

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 13
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

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Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PÁEZ

Asunto: Radicación: 13-187439- -7
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 15691
 Folios: 13

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2012/020606				
Fecha de Presentación Internacional	09/01/2012				

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:	Radicado No. 13-137489-00000-0000	06/Agosto/2013
Reivindicaciones:	Radicado No. 13-137489-00000-0000	06/Agosto/2013
	Radicado No. 13-137489-00006-0000	03/Junio/2014
Dibujos:	Radicado No. 13-137489-00000-0000	06/Agosto/2013
Oposición:	Radicado No. 13-137489-00000-0000	Lafrancol 28/Noviembre/2013
Respuesta de la solicitante	Radicado No. 13-137489-00006-0000	03/Junio/2014
Prioridad:	US 61/431,270	10/Enero/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta 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

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. Para la presente solicitud se estudió el capítulo reivindicatorio completo (Radicado No. 13-137489-00006-0000).

Título de la solicitud: “Métodos y productos de fármaco para tratar enfermedad de Alzheimer”

El problema planteado en esta solicitud consiste en la necesidad de proporcionar una terapia efectiva para la Enfermedad de Alzheimer.

Para resolver este problema técnico la solicitud en estudio proporciona una composición que comprende pioglitazona de dosis baja para utilizarse en el tratamiento de daño cognitivo del tipo Alzheimer.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/4436, C12Q 1/68.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 2 – 10, a pesar de que encabece como composición, está dirigido al tratamiento del Alzheimer y al régimen de dosificación de la pioglitazona en el tratamiento de esta enfermedad, por lo tanto, al referirse a un método de tratamiento en humanos no es patentable de acuerdo con el Art citado.

El objeto de las reivindicaciones 20 – 35 no es patentable de acuerdo con el Art citado puesto que hace referencia a un método de tratamiento en humanos.

El objeto de las reivindicaciones 36 – 80 hace referencia a un método de diagnóstico in vitro, el cual, así se lleve a cabo en una muestra fuera del cuerpo del paciente incluye un paso que consiste en determinar que el paciente tiene un riesgo incrementado de desarrollar daño cognitivo y Alzheimer o que necesita un tratamiento específico. En ese sentido, dichos métodos de diagnóstico permiten por sí solos tomar una decisión del tratamiento a seguir, tal como está descrito en el ejemplo 8 de la memoria descriptiva, y por lo tanto no son patentables de acuerdo con el Art citado.

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

Las reivindicaciones 1, 19 y 81, aunque encabezan como composición, no mencionan alguna característica técnica del objeto reclamado y se refieren al uso de la misma. Por lo tanto, el objeto de dichas reivindicaciones no es patentable, de acuerdo con el Art 14.

El uso de las reivindicaciones 11 – 18 no es patentable de acuerdo con el Art citado.

6. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 82 carece de claridad puesto que no se definen las características técnicas esenciales de la composición, esto es, la estructura del principio activo y el vehículo empleado. Se recuerda a la solicitante que la forma correcta de reclamar una composición farmacéutica es a partir de sus componentes (ingredientes activos y excipientes) y las proporciones de los mismos (reivindicación 82).

Las reivindicaciones 83 a 94 se consideran resultados a alcanzar puesto que hacen referencia al modo de presentación de la composición pero no mencionan las características técnicas de los excipientes empleados que permitan que la composición sea por ejemplo una tableta o una cápsula.

El término "...de dosis baja..." empleado en la reivindicación 82 carece de claridad puesto que no es limitativo y no permite tener certeza del alcance de la materia reclamada.

7. Análisis de la Oposición

Argumentaciones de Lafrancol S.A.S.

El opositor afirma que esta solicitud pretende obtener derecho de patente para el principio activo pioglitazona, el cual según argumenta, se encuentra plenamente identificado como se cita en los documentos EP193256 y US2507631 de 1986 y 1987 respectivamente.

Adicionalmente señala que los métodos de tratamiento quirúrgicos, terapéuticos o de diagnóstico aplicables al cuerpo humano no son susceptibles de aplicación industrial y por lo tanto no puede acceder a beneficios patentarios.

Por otra parte afirma que las combinaciones no se consideran invenciones y señala que un efecto sinérgico nuevo no se considera inventivo dado que puede ser obvio para la persona versada en la materia.

Finalmente presenta los siguientes documentos del estado del arte como relevantes para demostrar la falta de novedad y nivel inventivo de la presente solicitud:

US2007/0105859 (Fecha de publicación 10/Mayo/2007)

WO2010/019550 (Fecha de publicación

CINAPS Compound Dossier (Fecha de publicación 22/Diciembre/2009)

US 2010/0120842 (Fecha de publicación 13/Mayo/2010)

Respuestas del Solicitante

En respuesta al argumento de la solicitante según cual, la presente solicitud pretende obtener derecho de patente para el principio activo conocido pioglitazona, la solicitante afirma que la presente solicitud no reivindica la pioglitazona como tal sino una composición que la comprende, lo cual se considera una conclusión equivocada.

Adicionalmente señala que la sociedad opositora se refiere a la falta de nivel inventivo y de novedad de la presente solicitud pero que en el documento de la oposición no se presenta ningún análisis de las razones que permitan considerar el porqué de la carencia de tales requisitos.

Por otra parte, frente al argumento según el cual las combinaciones no se consideran invenciones, la solicitante argumenta que esto es una conclusión equivocada que contradice lo establecido por el Tribunal de Justicia y que de hecho no se puede descartar de plano la patentabilidad de una invención que se refiere a una combinación pues debe analizarse el caso concreto.

