

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
DILIA MARÍA RODRÍGUEZ D'ALEMÁN
notificaciones@clarkemodet.com.co

ASUNTO Radicación 10 054 082
 Trámite 011
 Evento 379
 Actuación 519
 Oficio **SS - 196 5 6**
 Folios 019

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (Fase Nacional)	☒	Capítulo I	☐
		Capítulo II	☒
No. Solicitud Internacional	PCT/EP2008/009451		
Fecha de Presentación Internacional	10/Noviembre/2008		

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 7 a 22	06/Mayo/2010
Reivindicaciones:	Folios 23 a 28	06/Mayo/2010
Prioridad:	MI2007A002225	23/Noviembre/2007

2. Análisis de la prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Composiciones farmacéuticas que contienen antagonistas de
Expediente 10 054 082 1er Art 45

bradiquinina y ácido hialurónico y sus usos".

El problema planteado en esta solicitud consiste en la necesidad de suministrar tratamientos efectivos para las enfermedades degenerativas articulares tales como osteoartritis.

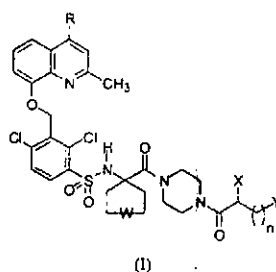
Para resolver este problema técnico la solicitud en estudio proporciona composiciones farmacéuticas, caracterizadas porque comprenden, como ingredientes activos, una mezcla de:

- a) Ácido hialurónico o sus sales seleccionadas de sodio y potasio
- b) Un antagonista del receptor de bradiquinina B2

Junto con portadores y excipientes farmacéuticamente aceptables, en donde:

El ácido hialurónico está en forma polimérica con un PM medio entre 0,5 y 10 millones de Dalton, y el antagonista de bradiquinina se selecciona de:

- Antagonistas del receptor B2, seleccionados del grupo de:
 - H-D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-F5F-Igl-Arg-OH
 - H-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-Igl-Oic-Arg-OH
 - H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH
 - 4-[2-[[[3-(3-Brom-2-metilo-imidazo[1,2-a]piridina-8-iloximetil)-2,4-dicloro-fenil]-metilo-carbamoil]-metilo)-carbamoil]-vinil]-N,N-dimetil-benzamida
 - 3-(6-Acetilamino-piridina-3-il)-N-[[[2,4-dicloro-3-(2-metilo-quinolina-8-iloximetil)-fenil]-metilo-carbamoil]-metilo)-acrilamida
 - [3-(4-carbamidoil-benzoilamino)-propil]-amida del ácido 1-[2,4-Dicloro-3-(2,4-dimetil-quinolin-8-iloximetil)-bencensulfonil-pirrolidin-2-carboxílico
 - 4-(4-[1-[2,4-Dicloro-3-(2,4-dimetil-quinolin-8-iloximetil)-bencensulfonil]-pirrolidina-2-carbonil]-piperazin-1-carbonil)-benzamida
 - 2-[5-(4-ciano-benzoil)-1-metilo-1H-pirrol-2-il]-N-[2,4-dicloro-3-(2-metilo-quinolin-8-iloximetil)-fenil]-N-metilo-acetamida
- Antagonistas del receptor B2, que tienen la fórmula general (I):



El objeto de la presente solicitud se relaciona con la clasificación internacional de patentes (CIP): A61K 9/08, A61K 31/728; A61K 31/726; A61K 31/70; C07K 7/00, C07D 215/00.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 17 – 19 no es patentable, de acuerdo con el Art 14.

5. De la Solicitud de Patente

Titulo

El título de la solicitud hace referencia al uso de las composiciones reclamadas, lo cual no es patentable de acuerdo con el Art 14 de la Decisión 486. Se sugiere eliminar toda referencia al uso en el título de la solicitud.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es concisa porque una composición debe estar caracterizada por sus componentes y las cantidades o proporciones de dichos componentes en la formulación. Se sugiere incluir las cantidades de ácido hialurónico y de los antagonistas de bradiquinina de acuerdo a lo soportado en la descripción. Se sugiere incluir el contenido de las reivindicaciones 5 y 9 dentro de la reivindicación independiente.

La reivindicación 1 no se encuentra soportada en la descripción, porque la descripción solo soporta y prueba composiciones que comprenden ácido hialurónico y 4-(S)-amino-5-(4-[[4-[2,4-dicloro-3-(2,4-dimetil-quinolin-8-iloximetil)-bencensulfonamido]-tetrahidropiran-4-carbonil]-piperazin-1-il-5-oxo-pentil]-trimetil-amonio o icatibant.

La reivindicación 13 no se encuentra soportada en la descripción porque la descripción solo soporta composiciones en las que los componentes se encuentran en jeringas prellenadas, adicionalmente la reivindicación hace referencia al proceso de reconstituir una formulación sólida, lo cual no es una característica de una formulación y se realiza de forma manual en el momento de la aplicación. Se sugiere eliminar la reivindicación 13 del capítulo reivindicatorio.

Las reivindicaciones 9-11 hacen referencia a las dosis de los antagonistas de bradiquinina, sin embargo la solicitud solo soporta y prueba composiciones que contienen 4-(S)-amino-5-(4-[[4-[2,4-dicloro-3-(2,4-dimetil-quinolin-8-iloximetil)-bencensulfonamido]-tetrahidropiran-4-carbonil]-piperazin-1-il-5-oxo-pentil]-trimetil-amonio o icatibant en dosis de 0.2-5mg. Se sugiere restringir la solicitud a lo efectivamente soportado en la descripción.

6. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el requisito de unidad de invención porque se refiere a 5, grupos inventivos¹.

Los grupos inventivos están definidos por las siguientes características **esenciales**:

¹ Guía para examen de solicitudes de patente de invención y modelo de utilidad. Pág. 101. Superintendencia de Industria y Comercio, Delegatura para la Propiedad Industrial, Dirección de Nuevas Creaciones.

1° Composiciones farmacéuticas que comprenden ácido hialurónico y un antagonista de receptor de bradiquinina B2 seleccionado del grupo de:

- H-D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-F5F-Igl-Arg-OH
- H-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-Igl-Oic-Arg-OH
- H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH

2° Composiciones farmacéuticas que comprenden ácido hialurónico y un antagonista de receptor de bradiquinina B2 seleccionado del grupo de:

- 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-metilo)-carbamoil-vinil]-N,N-dimetil-benzamida
- 3-(6-Acetilamino-piridina-3-il)-N-([2,4-dicloro-3-(2-metilo-quinolina-8-iloimetil)-fenil]-metilo-carbamoil)-metilo-acrilamida.

