3.84	9.6 $\pm$ 1.3
	20	7.69	4 $\pm$ 1.6
AGGREGATION HEIGHT			
Controls			103 $\pm$ 5
PCR 4099	2.5	2.14	86 $\pm$ 5
(racemate)	5	4.28	72 $\pm$ 8
	12.5	10.71	32 $\pm$ 9
SR 25989A	25	22.46	101 $\pm$ 1
SR 25990A	2.5	2.14	67 $\pm$ 7
	5	4.28	76 $\pm$ 5
	12.5	11.23	19 $\pm$ 4
	25	22.46	11 $\pm$ 1

*mean of results $\pm$ standard error of the mean (SEM)

**Student test

***aggregation height in mm; mean $\pm$ SEM (n = 7)

n.s. not significant

For the aggregation with collagen, the percentage of inhibition is the difference of the slopes of the curves representing the variation of the optical density as a function of time for the control and the product to be tested divided by the slope of the control multiplied by

100. The results shown in Table II demonstrate again that only the dextro-rotatory isomer is active whereas the salts have comparable activities.

TABLE II

PRO- DUCT	DOSE mg/Kg P.O.	QUAN- TITY of base ad- ministered	SLOPE	% IN- HIBI- TION P**
Controls			4.8 $\pm$ 0.1	
PCR 4099	4.48	3.84	3.6 $\pm$ 0.2	25 0.05
(racemate)	8.97	7.69	2.7 $\pm$ 0.5	44 0.01
	17.9	15.38	1.5 $\pm$ 0.1	69 0.001
SR 25989C	20	15.38	4.5 $\pm$ 0.2	30 n.s.
	40	30.76	4.0 $\pm$ 0.2	17 n.s.
SR 25990C	1.25	0.96	4.5 $\pm$ 0.5	6 n.s.
	2.5	1.92	4.1 $\pm$ 0.2	15 n.s.
	5	3.84	2.3 $\pm$ 0.1	52 0.001
	10	7.69	1.7 $\pm$ 0.1	65 0.001
Controls			1.5 $\pm$ 0.1	
SR 25990E	10	3.84	2.1 $\pm$ 0.1	40 0.01
	20	7.69	1.4 $\pm$ 0.4	60 0.01
Controls			1.97 $\pm$ 0.29	
PCR 4099	2.5	2.14	3.15 $\pm$ 0.33	21 n.s.
(racemate)	5	4.28	2.94 $\pm$ 0.50	26 0.05
	12.5	10.71	2.19 $\pm$ 0.42	45 0.01
SR 25989A	25	22.46	4.32 $\pm$ 0.29	10 n.s.
SR 25990A	2.5	2.14	3.05 $\pm$ 0.19	23 0.05
	5	4.28	1.24 $\pm$ 0.22	69 0.001
	12.5	11.23	0.36 $\pm$ 0.14	78 0.001
	25	22.46	0.74 $\pm$ 0.15	51 0.001

**Student test.
n.s. not significant

The antithrombotic activity of these compounds has also been studied in the test of venous thrombosis on a screw thread described by Kumada T. et al. in Thromb. Res 18 p. 189 (1980).

Female rats of the same type as those previously described, in groups of 10 animals, were anesthetized with diethyl ether and their vena cava was isolated after abdominal incision.

A metallic screw thread 21 mm long consisting of a dentist's drill, marketed by Dyna (France) size No. 30, was introduced into the lumen of this vein just below the renal bifurcation descending towards the iliac veins, without damaging the wall; 19 to 20 mm of the length of the screw thread are implanted and the remaining 1 mm protrudes through the closed stomach into the exterior.

The thrombi formed rapidly and five hours later, under pentobarbital anesthesia, the abdomen is re-opened and ligatures are placed above and below the screw thread which is withdrawn after longitudinal incision of the vein and the isolated thrombus is weighed.

The results which are presented in Table III show that the levo-rotatory isomer is inactive in this test, in contrast to the dextro-rotatory isomer and the racemate.