Finalmente, la solicitante presenta un nuevo capítulo reivindicatorio conformado por 94 reivindicaciones.

Respuesta del Examinador Técnico

En primer lugar, se aclara que la presente solicitud reclama una composición cuyo componente principal es la pioglitazona, el cual es un principio activo conocido en el estado de

técnica. Sin embargo, la composición reclamada en la reivindicación 82 no revela ninguna característica técnica de la composición y por lo tanto se han hecho observaciones al respecto en el presente examen.

Respecto a la objeción por materia exceptuada de patentabilidad de la presente solicitud se aclara que se ha tenido en cuenta en el presente examen.

En relación con el argumento de que las combinaciones no son invenciones, se aclara que esto no es cierto, pues siempre y cuando se demuestre un efecto sinérgico y se cumplan con requisitos de patentabilidad, las combinaciones pueden ser materia patentable.

Respecto a los documentos presentados por la sociedad opositora, el documento US2007/0105859 divulga derivados de 4- fenilamino – benzaloxima y composiciones farmacéuticas que comprenden los compuestos o sales farmacéuticamente aceptables de los mismos; el documento WO2010/019550 se refiere a métodos para la identificación de variables genéticas asociadas con el desarrollo de una condición de interés como el Alzheimer; el documento CINAPS Compound Dossier presenta la información del compuesto pioglitazona y lo referente a su farmacocinética y sus usos y aplicaciones; y el documento US 2010/0120842 enseña métodos para el tratamiento de condiciones relacionadas con el sistema nervioso, incluido el empleo del compuesto pioglitazona como un agente adicional de una composición.

Si bien la sociedad opositora no presentó un análisis con dichos documentos presentados, una vez revisados, los documentos US2007/0105859, WO2010/019550, CINAPS Compound Dossier y US2010/0120842 no desvirtúan la novedad (ó el nivel inventivo) del objeto de esta solicitud puesto que no se refieren a una composición farmacéutica que comprenda el compuesto pioglitazona.

8. 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: 10 de enero de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO/2000/032190A1	“Compositions an methods for the treatment of Alzheimer disease, central nervous system injury and inflamatory diseases”	08/Junio/2000
D2	Sato et al. 2011	“Efficacy of PPAR-y agonist pioglitazone in mild Alzheimer disease”	Publicación online: 18/Noviembre/2009

El documento D1¹ divulga métodos y composiciones útiles para el tratamiento de la enfermedad de Alzheimer (resumen).

El documento D2² divulga un estudio piloto que demuestra la efectividad del antagonista de PPAR-gama, la pioglitazona, en la recuperación funcional de pacientes con AD (Resumen).

9. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 82 consiste en “Una composición farmacéutica que comprende pioglitazona de dosis baja...”

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

1 http://www.lens.org/images/patent/WO/2000032190/A1/WO_2000_032190_A1.pdf
2 [http://www.neurobiologyofaging.org/article/S0197-4580\(09\)00339-X/abstract](http://www.neurobiologyofaging.org/article/S0197-4580(09)00339-X/abstract)

Características esenciales	D1	D2
Una composición farmacéutica	Una composición farmacéutica (Pág. 38)	
Que comprende pioglitazona de dosis baja	Que comprende pioglitazona (Pág. 39 línea 10) una dosis de 0.5 a 100mg (Pág. 43 líneas 21 - 30).	Dosis baja de pioglitazona 15 – 30 mg/día (Pág, 1627 col. izq. tercer párrafo)
O una sal farmacéuticamente aceptable de la misma	O una sal farmacéuticamente aceptable de la misma (Pág. 40)	
Y un vehículo farmacéuticamente aceptable	Que comprende un vehículo farmacéuticamente aceptable (Pág. 38 líneas 25 -26).	

El documento D1 se considera el estado de la técnica cercano a la definida en la reivindicación 82 porque divulga una composición farmacéutica útil en el tratamiento de la enfermedad de Alzheimer que comprende el compuesto pioglitazona y un vehículo farmacéuticamente aceptable. Dicho documento divulga una dosis que comprende de 0.5 a 100 mg que pueden estar presentadas en forma de tabletas, cápsulas, sólidos (Págs. 41 - 43).

La diferencia entre la invención, definida en la reivindicación 82 y la composición divulgada en el documento D1 consiste en que la dosis de pioglitazona del documento D1 puede tener valores altos de dosis cercanos a 100mg mientras que la composición reclamada en la presente solicitud reclama una dosis baja, aun cuando está indeterminada.

El efecto que confiere dicha diferencia es que se puede conseguir un efecto en el tratamiento de la enfermedad de Alzheimer a una dosis baja del compuesto.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar la composición conocida en el documento D1 para conseguir una composición que sea efectiva a dosis bajas”.

Sin embargo, el documento D2 ya había demostrado el efecto de una dosis baja de pioglitazona en pacientes con la enfermedad de Alzheimer (Pág, 1627 col. izq. tercer párrafo). Por lo tanto, para una persona medianamente versada en la materia, habría resultado previsible la selección de una dosis baja del compuesto de interés, como la empleada en el documento D2, dentro del amplio rango enseñado en el documento D1, para llegar al objeto reclamado en la presente solicitud. Por lo cual, la solución propuesta en la presente solicitud se considera obvia y sin altura inventiva.

Las reivindicaciones dependientes 83 – 94 tampoco cumplen con el requisito de nivel inventivo a la luz de los documentos D1 y D2.

10. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO/2000/032190A1	82 – 94	Nivel inventivo
D2	Sato et al. 2011	82 – 94	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 del día siguiente 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 de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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 2014-12-30

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Efficacy of PPAR- γ agonist pioglitazone in mild Alzheimer disease

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Abstract

To test the effects of the PPAR- γ agonist pioglitazone on cognition, regional cerebral blood flow (rCBF), and plasma levels of A β 40 and A β 42, we conducted a 6-month, randomized, open-controlled trial in patients with mild Alzheimer disease (AD) accompanied with type II diabetes mellitus. We randomly assigned 42 patients to either the group treated with 15–30 mg pioglitazone daily ($n = 21$, pioglitazone group) or not ($n = 21$, control group). The pioglitazone group improved cognition and rCBF in the parietal lobe, while the control group showed no such improvement. The plasma A β 40/A β 42 ratio increased in the control group, but showed no significant change in the pioglitazone group. Both groups showed good control of diabetes during the study. In addition, pioglitazone treatment resulted in a decrease in fasting plasma insulin levels, indicating enhanced insulin sensitivity. The results of this pilot study demonstrated that pioglitazone exhibited cognitive and functional improvements, and stabilization of the disease in diabetic patients with AD. Pioglitazone may offer a novel strategy for the treatment of AD.

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Keywords: Pioglitazone; Alzheimer's disease; Cognition; Cerebral blood flow; Plasma A β

1. Introduction

There is a growing body of evidence that conditions caused by insulin abnormalities, such as impaired glucose tolerance and type II diabetes mellitus (DM), have increased risk of age-related cognitive decline and Alzheimer's disease (AD) (Yoshitake et al., 1995; Ott et al., 1999; Peila et al., 2002; Luchsinger et al., 2001). Insulin resistance and peripheral hyperinsulinemia have been known to promote the neurodegeneration that occurs in AD, such as beta amyloid (A β) deposits and increase of tau hyperphosphorylation (Craft and Watson, 2004; Biessels et al., 2006; Cole et al., 2007; Neumann et al., 2008). Therefore, correcting insulin dysregulation may offer a novel strategy for the treatment of AD. A recent clinical trial performed by Reger et al. (2008) showed that the administration of intranasal insulin improved cognitive performance in elderly patients with early AD and amnesic mild cognitive impairment (MCI).

On the other hand, pharmacologic treatment with thiazolidinediones, such as rosiglitazone and pioglitazone, agonists of the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR- γ), may offer some therapeutic relief for AD by lowering peripheral insulin and enhancing insulin sensitivity. In addition, PPAR- γ agonists have been shown to inhibit inflammatory gene expression, alter A β homeostasis and exhibit neuroprotective effects (Landreth, 2007; Neumann et al., 2008; Jiang et al., 2008). It has recently been demonstrated that 6 months of treatment with rosiglitazone preserved cognitive function for patients with AD and amnesic MCI compared with a placebo-treated group (Watson et al., 2005).

To test the effects of the PPAR- γ agonist pioglitazone on cognition, regional cerebral blood flow (rCBF), and plasma levels of A β 40 and A β 42, we conducted a 6-month, randomized, open-controlled trial in patients with AD with type II DM. In the future, number of patients with AD and DM will increase steadily in the context of an aging population. The aim of this study was to determine whether pioglitazone may be effective for the treatment of these conditions.

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2. Methods

2.1. Subjects

We enrolled 42 patients with mild AD (a Clinical Dementia Rating score of 0.5 or 1) (Hughes et al., 1982) with type II DM from the Memory Clinic at the Tokyo Medical University Hospital. We established a diagnosis in each case using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984) for probable AD. Patients taking donepezil (a cholinesterase inhibitor) were enrolled, provided that the dose had remained unchanged from 6 months before participation through the study. No subjects were taking any medication known to affect cognition or behavior during the study, including neuroleptics, benzodiazepines, and antidepressants. In the present study, subjects were excluded if they had any evidence of other neurologic or psychiatric disorders sufficient to cause memory impairment, including depression or anxiety disorder as reflected by a score of more than 5 on the Geriatric Depression Scale-15 (Sheikh and Yesavage, 1986). Diabetes was defined as the use of an oral hypoglycemic drug or a casual (nonfasting) plasma glucose level of 200 mg/dl or more, or a fasting plasma glucose level of 126 mg/dl or more, according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997). Subjects were also excluded if they had clinically significant medical problems, including cancer within the preceding 3 years, chronic renal or heart failure, severe pulmonary disease, or poorly controlled diabetes or use of insulin.

Written informed consent was obtained from all subjects and from the legal representatives of the subjects with AD. This study was approved by the ethics committee of our institute.

2.2. Study protocol

The study was set up as a prospective randomized, open-controlled design. Subjects were randomly assigned to treatment with pioglitazone 15–30 mg daily [pioglitazone group, $n = 21$ (15 mg, $n = 19$; 30 mg, $n = 2$)] or not (control group, $n = 21$) with a simple sealed envelope method, in addition to their previous treatment for control of DM, including sulfonylureas, biguanides, and α -glucosidase inhibitors. Safety evaluations conducted throughout the study included physical examinations for edema or other adverse effects, vital sign measurement, and standard laboratory tests (hematology and liver function tests).