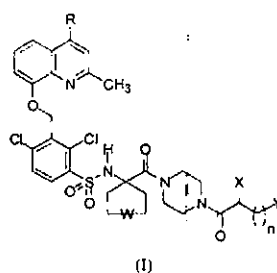
3° Composiciones farmacéuticas que comprenden ácido hialurónico y un antagonista de receptor de bradiquinina B2 seleccionado del grupo de:

- [3-(4-carbamidoil-benzoilamino)-propil]-amida del ácido 1-[2,4-Dicloro-3-(2,4-dimetil-quinolin-8-iloimetil)-bencensulfonil-pirrolidin-2-carboxílico
- 4-(4-[1-[2,4-Dicloro-3-(2,4-dimetil-quinolin-8-iloimetil)-bencensulfonil]-pirrolidina-2-carbonil]-piperazin-1-carbonil)-benzamida

4° Composiciones farmacéuticas que comprenden ácido hialurónico y un antagonista de receptor de bradiquinina B2 el cual es:

- 2-[5-(4-ciano-benzoil)-1-metilo-1H-pirrol-2-il]-N-[2,4-dicloro-3-(2-metilo-quinolin-8-iloimetil)-fenil]-N-metilo-acetamida.

5° Composiciones farmacéuticas que comprenden ácido hialurónico y antagonistas del receptor B2, que tienen la fórmula general (I):



En donde:

- R es hidrógeno o metilo
- W representa un enlace simple o un átomo de O
- n = 3, 4

- X es hidrógeno o un grupo amino -NR1R2, en el que R1 y R2 pueden ser de modo independiente entre sí hidrógeno o un grupo seleccionado de metilo, etilo, n-propilo, isopropilo,
- Y es un amonio cuaternario (-NR3R4R5)⁺A⁻ en el que R3, R4, R5 pueden ser de modo independiente entre sí metilo, etilo, n-propilo, isopropilo, butilo, isobutilo, n-pentilo y A- es un anión de un ácido farmacéuticamente aceptable.

Se sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: Noviembre 23 de 2007 y se encontraron los siguientes documentos que anticipen el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	Advanced Drug Delivery Reviews 58 (2006) 226– 242	Intraarticular drug delivery in osteoarthritis	2006
D2	British Journal of Pharmacology (1997) 122, 1436 ± 1440	Effects of a nonpeptide bradykinin B2 receptor antagonist, FR167344, on different in vivo animal models of inflammation	1997

D1 <http://www.med.nyu.edu/pmr/residency/resources/injections%20and%20procedures/intra-art%20drugs%20in%20OA.pdf> Divulga que el ácido hialurónico nativo pesa entre 4 y 10 millones de Daltons y forma una red extensa a concentraciones sobre 0.3mg/mL. Establece que en el fluido de las articulaciones, las altas concentraciones de ácido hialurónico aproximadamente (0.35g/100mL) es responsable por las propiedades viscoelásticas del fluido sinovial (Pág. 235, columna de la derecha). Enseña que muchas preparaciones de ácido hialurónico, como formulaciones en hidrogel de ácido hialurónico en agua para inyección y que la mayoría de las formulaciones contienen ácido hialurónico de bajo peso molecular (0.5-1.5 millones de Daltons) (Pág. 236, párrafo 4.2.2). Adicionalmente el documento D1 divulga que Icatibant es un antagonista potente y específico del receptor B2 de bradiquinina, el cual provee gran alivio del dolor en osteoartritis (Pág. 239, columna de la derecha).

D2 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1565090/pdf/122-0701534a.pdf> revela que el compuesto 4-[2-[[[3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloximetil)-2,4-dicloro-fenil]-metilo-carbamoil]-metilo)-carbamoil]-vinil]-N,N-dimetil-benzamida (denominado FR167344) es un potente y selectivo antagonista del receptor de bradiquinina B2, de larga acción y activo oralmente (Pág. 1436, columna de la derecha). Adicionalmente, el documento D2 establece que la bradiquinina puede estar envuelta

en enfermedades inflamatorias como la artritis (Pág. 1436, columna de la izquierda) y señala que los antagonistas del receptor B2 pueden tener potencial terapéutico contra enfermedades inflamatorias y que el FR167344 puede tener potencial terapéutico contra enfermedades inflamatorias por vía oral, en el caso de enfermedades crónicas como artritis reumatoidea (Pág. 1438, dos columnas).

8. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en: Composiciones farmacéuticas, caracterizadas porque comprenden, como ingredientes activos, una mezcla de:

- a) Ácido hialurónico o sus sales seleccionadas de sodio y potasio
- b) Un antagonista del receptor de bradiquinina B2

Junto con portadores y excipientes farmacéuticamente aceptables, en donde:

El ácido hialurónico está en forma polimérica con un PM medio entre 0,5 y 10 millones de Dalton, y el antagonista de bradiquinina se selecciona de:

- 4-[2-[[[3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloximetil)-2,4-dicloro-fenil]-metilo-carbamoil]-metilo)-carbamoil]-vinil]-N,N-dimetil-benzamida

Comparación de las características técnicas **esenciales**, con las del estado de la técnica:

Características esenciales	D1	D2
Composiciones farmacéuticas Comprende como ingredientes activos, una mezcla de	Composiciones farmacéuticas	Composiciones farmacéuticas
a) Ácido hialurónico o sus sales seleccionadas de sodio y potasio, El ácido hialurónico está en forma polimérica con un PM medio entre 0,5 y 10 millones de Dalton.	Establece que el ácido hialurónico nativo pesa entre 4 y 10 millones de Daltons y forma una red extensa a concentraciones sobre 0.3mg/mL. Establece que en el fluido de las articulaciones, las altas concentraciones de ácido hialurónico aproximadamente (0.35g/100mL) es responsable por las propiedades viscoelásticas del fluido sinovial (Pág. 235, columna de la derecha). Enseña que existen muchas preparaciones de ácido hialurónico, como formulaciones en hidrogel de ácido hialurónico en agua para inyección y que la mayoría de las formulaciones contienen	No lo menciona

	ácido hialurónico de bajo peso molecular (0.5-1.5 millones de Daltons) (Pág. 236, párrafo 4.2.2).	
b) 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-vinil]-N,N-dimetil-benzamida	Establece que Icatibant es un antagonista potente y específico del receptor B2 de bradiquinina, el cual provee gran alivio del dolor en osteoartritis (Pág. 239, columna de la derecha).	FR167344, 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-vinil]-N,N-dimetil-benzamida es un potente y selectivo antagonista del receptor de bradiquinina B2, de larga acción y activo oralmente (Pág. 1436, columna de la derecha). Establece que la bradiquinina puede estar envuelta en enfermedades inflamatorias como la artritis (Pág. 1436, columna de la izquierda). Señala que los antagonistas del receptor B2 puede tener potencial terapéutico contra enfermedades inflamatorias y que el FR167344 puede tener potencial terapéutico contra enfermedades inflamatorias por vía oral, en el caso de enfermedades crónicas como artritis reumatoidea, la actividad oral de FR167344 puede ser de mérito considerable (Pág. 1438, dos columnas).
Junto con portadores y excipientes farmacéuticamente aceptables	Las formulaciones pueden contener adicionalmente excipientes como cloruro de sodio (Pág. 236, párrafo 4.2.2).	FR167344 fue disuelto en 0.05 N HCl y administrado oralmente a un volumen de 5 ml/kg (Pág. 1437, métodos).