TABLE III

PRO- DUCT	DOSE mg/Kg P.O. admin.	QUAN- TITY of base	WEIGHT of thrombus*	VARI- ATION %	P**
Controls			1.9 $\pm$ 0.1		
PCR 4099	4.48	3.84	2.17 $\pm$ 0.24	44	0.001
(racemate)	8.97	7.69	1.39 $\pm$ 0.15	64	0.001
	17.9	15.38	1.00 $\pm$ 0.19	74	0.001
SR 25989C	40	30.76	1.17 $\pm$ 0.42	-7	n.s.
SR 25990C	1.25	0.96	1.11 $\pm$ 0.32	20	n.s.
	2.5	1.92	2.29 $\pm$ 0.22	31	0.01
	5	3.84	1.71 $\pm$ 0.24	56	0.01
	10	7.69	1.26 $\pm$ 0.19	67	0.01
	20	15.38	1.20 $\pm$ 0.13	69	0.01

60

65

TABLE III-continued

PRO- DUCY	DOSE mg/Kg P.O. Admin.	QUAN- TITY of base	WEIGHT of thrombi*	VARIA- TION %	P**
Controls			3.73 $\pm$ 0.36		
SR 25990E	10	1.34	1.48 $\pm$ 0.15	60	0.001
	20	7.59	1.13 $\pm$ 0.15	68	0.001

* = weight of thrombi in mg $\pm$ standard error of the mean
 ** = 1) test of Kruskal-Wallis

For the toxicological study, the compounds were administered by the oral route in the form of a suspension in the same volume of water made up to 10% (wt/v) with gum arabic to groups of 10 fasted female rats of the Sprague Dawley strain weighing 120 to 135 grams.

The number of dead animals was determined 14 days after the administration of the compound under study. The lethal doses thus determined, expressed in weight of the salt administered, are presented in Table IV; these results show on the one hand that the toxicity of the (acetic mixture is similar to that of the levo-rotatory isomer whereas the dextro-rotatory isomer is markedly less toxic, and, on the other hand, that the toxicity depends on the nature of the acid used to form the salt.

TABLE IV

PRODUCTS	D 10	D 50 (1)	D 90	ABSOLUTE LETHAL DOSE
PCR 4099 (racemate)	1318	1615 (1443-1747)	1975	2000
SR 25989 A	1219	1792 (1443-1797)	2299	2000
SR 25990 A	3055	4316 (3569-5705)	5127	5000
SR 25990 C	2277	2591 (2372-2805)	2974	4000
SR 25990 D	2634	4268 (3381-6012)	6914	5000

(1) = confidence interval

The pharmacological study just presented has demonstrated the interesting inhibitory properties towards platelet aggregation of the compound 1d and the absence of any activity of its isomer 1l.

The medicine of the invention can be made available for oral administration in the form of tablets, sugar-coated tablets, capsules, drops, granules or a syrup. It can also be made available for rectal administration in the form of suppositories or for parenteral administration in the form of an injectable solution.

Each unit dose contains advantageously from 0.001 g to 0.100 g of the derivative of the invention, and the daily doses to be administered may vary from 0.001 g to 0.500 g of active ingredient depending on the age of the patient and the severity of the disorder to be treated. Some pharmaceutical formulations of the medicine of

the invention will be given below as non-restrictive examples.

(1) Tablets

Active ingredient: 0.010 g

Excipient: lactose, powdered sugar, rice-starch, alginate acid, magnesium stearate

(2) Sugar-coated tablets

Active ingredient: 0.005 g

Excipient: magnesium stearate, maize starch, gum arabic, shellac, white sugar, glucose, white wax, carnauba wax, paraffin, croscollin.

(3) Capsules

Active ingredient: 0.025 g

Excipient: magnesium stearate, maize starch, lactose.

(4) Injectable solution

Active ingredient: 0.050 g

Isotonic saline q.s.p. 3 ml

(5) Suppositories

Active ingredient: 0.030 g

Semi-synthetic triglycerides q.s.p. 1 suppository.

On account of its interesting inhibitory properties towards platelet aggregation and its interference in the mechanism of formation of arterial and venous thromboses, the medicine of the invention can be usefully administered in the treatment and prevention of platelet disorders due to extracorporeal blood circuits or the consequence of complications in atheroma.

