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HERRERA
RUIZ

DELEGATURA DE NUEVAS CREACIONES

SOLICITUD
FASE NACIONAL -PCT

21. EXPEDIENTE No. _____

54. TITULO: FORMULACIÓN LÍQUIDA ESTABLE DE ETANERCEPT

51. CLASIFICACION INTERNACIONAL:
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71. SOLICITANTE: LG LIFE SCIENCE LTD.

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22. BOGOTÁ D.C., DICIEMBRE 2 DE 2013

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DIRECCIÓN DE NUEVAS CREACIONES
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SOLICITANTE (S) Esta persona también es inventor. Para datos adicionales utilizar hoja de información complementaria

	APELLIDOS O RAZÓN SOCIAL	NOMBRE	IDENTIFICACIÓN	TIPO
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6 DATOS DEL SOLICITANTE

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NACIONALIDAD O LUGAR DE CONSTITUCIÓN COREA DEL SUR

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OTRO(S) SOLICITANTE(S) Y/O (OTRO(S)) INVENTOR(ES)

[ ] Los demás solicitantes y/o (demás) inventores se indican en una hoja a continuación.

9 [ ] **REPRESENTANTE LEGAL** [X] **APODERADO**

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10 DECLARACIONES DE PRIORIDAD [X] SI [ ] NO

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11	DECLARACIÓN SOBRE USO DE RECURSOS GENÉTICOS O BIOLÓGICOS

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de recursos genéticos o biológicos de los que cualquiera de los países miembros de la Comunidad Andina es país de origen.

☐ SI ☒ NO

12	DECLARACIÓN SOBRE USO DE CONOCIMIENTOS TRADICIONALES

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de conocimientos tradicionales de comunidades indígenas, afroamericanas o locales de países miembros de la Comunidad Andina.

☐ SI ☒ NO

13	REDUCCIÓN DE TASAS

Declaro que carezco de medios económicos para presentar la solicitud de patente.

☐ SI ☒ NO

Micro, pequeñas y medianas empresas ☐
Universidades públicas o privadas ☐
Entidades sin ánimo de lucro ☐

Debe aportar los documentos que se indican en el numeral 17 de anexos

14	AUTORIZACIÓN DE NOTIFICACIÓN EN LÍNEA ☒ SI ☐ NO

Manifiesto que he leído y entendido perfectamente los términos y condiciones de uso de medios electrónicos para las notificaciones en línea a través de Internet de los actos administrativos proferidos por la Superintendencia de Industria y Comercio que deben ser notificados personalmente y, en consecuencia, autorizo el servicio de notificación a través de internet.

15	COMPROBANTE DE PAGO O PAGO ELECTRÓNICO	N°	Fecha

16	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL *Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)*

Ernesto Cavelier

ERNESTO CAVELIER FRANCO

17	ANEXOS

Documentación Técnica
Solicitud Internacional en castellano

1. ☒ Descripción N° de folios:
2. ☒ Reivindicaciones N° Reivindicaciones:
3. ☒ Dibujos y/o figuras N° folios:
4. ☒ Resumen.
5. ☐ Certificado de depósito de material biológico si fuera el caso.
6. ☐ Listado de secuencias de nucleótidos y/o aminoácidos en forma digital si fuera el caso.
7. ☒ Arte final 12 x 12.
8. ☐ Anexo formato digital.

Documentación Jurídica

9. ☐ Poderes, si fuera el caso,
10. ☐ Documento que legalmente pruebe la cesión del inventor al solicitante o a su causante.
11. ☐ Copia del contrato de acceso de recursos genéticos o productos derivados, *certificado o número de registro, si fuera el caso.*
12. ☐ Copia de la licencia o autorización de Conocimientos Tradicionales, certificado o número de registro, si fuera el caso.
13. **Reducción de tasas**

Micro, pequeñas o medinas empresas

☐ Copia simple de la declaración de renta del año inmediatamente anterior, o en su defecto prueba documental idónea.

☐ Documento de constancia de cumplimiento con lo establecido en la ley 905 de 2004.

Universidades públicas o privadas

☐ Copia acto de reconocimiento institucional emitido por el Ministerio de Educación

Entidades sin ánimo de lucro

☐ Copia de registro vigente en Cámara de comercio.
☐ Hoja de información complementaria.
☐ Otros, especificar

14. ☒ Comprobante de pago de la tasa de presentación de la solicitud.
15. ☐ Comprobante de pago por reivindicación de prioridad.
16. ☒ Comprobante de pago de la tasa por concepto de excedente de palabras en la publicación.
17. ☒ Comprobante de pago por reivindicación adicional a 10.

DESCRIPCIÓN

Título de la invención

Formulación líquida estable de etanercept

Campo técnico

La presente invención se refiere a una formulación líquida de etanercept (proteína de fusión sTNFR:Fc recombinante p75) y más particularmente a una formulación química que comprende uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas en una cantidad suficiente para reducir subproductos de etanercept durante el almacenamiento.

Técnica anterior

El etanercept es un modulador biológico de la inflamación que funciona como inhibidor competitivo de TNF-α, que se une al receptor de TNF-α de la superficie celular, para inhibir respuestas inmunitarias mediadas por TNF-α. El etanercept es una macromolécula con un peso molecular de aproximadamente 150 kDa, y es un homodímero de dos proteínas de fusión Fc unidas mediante un enlace disulfuro, consistiendo cada proteína de fusión Fc en un receptor de TNF p75 soluble humano acoplado a la porción Fc de la inmunoglobulina humana G subclase 1 (Goldenberg, Clinical Therapeutics, 21(1): 75-87, 1999; Moreland y col., Ann. Intern. Med., 130(6): 478-486, 1999).

Esta proteína de fusión prototípica se sintetizó en primer lugar en los primeros años de la década de 1990 por parte de Bruce A. Beutler en el Centro Médico de la Universidad del Suroeste de Texas, Estados Unidos, y se comercializó por Amgen, con la denominación comercial de Enbrel, in 2002. El etanercept es un inhibidor de TNF-α que se usa para tratar artritis reumatoide,

psoriasis y espondilitis anquilosante y se encuentra sometido a ensayos clínicos para el tratamiento de vasculitis, enfermedad de Alzheimer y enfermedad de Crohn.

Como los fármacos de proteínas, los fármacos de anticuerpos tienen una semivida corta y puede causarse fácilmente su desnaturalización química y física mediante temperatura desfavorable, estrés por cizallamiento, vibración, congelación-descongelación, exposición a UV, cambio excesivo del pH, disolventes orgánicos y contaminación microbiana. La desnaturalización química incluye disociación de dímeros, oxidación, desaminación, isomerización y polimerización, que están influenciadas por los aminoácidos que constituyen el anticuerpo y por condiciones del disolvente que contiene el anticuerpo (sal, pH y temperatura). La desnturalización química incluye pérdida de estructura terciaria, agregación covalente/no covalente y adhesión de monómeros, que están influenciadas por parches hidrófobos en la superficie de la proteína cambiados por entornos circundantes que contienen anticuerpo, tales como disolventes, estructuras de proteínas complejas tales como distribución de carga y estabilidad térmica. La desnaturalización física o química de un anticuerpo causa una pérdida de su actividad fisiológica. Debido a que la desnaturalización es un proceso irreversible, los anticuerpos, una vez desnaturalizados, no pueden recuperar sus propiedades nativas del estado inicial, lo que causa una reducción de sus eficacias terapéuticas. Se ha sugerido también que la agregación de monómeros provoca respuestas inmunitarias. De este modo, se han realizado muchos estudios sobre formulaciones de anticuerpos que contenían una cantidad fisiológicamente eficaz sin agregados (Ishikawa y col., Biol. Pharm. Bull., 33(8): 1413-1417, 2010; Levin y col., The Development of Therapeutic Monoclonal Antibody Products, editores.

Boston (MA): BioProcess Technology Consultants, Inc, capítulo 9).