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga que el ácido hialurónico e inhibidores del receptor B2 de bradiquinina son útiles en el tratamiento de osteoartritis. Establece que el ácido hialurónico nativo pesa entre 4 y 10 millones de Daltons y forma una red extensa a concentraciones sobre 0.3mg/mL. Establece que en el fluido de las articulaciones, las altas concentraciones de ácido hialurónico aproximadamente (0.35g/100mL) es responsable por las propiedades viscoelásticas del fluido sinovial (Pág. 235, columna de la derecha). Enseña que muchas preparaciones de ácido hialurónico, como formulaciones en hidrogel de ácido hialurónico en agua para inyección y que la mayoría de las formulaciones contienen ácido hialurónico de bajo peso molecular (0.5-1.5

millones de Daltons) (Pág. 236, párrafo 4.2.2).

Adicionalmente el documento D1 divulga que Icatibant es un antagonista potente y específico del receptor B2 de bradiquinina, el cual provee gran alivio del dolor en osteoartritis (Pág. 239, columna de la derecha).

La diferencia entre la invención, definida en la reivindicación 1 y las enseñanzas del documento D1 consisten en que la presente solicitud reclama composiciones que comprenden una combinación de ácido hialurónico y 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-metilo)-carbamoil-vinil]-N,N-dimetil-benzamida, y la anterioridad solo habla de composiciones de ácido hialurónico e inhibidores del receptor B2 de bradiquinina, como el icatibant, en forma separada.

No se evidencia algún efecto logrado por el hecho de diseñar una composición que comprende ácido hialurónico y 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-metilo)-carbamoil-vinil]-N,N-dimetil-benzamida, porque los ensayos presentados solamente prueban las composiciones que comprenden ácido hialurónico e icatibant y ácido hialurónico y (4-(S)-amino-5-(4-{4-[2,4-dicloro-3-(2,4-dimetil-quinolin-8-iloimetil)-bencensulfonilamino]-tetrahidro-piran-4-carbonil}-piperazin-1-il-5-oxo-pentil)-trimetil-amonio).

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: ¿Cómo modificar las composiciones reveladas en el documento D1 para obtener una composición alternativa para el tratamiento de osteoartritis?

Sin embargo, el documento D2 revela que el compuesto 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-metilo)-carbamoil-vinil]-N,N-dimetil-benzamida (denominado FR167344) es un potente y selectivo antagonista del receptor de bradiquinina B2, de larga acción y activo oralmente (Pág. 1436, columna de la derecha). Adicionalmente, el documento D2 establece que la bradiquinina puede estar envuelta en enfermedades inflamatorias como la artritis (Pág. 1436, columna de la izquierda) y señala que los antagonistas del receptor B2 pueden tener potencial terapéutico contra enfermedades inflamatorias y que el FR167344 puede tener potencial terapéutico contra enfermedades inflamatorias por vía oral, en el caso de enfermedades crónicas como artritis reumatoidea (Pág. 1438, dos columnas).

En consecuencia, la persona del oficio normalmente versada en la materia, se vería motivada por lo revelado en el documento D2 que revela que el compuesto 4-[2-([3-(3-Bromo-2-metilo-imidazo[1,2-a]piridina-8-iloimetil)-2,4-dicloro-fenil]-metilo-carbamoil)-metilo)-carbamoil-vinil]-N,N-dimetil-benzamida (denominado FR167344) es un potente y selectivo antagonista del receptor de bradiquinina B2 (que puede ser usado para enfermedades inflamatorias como la artritis) a combinar el ácido hialurónico el cual se divulga en el documento D1 que puede ayudar a restaurar la viscoelasticidad del fluido sinovial y que reduce el dolor asociado con osteoartritis (Pág. 235, columna de la derecha y Pág. 236, párrafo 4.2.3) y formularlos en una composición farmacéutica, porque el documento D1 enseña que los compuestos antagonistas de receptor B2 de

bradiquinina como el icatibant proveen gran alivio del dolor en osteoartritis (Pág. 239, columna de la derecha). Por lo tanto, ante la falta de ensayos que demuestren un efecto inesperado causado por la composición que comprende ácido hialurónico y FR167344, se considera que la composición reclamada en la reivindicación 1 de la presente solicitud no presenta nivel inventivo.

Conclusión:

La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

9. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Advanced Drug Delivery Reviews 58 (2006) 226– 242	1, 2, 5-8 y 12-14	Nivel Inventivo
D2	British Journal of Pharmacology (1997) 122, 1436 ± 1440	1, 2, 5-8 y 12-14	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para dar respuesta y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 30 OCT. 2012

Realizado por: Juanita Catalina Rico
Revisado por: Consuelo Leguizamón 



Intraarticular drug delivery in osteoarthritis[☆]

Nicole Gerwin^{a,*}, Caroline Hops^b, Andrea Lucke^{b,1}

^a Bone and Cartilage Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, WKL-125.13.59, CH-4002 Basel, Switzerland

^b Pharmaceutical Sciences Department, Sanofi-Aventis Deutschland GmbH, Industriepark Hoechst, 65926 Frankfurt am Main, Germany

Received 20 November 2005; accepted 30 January 2006

Available online 23 February 2006

Abstract

Osteoarthritis (OA) is a primarily non-inflammatory, degenerative joint disease characterized by progressive loss of articular cartilage, subchondral bone sclerosis, osteophyte formation, changes in the synovial membrane, and an increased volume of synovial fluid with reduced viscosity and hence changed lubrication properties. As OA is the most common type of arthritis and a leading cause of disability, there is a largely unmet medical need for disease-modifying and symptomatic treatment. Due to the localized nature of the disease, intraarticular (IA) drug injection is an attractive treatment approach for OA. The various glucocorticoid and hyaluronic acid (HA) formulations, which are currently available on the market for IA treatment, provide only short-term pain relief or/and often do not provide adequate pain relief. The available oral drugs for symptomatic treatment also have shortcomings, most notably side effects. Therefore, there is still a large unmet need for novel OA drugs, which provide effective long-term pain relief and/or have disease-modifying properties. To achieve long-term drug exposure, different established formulations such as suspensions and hydrogels, and also novel approaches such as lipid based formulations and nano- or microparticles are currently in development. The development of novel drugs in combination with new formulations for IA treatment of OA, represents a promising approach in this challenging area of research.

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Keywords: Osteoarthritis; Intraarticular; Sustained release; Hyaluronic acid; Glucocorticoid

[☆] This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Drug Delivery in Degenerative Joint Disease", Vol. 58/2, 2006.

* Corresponding author. Tel.: +41 61 6962652; fax: +41 61 6964509.

E-mail address: nicole.gerwin@novartis.com (N. Gerwin).

¹ Current address: Pharmaceutical Research and Development, Boehringer Ingelheim Pharma GmbH and Co. KG, Birkendorfer Straße 65, 88397 Biberach, Germany.

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1. Introduction

Osteoarthritis (OA) is the most common form of arthritis, with a prevalence after the age of 65 years of about 60% in men and 70% in women [1]. Current treatment options for OA are limited. They include symptomatic treatment with simple analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) or intra-articular (IA) injected glucocorticoids and hyaluronic acid (HA) preparations. Non-pharmacological measures range from physical exercise and weight loss to joint lavage, and eventually surgical joint replacement.