We claim:

1. Dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer and its pharmaceutically acceptable salts.

2. Hydrochloride of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

3. Hydrogen sulfate of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

4. Hydrobromide of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

5. Taurocholate of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

6. Pharmaceutical composition which comprises an effective platelet aggregation inhibiting amount of a compound of claim 1 and a pharmaceutically acceptable carrier.

7. Composition according to claim 6, comprising unit doses containing from 0.001 g to 0.100 g of active ingredient.

Active Ingredient Search Results from "OB_Rx" table for query on "clopidogrel."

Appl No	TE Code	RLD	Active Ingredient	Dosage Form; Route	Strength	Proprietary Name	Applicant
<u>076274</u>	AB	No	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 75MG BASE	CLOPIDOGREL BISULFATE	APOTEX INC
<u>076273</u>	AB	No	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 75MG BASE	CLOPIDOGREL BISULFATE	DR REDDYS LABS INC
<u>020839</u>		No	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 300MG BASE	PLAVIX	SANOFI AVENTIS US
<u>020839</u>	AB	Yes	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 75MG BASE	PLAVIX	SANOFI AVENTIS US

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<u>076273</u>	AB	No	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 75MG BASE	CLOPIDOGREL BISULFATE	DR REDDYS LABS INC
<u>020839</u>		No	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 300MG BASE	PLAVIX	SANOFI AVENTIS US
<u>020839</u>	AB	Yes	CLOPIDOGREL BISULFATE	TABLET; ORAL	EQ 75MG BASE	PLAVIX	SANOFI AVENTIS US

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Search results from the "OB_Rx" table for query on "020839."

Active Ingredient: CLOPIDOGREL BISULFATE
Dosage Form/Route: TABLET; ORAL
Proprietary Name: PLAVIX
Applicant: SANOFI AVENTIS US
Strength: EQ 75MG BASE
Application Number: 020839
Product Number: 001
Approval Date: Nov 17, 1997
Reference Listed Drug: Yes
RX/OTC/DISCN: RX
TE Code: AB
Patent and Exclusivity Info for this product: [View](#)

Active Ingredient: CLOPIDOGREL BISULFATE
Dosage Form/Route: TABLET; ORAL
Proprietary Name: PLAVIX
Applicant: SANOFI AVENTIS US
Strength: EQ 300MG BASE
Application Number: 020839
Product Number: 002
Approval Date: Sep 20, 2007
Reference Listed Drug: No
RX/OTC/DISCN: RX
TE Code:
Patent and Exclusivity Info for this product: [View](#)

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Search results from the "OB_Rx" table for query on "020839."

30
150

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Patent and Exclusivity Info for this product: [View](#)

Active Ingredient: CLOPIDOGREL BISULFATE
Dosage Form/Route: TABLET; ORAL
Proprietary Name: PLAVIX
Applicant: SANOFI AVENTIS US
Strength: EQ 300MG BASE
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Lista de resultados combinados (PubMed (6) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBM (6) | Medline - OVID (8) | OVID - Journals@Ovid (1) | Google Scholar (14) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (6))

1

Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: MH Denninger; J Nacciani; E Serre-Lacroix

Fuente: Semin Thromb Hemost, 1999

En: Google Scholar

2

Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: Denninger MH; Nacciani J; Serre-Lacroix E; Sissmann J

Fuente: Seminars in Thrombosis & Hemostasis, 25 Suppl 2:41-5, 1999.

En: Medline - OVID

3

Registro Completo  

Título: ... Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on ...

Autores: G Helft; JJ Osende; SG Worthley; AG Zaman; OJ

Fuente: Arteriosclerosis, Thrombosis, and Vascular Biology, 2000

En: Google Scholar

4

Registro Completo  

Título: Complementary, additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis Journal of the American College of Cardiology, 35(Suppl A):326A, 2000

Autores: Bhatt DL, Feody JM, Hirsch AT, Ringleb F, Hacke W, Topol EJ

Fuente: Journal of the American College of Cardiology, 35(Suppl A):326A, 2000. AN: CN-00371090 Complete Reference

En: Evidence Based Medicine Review - EBM

5

Registro Completo  

Título: Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

Autores: Helft, Gerard; Osende, Julio J; Worthley, Stephen G; Zaman, Azfar G; Rodriguez, Oswaldo J; Lev, Eli I; Farkouh, Michael E; Fuster, Valentin; Badimon, Juan J; Chesebro, James H