Existen muchos procedimientos disponibles para evitar la desnaturalización de proteínas en formulaciones líquidas. En algunos fármacos de proteínas, los problemas de estabilidad se abordan mediante la liofilización. Por ejemplo, la patente de Estados Unidos Nº 7.592.004 divulga una formulación liofilizada de daclizumab que se estabiliza usando 5 a 25 mM de una solución tampón de histidina (pH de 5,5 a 6,5), el 0,005 al 0,03 % de un tensioactivo no iónico, polisorbato y 100 a 300 mM de un azúcar no reductor, sacarosa. La publicación de patente de Estados Unidos Nº 2010-0158925 divulga una formulación liofilizada de cetuximab que se estabiliza usando una solución tampón de histidina y ácido lactobiónico. No obstante, el procedimiento de liofilización genera estreses por congelación y por secado tales como formación de cristales de hielo, cambios en el pH y concentración alta de soluto y estos estreses pueden causar la desnaturalización del anticuerpo. Además, debido a que se necesita un liofilizador de capacidad elevada para el procedimiento de liofilización durante la producción, los costes de producción aumentan durante una producción a gran escala. Disolver el producto liofilizado en medio acuoso estéril para su reconstitución antes de su uso también representa un inconveniente.

Como alternativa para solucionar estas limitaciones, se añade un estabilizante a formulaciones líquidas para mejorar la estabilidad del anticuerpo. Son conocidos como estabilizantes para proteínas, incluidos anticuerpos, los tensioactivos, albúminas de suero, polisacáridos, aminoácidos, polímeros, sales o similares (Wang, Int. J. Pharm., 185: 129-188, 1999; Wang y col., J. Pharm. Sci., 96(1): 1-26, 2007).

La publicación de patente de Estados Unidos Nº 2011-0070231 divulga una

composición líquida estable de anticuerpo IgG, en la que la formulación líquida farmacéutica estable incluye 50 mg/ml o más de daclizumab en 20 a 60 mM de una solución de tampón de succinato (pH de 5,5 a 6,5), del 0,02 al 0,04 % de polisorbato y 75 a 150 mM de cloruro de sodio.

La publicación de patente de Estados Unidos Nº 2011-0020328 divulga una cantidad terapéuticamente eficaz de formulación de anticuerpo anti-CD20, en la que la composición farmacéuticamente estable incluye 10 a 100 mM de acetato de sodio, 25 a 100 mM de cloruro de sodio, del 0,5 al 5 % de base libre de arginina, 0,02 al 0,2 mM de EDTA y del 0,01 al 0,2 % de polisorbato 80, y su pH es de 5,0 a 7,0.

La patente de Estados Unidos Nº 7.785.592 divulga una formulación estable, en la que se estabilizan 75 mg/ml o más de palivizumab usando una solución tampón de histidina y glicina sin sales iónicas y un tensioactivo y su estabilidad se mantiene a 2 a 8 ºC durante al menos 15 meses.

La patente de Estados Unidos Nº 6.991.790 divulga una formulación farmacéutica acuosa estable que incluye una cantidad terapéuticamente eficaz de un anticuerpo, una solución de tampón de acetato de un pH de aproximadamente 4,8 a 5,5, un tensioactivo y un poliol sin un agente isotónico tal como cloruro de sodio.

La patente de Estados Unidos Nº 7.648.702 divulga una formulación líquida en la que una proteína de fusión del receptor p75 del factor de necrosis tumoral humano unida a la porción Fc de la inmunoglobulina G1 (IgG1) humana se estabiliza usando una concentración de aproximadamente 10 a 200 mM de L-arginina como agente de prevención de la agregación. Esta patente es la única técnica de uso de etanercept como ingrediente activo.

Para preparar formulaciones estables, no obstante, deberían usarse estabilizantes apropiados considerando las propiedades fisicoquímicas de cada ingrediente activo. Cuando los estabilizantes se usan en combinación, la competencia entre los mismos y los efectos adversos pueden provocar efectos no deseados. Además, las concentraciones de anticuerpos deberían encontrarse dentro del intervalo adecuado para la estabilización, y sus concentraciones son relativamente más altas que las de fármacos de proteínas. Así, se requiere mucho esfuerzo y precaución para estabilizar anticuerpos en soluciones (Shire y col., J. Pharm. Sci., 93(6): 1390-1402, 2004).

Existen unos pocos estudios sobre las formulaciones líquidas estables de etanercept, y el único procedimiento para estabilizar etanercept del que son conscientes los inventores es el uso de L-arginina en la formulación líquida. Por lo tanto, existe la necesidad urgente de desarrollar una formulación líquida nueva que sea capaz de mantener de forma estable la actividad de etanercept durante un periodo largo y que sea más eficaz la estabilización de etanercept que la formulación conocida que contiene L-arginina.

Divulgación

Problema técnico

Los presentes inventores han realizado muchos esfuerzos para desarrollar un procedimiento para preparar una formulación líquida capaz de mantener de forma estable la actividad de etanercept. Como resultado, encontraron que uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina e histidina muestran efectos significativos en la estabilización de etanercept en una solución, completando, de este modo, la presente invención.

Solución técnica

Un objeto de la presente invención es proporcionar una formulación acuosa que comprende estabilizantes para reducir la formación de subproductos de etanercept durante el almacenamiento en solución y mantener su actividad durante un periodo de tiempo largo.

Efectos ventajosos

La formulación líquida según la presente invención puede prevenir eficazmente la formación de subproductos de etanercept y mantener de forma estable su eficacia farmacéutica durante un almacenamiento a largo plazo. Por lo tanto, no se requiere un procedimiento de reconstitución antes de la administración y la formulación estéril puede administrarse a pacientes asegurando la seguridad del paciente. De este modo, se espera aplicar a los sectores con necesidad de tratamiento de etanercept.

Mejor modo

En un aspecto para lograr el objeto anterior, la presente invención proporciona una formulación líquida que comprende uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas.

La formulación de la presente invención puede ser una formulación líquida de etanercept que comprende etanercept y uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas.

La formulación muestra una estabilidad en almacenamiento mejorada reduciendo los subproductos de etanercept que se producen debido a su desnaturalización durante el almacenamiento.

Como estabilizante, pueden estar presentes en la formulación metionina,

lisina, histidina o sales farmacéuticamente aceptables de las mismas en una cantidad de 0,1 a 250 mM.

El etanercept puede estar presente en la formulación en una cantidad de 1 a 100 mg/ml.

La formulación puede incluir también uno o más materiales seleccionados del grupo que consiste en un tampón, un agente isotónico, un excipiente y un conservante.

El tampón puede seleccionarse del grupo que consiste en citrato, fosfato, succinato, tartrato, fumarato, gluconato, oxalato, lactato, acetato, histidina y Tris, y puede estar presente en una cantidad de 0,1 a 100 mM.

El agente isotónico puede seleccionarse del grupo que consiste en cloruro de sodio, cloruro de potasio, ácido bórico, borato de sodio, manitol, glicerina, propilenglicol, polietilenglicol, maltosa, sacarosa, eritritol, arabitol, xilitol, sorbitol y glucosa y puede estar presente en una cantidad de 1 a 1000 mM.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, de 0,1 a 100 mM tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de

tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

La formulación de etanercept según la presente invención puede ser una formulación líquida que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

A continuación, la presente invención se describirá en detalle.

La presente invención ha demostrado por primera vez que la metionina, la lisina, la histidina y las sales farmacéuticamente aceptables de las mismas tienen los efectos de reducir la formación de subproductos de etanercept en solución y mantener de forma estable su actividad durante un almacenamiento a largo plazo y, así, sugiere un uso novedoso de las mismas como estabilizantes de una cantidad farmacéuticamente eficaz de etanercept.

Para preparar formulaciones estables, deberían usarse estabilizantes apropiados considerando las propiedades fisicoquímicas de cada ingrediente

activo. En función de la diferencia de tipos, concentración y combinaciones de materiales incluidos en una formulación, la competencia entre los materiales presentes en la formulación puede provocar efectos no deseados. Por lo tanto, es difícil preparar una formulación específica de un fármaco estable.

A la luz de los antecedentes anteriores, los inventores de la presente invención han hallado que la formulación líquida que comprende uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina e histidina y sales farmacéuticamente aceptables de las mismas aumenta la estabilidad en almacenamiento de etanercept en comparación con la formulación que no tiene estabilizantes, reduciendo los subproductos de etanercept en solución durante el almacenamiento.