2.3. Metabolic, neuropsychological measures, and A β measurements

We administered the Mini-Mental State Examination (MMSE) (Folstein et al., 1975), Alzheimer's Disease

Assessment Scale-Cognitive Subscale Japanese version (ADAS-Jcog) (Honma et al., 1992), Wechsler Memory Scale-revised (WMS-R) logical memory-I (Wechsler, 1987), Frontal Assessment Battery (FAB) (Dubois et al., 2000), and category fluency at baseline and 6 months after treatment. For category fluency, subjects were asked to generate as many items as possible from the category animals, and then vegetables, with 60 s allowed for each semantic category. The total number of correct words (animals and vegetables) was summed to obtain the total category fluency score. All tests were administered by an experienced psychometrist, blinded to the clinical data.

Additional measures included fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), fasting immunoreactive insulin (FIRI), and homeostasis model assessment ratio (HOMA-R). Plasma insulin levels were measured by radioimmunoassay. Plasma A β 40 and A β 42 concentrations were determined by sandwich enzyme-linked immunosorbent assays (ELISA) using the BAN50 capture antibody and C-terminal specific detector antibodies BA27 and BC05 (Kawarabayashi et al., 2001; Fukumoto et al., 2003). We have demonstrated this ELISA system to detect A β 40 or A β 42 at concentrations as low as 2 pmol/L.

2.4. rCBF study

We underwent SPECT studies at baseline and 6 months after treatment to measure rCBF deficits. The intervals between the cognitive assessment and rCBF study in all subjects were within 2 weeks. SPECT studies after intravenous injection of 222 MBq of N-isopropyl-p-[¹²³I] iodoamphetamine were performed using a triple-head rotating gamma camera (PRISM 3000 XP, Philips Medical System, Cleveland, USA) with a fan-beam collimator permitting a spatial resolution of 6.8 mm full width at half maximum.

Three-dimensional stereotactic surface projections (3D-SSP) created with the Neurological Statistical Image Analysis Software (Neurostat) developed by Minoshima et al. (1995) were used to evaluate the spatial distribution of abnormal CBF. Each image set was realigned to the bicommissure stereotactic coordinate system (Talairach and Tournoux, 1998). Each brain was standardized anatomically to match a standard atlas brain while preserving rCBF activity. Subsequently, maximum cortical activity was extracted to adjacent predefined surface pixels on a pixel-by-pixel basis using a 3D-SSP technique. Data sets were normalized to the mean cortical activity. To quantify rCBF deficits, the normalized brain activity of each patient was compared with that of 28 normal controls (12 men and 16 women, mean age 75.1 ± 6.4 years) using pixel-by-pixel z -score analysis: $[(\text{normal mean}) - (\text{individual value})]/(\text{normal SD})$. A positive z -score represents a reduced rCBF in the patient relative to the normal control mean.

To demonstrate rCBF alterations, two-sample t -test values were calculated on a pixel-by-pixel basis between controls

and each group at initial SPECT, and then transformed to z -values by a probability integral transformation. Furthermore, to assess rCBF changes, differences between the initial and last SPECT data were compared in each group. Next, to measure rCBF reduction, the mean z -score for each patient was calculated by the stereotactic extraction estimation method (Mizumura et al., 2003). Mean z -scores for each gyrus of the frontal, parietal, temporal, occipital, and limbic lobe of the right and left hemispheres were automatically measured (average z -value of the coordinates with a z -value that exceeds 0 of the threshold value). Each mean value for a patient was compared to the corresponding mean values of 28 control subjects on a region-by-region basis and the magnitude of CBF reduction was expressed as a z -score. The method of rCBF measurement was described elsewhere in detail (Hanyu et al., 2008).

2.5. ApoE phenotyping

ApoE phenotypes were determined from plasma samples by isoelectric electrophoresis and immunoblotting. A detailed procedure has been described previously by Kataoka et al. (1994).

2.6. Statistical analysis

Values were expressed as means $\pm$ standard deviation. Statistical analysis was performed using paired and unpaired t -test, the chi-square test, Mann–Whitney U test, and Wilcoxon signed-rank test. A comparison of mean z -scores for cerebral subregions between the two groups was performed by one-way ANOVA and post hoc t -tests with Bonferroni's corrections. A p value of less than 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Comparisons of subjects characteristics (Table 1)

There were no significant differences between the pioglitazone group and the control group in age, gender, education, duration of symptoms, numbers using donepezil, number of ApoE ϵ 4 carriers, or treatment of DM. The frequency of medical comorbidity, such as hypertension, hyperlipidemia, and ischemic heart disease, was similar in the groups.

3.2. Metabolic, neuropsychological, and plasma A β 40 and A β 42 changes (Table 2)

At baseline, no significant differences between the two groups were found in metabolic measurements, including FPG, HbA1c, FIRI, and HOMA-R and in neuropsychological performance, including the MMSE, ADAS-Jcog, WMS-R logical memory-I, FAB, and category fluency. At month 6, FPG and HbA1c decreased significantly than those at base-

Table 1
Comparisons of subject characteristics.