Fuente: Arteriosclerosis, Thrombosis & Vascular Biology, 20(10):2316-2321, October 2000. AbstractComplete ReferenceTable of Contents

En: OVID - Journals@Ovid

6

Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: Helft G, Osende JJ, Worthley SG, Zaman AG, Rodriguez OJ, Lev EI, Farkouh ME, Fuster V, Badimon JJ, Chesebro JH

Fuente: Arteriosclerosis, thrombosis, and vascular biology, 20(10):2316-21, 2000 Oct. AN: CN-00326948 AbstractComplete Reference

En: Evidence Based Medicine Review - EBM

7

Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: Helft G; Osende JJ; Worthley SG; Zaman AG; Rodriguez OJ; Lev EI; Farkouh ME; Fuster V; Badimon JJ; Chesebro JH

37
182
132

Fuente: Arteriosclerosis, Thrombosis & Vascular Biology. 20(10):2316-21, 2000 Oct.

En: Medline - OVID

8

Registro Completo  

Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: Cuffetti G, Lombardini R, Pirro M, Lupattelli G, Mannarino E

Fuente: Clinical hemorheology and microcirculation. 25(1):31-9, 2001. AN: CN-00377053 AbstractComplete Reference

En: Evidence Based Medicine Review - EBM

9

Registro Completo  

Título: Clopidogrel: Hemorheological effects in subjects with subclinical atherosclerosis

Autores: G Cuffetti

Fuente: Clinical Hemorheology and Microcirculation, 2001

En: Google Scholar

10

Registro Completo  

Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: Cuffetti G; Lombardini R; Pirro M; Lupattelli G; Mannarino E

Fuente: Clinical Hemorheology & Microcirculation. 25(1):31-9, 2001.

En: Medline - OVID

11

Registro Completo  

Título: [Effect of therapy with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis of lower extremities and intermittent claudication]. [Russian]

Autores: Komarov AL; Kropacheva ES; Tarasova TN; Dobrovolskii AB; Titova EV; Mironova IY; Naumov VG; Panchenko EF

Fuente: Kardiologiya. 42(3):45-50, 2002.

En: Medline - OVID

12

Registro Completo  

Título: ... with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis ...

Autores: AL Komarov; ES Kropacheva; TN Tarasova; AB

Fuente: Kardiologiya, 2002

En: Google Scholar

13

Registro Completo  

Título: Management of atherosclerosis with clopidogrel in high-risk patients with recent transient ischaemic attack or ischemic stroke (MATCH): study results Stroke. 35(6):e181, 2004

Autores: Diener H-C

Fuente: Stroke. 35(6):e181, 2004. AN: CN-00487968 Complete Reference

En: Evidence Based Medicine Review - EBM

14

Registro Completo  

Título: ... effects of aspirin, clopidogrel, and their combination on the progression of atherosclerosis in ...

Autores: Q Gu; JL Chen; YM Ruan

Fuente: Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 2005

En: Google Scholar

15

Registro Completo  

Título: [Inhibitory effects of aspirin, clopidogrel, and their combination on the progression of

atherosclerosis in rabbits], [Chinese]

Autores: Gu Q; Chen JL; Ruan YM

Fuente: Chung-Kuo I Hsueh Ko Hsueh Yuan Hsueh/Pao Acta Academiae Medicinae Sinicae. 27(1):87-91, 2005 Feb.

En: Medline - OVID

☐ 16

Registro Completo  

Título: Ask the doctors. My mother in her early 90s has been diagnosed with atherosclerosis, had a successful angioplasty a year ago for severe chest pain, takes clopidogrel and aspirin, and in general eats a low-fat diet, although she really likes melted ice cream. But she doesn't like to walk. What else should she be taking, and what can I say when she says she's too old to exercise?.

Autores: Anonymous

Fuente: Heart Advisor. 8(5):8, 2005 May.

En: Medline - OVID

☐ 17

Registro Completo  

Título: Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. [see comment].

Autores: Husted S; Emanuelsson H; Heptinstall S; Sandset PM; Wickens M; Peters G

Fuente: European Heart Journal. 27(9):1038-47, 2006 May.

En: Medline - OVID

☐ 18

Registro Completo  

Título: Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin.