Específicamente, en una realización de la presente invención, para investigar el estabilizante óptimo para la preparación de la formulación líquida estable de etanercept, se añaden una variedad de aminoácidos, tensioactivos y polímeros para examinar sus efectos de estabilización. Como resultado, se halló que la lisina, la histidina, la metionina o las combinaciones de las mismas muestran efectos significativos en la estabilización de etanercept previniendo su desnaturalización durante el almacenamiento en solución a temperatura alta.

Además, se halló que la formulación según la presente invención mostraba efectos estabilizantes del etanercept que eran equivalentes o superiores a los de la formulación que comprende L-arginina, que es la formulación estable conocida de etanercept (patente de Estados Unidos Nº 7.648.702). En particular, se encontró que la metionina mostraba un efecto muy excelente de estabilización de etanercept, mejor que la L-arginina.

Específicamente, los subproductos de etanercept formados durante el

almacenamiento se examinaron por HPLC de exclusión por tamaño (SE-HPLC). Como resultado, en comparación con la cantidad total de impurezas de la formulación de etanercept que se preparó usando L-arginina como estabilizante en la patente de Estados Unidos Nº 7.648.702, la formulación preparada usando lisina como estabilizante mostró una cantidad similar de impurezas y la formulación preparada usando histidina o metionina como estabilizante mostró una cantidad inferior de impurezas (véase la Tabla 2). El análisis por HPLC de interacción hidrófoba (HI-HPLC) también mostró resultados similares. Estos resultados indican que la histidina, la lisina y la metionina previenen la desnaturalización de etanercept inhibiendo la formación de subproductos del mismo (véase la Tabla 3).

Por lo tanto, la formulación líquida según la presente invención puede mantener de forma estable la eficacia farmacéutica de etanercept durante un periodo de tiempo largo reduciendo eficazmente la formación de subproductos de etanercept.

Como se usa en el presente documento, el término "etanercept (proteína de fusión sTNFR:Fc recombinante p75)" se refiere a una proteína que es una forma homodímera de dos proteínas de fusión Fc unidas mediante enlaces disulfuro, consistiendo cada proteína de fusión Fc en un receptor del FNT (factor de necrosis tumoral) de 75 kilodalton (p75) soluble humano acoplado a la porción Fc de la IgG1 humana.

Específicamente, etanercept es una forma homodímera de dos proteínas de fusión Fc unidas mediante 3 enlaces disulfuro, consistiendo cada proteína de fusión Fc en una porción de unión a ligando extracelular del receptor p75 del FNT soluble humano unida a la porción Fc de la IgG1 humana. El componente Fc de

etanercept contiene el dominio CH2, el dominio CH3 y la región bisagra, pero no el dominio CH1 de la IgG1. El etanercept puede tener un peso molecular de aproximadamente 150 kilodaltons (kDa). Este etanercept puede comercializarse actualmente con la denominación comercial ENBREL® (Amgen Inc., Thousand Oaks, CA.), y tiene el número CAS 185243-69-0.

El etanercept puede producirse mediante tecnología de ADN recombinante, pero no está limitado a la misma.

El etanercept de la presente invención es un modulador de la inflamación biológico que funciona como inhibidor competitivo de TNF-α, que se une a la superficie celular del receptor de TNF-α, para inhibir respuestas inmunitarias mediadas por el TNF-α y se usa para tratar artritis reumatoide, psoriasis y espondilitis anquilosante, y está siendo sometido a ensayos clínicos para el tratamiento de vasculitis, enfermedad de Alzheimer y enfermedad de Crohn.

En la formulación líquida según la presente invención, el etanercept está contenido en una cantidad terapéuticamente eficaz. Preferentemente, el etanercept está presente en una cantidad de 1 a 100 mg/ml.

Tal como se usa en el presente documento, la expresión “formulación líquida estabilizada” o "formulación líquida estable" se refiere a una formulación que mantiene la identidad física y química y la integridad del ingrediente activo terapéutico, etanercept, durante el almacenamiento en solución. Puede realizarse una medición analítica de la estabilidad de etanercept mediante ensayos de estabilidad de proteínas ampliamente conocidos en la técnica. La estabilidad puede medirse a una temperatura predeterminada durante un periodo de tiempo predeterminado. Para un ensayo rápido, la formulación puede almacenarse a una temperatura más alta o “elevada”, por ejemplo, 40 °C durante 2 semanas a un mes

o durante más tiempo, y medirse su estabilidad en función del tiempo para este periodo.

Como se usa en el presente documento, el término "estabilizante" se refiere a un producto químico específico que interactúa con una molécula biológica y/o un excipiente farmacéutico general en una formulación para mejorar su estabilidad. Generalmente, el estabilizante protege proteínas del estrés inducido en la superficie de contacto aire/solución y el estrés inducido en la solución/superficie que causan agregación de proteínas. En la presente invención, el estabilizante es un componente que reduce la formación de subproductos de etanercept durante el almacenamiento en solución para mantener su actividad durante un periodo de tiempo largo, y es preferentemente uno o más de las sustancias del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas.

Como para los aminoácidos, se incluyen en el ámbito de la invención tanto las formas L como las formas D. No solo los aminoácidos mismos tales como metionina, lisina e histidina, sino también análogos, solvatos, hidratos y estereoisómeros y las sales farmacéuticamente aceptables de los mismos están dentro del ámbito de la presente invención mientras muestren sustancialmente el mismo efecto.

Como se usa en el presente documento, la expresión "sales farmacéuticamente aceptables" se refiere a compuestos que se preparan en forma de sales de los aminoácidos, tales como metionina, lisina e histidina, dentro del intervalo que mantiene sus funciones como estabilizantes de la presente invención. Específicamente, puede formar una sal por adición de ácidos y, por ejemplo, puede formar una sal con un ácido inorgánico (por ejemplo, ácido

clorhídrico, ácido bromhídrico, ácido fosfórico, ácido nítrico, ácido sulfúrico, etc.), ácido carboxílico orgánico (por ejemplo, ácido acético, ácido haloacético tal como ácido trifluoroacético, ácido propiónico, ácido maleico, ácido succínico, ácido málico, ácido cítrico, ácido tartárico, ácido salicílico), ácido de azúcar (ácido glucurónico, ácido galacturónico, ácido glucónico, ácido ascórbico), polisacárido ácido (por ejemplo, ácido hialurónico, sulfato de condroitina, ácido de arginina), ácido sulfónico orgánico, incluido el éster de azúcar de ácido sulfónico tal como sulfato de condroitina (por ejemplo, ácido metanosulfónico, ácido p-toluenosulfónico).

Como se usa en el presente documento, el término "análogos" se refiere a compuestos que muestran las mismas funciones que los aminoácidos, tales como metionina, lisina e histidina, que se usan como estabilizantes de la presente invención. En la presente invención, los aminoácidos tales como metionina, lisina e histidina incluyen los análogos de los mismos. En la presente invención, los ejemplos de los análogos pueden incluir análogos de metionina, a saber, selenometionina, ácido hidroximetilbutanoico, etionina y trifluorometionina, pero no están limitados a los mismos.

Como se usa en el presente documento, el término "subproductos" se refiere a los productos no deseados que reducen la proporción del ingrediente terapéuticamente activo, etanercept, en la formulación. Los subproductos típicos incluyen "productos de peso molecular bajo" resultantes de la desnaturalización de etanercept por desaminación o hidrólisis, "productos de peso molecular alto" tales como oligómeros y agregados o mezclas de los mismos.

Como se usa en el presente documento, la expresión "productos de peso molecular alto" incluye, etanercept, fragmentos que se han agregado

subsiguientemente mediante desnaturalización (por ejemplo, producidos mediante degradación de polipéptidos debido a desaminación o hidrólisis) o mezclas de los mismos. Normalmente, los productos de peso molecular alto son complejos que tienen pesos moleculares más altos que el monómero terapéutico etanercept, y pueden tener un peso molecular superior a aproximadamente 150 kDa.