	Pioglitazone group	Control group
No. of patients	21	21
Age (years)	77.4 $\pm$ 6.2	77.6 $\pm$ 6.5
Gender (men/women)	11/10	9/12
Education (years)	11.9 $\pm$ 2.5	11.4 $\pm$ 2.6
Duration (years)	2.8 $\pm$ 0.7	2.9 $\pm$ 0.8
No. using donepezil	10 (48%)	10 (48%)
ApoE ϵ 4 status	10 carriers/11 non-carriers	9 carriers/12 non-carriers
Treatment for diabetes mellitus		
Diet only	9 (43%)	8 (38%)
Hypoglycemic agents	12 (57%)	13 (62%)
Sulfonylureas	8	10
Biguanides	2	3
α -Glucosidase inhibitors	4	4
Medical complications		
Hypertension	8 (38%)	9 (43%)
Hyperlipidemia	4 (19%)	4 (19%)
Ischemic heart disease	2 (10%)	3 (14%)

line in both groups, while FIRI and HOMA-R decreased significantly in the pioglitazone group, but not in the control group. The MMSE, ADAS-Jcog, and WMS-R logical memory-I improved significantly in the pioglitazone group, while the ADAS-Jcog worsened significantly in the control group. Neither group showed any significant change in the FAB and category fluency.

We measured plasma A β 40 and A β 42 levels in 17 subjects with the pioglitazone group and 18 subjects with the control group. At baseline, no significant differences were observed in A β 40, A β 42, and A β 40/A β 42 ratio between the groups. At month 6, there was a tendency toward decrease of A β 42 in the control group. As a result, the A β 40/A β 42 ratio increased significantly in the control group, while it showed no significant change in the pioglitazone group.

3.3. rCBF changes

Table 3 shows the z -scores of each region at baseline and month 6 in both groups. Fig. 1a and b shows relative decrease of rCBF in the pioglitazone group and control group compared to normal controls at baseline, respectively. Both groups showed significant decreases in rCBF of the parietotemporal lobe, posterior cingulate, and frontal lobe, which are considered characteristic features of AD. There were no significant differences of z -score in each region between the two groups. Fig. 2a and b shows rCBF changes from baseline at month 6 in both groups. The pioglitazone group showed an improvement of rCBF in the right and left parietal lobe and some regions of the frontal lobe. In contrast, the control group showed no improvement of rCBF in any brain regions. Quantitative z -score analysis showed a significant increase of rCBF in the right superior parietal lobule, right and left inferior parietal lobules, and left postcentral gyrus in the pioglitazone group. In contrast, there was no significant

Table 2
Metabolic, neuropsychological, and plasma A β 40 and A β 42 changes.

	Pioglitazone group		Control group	
	Baseline	Month 6	Baseline	Month 6
Metabolic changes				
Fasting plasma glucose (mg/dl)	137 $\pm$ 41	121 $\pm$ 27*	148 $\pm$ 40	131 $\pm$ 29*
Hemoglobin A1c (%)	6.8 $\pm$ 1.2	6.4 $\pm$ 1.1**	6.9 $\pm$ 1.0	6.5 $\pm$ 0.8*
Fasting immunoreactive insulin (μ U/ml)	7.4 $\pm$ 3.6	5.7 $\pm$ 2.3*	6.6 $\pm$ 4.8	6.5 $\pm$ 2.7
HOMA-R	2.4 $\pm$ 1.2	1.6 $\pm$ 0.8**	2.2 $\pm$ 1.5	1.9 $\pm$ 0.8
Neuropsychological change				
MMSE	22.1 $\pm$ 3.5	23.1 $\pm$ 4.1*	21.9 $\pm$ 3.4	21.6 $\pm$ 3.0
ADAS-Jcog	15.5 $\pm$ 5.9	14.2 $\pm$ 6.5*	15.3 $\pm$ 4.7	17.5 $\pm$ 5.2**
WMS-R logical memory I	6.5 $\pm$ 4.1	7.8 $\pm$ 4.7**	5.7 $\pm$ 3.2	5.4 $\pm$ 3.6
Frontal assessment battery	11.8 $\pm$ 3.0	11.8 $\pm$ 3.0	10.9 $\pm$ 2.1	10.2 $\pm$ 2.6
Category fluency	20.2 $\pm$ 6.4	21.0 $\pm$ 6.4	19.6 $\pm$ 3.8	19.5 $\pm$ 3.9
Plasma Aβ40 and Aβ42 changes				
A β 40 (pmol/L)	54.8 $\pm$ 18.9	53.5 $\pm$ 19.7	54.9 $\pm$ 21.6	53.6 $\pm$ 27.1
A β 42 (pmol/L)	6.2 $\pm$ 2.0	6.3 $\pm$ 2.2	7.7 $\pm$ 4.2	6.0 $\pm$ 2.6
A β 40/A β 42	9.3 $\pm$ 3.2	9.1 $\pm$ 3.5	7.6 $\pm$ 2.2	9.5 $\pm$ 3.9*

Values are mean $\pm$ SD.

* $p < 0.05$ compared with baseline.

** $p < 0.01$ compared with baseline.

increase of rCBF in any regions of the control group. No significant decrease of rCBF was found in any regions of both groups.

3.4. Adverse events

Three patients in the pioglitazone group had mild peripheral edema, but the adverse effect was tolerated without discontinuation of the study medication.

4. Discussion

We found that the pioglitazone group showed improvements of scores on the MMSE, ADAS-J-cog, and WMS-R logical memory-I, while the control group showed worsened scores on the ADAS-Jcog after 6 months of treatment. In addition, our SPECT study demonstrated that the pioglitazone group showed an improvement of rCBF in the parietal lobe, while the control group showed no significant changes of rCBF in any brain region after 6 months of treatment, compared with baseline values. Although both groups showed improvements of FPG and HbA1c levels after 6 months of treatment, pioglitazone treatment resulted in a significant decrease in FIRI and HOMA-R, indicating enhanced insulin sensitivity. Our results suggest beneficial effects of pioglitazone in patients with mild AD with DM.