There are major unmet needs in OA treatment, for disease-modifying OA drugs (DMOADs), which are

not available yet, and also for efficacious pain treatments with long-lasting effects. Furthermore, there is a significant unmet need for OA treatments which do not have any major side effects, due to the chronic nature of the disease often requiring treatment of extended duration. The currently available systemic drugs for relief of OA pain, e.g., the non-selective NSAIDs and selective cyclooxygenase 2 (COX-2) inhibitors, are effective in the early-mid stages of OA, but often fail to provide adequate pain relief as the joint deteriorates. In addition, NSAIDs cause gastrointestinal complications in a significant number of patients, and the COX-2 inhibitors have recently raised concerns regarding cardiovascular side effects/risks, resulting in the with-

The duration of action of glucocorticoids is prolonged relative to their short residence time in SF, which is probably due to their mechanism of action: IA injected glucocorticoids in solution can pass the cell membrane directly and bind to their receptors in the cytoplasm. The hormone-receptor complex then migrates to the nucleus, where it regulates the transcription of pro-inflammatory and pain mediators [27] and thus exhibits its effects beyond the half-life of the compound alone.

4.1.4. Side effects

Side effects of steroid injections are infrequent. The incidence of infection is low and is clearly less with the use of glucocorticoid packaged in pre-filled syringes. The incidence of post-injection flares, which begin 6–12 h after injection and resolve spontaneously in 1 to 3 days, is unclear. The underlying mechanism may be a mild synovitis due to a reaction to the crystalline steroid suspensions.

Early studies reported the possibility of marked destructive cartilage changes resulting from excessive IA use of steroids. In 1995, ACR guidelines recommended that injection of corticosteroids in a given knee joint not be performed more than three or four times in a given year. However, based on recent studies this risk of cartilage degradation seems minimal [19].

Recent findings suggest that chondrolysis occurs during OA flares, defined by episodes of exacerbation of pain with synovial effusion. Since IA glucocorticoids may be particularly indicated and efficient in the treatment of inflammatory flares and to reduce joint effusion, it is now recommended that steroids be used for the short-term treatment of disease flares, i.e., exacerbation of pain accompanied by effusion [19].

4.2. Hyaluronic acid (HA) injections

4.2.1. Possible mechanisms of action of HA in OA

Hyaluronic acid (HA) is a high molecular weight polysaccharide of *N*-acetyl glucosamine and glucuronic acid building blocks. It is produced by synovio-cytes, fibroblasts and chondrocytes, and represents a main constituent of SF and hyaline cartilage. In articular cartilage, HA provides a backbone for the attachment of aggrecan side chains by link proteins. This HA-aggrecan complex interacts with water and

type II collagen fibers to form the extracellular matrix of articular cartilage.

Native HA has a molecular weight of 4–10 million Da and forms an extensive network at concentrations over 0.3 mg/ml in aqueous solutions [9,28–30]. In joint fluid, the high concentration of HA (approximately 0.35 g/100 ml) (Table 1) is responsible for the viscoelastic properties of SF.

The viscoelasticity of HA is crucial for the maintenance of joint homeostasis. Since HA behaves as a viscous liquid at low shear rates and as an elastic solid at high shear rates, SF acts as a viscous lubricant during low impact movements of the joint and as an elastic shock absorber during high impact movement. HA also has barrier and exclusion effects that limit the movement of cells and large molecules through normal SF [9]. In joints affected by OA, the molecular weight and concentration of HA are diminished. This low HA concentration may be attributed both to reduced synthesis of HA and also increased volume of SF. This adversely affects the ability of SF to lubricate and protect articular tissues, and to absorb joint loads, which contributes to further progression of OA [9].

The concept of viscosupplementation suggests that IA injection of HA could help restore the viscoelasticity of the SF, but HA has also been demonstrated to have a multiplicity of biological actions on cells in vitro. For example, it inhibits prostaglandin E₂ synthesis induced by interleukin (IL)-1 in human OA synovial cells, and influences leukocyte adherence, proliferation, migration and phagocytosis (reviewed by Brandt et al. [30]). These anti-inflammatory effects of HA can be expected to have an indirect anti-nociceptive effect. Besides HA seems to have direct anti-nociceptive effects. For example there is evidence for inhibition of PGE₂ and bradykinin synthesis in joint tissues by HA, entrapment of endogenous pain substances by HA and decreased activation of joint pain fibers secondary to coating of their receptor endings with viscous HA (reviewed by Marshall [9]).

Chondroprotective effects of HA were observed in vitro, e.g., that it stimulates production of tissue inhibitors of matrix metalloproteinases (TIMP-1) by chondrocytes, inhibits neutrophil-mediated cartilage degradation and attenuates IL-1 induced matrix degeneration and chondrocyte cytotoxicity [9]. In several studies HA enhanced PG synthesis and decreased PG breakdown and release from cartilage

matrix [31]. Furthermore, it was shown to normalize endogenous HA synthesis by synoviocytes [32].

4.2.2. Overview of HA products for IA injection

Several HA preparations are currently available. They are hydrogel formulations of HA in water for injection and may contain additional excipients, such as sodium chloride for isotonicization and sodium hydrogenphosphate for pH adjustment.

Most of these formulations contain HA of a comparatively low molecular weight (0.5–1.5 million Da), while one formulation (Synvisc®) contains cross-linked HA (hylan) with 6–7 million Da molecular weight, the latter being close to the molecular weight of native HA.

So far, differences in half-life of low molecular weight HA and hylan were demonstrated only in animal studies. Rabbit studies demonstrated that the injected hylan is cleared from the joint by 28 days after the last injection [9].

The low molecular weight HA can be obtained from fermentation (e.g., *Streptococcus equi*) or by isolation, e.g., from cockcombs.

In contrast to the IA application of glucocorticoid formulations, the dosing regimen of HA formulations is different for different preparations. For the cross-linked HA preparation (Synvisc®) up to 3 injections in weekly intervals are recommended. A repetition of this treatment cycle is not recommended earlier than 4 weeks after the last injection and with not more than 6 injections per 6 months [22]. For low molecular weight HA preparations, up to 5 injections in weekly intervals are recommended [22].

4.2.3. Evidence for symptomatic and disease-modifying efficacy of HA injections

A large number of clinical trials have been conducted with different HA preparations over the past 25 years. The majority of these studies have provided evidence that the HA preparations are more effective than placebo in reducing pain associated with OA. A recently published meta-analysis of 20 blinded, placebo-controlled randomized studies confirms the symptomatic efficacy and showed that older patients (>65 years) and patients with endstage disease are less likely to benefit from IA HA injections [33].

HA acts as a slow acting symptomatic drug with a delayed onset of efficacy at 2 to 5 weeks after injection

and a long-lived benefit on pain, which may persist until 6 to 12 months. Depending on the HA preparation, a course of 3 to 5 weekly injections is recommended. A new treatment cycle is usually proposed when symptoms reappear [19].

For the high molecular weight hylan (Synvisc®) compared to the lower weight HA preparations, superior viscoelastic properties and prolonged half-life in the joint space have been postulated [28,34]. Some evidence from pre-clinical and clinical studies suggests that the greater the viscoelasticity of the HA preparation, the greater the effectiveness of the treatment [35]. However, the available clinical study results do not prove a significant superiority in efficacy for hylan [33].

When comparing hylan with various steroids, Ayral [19] found 3 randomized controlled trials reporting similar benefits of HA and steroids at 1 month, but with superiority of HA thereafter. Therefore, Ayral recommends that fast-acting but short-lived steroids and the delayed but long-acting HAs should be considered as complementary therapies.