Como se usa en el presente documento, la expresión "productos de peso molecular bajo" incluye, por ejemplo, polipéptidos terapéuticos producidos por desaminación o hidrólisis, es decir, fragmentos de etanercept. Normalmente, los productos de peso molecular bajo son complejos que tienen pesos moleculares más bajos que el monómero terapéutico etanercept, y pueden tener un peso molecular inferior a aproximadamente 150 kDa.

La formulación líquida de la presente invención incluye etanercept y uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas. Específicamente, la formulación líquida comprende metionina, lisina o histidina como estabilizante, o dos estabilizantes tales como metionina y lisina, metionina e histidina, o lisina e histidina, o tres estabilizantes tales como metionina, lisina e histidina. En las formulaciones anteriores que comprenden combinaciones de los aminoácidos, pueden añadirse posteriormente las sales farmacéuticamente aceptables de metionina, lisina, histidina, o la metionina, la lisina y la histidina pueden ser las formas modificada de las sales farmacéuticamente aceptables, respectivamente. La formulación líquida comprende preferentemente metionina, lisina, histidina, metionina y lisina, metionina e histidina, o lisina e histidina, y más preferentemente metionina como estabilizante.

La metionina es un aminoácido esencial y tiene la fórmula química

$C_5H_{11}NO_2S$ y un peso molecular de 149,21. La metionina es un aminoácido no polar y contiene un grupo tioéter (-S-CH_3) en su cadena lateral. Tiene un valor de pK_1 de 2,28, un valor de pK_2 de 9,21 y un valor del punto isoeléctrico (PI) de 5,74. La metionina previene la oxidación de anticuerpos en formulaciones líquidas para estabilizar anticuerpos compitiendo con los residuos de metionina de los anticuerpos por la reacción con los radicales de hidroxilo libres (Lam y col., J. Pharm. Sci., 86(11): 1250-1255, 1997; Wang, Int. J. Pharm., 185: 129-188, 1999).

La lisina es un aminoácido esencial y tiene la fórmula química $C_6H_{14}N_2O_2$ y un peso molecular de 146,19. La lisina es un aminoácido básico y tiene un valor de pK_1 de 2,18, un valor de pK_2 de 8,95, un valor de pK_3 de 10,53 y un valor de PI de 9,74.

La histidina es un aminoácido esencial con un grupo funcional de imidazol que tiene carga positiva en su cadena lateral y tiene una fórmula química de $C_6H_9N_3O_2$ y un peso molecular de 155,15. La histidina es un aminoácido básico y tiene un valor de pK_1 de 1,82, un valor de pK_2 de 9,17, un valor de pK_3 de 6,0 y un valor del PI de 7,59.

Por el contrario, la L-arginina contenida en la formulación disponible comercialmente conocida de etanercept es un aminoácido esencial y tiene la fórmula química $C_6H_{14}N_4O_2$ y un peso molecular de 174,2. La arginina es un aminoácido básico y tiene un valor de pK_1 de 2,17, un valor de pK_2 de 9,04, un valor de pK_3 de 12,48 y un valor de PI de 10,76. Es decir, la metionina, la lisina y la histidina usadas como estabilizante en la presente invención son totalmente diferentes de la arginina como estabilizante conocido para etanercept en construcción, fórmula química, propiedades fisicoquímicos y tendencia a la ionización. La presente invención ha demostrado por primera vez que los

aminoácidos tales como la metionina, la lisina y la histidina son los estabilizantes apropiados para el etanercept.

El contenido del estabilizante en la formulación líquida de la presente invención es 0,1 a 250 mM, preferentemente 1 a 100 mM y más preferentemente 5 a 50 mM.

La formulación líquida de la presente invención puede incluir también cualquier material que están contenido generalmente en formulaciones de fármacos de proteínas o fármacos de anticuerpos para aumentar la estabilidad del etanercept según uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas, excepto las que deterioran la función de la presente invención. Por ejemplo, puede añadirse a la formulación líquida de la presente invención L-arginina conocida como estabilizante para el etanercept.

La formulación líquida según la presente invención puede incluir también un tampón además del estabilizante. Tal como se usa en el presente documento, el término "tampón" se refiere al componente que mejora la isotonicidad y la estabilidad química de la formulación y funciona manteniendo un pH fisiológicamente adecuado. El tampón evita un cambio rápido del pH de la formulación líquida manteniendo un pH de la solución que produzca la estabilización de etanercept. Los ejemplos preferentes del tampón incluyen citrato, fosfato, succinato, tartrato, fumarato, gluconato, oxalato, lactato, acetato, histidina y Tris, pero no están limitados a los mismos. En la realización específica, el tampón es un tampón de fosfato. El tampón puede usarse bien solo o bien en combinación de dos o más de los mismos.

En las diversas realizaciones de la presente invención, la formulación

debería tener un pH de aproximadamente 5 a 7,5 o un pH de aproximadamente 5,8 a 6,8. En la realización específica, la formulación tiene un pH de aproximadamente 6,0 a 6,6. También se pretende que los intervalos desde un valor intermedio a los niveles de pH mencionados anteriormente, por ejemplo, aproximadamente un pH de 5,2 a aproximadamente un pH de 6,4 (por ejemplo, un pH de 6,2) sean parte de la invención. Por ejemplo, se pretende que estén incluidos intervalos de valores que usan una combinación de cualquiera de los valores mencionados anteriormente como límites inferior y/o superior. Si es necesario, el pH puede ajustarse mediante técnicas conocidas en la técnica. Por ejemplo, el pH puede ajustarse a cualquier intervalo deseado mediante la adición de HCl o mediante la adición de histidina, si es necesario.

En otra realización, el tampón está presente a una concentración de 0,1 a 100 mM, preferentemente 1 a 50 mM y más preferentemente 5 a 25 mM. Los intervalos para un valor intermedio a las concentraciones mencionadas anteriormente también se pretende que sean parte de la presente invención. Por ejemplo, se pretende que estén incluidos intervalos de concentraciones que usan una combinación de cualquiera de las concentraciones mencionadas anteriormente como límites inferior y/o superior. En la realización específica, el tampón debería estar presente en una cantidad suficiente para mantener un pH fisiológicamente suficiente.

La formulación líquida según la presente invención puede incluir también un agente isotónico además del estabilizante. Tal como se usa en el presente documento, la expresión "agente isotónico" se refiere a un componente que funciona manteniendo parcialmente la isotonicidad de la formulación y el nivel de proteínas, y mantiene parcialmente el nivel, la relación o la proporción del

polipéptido terapéuticamente activo presente en la formulación. El agente isotónico posee la misma presión osmótica que el plasma sanguíneo y, por lo tanto, puede infundirse por vía intravenosa a un sujeto sin cambiar la presión osmótica del plasma sanguíneo del sujeto. De hecho, en una realización según la presente invención, el agente isotónico está presente en una cantidad suficiente para proporcionar la formulación adecuada para la infusión por vía intravenosa. A menudo, el agente isotónico sirve también como agente voluminizador. Como tal, el agente isotónico puede permitir que la proteína supere diversos estreses tales como congelación y cizallamiento.

El agente isotónico sirve para mantener la presión osmótica adecuada en el organismo, cuando se administra al organismo etanercept en solución. Los ejemplos de agente isotónico pueden incluir los comúnmente usados cloruro de sodio, cloruro de potasio, ácido bórico, borato de sodio, manitol, glicerina, propilenglicol, polietilenglicol, maltosa, sacarosa, eritritol, arabitol, xilitol, sorbitol y glucosa, pero no están limitados a los mismos. En la realización específica, el agente isotónico es NaCl. Estos agentes isotónicos pueden usarse bien solos o bien en combinación de dos o más de los mismos.

En otra realización más, el agente isotónico (por ejemplo, NaCl) están presente en una concentración de 1 a 1000 mM, preferentemente 10 a 500 mM y más preferentemente 50 a 250 mM. Los intervalos desde un valor intermedio a las concentraciones mencionadas anteriormente también se pretende que sean parte de la presente invención. Por ejemplo, se pretende que estén incluidos intervalos de concentraciones que usen una combinación de cualquiera de las concentraciones mencionadas anteriormente como límites inferior y/o superior. El agente isotónico debería estar presente en una cantidad suficiente para mantener

la ósmosis de la formulación.