A number of studies have examined potential mechanisms through which PPAR- γ agonists might act to ameliorate AD pathogenesis and progression (Landreth, 2007; Neumann et al., 2008; Jiang et al., 2008). As shown in the present study, PPAR- γ agonists improve sensitivity to insulin by decreasing circulating insulin and increasing insulin mediated glucose uptake. They enhanced insulin action in the brain, resulting

in cognitive improvement. AD has a significant inflammatory component which has been linked to A β accumulation and neuronal loss. PPAR- γ agonists also demonstrate to reduce the levels of multiple inflammatory mediators. Some studies have showed that they suppress the A β -mediated activation of microglia (Combs et al., 2000) and inhibit A β production stimulated by inflammatory cytokines (Sastre et al., 2003, 2006). In addition, the involvement of PPAR- γ agonists in the regulation of A β production and metabolism has recently been investigated. Heneka et al. (2005) reported that pioglitazone treatment decreased β -secretase-1 (BACE1) associated with lower plaque burden in an animal model of AD. Pedersen et al. (2006) reported that rosiglitazone attenuated reductions in insulin-degrading enzyme activity and reduced brain levels of A β 42 in Tg2576 mice. PPAR- γ activation has also been associated with stimulation of A β clearance (Camacho et al., 2004). Furthermore, PPAR- γ agonists could reduce phosphorylation of tau protein, resulting in inhibition neurofibrillary tangle accumulation in AD (d'Abramo et al., 2006). A recent experimental study showed that pioglitazone not only overcomes cerebrovascular dysfunction and altered neurometabolic coupling in AD transgenic mice, but also counteracts cerebral oxidative stress and glial activation (Nicolakakis et al., 2008). Considering these potentially efficacious effects, PPAR- γ agonists, such as rosiglitazone and pioglitazone, may offer a novel strategy for the treatment of AD.

Recently, a randomized, double-blind pilot trial demonstrated that 6 months of treatment with rosiglitazone showed improvements in memory and selective attention, associated with less reduction in plasma A β 40 and A β 42 of 20 patients with AD and MCI compared with 10 control patients (Watson et al., 2005). Another study on 511 AD patients demonstrated that ApoE ϵ 4 non-carriers exhibited cognitive and

Table 3

Mean z-scores for cerebral subregions at initial and last SPECT studies for each group.