Three randomized controlled trials comparing HAs with NSAIDs found that the benefit obtained with HA was similar to that of NSAIDs for pain, and with fewer gastrointestinal side effects [19].

There has been much debate whether HA also has structure-modifying effects. Recently Goldberg and Buckwalter [36] reviewed the available evidence. While effects of HA on cultured cells suggest that HA promotes joint repair and inhibits destruction (see 4.2.1), studies in OA animal models evaluating potential structure-modifying effects in the OA joint produced conflicting results [30,36]. Studies in dogs and rabbits which had undergone anterior cruciate ligament transection (ACLT) or partial meniscectomy suggested that HA injections reduced gross cartilage damage and abnormalities in cartilage biochemistry, or even produced pronounced tissue repair. In contrast, injection of HA into sheep joints subjected to meniscectomy resulted in significantly more extensive osteophytosis and cartilage fibrillation and in reduction in proteoglycan synthesis. A striking reduction in proteoglycan concentration in cartilage was also observed in dogs with ACLT which were prophylactically treated with a series of HA injections. These findings raised concerns that HA treatment could accelerate joint damage in OA.

However, the release of lipophilic low molecular weight compounds (e.g., prednisolone, triamcinolone) was also demonstrated from larger albumin microparticles (~23 µm) [50].

The use of PLGA microparticles for the release of diclofenac sodium enabled a release over several days, but with no positive effect in a rabbit arthritis model [47]. In contrast, the efficacy of IA PLGA nanoparticles (300–490 nm) loaded with betamethasone phosphate for about 6 weeks was shown in another study [43]. In this study, also the uptake of the nanoparticles by synovial macrophages was demonstrated. A further study with fluorescence-labeled PLGA micro- and nanoparticles demonstrated that microparticles (~30 µm) were not taken up by phagocytosis in contrast to the nanoparticles [44].

For the release of macromolecular and well soluble compounds, microparticles produced by complex coacervation of gelatine and chondroitine sulfate were used. The manufacturing procedure required neither the use of organic solvents nor increased temperatures. Compared to the typical manufacturing procedures for PLGA micro- and nanoparticles, this may be beneficial especially for peptide and protein drugs [48].

Further polymer-based explorative approaches for cartilage therapy and related strategies for controlled release of compound comprise the use of polymer microparticles and hydrogels based on various materials, such as collagen, fibrin, chitosan and hyaluronan [49].

5.2. Candidate drugs and drug targets for symptomatic and disease-modifying IA treatment

There is still a large unmet medical need for novel drugs for IA injection, which provide efficient long-term pain relief. Candidates for future IA symptomatic treatments are for example oral drugs with proven analgesic effects, which could be formulated to achieve sustained release into the joint. Furthermore, some of the analgesics with novel mechanisms of action which are currently in development, are ideal candidates for IA treatment, because they are not orally bioavailable or have severe side effects when applied systemically.

One example is the development of capsaicin in a special formulation for IA injection (www.algorx.com). Capsaicin, the active ingredient in hot chilli peppers, activates neurons which transmit pain signals

to the brain. At high concentrations capsaicin causes a temporary degradation of pain-sensing endings and thereby prevents neurons from transmitting pain, which is expected to cause long-lasting pain relief. Injection of capsaicin directly into the limited joint space is expected to yield high local concentrations of capsaicin at the site of action and low systemic distribution. Phase II clinical trials with IA capsaicin for the treatment of knee OA are currently ongoing.

Another example is the decapeptide Icatibant (HOE140), a specific and potent bradykinin B2 receptor antagonist, which is not suitable for oral treatment due to its peptidic nature. In a first proof-of-mechanism study in patients with knee OA a single IA injection of HOE140 provided greater pain relief than placebo and was well tolerated. A Phase IIa trial for symptomatic effects in OA is currently ongoing [52].

Despite a large medical need, no disease-modifying OA drug (DMOAD) is currently available. IA application will enable the development of novel disease-modifying small chemical molecules and biologicals, which would not be tolerated when applied systemically or would be much less effective.

An example is the IA injection of the protein drug anakinra in a recently conducted small safety study in patients with painful knee OA [53]. Anakinra is a recombinant form of IL-1 receptor antagonist (IL-1Ra) and acts as a competitive antagonist of IL-1, which is a key mediator of cartilage degrading processes and induction of pain in OA. Previously, anakinra had provided significant symptomatic improvement and slowed the radiographic progression in clinical trials with rheumatoid arthritis patients upon daily subcutaneous administration. The safety study in knee OA patients showed that anakinra is well tolerated in patients with painful knee OA. Long-term benefit on pain in the responders may suggest a therapeutic effect, but remains speculative since there was no control arm in this study. A long-term randomized placebo-controlled study will be required to determine the efficacy of IA anakinra for pain relief and improvement of joint structure, which was not assessed in this study.

Due to the short half-life of most IA injected molecules in SF and the discomfort and risk of infection associated with IA injections, novel IA treatments for OA can only be developed in conjunction with appropriate sustained release formulations.



Effects of a nonpeptide bradykinin B₂ receptor antagonist, FR167344, on different *in vivo* animal models of inflammation

Masayuki Asano, Chie Hatori, Noriaki Inamura, Hiroe Sawai, Jiro Hirosumi, Tatsujiro Fujiwara & Kunio Nakahara

Department of Pharmacology, Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-3, 5-chome, Tokodai, Tsukuba, Ibaraki 300-26, Japan

1 The effects of a novel, potent and orally active nonpeptide bradykinin B₂ receptor antagonist, FR167344 (N-[N-[3-[(3-bromo-2-methylimidazo[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-N-methylaminocarbonylmethyl]-4-(dimethylaminocarbonyl) cinnamylamide hydrochloride) were tested in three different *in vivo* models of inflammation.

2 Oral administration of FR167344 inhibited carrageenin-induced paw oedema in rats (carrageenin: 1%, 0.1 ml per animal, intraplantar), with an ID₅₀ of 2.7 mg kg⁻¹ at 2 h after carrageenin injection (*n* = 10 or 11).

3 Oral administration of the compound also inhibited kaolin-induced writhing (kaolin: 250 mg kg⁻¹, i.p.) in mice, with ID₅₀ of 2.8 mg kg⁻¹ in 10 min writhing and 4.2 mg kg⁻¹ in 15 min writhing (*n* = 19 or 20).

4 Additionally, oral administration of FR167344 inhibited caerulein-induced pancreatic oedema with an ID₅₀ of 13.8 mg kg⁻¹ as well as increases in amylase and lipase of blood samples with ID₅₀ of 10.3 and 7.4 mg kg⁻¹, respectively, in rats (*n* = 10).

5 These results show that FR167344 is an orally active, anti-inflammatory and anti-nociceptive agent in carrageenin-induced paw oedema, kaolin-induced writhing and caerulein-induced pancreatitis. FR167344 may have therapeutic potential against inflammatory diseases by oral administration and it may be a useful tool for studying the involvement of B₂ receptors in various *in vivo* models of inflammation.