La formulación líquida según la presente invención puede incluir también un excipiente farmacéuticamente aceptable y los ejemplos del excipiente pueden incluir azúcares y polioles, tensioactivos, polímeros o similares. Los ejemplos de los azúcares y polioles pueden incluir sacarosa, trehalosa, lactosa, maltosa, galactosa, manitol, sorbitol, glicerina, los ejemplos de los tensioactivos pueden incluir tensioactivos no iónicos tales como polisorbato 20, polisorbato 80 y poloxámeros y los ejemplos de los polímeros pueden incluir dextrano, polietilenglicol, carboximetilcelulosa, ácido hialurónico y ciclodextrina.

La formulación líquida según la presente invención puede incluir también un conservante. Conservante significa un producto químico que se añade a formulaciones farmacéuticas como agente antimicrobiano. Los ejemplos del conservante pueden incluir cloruro de benzalconio, bencetonio, clorhexidina, fenol, m-cresol, alcohol bencílico, metilparabeno, propilparabeno, clorobutanol, o-cresol, p-cresol, cloro-cresol, nitrato fenilmercúrico, timerosal y ácido benzoico, pero no están limitados a los mismos. Los conservantes pueden usarse bien solos o bien en combinación de dos o más de los mismos.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 100 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y 1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 100 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y 1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 100 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y 1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 1 a 50 mM de histidina o sales farmacéuticamente aceptables de la misma, 1 a 100 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y

1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 1 a 50 mM de histidina o sales farmacéuticamente aceptables de la misma, 1 a 100 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y 1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

En la realización específica, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio. Más específicamente, la formulación de la presente invención es una formulación líquida estable que comprende 1 a 100 mg/ml de etanercept, 1 a 50 mM de metionina o sales farmacéuticamente aceptables de la misma, 1 a 50 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 50 mM de tampón de fosfatos y 1 a 500 mM de cloruro de sodio a pH de 6,0 a 6,6.

La formulación de la presente invención puede usarse para el tratamiento de un enfermedad en la que etanercept sea terapéuticamente eficaz. El etanercept es un modulador de la inflamación biológico que inhibe respuestas mediadas por TNF-α y la formulación de la presente invención se usa para tratar artritis

reumatoide, psoriasis, espondilitis anquilosante, vasculitis, enfermedad de Alzheimer o enfermedad de Crohn, sin estar limitada a las mismas. La formulación de la presente invención puede administrarse por vía oral o parenteral, es decir, por vía subcutánea, intramuscular, intraperitoneal, intraabdominal, transdérmica y/o intravenosa, pero no está limitada a las mismas.

En otro aspecto, la presente invención proporciona un procedimiento para aumentar la estabilidad de etanercept usando una formulación líquida que comprende uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticarnente aceptables de las mismas.

La formulación líquida puede aumentar la estabilidad en almacenamiento de etanercept reduciendo los subproductos de etanercept que se producen debido a la desnaturalización durante el almacenamiento.

Modo de la invención

A continuación, la presente invención se describirá con más detalle con referencia a los Ejemplos. No obstante, estos Ejemplos se presentan solo con fines ilustrativos y no se pretende que la invención esté limitada por estos Ejemplos.

<Ejemplo 1> Ensayo de estabilidad de una formulación acuosa de etanercept según la adición de estabilizante

Para investigar el estabilizante óptimo para la preparación de la formulación acuosa estable de etanercept, se añadió histidina como estabilizante a una solución de fosfato 5 mM a la que se añadió etanercept a una concentración de 50 mg/ml, y se añadió cloruro de sodio a la misma como agente isotónico para preparar la Formulación 1.

Además, se añadió etanercept a una solución de fosfato 10 mM a una

concentración de 50 mg/ml, y se añadió cloruro de sodio a la misma como agente isotónico. Después, se añadió en cada caso un compuesto de entre lisina, arginina, metionina, glicina, polisorbato 20, polisorbato 80, poloxámero 188, propilenglicol, sulfato de protamina, sacarosa y guanidina-HCl para preparar las Formulaciones 2 a 12.

Finalmente se añadió cloruro de sodio como agente isotónico a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de 50 mg/ml para preparar una formulación exenta de estabilizante. Las composiciones de estabilizante de las formulaciones se muestran en la Tabla 1 siguiente. Se dispusieron 0,5 ml de la formulación preparada en cada jeringa de vidrio de 1,0 ml, se sellaron y se almacenaron a 50 °C.

Tabla 1

	Composición del estabilizante
Formulación 1	Histidina 10 mM
Formulación 2	Lisina 25 mM
Formulación 3	Arginina 25 mM
Formulación 4	Metionina 25 mM
Formulación 5	Glicina al 1 %
Formulación 6	Tween 20 al 0,1 %
Formulación 7	Tween 80 al 0,1 %
Formulación 8	F68 al 0,1 %
Formulación 9	Propilenglicol al 0,1 %
Formulación 10	Sulfato de protamina al 0,03 %
Formulación 11	Sacarosa al 2 %
Formulación 12	Guanidina-HCl al 0,2 %

formulación exenta de estabilizante	-

A los 7 días después del almacenamiento a 50 °C, se analizaron las Formulaciones 1 a 12 y la formulación exenta de estabilizante de la Tabla 1 por SE-HPLC para examinar los tipos de productos de peso molecular bajo y de productos de peso molecular alto tales como oligómeros y agregados resultantes de la desnaturalización de etanercept y la cantidad total de estos productos se representó como Impurezas totales. Además, se examinaron cambios estructurales de etanercept por HI-HPLC En la HI-HPLC, se separaron las sustancias relacionadas con etanercept en 4 picos, incluidos pico previo, pico 1, pico 2 y pico 3. El pico previo y el pico 1 representan productos de bajo peso molecular, el pico 2 representa el etanercept y el pico 3 representa dímeros con agregación o actividad baja. Por lo tanto, la cantidad total del pico previo, el pico 1 y el pico 3 se representó como Impurezas totales. Los resultados se muestran en las Tablas 2 y 3, respectivamente.

Tabla 2

	Impurezas totales por SE-HPLC (%)	
	Día 0	Día 7
Formulación 1	4,6	22,2
Formulación 2	4,7	24,8
Formulación 3	4,6	24,2
Formulación 4	4,6	20,8
Formulación 5	4,6	28,5
Formulación 6	4,7	29,4
Formulación 7	4,6	29,0

Formulación 8	4,6	28,8
Formulación 9	4,7	27,8
Formulación 10	4,6	29,4
Formulación 11	4,8	28,4
Formulación 12	4,7	27,5
formulación exenta de estabilizante	4,7	30,2

Tabla 3

	Impurezas totales por HI-HPLC (%)	
	Día 0	Día 7
Formulación 1	14,7	33,7
Formulación 2	14,6	34,9
Formulación 3	15,0	34,3
Formulación 4	15,2	30,3
Formulación 5	14,8	36,6
Formulación 6	15,2	35,5
Formulación 7	15,2	36,2
Formulación 8	15,4	35,5
Formulación 9	15,2	35,1
Formulación 10	15,0	36,9
Formulación 11	14,9	35,1

Formulación 12	15,1	34,8
formulación exenta de estabilizante	14,4	41,9

Como muestran los resultados por SE-HPLC de la Tabla 2, cuando se almacenó a 50 ºC durante 7 días, la Formulación 1, que contenía histidina como estabilizante, mostró unas impurezas totales del 22,2 %, la Formulación 2, que contenía lisina como estabilizante, mostró unas impurezas totales del 24,8 %, la Formulación 3, que contenía arginina como estabilizante, mostró unas impurezas totales del 24,2 %, la Formulación 4, que contenía metionina como estabilizante ,mostró unas impurezas totales del 20,8 %. Todas ellas mostraron unas Impurezas totales significativamente bajas, en comparación con la formulación exenta de estabilizante, que mostró unas Impurezas totales del 30,2 %. Cuando estas formulaciones se compararon con la Formulación 3, que usa L-arginina como estabilizante, (patente de Estados Unidos Nº 7.648.702), la Formulación 2, que contenía lisina, mostró unas impurezas totales similares del 24,8 % y la Formulación 1, que contenía histidina, y la Formulación 4, que contenía metionina, mostraron unas Impurezas totales reducidas del 22,2 % y del 20,8 %, respectivamente.