			Pioglitazone group		Control group	
			Baseline	Month 6	Baseline	Month 6
Frontal lobe						
Superior frontal gyrus	L		0.85 ± 0.46	0.74 ± 0.32	0.90 ± 0.34	0.96 ± 0.46
	R		0.71 ± 0.26	0.70 ± 0.28	0.96 ± 0.40	0.96 ± 0.41
Middle frontal gyrus	L		0.84 ± 0.35	0.75 ± 0.28	0.89 ± 0.36	1.05 ± 0.53
	R		0.88 ± 0.36	0.78 ± 0.27	1.04 ± 0.48	1.06 ± 0.57
Inferior frontal gyrus	L		0.81 ± 0.37	0.83 ± 0.37	1.01 ± 0.48	1.16 ± 0.70
	R		0.77 ± 0.49	0.72 ± 0.34	0.93 ± 0.37	0.95 ± 0.55
Medial frontal gyrus	L		0.89 ± 0.42	0.83 ± 0.34	0.98 ± 0.41	1.00 ± 0.43
	R		0.84 ± 0.31	0.75 ± 0.39	0.96 ± 0.44	0.95 ± 0.40
Orbital gyrus	L		0.65 ± 0.66	0.74 ± 0.67	1.18 ± 0.83	1.17 ± 0.72
	R		0.83 ± 0.84	0.88 ± 0.82	1.04 ± 0.88	0.96 ± 0.82
Rectal gyrus	L		0.63 ± 0.46	0.72 ± 0.59	0.89 ± 0.53	0.89 ± 0.58
	R		0.75 ± 0.49	0.67 ± 0.62	0.80 ± 0.57	0.95 ± 0.53
Paracentral lobule	L		0.52 ± 0.34	0.44 ± 0.32	0.61 ± 0.39	0.59 ± 0.37
	R		0.41 ± 0.33	0.38 ± 0.32	0.55 ± 0.33	0.49 ± 0.31
Precentral gyrus	L		0.71 ± 0.22	0.70 ± 0.26	0.71 ± 0.38	0.76 ± 0.45
	R		0.63 ± 0.34	0.66 ± 0.44	0.68 ± 0.31	0.70 ± 0.35
Subcallosal gyrus	L		0.50 ± 0.44	0.53 ± 0.46	0.72 ± 0.65	0.94 ± 0.61
	R		0.35 ± 0.37	0.64 ± 0.52	0.75 ± 0.66	0.66 ± 0.47
Parietal lobe						
Superior parietal lobule	L		0.91 ± 0.47	0.80 ± 0.36	0.82 ± 0.35	0.85 ± 0.42
	R		0.95 ± 0.40	0.76 ± 0.31*	0.82 ± 0.50	0.77 ± 0.37
Inferior parietal lobule	L		1.24 ± 0.64	0.97 ± 0.40*	1.14 ± 0.39	1.17 ± 0.58
	R		1.44 ± 0.95	1.03 ± 0.49*	1.16 ± 0.54	1.17 ± 0.65
Angular gyrus	L		1.38 ± 1.03	1.29 ± 0.56	1.25 ± 0.62	1.18 ± 0.78
	R		1.53 ± 0.85	1.45 ± 1.00	1.18 ± 0.94	1.22 ± 0.97
Postcentral gyrus	L		0.83 ± 0.38	0.59 ± 0.26*	0.78 ± 0.47	0.82 ± 0.61
	R		0.81 ± 0.67	0.71 ± 0.51	0.85 ± 0.43	0.67 ± 0.43
Precuneus	L		1.18 ± 0.54	1.05 ± 0.47	0.98 ± 0.52	1.12 ± 0.43
	R		0.91 ± 0.44	0.89 ± 0.46	0.85 ± 0.48	0.90 ± 0.39
Supramarginal gyrus	L		1.35 ± 1.01	1.02 ± 0.47	1.13 ± 0.69	1.06 ± 0.59
	R		1.31 ± 0.89	1.08 ± 0.55	1.29 ± 0.82	1.23 ± 0.90
Temporal lobe						
Superior temporal gyrus	L		0.92 ± 0.39	0.95 ± 0.38	1.06 ± 0.34	0.98 ± 0.45
	R		1.04 ± 0.49	0.96 ± 0.48	1.00 ± 0.40	1.04 ± 0.39
Middle temporal gyrus	L		1.03 ± 0.51	1.10 ± 0.34	1.10 ± 0.29	1.12 ± 0.40
	R		1.17 ± 0.54	1.07 ± 0.63	0.95 ± 0.35	1.02 ± 0.45
Inferior temporal gyrus	L		0.84 ± 0.35	1.01 ± 0.57	1.02 ± 0.45	1.06 ± 0.50
	R		0.99 ± 0.72	0.95 ± 0.82	0.90 ± 0.42	0.97 ± 0.51
Transverse temporal gyrus	L		0.66 ± 0.57	0.60 ± 0.57	0.46 ± 0.96	0.65 ± 1.06
	R		0.57 ± 0.58	0.63 ± 0.68	0.59 ± 1.07	0.56 ± 0.68
Occipital lobe						
Superior occipital gyrus	L		0.87 ± 0.76	0.88 ± 0.60	0.70 ± 0.66	0.61 ± 0.64
	R		0.91 ± 0.86	0.98 ± 0.87	0.79 ± 0.93	0.84 ± 1.05
Middle occipital gyrus	L		0.83 ± 0.48	1.04 ± 0.48	0.79 ± 0.49	0.67 ± 0.46
	R		0.88 ± 0.42	0.92 ± 0.37	0.63 ± 0.52	0.69 ± 0.65
Inferior occipital gyrus	L		0.53 ± 0.34	0.66 ± 0.44	0.73 ± 1.09	0.53 ± 0.86
	R		0.58 ± 0.41	0.73 ± 0.73	0.43 ± 0.60	0.37 ± 0.63
Cuneus	L		0.77 ± 0.44	0.78 ± 0.48	0.65 ± 0.66	0.56 ± 0.56
	R		0.51 ± 0.28	0.62 ± 0.45	0.56 ± 0.63	0.54 ± 0.60
Fusiform gyrus	L		0.84 ± 0.27	1.09 ± 0.48	1.06 ± 0.71	1.07 ± 0.63
	R		0.73 ± 0.60	0.83 ± 0.70	0.66 ± 0.31	0.80 ± 0.44
Lingual gyrus	L		0.53 ± 0.31	0.70 ± 0.72	0.60 ± 0.91	0.50 ± 0.82
	R		0.35 ± 0.22	0.50 ± 0.65	0.37 ± 0.63	0.28 ± 0.58
Limbic lobe						
Thalamus	L		0.37 ± 0.32	0.38 ± 0.29	0.38 ± 0.32	0.65 ± 0.33
	R		0.48 ± 0.43	0.37 ± 0.51	0.46 ± 0.38	0.48 ± 0.42
Cingulate gyrus	L		0.93 ± 0.32	0.92 ± 0.32	0.97 ± 0.43	1.07 ± 0.37
	R		0.80 ± 0.21	0.86 ± 0.21	0.93 ± 0.40	0.92 ± 0.38

Table 3 (Continued)

		Pioglitazone group		Control group	
		Baseline	Month 6	Baseline	Month 6
Parahippocampal gyrus	L	0.83 ± 0.53	0.88 ± 0.59	0.85 ± 0.45	0.96 ± 0.47
	R	0.80 ± 0.73	0.83 ± 0.72	0.74 ± 0.35	0.75 ± 0.39
Anterior cingulate	L	0.96 ± 0.48	0.89 ± 0.43	0.95 ± 0.46	1.12 ± 0.37
	R	0.84 ± 0.35	0.83 ± 0.38	0.88 ± 0.42	1.00 ± 0.38
Posterior cingulate	L	1.00 ± 0.48	0.94 ± 0.47	0.99 ± 0.51	0.93 ± 0.39
	R	0.94 ± 0.41	0.94 ± 0.39	0.86 ± 0.44	0.82 ± 0.43
Uncus	L	0.82 ± 0.55	0.96 ± 0.67	1.23 ± 0.76	1.25 ± 0.77
	R	0.85 ± 0.83	0.95 ± 1.02	0.85 ± 0.54	0.82 ± 0.49

* $p < 0.05$ as compared with each score at baseline SPECT.