Keywords: Bradykinin; antagonist; B₂ receptor; nonpeptide; orally active; FR167344; oedema; pain; pancreatitis

Introduction

Bradykinin, an endogenous nonapeptide produced by kallikrein, elicits various biological effects including oedema, pain, inflammation and hypotension (Burch *et al.*, 1990; Bhoola *et al.*, 1992). It thus seems that bradykinin could be involved in such inflammatory diseases as asthma, rhinitis, arthritis and pancreatitis (Bhoola *et al.*, 1992; Hall, 1992). Two subtypes of bradykinin receptor, designated B₁ and B₂, have been identified by molecular cloning and pharmacological means (Regoli & Barabé, 1980; Hess *et al.*, 1992; Menke *et al.*, 1994). To investigate the pathophysiological role of bradykinin, many bradykinin receptor antagonists (mainly B₂ receptor antagonists) have been synthesized (Burch *et al.*, 1990; Stewart, 1995). Recently, 'second-generation' B₂ receptor antagonists including Hoe 140 (D-Arg-[I¹Hyp³, Thi⁵, D-Tic⁷, Oic⁸]bradykinin) and S 16118 (p-guanidobenzoyl-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]bradykinin) have been obtained. They are highly potent and long-acting against bradykinin-induced responses and can be used to inhibit inflammation *in vivo* (Hock *et al.*, 1991; Wirth *et al.*, 1991b; Cherónis *et al.*, 1992; Félétou *et al.*, 1995b). Hoe 140 and S 16118 showed inhibitory effects on various animal models, such as carrageenin-induced paw oedema, caerulein-induced pancreatitis and bronchial microvascular leakage (Wirth *et al.*, 1991b; Griesbacher & Lembeck, 1992; Bertrand *et al.*, 1993; Félétou *et al.*, 1995a). These observations indicate the involvement of bradykinin in inflammatory reactions. However, these antagonists are all peptide analogues of limited therapeutic use owing to their poor oral bioavailability. Recently, a nonpeptide bradykinin B₂ receptor antagonists, WIN 64338 ([4-[2-[[bis(cyclohexylamino)methylene]amino]-3-(2-naphthyl)-1-oxopropyl]amino]phenyl)methyltributylphosphonium-

chloride monohydrochloride), has been described (Sawutz *et al.*, 1994; Hall *et al.*, 1995), but this antagonist has not been shown to be orally active.

We have previously described a novel, potent, selective, orally active and long acting nonpeptide bradykinin B₂ receptor antagonist, FR167344 (N-[N-[3-[(3-bromo-2-methylimidazo[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-N-methylaminocarbonylmethyl]-4-(dimethylaminocarbonyl)cinnamylamide hydrochloride; Figure 1) (Aramori *et al.*, 1997; Inamura *et al.*, 1997). FR167344 antagonized [³H]-bradykinin binding to B₂ receptors in guinea-pigs, rats and man with IC₅₀ values of 6.6 × 10⁻¹⁰, 1.2 × 10⁻⁹ and 1.3 × 10⁻⁸ M, respectively. However, it had no effect on [³H]-[des-arg¹⁰]-kallidin (a high-affinity bradykinin B₁ receptor ligand) binding to B₁ receptors even at 10⁻⁵ M (Inamura *et al.*, 1997). Moreover this compound potently antagonized bradykinin-induced contractions with a pA₂ value of 9.3 in guinea-pig isolated ileum, but it did not inhibit [des-Arg⁹]-bradykinin-induced rabbit aorta contractions (mediated by B₁ receptors) or acetylcholine or histamine-induced guinea-pig ileum contractions even at 10⁻⁶ M (Inamura *et al.*, 1997). *In vivo*, oral administration of FR167344 inhibited bradykinin-induced bronchoconstriction in guinea-pigs and the bradykinin-induced hypotensive response in rats at a dose of 1 mg kg⁻¹ (Inamura *et al.*, 1997). Furthermore, FR167344 is highly B₂ selective not only *in vitro* but also *in vivo* and, unlike some peptide B₂-receptor antagonists, it is not converted into a B₁ antagonistic compound by peptidases such as carboxypeptidases which can convert peptidic B₂ receptor antagonists into B₁-receptor selective [des-Arg⁹]-BK-forms (Drapeau *et al.*, 1991; Wirth *et al.*, 1991a).

The purpose of this study was to show that FR167344 is an orally active, anti-inflammatory and anti-nociceptive agent in carrageenin-induced paw oedema, kaolin-induced writhing and caerulein-induced pancreatitis, and to confirm the involvement of B₂ receptors in these models.

¹ Author for correspondence.

Methods

Carrageenin-induced paw oedema

The carrageenin-induced paw oedema model was performed by the method previously described (Winter *et al.*, 1962). Male Sprague-Dawley rats (8 weeks old, from Clea Japan, Inc.) were deprived of food overnight and treated orally with FR167344, 15 min before carrageenin was injected into the right hind paw intraplantar. Paw volume was measured by water plethysmometer before and 1, 2, 3 and 4 h after the injection of carrageenin. FR167344 was dissolved in 0.05 N HCl and administered orally at a volume of 5 ml kg⁻¹. Carrageenin was made up as 1% solution in saline. Each rat received 0.1 ml of the irritant. As saline-control, saline was administered in the same manner as carrageenin.

Kaolin-induced writhing

Male ICR mice (Slc:ICR, 5 weeks old, from Japan SLC, Inc.) were fasted overnight and used. Writhing responses were induced by an intraperitoneal injection of kaolin (250 mg kg⁻¹, 50 ml kg⁻¹). The responses were counted over a 10 or 15 min period by a trained observer. FR167344 dissolved in 0.05 N HCl or vehicle (10 ml kg⁻¹) was administered orally 30 min before the intraperitoneal injection of kaolin. As saline-control, saline was administered in the same manner as kaolin.

Caerulein-induced pancreatitis

Pancreatitis was induced according to the method previously described (Shimizu *et al.*, 1993) with minor modifications. Female Sprague-Dawley rats (9–10 weeks old, from Clea Japan, Inc.) were used. After the rats has been deprived of food for 18 h, caerulein (20 µg kg⁻¹) was injected intraperitoneally four times at hourly intervals over a 3 h period. Saline-injected (i.p.) animals served as saline-control. FR167344 dissolved in 0.05 N HCl or vehicle (5 ml kg⁻¹) was administered orally 30 min before the first caerulein injection. Three hours after the last caerulein injection, a blood sample was taken from the abdominal artery with heparin under anaesthesia (diethyl-ether inhalation), and the animals were killed by exsanguination, the pancreas was taken and weighed and the plasma was removed by centrifugation. Amylase and lipase levels were determined by the modified 4-nitrophenylmaltoheptaoside method (Denka Seiken Co., Ltd., Tokyo, Japan) (Dupuy *et al.*, 1987) and the monoglyceridelipase method (Nippon Shoji Co., Ltd., Osaka, Japan), respectively. Both enzyme assays were performed by use of an automatic analyser (model TBA-20R, Toshiba Co., Tokyo, Japan).