Los resultados de la HI-HPLC de la Tabla 3 son similares a los resultados de la SE-HPLC. La formulación exenta de estabilizante mostró unas Impurezas totales del 41,9 %. Por el contrario, cada una de las Formulaciones 1, 2, 3 y 4 mostraron unas Impurezas totales del 33,7 %, 34,9 %, 34,3 % y 30,3 % respectivamente, lo que indica que la histidina, la lisina, la L-arginina y la metionina añadidas a cada formulación tienen efectos de prevención de la

desnaturalización de etanercept. En particular, la Formulación 4, que contiene metionina,s mostró las Impurezas totales más reducidas, lo que indica que la metionina es la más eficaz en la estabilización del etanercept.

En consecuencia, la presente invención demostró que la lisina, la histidina y la metionina tienen efectos de estabilización de etanercept que previenen su desnaturalización, que son equivalentes o superiores a los de la L-arginina y, en particular, la metionina es más eficaz en la estabilización de etanercept que la L-arginina.

<Ejemplo 2> Ensayo de estabilidad de una formulación acuosa de etanercept según la concentración de metionina

Para investigar la concentración óptima de metionina para la estabilización de etanercept, se añadió cloruro de sodio 120 mM a soluciones de fosfato 10 mM, y se añadió a cada una de las mismas metionina 25 mM y 12,5 mM, después de lo cual se añadió etanercept a una concentración de 50 mg/ml para preparar las Formulaciones 13 y 14, respectivamente. Según la preparación de una formulación acuosa de etanercept disponible comercialmente, se añadió L-arginina 25 mM a una solución de cloruro de sodio 100 mM y sacarosa al 1 % en fosfato 10 mM, y se añadió etanercept a una concentración de 50 mg/ml para preparar el control. Se dispusieron en cada jeringa de vidrio 0,5 ml de la formulación de 1,0 ml, se sellaron y se almacenaron a 40 °C, 25 °C y 4 °C. Las composiciones de las formulaciones acuosas preparadas se muestran en la Tabla 4 siguiente.

Tabla 4

	Composición
Formulación 13	50 mg/ml de etanercept, solución de fosfato 10 mM, NaCl 120 mM, metionina 25 mM (pH 6,3)

Formulación 14	50 mg/ml de etanercept, solución de fosfato 10 mM, NaCl 120 mM, metionina 12,5 mM (pH 6,3)
Control	50 mg/ml de etanercept, solución de fosfato 25 mM, NaCl 100 mM, sacarosa al 1 %, L-arginina 25 mM (pH 6,3)

Después la Formulación 13, la Formulación 14 y el control se almacenaron a 40 °C durante 1 y 3 semanas, y a 25 °C y a 4 °C durante 4 y 8 semanas cada una, se midieron sus Impurezas totales mediante SE-HPLC y HI-HPLC, como en el Ejemplo 1. Los resultados se muestran en las Tablas 5 y 6, respectivamente.

Tabla 5

	Impurezas totales por SE-HPLC (%)								
	40 °C			25 °C			4 °C		
	0 semanas	1 semana	3 semanas	0 semanas	4 semanas	8 semanas	0 semanas	4 semanas	8 semanas
Formulación 13	5,5	11,5	20,5	5,5	14,8	18,8	5,5	8,8	10,2
Formulación 14	5,5	12,5	22,0	5,5	16,0	19,9	5,5	8,9	10,5
Control	5,5	13,4	23,3	5,5	16,2	20,4	5,5	8,8	10,3

Tabla 6

	Impurezas totales por HI-HPLC (%)								
	40 °C			25 °C			4 °C		
	0 semanas	1 semana	3 semanas	0 semanas	4 semanas	8 semanas	0 semanas	4 semanas	8 semanas
Formulación 13	10,0	15,9	21,6	10,0	15,3	18,5	10,0	11,0	11,9
Formulación 14	9,9	17,0	23,3	9,9	16,7	19,8	9,9	11,3	11,6
Control	10,2	18,0	25,5	10,2	17,4	20,6	10,2	10,0	11,9

Como se muestra en los resultados de las Tablas 5 y 6, la Formulación 13 y la Formulación 14, que contienen metionina como estabilizante, mostraron una producción de impurezas baja en todas las condiciones de almacenamiento de 40 °C, 25 °C y4 °C, en comparación con el grupo de control preparado según el procedimiento de preparación de una formulación acuosa de etanercept disponible comercialmente. Específicamente, los resultados de la SE-HPLC y la HI-HPLC mostraron que la Formulación 13, que contenía metionina 25 mM, y la Formulación 14, que contenía metionina 12,5 mM, mostraron una producción de

impurezas más reducida a 40 °C y 3 semanas y a 25 °C y 8 semanas, y una producción de impureza similar a 4 °C y 8 semanas, en comparación con el control. Estos resultados indican que la formulación que contiene metionina de la presente invención es una formulación más estable para mantener la actividad de etanercept durante un periodo de tiempo largo que la formulación disponible comercialmente.

En conclusión, la presente invención demostró que la formulación acuosa de etanercept que contenía metionina previene la desnaturalización de etanercept para mantener su actividad durante un periodo de tiempo largo, y la metionina es muy eficaz como estabilizante en la formulación acuosa de etanercept.

<Ejemplo 3> Ensayo de estabilidad de la formulación acuosa de etanercept que comprende histidina y lisina, o histidina y metionina

Se añadieron histidina 5 mM y lisina 25 mM como estabilizante a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de 50 mg/ml y se añadió cloruro de sodio a la misma como agente isotónico para preparar la Formulación 15.

Además, se añadieron histidina 5 mM y metionina 25 mM como estabilizante a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de 50 mg/ml y se añadió cloruro de sodio a la misma como agente isotónico para preparar la Formulación 16.

Finalmente, se añadió cloruro de sodio como agente isotónico a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de 50 mg/ml para preparar una formulación exenta de estabilizante.

Se dispusieron 0,5 ml de la formulación preparada en cada jeringa de vidrio de 1,0 ml, se sellaron y se almacenaron a 50 °C.

Las composiciones con estabilizante de las formulaciones se muestran en la Tabla 7 siguiente.

Tabla 7

	Composición con estabilizante
Formulación 15	Histidina 5 mM + Lisina 25 mM
Formulación 16	Histidina 5 mM + Metionina 25 mM
formulación exenta de estabilizante	-

A los 7 días después del almacenamiento a 50 ºC, las Formulaciones 15, 16 y la formulación exenta de estabilizante de la Tabla 7 se analizaron por SE-HPLC como en el Ejemplo 1 para medir sus Impurezas totales. Los resultados se muestran en la Tabla 8.

Tabla 8

	Impurezas totales por SE-HPLC (%)	
	día 0	7 días
Formulación 15	4,7	21,5
Formulación 16	4,7	20,9
formulación exenta de estabilizante	4,7	30,2

Como se muestra en los resultados de SE-HPLC de la Tabla 8, cuando se almacenan a 50 ºC durante 7 días, la Formulación 16, que contiene histidina como estabilizante, mostró unas impurezas totales del 21,5 % y mostró unas Impurezas totales significativamente bajas en comparación con la formulación exenta de estabilizante que mostró unas Impurezas totales del 30,2 %. Y la Formulación 16 mostró unas Impurezas totales significativamente bajas en comparación con la

Formulación 1, que contenía histidina 10 mM, que mostró unas Impurezas totales del 22,2 % y la Formulación 2, que contenía lisina 25 mM, que mostró unas Impurezas totales del 24,8 %.

Es decir, la presente invención demostró que la formulación que comprendía histidina y lisina tiene efectos significativamente excelentes de prevención de la desnaturalización de etanercept reduciendo la cantidad de impurezas totales durante el almacenamiento, en comparación con la formulación exenta de estabilizante y cada formulación que comprendía histidina, lisina y metionina, respectivamente.