Autores: Husted S; Emanuelsson H; Heptinstall S; Sandset PM; Wickens M; Peters G

Fuente: European heart journal. 27(9):1038-47, 2006 May. AN: CN-00562594 AbstractComplete Reference

En: Evidence Based Medicine Review - EBMR

☐ 19

Registro Completo  

Título: Is there a different effect of clopidogrel plus aspirin versus clopidogrel alone on stroke patients with small vessel disease and large artery atherosclerosis? Analysis from MATCH Stroke. 38(2):522, 2007

Autores: Benavente O; Pearce L; Amarenco P; Streifler J; Kase C

Fuente: Stroke. 38(2):522, 2007. AN: CN-00602957 Complete Reference

En: Evidence Based Medicine Review - EBMR

☐ 20

Registro Completo  

Título: Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model.

Autores: Li M; Zhang Y; Ren H; Zhang Y; Zhu X

Fuente: Atherosclerosis. 194(2):348-56, 2007 Oct.

En: Medline - OVID

☐ 21

Registro Completo  

Título: ... , additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis

Autores: OL Bhatt; JM Foody; AT Hirsch; P Ringleb; W Hacke

En: Google Scholar

☐ 22

Registro Completo  

Título: ... agent, clopidogrel, in the young and the elderly with and without symptomatic atherosclerosis

Autores: MC Guillin; G Bonnet; J Sissmann; J Neccian; JP

En: Google Scholar

34
154
154

13

Registro Completo  **Título:** Effects of clopidogrel on the formation of experimental atherosclerosis**Autores:** Q Gu; JL Chen; YM Ruan; WX; QZ Wang**En:** Google Scholar

24

Registro Completo  **Título:** ... oral reversible ADP receptor antagonist, compared with clopidogrel in patients with atherosclerosis**Autores:** S Husted; H Emanuelsson; S Heptinstall; S Clark**En:** Google Scholar

25

Registro Completo  **Título:** ... front-loaded regimen of clopidogrel on arterial thrombus formation in patients with atherosclerosis ...**Autores:** G Hellft; JJ Osenda; SG Worthley; Q Rodríguez; AG**En:** Google Scholar

26

Registro Completo  **Título:** ... oral reversible ADP receptor antagonist, compared with clopidogrel in patients with atherosclerosis**Autores:** E Husted; C Heptinstall**En:** Google Scholar

27

Registro Completo  **Título:** ... , additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis [...**Autores:** DL Bhatt; JM Foody; AT Hirsch**En:** Google Scholar

28

Registro Completo  **Título:** ... reduces P-selectin and formation of platelet-leukocyte aggregates in patients with atherosclerosis ...**Autores:** U Klinkhardt; R Bauersachs; J Adams; J Graff**En:** Google Scholar

29

Registro Completo  **Título:** ... agent, clopidogrel, in the young and the elderly with and without symptomatic atherosclerosis [...**Autores:** MC Guillin; G Bonnet; J Sisemann**En:** Google Scholar

30

Registro Completo  **Título:** Ask the doctors. My mother in her early 90s has been diagnosed with atherosclerosis, had a successful angioplasty a year ago for severe chest pain, takes clopidogrel and aspirin, and in general eats a low-fat diet, although she really likes melted ice cream. But she doesn't like to walk. What else should she be taking, and what can I say when she says she's too old to exercise?**En:** MEDLINE with Full Text

31

Registro Completo  **Título:** (Inhibitory effects of aspirin, clopidogrel, and their combination on the progression of atherosclerosis in rabbits)**Autores:** (chi)**En:** MEDLINE with Full Text

38
155
35
185☐ 32Registro Completo  

Título: [Effect of therapy with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis of lower extremities and intermittent claudication]

Autores: Vliianie terapii klopidogrelem na tolerantnost' k khod'be i pokazateli gemostaza u bol'nykh s ateroskleroticheskimi porazheniyami arterii nizhnikh konchanoostei i peremeshkivaniyami khrokhotoi

En: MEDLINE with Full Text

☐ 33Registro Completo  

Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: (eng)

En: MEDLINE with Full Text

☐ 34Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: (eng)

En: MEDLINE with Full Text

☐ 35Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: (eng)

En: MEDLINE with Full Text