functional improvement in response to rosiglitazone (Risner et al., 2006). Consistent with these clinical trials, our results indicate favorable effects of pioglitazone on cognition. In addition, we demonstrated an improvement of rCBF deficits in the parietal lobe by pioglitazone treatment. Posterior association cortices, including the parietal lobe, are associated

with occurrence of AD pathology. Indeed, a decreased rCBF in these regions is considered a radiological marker for the diagnosis of AD (Matsuda, 2001). Therefore, our SPECT study suggests that pioglitazone might act in some regions relevant to neurodegeneration in AD, resulting in cognitive improvement and slowing of clinical progression. Unlike the results of Watson et al. (2005) we failed to show significant changes in A β 40 and A β 42 levels after treatment. However, there was a non-statistically significant tendency toward a decrease in A β 42 and subsequently a significant increase of A β 40/A β 42 ratio in the control group, but not in the pioglitazone group. Considering that plasma A β 42 decreases with AD progression (Mayeux et al., 2003), our results support the concept of stabilization of the disease by pioglitazone treatment.

Although a previous large trial of rosiglitazone demonstrated that ApoE ϵ 4 positive patients showed no improvement on cognition (Risner et al., 2006), we did not find a correlation between the therapeutic effects of pioglitazone and ApoE ϵ 4 status, perhaps because of the small sample size. Two ongoing trials of rosiglitazone in AD and MCI in which participants will be stratified by ApoE ϵ 4 carrier status will provide a more definitive data of ApoE-dependent effects. Since the basic actions of pioglitazone are the ability of PPAR- γ receptor to regulate the sensitivity of peripheral tissues and fat to insulin action, other factors, such as the degree of insulin resistance, plasma glucose or insulin levels, and obesity, may affect therapeutic effects of pioglitazone. Further large group studies in a large sample will be required to confirm the correlation of effects of pioglitazone with these factors.

Our study naturally has some limitations. First, because this study was not blinded, the possibility of a placebo effect cannot be excluded. In addition, the numbers of subjects in each group were small. Secondly, it remains uncertain whether the 6 months of study duration is appropriate to assess the clinical efficacy in patients with AD. In general, the rate of decline in neuropsychological function can be quite variable. There may be a plateau of stabilization on the cognitive impairment, especially at the very early stages of AD (Haxby et al., 1992). Since our patients had mild stage defined by CDR 0.5 or 1 and a mean MMSE score of around 22 at initial evaluation, most patients in this group may have had

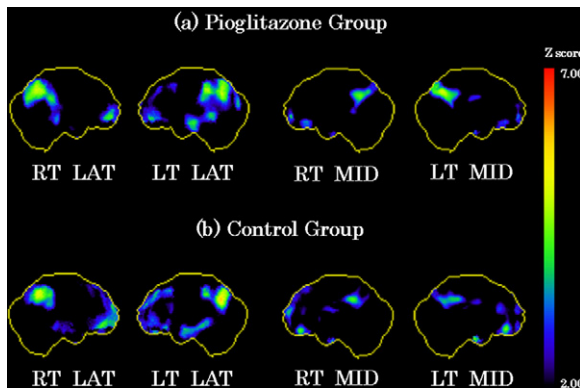


Fig. 1. Three-dimensional views of decreased rCBF in the pioglitazone group (a) and control group (b) compared to normal controls at baseline, respectively. Both groups showed significant decrease of rCBF in the parietotemporal, posterior cingulate, and frontal lobe.

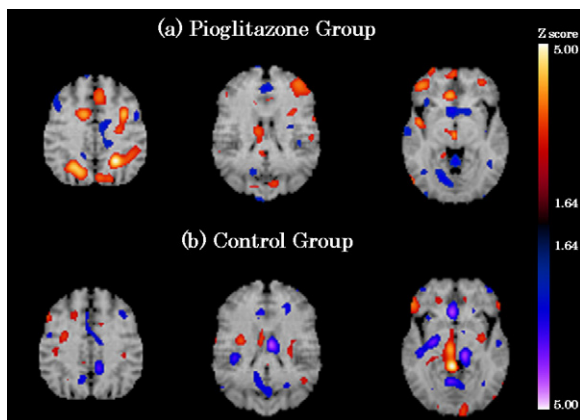


Fig. 2. Statistical maps showing relative changes in rCBF on repeated SPECT studies in the pioglitazone group (a) and control group (b). The pioglitazone group showed an improvement of rCBF in the right and left parietal lobe and some regions of the frontal lobe, while the control group showed no improvement of rCBF in any brain regions.

a steady course of decline. Thirdly, we used 3D-SSP, but not statistical parametric mapping (SPM), as an analysis of functional brain imaging. Both methods show possible deformation artifacts. Although 3D-SSP technique is less affected by the presence of atrophy than SPM (Ishii et al., 2001), it does not have several statistical functions applicable in SPM. Therefore, we compared the mean z -score for each gyrus between the groups and mean z -score changes between initial and last SPECT studies automatically measured by the stereotactic extraction estimation method (Mizumura et al., 2003).

Nevertheless, our findings that pioglitazone has beneficial effects on cognitive and functional improvements in patients with AD warrants further large-scale, double-blinded, long-term studies to confirm the clinical efficacy of pioglitazone treatment.

5. Conclusion

PPAR- γ agonist pioglitazone treatment resulted in improvements of cognition and rCBF, and in stabilization of the disease in diabetic patients with AD. In addition, pioglitazone improved not only FPG and HbA1c but also FIRI and HOMA-R, indicating enhanced insulin sensitivity. Our results suggest that pioglitazone is a new and potentially efficacious treatment of AD with DM.

Conflict of interest

No author has any conflict of interest to declare.

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