Además, tal como se muestra en los resultados de SE-HPLC de la Tabla 8, cuando se almacenan a 50 °C durante 7 días, la Formulación 16, que contiene histidina como estabilizante, mostró unas impurezas totales del 20,9 % que es un nivel significativamente bajo en comparación con la formulación exenta de estabilizante que mostró unas Impurezas totales del 30,2 %. En particular, la Formulación 16 mostró unas Impurezas totales bajas en comparación con la Formulación 15, que contenía histidina y lisina como estabilizante.

Es decir, la presente invención demostró que la formulación que comprendía histidina y metionina tiene efectos significativos en la prevención de la desnaturalización de etanercept reduciendo la cantidad de las Impurezas totales durante el almacenamiento, en comparación con la formulación exenta de estabilizante.

<Ejemplo 4> Ensayo de estabilidad de una formulación acuosa de etanercept que comprende metionina y lisina

Se añadieron metionina 12,5 mM y lisina 12,5 mM como estabilizante a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de

50 mg/ml y se añadió cloruro de sodio a la misma como agente isotónico para preparar la Formulación 17.

Además, se añadió cloruro de sodio como agente isotónico a una solución de fosfato 10 mM a la que se añadió etanercept a una concentración de 50 mg/ml para preparar una formulación exenta de estabilizante.

Se dispusieron 0,5 ml de la formulación preparada en cada jeringa de vidrio de 1,0 ml, se sellaron y se almacenaron a 50 °C. Las composiciones con estabilizante de las formulaciones se muestran en la Tabla 9 siguiente.

Tabla 9

	Composición con estabilizante
Formulación 17	Metionina 12,5 mM + Lisina 12,5 mM
formulación exenta de estabilizante	-

A los 7 días después del almacenamiento a 50 °C, la Formulación 17 y la formulación exenta de estabilizante de la Tabla 9 se analizaron por SE-HPLC como en el Ejemplo 1 para medir sus Impurezas totales. Los resultados se muestran en la tabla 10.

Tabla 10

	Impurezas totales por SE-HPLC (%)	
	día 0	7 días
Formulación 17	5,2	27,0
formulación exenta de estabilizante	5,2	32,8

Como se muestra en los resultados de SE-HPLC de la Tabla 10, cuando se

almacenan a 50 ºC durante 7 días, la Formulación 17, que contenía metionina y lisina como estabilizante, mostró unas impurezas totales del 27 %, que es un nivel significativamente bajo en comparación con la formulación exenta de estabilizante que mostró unas Impurezas totales del 32,8 %.

Es decir, la presente invención demostró que la formulación que comprendía metionina y lisina tiene efectos significativos en la estabilización de etanercept reduciendo la cantidad de las Impurezas totales, en comparación con la formulación exenta de estabilizante.

REIVINDICACIONES

[Reivindicación 1]

Una formulación líquida de etanercept que comprende etanercept y uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas.

[Reivindicación 2]

La formulación líquida según la reivindicación 1, en la que el estabilizante es metionina y/o sales farmacéuticamente aceptables de la misma.

[Reivindicación 3]

La formulación líquida según la reivindicación 1, en la que el estabilizante está presente en una cantidad de 0,1 a 250 mM.

[Reivindicación 4]

La formulación líquida según la reivindicación 1, en la que el etanercept está presente en una cantidad de 1 a 100 mg/ml.

[Reivindicación 5]

La formulación líquida según la reivindicación 1, que tiene una estabilidad aumentada de almacenamiento de etanercept, en comparación con la formulación que no tiene estabilizantes, reduciendo los subproductos de etanercept que se producen debido a la desnaturalización durante el almacenamiento.

[Reivindicación 6]

La formulación líquida según la reivindicación 1, que además comprende uno o más materiales seleccionados del grupo que consiste en un tampón, un agente isotónico, un excipiente o un conservante.

[Reivindicación 7]

La formulación líquida según la reivindicación 6, en la que el tampón está seleccionado del grupo que consiste en citrato, fosfato, succinato, tartrato, fumarato, gluconato, oxalato,lactato,acetato, histidina y Tris.

[Reivindicación 8]

La formulación líquida según la reivindicación 6, en la que el tampón está presente en una cantidad de 0,1 a 100 mM.

[Reivindicación 9]

La formulación líquida según la reivindicación 6, en la que el agente isotónico está seleccionado del grupo que consiste en cloruro de sodio, cloruro de potasio, ácido bórico, borato de sodio, manitol, glicerina, propilenglicol, polietilenglicol, maltosa, sacarosa, eritritol, arabitol, xilitol, sorbitol y glucosa.

[Reivindicación 10]

La formulación líquida según la reivindicación 6, en la que el agente isotónico está presente en una cantidad de 1 a 1000 mM.

[Reivindicación 11]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de

etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

[Reivindicación 12]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

[Reivindicación 13]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

[Reivindicación 14]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

[Reivindicación 15]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de histidina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

[Reivindicación 16]

La formulación líquida según la reivindicación 1, que comprende 1 a 100 mg/ml de etanercept, 0,1 a 250 mM de metionina o sales farmacéuticamente aceptables de la misma, 0,1 a 250 mM de lisina o sales farmacéuticamente aceptables de la misma, 0,1 a 100 mM de tampón de fosfatos y 1 a 1000 mM de cloruro de sodio.

RESUMEN

La presente invención se refiere a una formulación líquida estable de etanercept (proteína de fusión sTNFR:Fc p75 recombinante) y más particularmente a una formulación líquida que comprende uno o más estabilizantes seleccionados del grupo que consiste en metionina, lisina, histidina y sales farmacéuticamente aceptables de las mismas en una cantidad suficiente para reducir la formación de subproductos de etanercept durante el almacenamiento. La formulación líquida según la presente invención reduce eficazmente la producción de subproductos de etanercept y mantiene de forma estable su eficacia farmacéutica durante un almacenamiento a largo plazo. Por lo tanto, no se requiere un procedimiento de reconstitución antes de su administración y la formulación estéril puede administrarse a pacientes para garantizar la seguridad del paciente. Así, puede aplicarse a sectores con necesidad de tratamiento con etanercept.

42

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【발명의 영문명칭】 Etanercept Stable Aqueous Formulation

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45

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【명세서】

【발명의 명칭】

에타너셉트의 안정한 용액 제형{Etanercept Stable Aqueous Formulation}

【기술분야】

<1> 본 발명은 에타너셉트(Etanercept, recombinant p75 sTNFR:Fc fusion protein)의 안정한 용액 제형에 관한 것으로서, 보다 상세하게는 저장 동안에 에타너셉트의 부산물 형성을 감소시키기에 충분한 양의 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상의 안정화제를 포함하는 용액 제형에 관한 것이다.

【배경기술】

<2> 에타너셉트는 체내에서 TNF-α 리셉터(TNF-α receptor)의 세포 표면과 TNF-α가 결합하는 것을 경쟁적으로 저해하여, TNF-α와 관계된 면역반응을 억제하는 역할을 하는 생물학적 염증 조절제(biological inflammation modulator)이다. 에타너셉트는 수용성의 p75 TNF 리셉터와 인간 재조합 면역글로불린 서브클래스(subclass) 1의 Fc가 결합된 Fc 융합 단백질 2개가 이황화 결합(Disulfide bond)으로 연결된 동형이량체(Homodimer)의 형태를 갖으며, 분자량이 150 kDa에 이르는 거대분자이다(Goldenberg, *Clinical Therapeutics*, 21(1): 75-87, 1999; Moreland et al., Ann. Intern. Med., 130(6): 478-486, 1999).

<3> 이러한 융합 단백질의 전형적인 형태는 1990년대 초반에 텍사스대학교, 사우스웨스턴 메디컬센터(University of Texas Southwestern Medical Center)의 브루스 A 메틀러(Bruce A. Beutler) 등에 의해 처음 합성되었고, 2002년에 암젠(Amgen) 사에 의해 엔브렐(Enbrel)이라는 상품명으로 출시되었다. 에타너셉트는 TNF-α 저해제로 류마티스 관절염, 건선, 강직성 척추염 등에 사용되고 있으며, 혈관염, 알츠하이머병 및 크론병에 적용하기 위한 임상 연구가 진행 중에 있다.