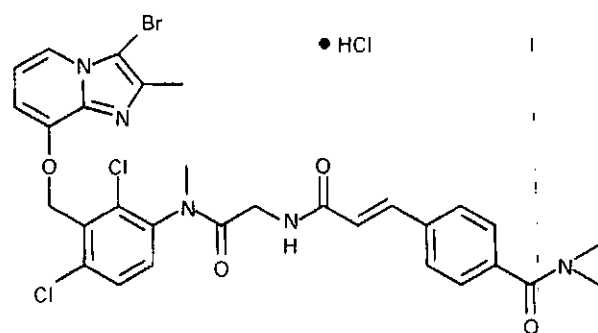


Figure 1 Structure of FR167344: N-[N-[3-[(3-bromo-2-methylimidazo[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-N-methylamino-carbonylmethyl]-4-(dimethylaminocarbonyl) cinnamylamide, hydrochloride.

Materials

FR167344 was chemically synthesized in Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Carrageenin was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Kaolin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Caerulein was purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan).

Statistical analysis

The results are expressed as the mean $\pm$ s.e. mean and the statistical significance of differences between groups was analysed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. ID₅₀ was obtained by using the non-linear curve fitting methods with an in-house computer programme.

Results

Carrageenin-induced paw oedema

An intraplantar injection of carrageenin (1%, 0.1 ml per animal) caused time-dependent paw oedema in the rat (Figure 2), although intraplantar injection of 0.1 ml saline caused almost no swelling (data not shown). In carrageenin-induced paw oedema in rats, oral administration of FR167344 inhibited paw swelling dose-dependently at 1, 2, 3 and 4 h after carrageenin injection (Figure 2). The ID₅₀ of FR167344 was 2.7 mg kg⁻¹ at the 2 h time point.

Kaolin-induced writhing

An intraperitoneal injection of kaolin (250 mg kg⁻¹, 50 ml kg⁻¹) caused time-dependent writhing in mice (Figure 3), although an intraperitoneal injection of saline (50 ml kg⁻¹) did not cause writhing (data not shown). Oral administration of FR167344 inhibited the kaolin-induced writhing response dose-dependently during 10 or 15 min after kaolin injection in mice (Figure 3). The ID₅₀ values were 2.8 mg kg⁻¹ in 10 min writhing and 4.2 mg kg⁻¹ in 15 min writhing.

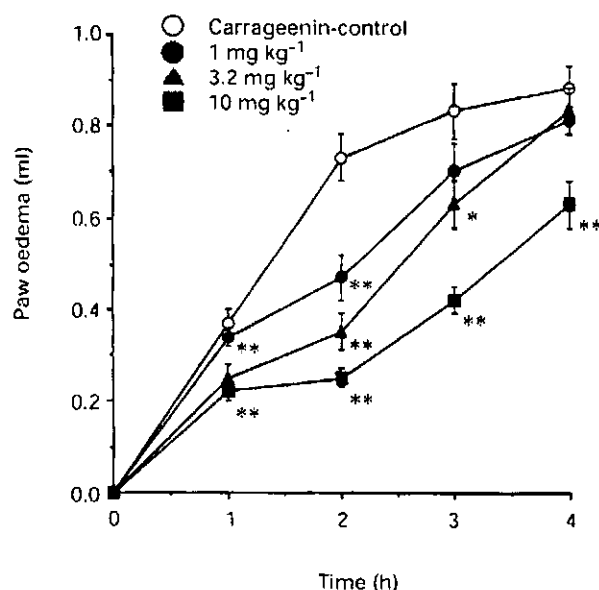


Figure 2 Inhibition of carrageenin-induced paw oedema by oral administration of FR167344 (1 mg kg⁻¹–10 mg kg⁻¹) in rats. Data are expressed as mean and vertical lines show s.e. mean (*n* = 10 or 11). **P* < 0.05, ***P* < 0.01 vs control (Dunnett's test).

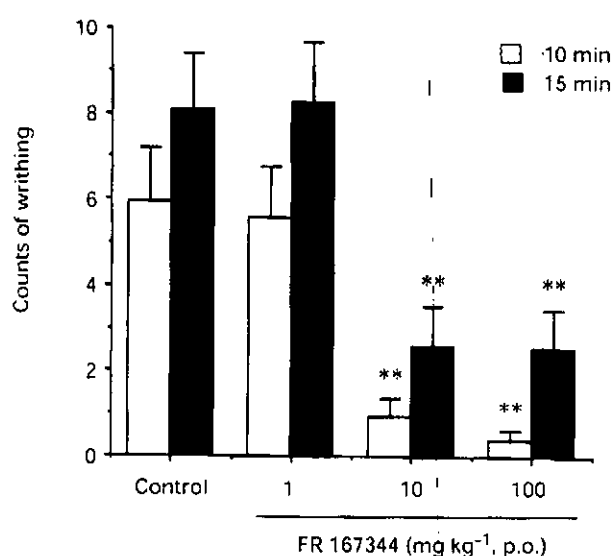


Figure 3 Inhibition of kaolin-induced writhing response by oral administration of FR167344 in mice. Data are expressed as mean and vertical lines show s.e.mean ($n=19$ or 20). Open columns and solid columns show counts of writhing in 10 min period and 15 min period after kaolin injection, respectively. $**P<0.01$ vs control (Dunnett's test).

Caerulein-induced pancreatitis

Oral administration of FR167344 inhibited caerulein-induced pancreatic oedema with an ID_{50} of 13.8 mg kg^{-1} (Figure 4). It also inhibited the increases in amylase and lipase of blood samples, and the ID_{50} estimates were 10.3 mg kg^{-1} and 7.4 mg kg^{-1} , respectively (Figure 5).

Discussion

In our investigation of the pathophysiological role of bradykinin and the development of drugs for inflammatory diseases, we have obtained the orally active nonpeptide bradykinin B₂ receptor antagonist, FR167344 (Inamura *et al.*, 1997) and FR173657 (Asano *et al.*, 1997) by optimization of a lead compound discovered by random screening. The present study demonstrates that oral administration of FR167344 inhibits carrageenin-induced paw oedema in rats, kaolin-induced writhing in mice, and caerulein-induced pancreatitis in rats. Our findings indicate that FR167344 may have therapeutic potential against inflammatory diseases by oral administration and that it may be a useful tool for studying the involvement of B₂ receptors in various *in vivo* models of inflammation.

Most biological actions of bradykinin are thought to be mediated by B₂ receptors (Burch *et al.*, 1990; Bhoola *et al.*, 1992). However, the active carboxypeptidase metabolites of bradykinin and kallidin, [des-Arg⁹]bradykinin and [des-Arg¹⁰]kallidin, preferentially stimulate B₁ receptors, which are generally absent in normal tissues, but inducible and functionally expressed in response to interleukin-1 β (deBlois *et al.*, 1991), bacterial lipopolysaccharides (Regoli *et al.*, 1981), *in vitro* tissue incubation (Regoli & Barabé, 1980; Regoli *et al.*, 1981) and *in vivo* arterial trauma (Pruneau *et al.*, 1994). Although the importance of B₁ receptors remains to be fully established, B₁ receptors are clearly involved in persistent inflammatory hyperalgesia (Perkins *et al.*, 1993; Perkins & Kelly, 1993) and other pathological conditions such as arthritis (Cruwys *et al.*, 1994), septic shock and hypotension (Hall, 1992). FR 167344 is highly B₂ selective *in vitro* (Inamura *et al.*, 1997), and it is not converted into a B₁ antagonistic compound by peptidases *in vivo*.

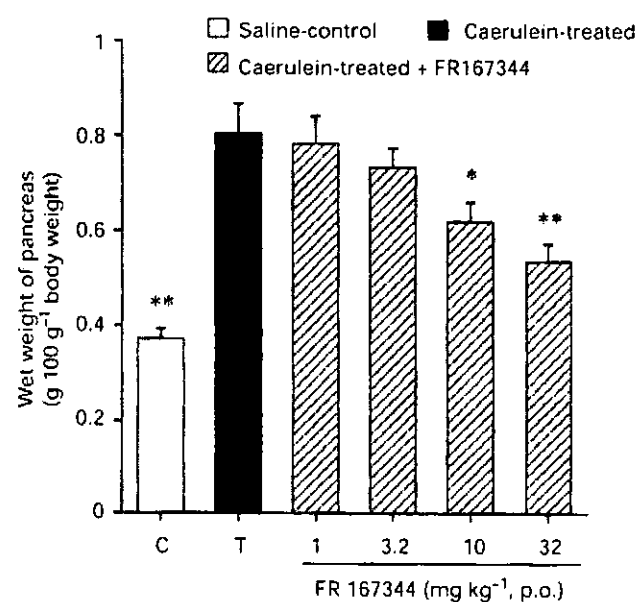


Figure 4 Inhibition of caerulein-induced pancreatic oedema by oral administration of FR167344 in rats. Data are expressed as mean and vertical lines show s.e.mean ($n=10$). $*P<0.05$, $**P<0.01$ vs caerulein-control (Dunnett's test).

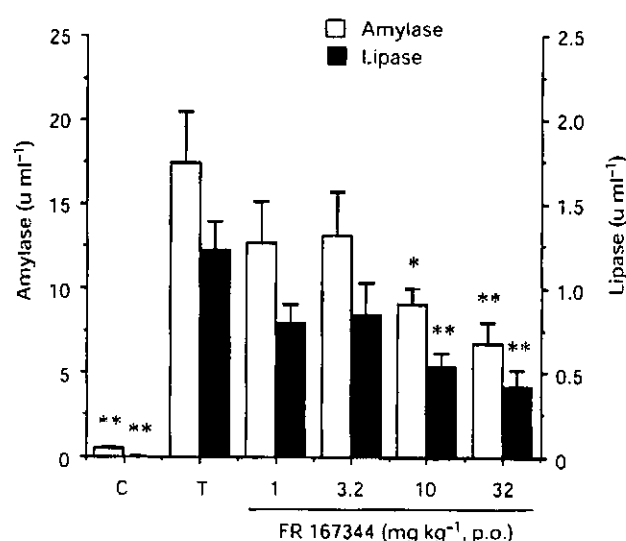


Figure 5 Inhibition of increase in amylase and lipase of blood samples by oral administration of FR167344 in caerulein-induced pancreatitis model. Data are expressed as mean and vertical lines shown s.e.mean ($n=10$). C and T show values in saline-control and caerulein-treated animals, respectively. $*P<0.05$, $**P<0.01$ vs caerulein-treated (Dunnett's test).

Previously, it has been shown that intravenous injection of B₂ receptor antagonists (Hoe 140, S 16118 and NPC 567 (D-Arg-[Hyp¹, D-Phe⁷]bradykinin)) reduces carrageenin-induced paw oedema (Costello & Hargreaves, 1989; Wirth *et al.*, 1991b; Félétou *et al.*, 1995a). Inhibition of carrageenin-induced inflammation has been shown to be highly predictive of anti-inflammatory drug activity in human inflammatory diseases (Wirth *et al.*, 1991b). Therefore, these B₂ receptor antagonists may have therapeutic potential against inflammatory diseases. Our data suggest that FR167344 may also have therapeutic potential against inflammatory diseases by oral administration. In the case of a chronic disease such as rheumatoid arthritis or asthma, oral activity of FR167344 would be of considerable merit in regard to patient's compliance with therapy. Our data also confirm that B₂ receptors (not B₁ re-

ceptors) play an important role in carrageenin-induced paw oedema. However, it has recently been shown that B₁ receptors are involved in intra-articular plasma extravasation in chronic antigen-induced arthritis (Cruwys *et al.*, 1994), which suggests that B₁ receptor antagonists may also reduce joint swelling in inflammatory arthritis.

Oral administration of FR167344 inhibited kaolin-induced writhing response in mice. Kaolin activates the kallikrein system (Fujiyoshi *et al.*, 1989) and induces the release of kinins (Fujiyoshi *et al.*, 1990). Subcutaneous administration of a potent peptidic B₂ antagonist, Hoe 140 (Hock *et al.*, 1991), also inhibits kaolin-induced writhing (Heapy *et al.*, 1993). Our results and these findings confirm the involvement of bradykinin in kaolin-induced writhing and suggest that FR167344 may be an effective drug for treatment of inflammatory pain by oral administration. Although FR167344 produced an almost complete inhibition in 10 min writhing, it produced only partial inhibition in 15 min writhing, and a similar result was obtained with Hoe 140 (Heapy *et al.*, 1993). These results indicate that bradykinin is a major mediator in the early phase of kaolin-induced writhing, but other mediators contribute to the late phase response. Compared with B₂ receptors, B₁ receptors do not seem to be involved in pain and hyperalgesia (Whalley *et al.*, 1987; Mizumura *et al.*, 1990), but it has recently been shown that the B₁ receptors are involved in chronic inflammatory hyperalgesia (Perkins *et al.*, 1993; Perkins & Kelly, 1993). Potent B₁ receptor antagonists may therefore be useful for the relief of chronic pain.

Additionally, FR167344 inhibited caerulein-induced pancreatic oedema and the increases in amylase and lipase of blood samples in rats. Hyperstimulation of exocrine function of the pancreas by the cholecystokinin analogue, caerulein, causes pancreatic oedema and increases in serum amylase and lipase (Steer & Meldolesi, 1987; Griesbacher & Lembeck, 1992; Félétou *et al.*, 1995a). This method is widely used for inducing morphological and biochemical changes in the pancreas similar to those observed in human oedematous pancreatitis (Willemer *et al.*, 1990; Griesbacher & Lembeck, 1992). In this model, other B₂ receptor antagonists (Hoe 140 and S 16118) also prevented pancreatic oedema (Griesbacher & Lembeck, 1992; Félétou *et al.*, 1995a). Our results confirm that B₂ receptors are involved in the caerulein-induced pancreatic oedema. FR167344 also inhibited the caerulein-induced increases in amylase and lipase in blood. However, Hoe 140 augmented those in blood (Griesbacher & Lembeck, 1992) and inhibited those in the pancreas (Griesbacher *et al.*, 1993). The cause of this discrepancy may be differences in the experimental conditions. Further investigations are required to elucidate the exact inhibitory mechanism of B₂ antagonists in the pancreatitis model.

In conclusion, this study shows that a nonpeptide B₂ receptor antagonist, FR167344, has inhibitory effects on some animal models of inflammation by oral administration. This compound would be not only a good tool for studying the pathophysiological role of B₂ receptors but also a useful oral treatment for inflammatory diseases.

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(Received June 13, 1997

Revised August 29, 1997

Accepted September 12, 1997)