<4> 일반적으로, 항체 의약품은 단백질 의약품과 마찬가지로 유효성분의 반감기가 무척 짧으며 적당하지 않은 온도, 전단 응력(Shear stress), 진동, 냉해동(Freeze-thawing), UV 노출, 과도한 pH 변화, 유기용매 및 미생물에 의한 오염 등에 의해 화학적 및 물리적으로 변성이 쉽게 일어난다. 화학적 변성으로는 이황체의 해리, 산화, 탈아미드화(Deamidation), 이성화(Isomerization) 및 다량체화(Polymerization) 등이 포함되고, 이들은 항체의 아미노산 조성과 항체를 포함하는 용매의 상태(염, pH 및 온도)에 영향을 받는다. 물리적 변성으로는 3차 구조의 손실, 단량체의 공유/비공유적 응집, 흡착 등이 포함되고, 이들은 용매 등과 같은 항체를 포함하고 있는 주변 환경에 의해 변하게 되는 단백질 표면에 있는 소수성 부분(Hydrophobic patchs), 전하 분포(Charge distribution)와 같은 단백질의 복잡한 구조와 열안정성에 영향을 받는다. 항체의 물리적 또는 화학적 변성은 생리활성 효과를 소실시키며, 이러한 변성은 비가역적이기 때문에, 일단 변성된 항체는 원래의 특성을 거의 회복할 수 없어 치료학적 효능이 떨어지게 된다. 또한 단량체의 응집

과 같은 현상은 면역반응을 일으킨다는 보고가 꾸준히 제기되고 있어 이러한 응집을 포함하지 않고, 생리학적 유효량을 가지는 제형에 관한 연구가 많이 진행되고 있다(Ishikawa et al., *Biol. Pharm. Bull.*, 33(8): 1413-1417, 2010; Levin et al., The Development of Therapeutic Monoclonal Antibody Products, editors. Boston (MA): BioProcess Technology Consultants, Inc, Chapter 9).

<5> 용액 중의 단백질 변성을 막기 위하여 여러가지 방법들이 연구되어 왔고, 일부 단백질 약품은 동결 건조방법으로 안정성 문제를 해결하고 있다. 실례로, 미국 등록특허 제7,592,004호에서는 다클리주맙(daclizumab)을 pH 5.5 내지 6.5의 5 내지 25 mM의 히스티딘 완충액과 0.005 내지 0.03% 비이온성 계면활성제인 폴리소르베이트 및 100 내지 300 mM의 비환원성 당인 수크로오스로 안정화하여 동결 건조한 제형을 기술하고 있으며, 미국 공개특허 제2010-0158925호에서는 세툭시맙(cetuximab)을 히스티딘 완충액과 락토비온산(lactobionic acid)로 안정화하여 동결 건조한 제형을 기술하고 있다. 그러나, 동결 건조 과정 중 얼음 결정의 형성, pH의 변화 및 용질의 농축 등과 같은 동결 및 건조 스트레스가 발생하기 때문에 항체의 변성을 초래할 수 있고, 생산 공정에 동결 건조가 포함되어 있어 대용량의 동결 건조기를 사용해야만 하는 등 대규모의 투자가 필요한 단점이 있다. 또한, 동결 건조 제품은 사용시에 다시 주사용수에 녹이야 하는 불편이 있다.

<6> 이와 같은 한계를 해결하는 대안으로서, 용액 상태인 항체에 안정화제를 첨가하여 안정성을 향상시키기도 한다. 항체를 포함한 단백질의 안정화제로는 계면활성제, 혈청 알부민, 다당류, 아미노산, 고분자, 염류 등이 알려져 있다(Wang, *Int.*

J. Pharm., 185: 129-188, 1999; Wang et al., *J. Pharm. Sci.*, 96(1): 1-26, 2007).

<7> 미국 공개특허 제2011-0070231호에서는 IgG 항체의 안정한 액상 조성물을 제시하고 있는데, 상기 특허는 50 mg/ml 이상의 다클리주맙이 pH 5.5 내지 6.5의 20 내지 60 mM 숙시네이트 완충액, 0.02 내지 0.04% 폴리소르베이트 및 75 내지 150 mM의 염화나트륨을 포함하는 안정한 액상의 약학 제형물에 대해 게시하고 있다.

<8> 미국 공개특허 제2011-0020328호에서는 치료학적 유효량의 항-CD20 항체의 제형을 제시하고 있는데, 이는 10 내지 100 mM의 소듐 아세테이트, 25 내지 100 mM의 염화나트륨, 0.5 내지 5%의 아르기닌 유리 염기, 0.02 내지 0.2 mM의 EDTA 및 0.01 내지 0.2%의 폴리소르베이트 80를 포함하는 것으로 pH가 5.0 내지 7.0인 약학적으로 안정한 조성물에 관한 것이다.

<9> 미국 등록특허 제7,785,592호에서는 75 mg/ml 이상의 팔리비주맙(palivizumab)이 이온성 염류와 계면활성제 없이 히스티딘 완충액과 글리신을 이용하여 안정화되어 2 내지 8℃에서 적어도 15개월이상 안정한 제형에 대해 소개하고 있다.

<10> 미국 등록특허 제6,991,790호에서는 염화나트륨과 같은 등장화제가 없는 치료학적 유효량의 항체, 약 pH 4.8 내지 약 5.5의 아세테이트 완충액, 계면활성제, 및 폴리올을 함유하는 안정한 수성 약학 제형물에 대해 게시하고 있다.

<11> 미국 등록특허 제7,648,702호에서는 인간 p75 종양 괴사 인자 수용체와 인간

IgG1의 Fc 부분의 융합체를 응집 방지제로서 L-아르기닌을 약 10 에서 200 mM의 농도로 사용하여 안정화시킨 액상 제형에 관해 기술하고 있다. 상기 특허는 유효약물로 에타너셉트를 포함한 유일한 공지 기술이다.

<12> 그러나, 이러한 안정화 제형을 제조하기 위해서는 유효 성분 각각의 물리화학적 특성에 따라 적절한 안정화제를 사용해야만 하며, 안정화제들을 병용할 경우 상호간의 경쟁작용 및 부작용으로 인하여 목적한 바와는 다른 역효과를 가져올 수 있다. 또한, 항체를 안정화하는데 적합한 범위의 농도가 존재하고, 단백질 의약품에 비해 농도가 상대적으로 높기 때문에 용액 중의 항체를 안정화하기 위해서는 많은 노력과 주의가 필요하다(Shire et al., *J. Pharm. Sci.*, 93(6): 1390-1402, 2004).

<13> 상기 종래의 항체 안정화 제형 기술들에서 보는 바와 같이, L-아르기닌을 포함한 액상 제형이 에타너셉트를 안정화한 유일한 기술로서 에타너셉트에 한정된 안정한 액상 제형에 관한 연구가 미비하여 에타너셉트의 활성을 안정하게 유지할 수 있는 새로운 용액 제형의 개발에 대한 필요성이 높은 실정이다.

<14> 이에 본 발명자들은 에타너셉트의 활성을 안정하게 유지할 수 있는 용액 제형의 제조 방법에 대해 연구한 결과, 메티오닌, 라이신 및 히스티딘이 수용액 상에서 에타너셉트의 안정화에 현저히 효과가 있음을 발견함으로써 본 발명을 완성하였다.

【발명의 내용】

【해결하려는 과제】

<15> 따라서, 본 발명의 목적은 저장 동안에 에타너셉트의 부산물 형성을 감소시키기고, 이의 활성을 장기간 유지하기에 충분한 양의 안정화제를 포함하는 용액 제형을 제공하는 것이다.

【과제의 해결 수단】

<16> 상기와 같은 목적을 달성하기 위해서, 본 발명은 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상의 안정화제를 포함하는 용액 제형을 제공한다.

발명의 일 양태에서, 본 발명은 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상을 안정화제로 포함하는 에타너셉트(etanercept)의 안정화 용액 제형을 제공한다.

<18> 발명의 다른 양태에서, 본 발명은 메티오닌이 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<19> 발명의 다른 양태에서, 본 발명은 라이신이 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<20> 발명의 다른 양태에서, 본 발명은 히스티딘이 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<21> 발명의 다른 양태에서, 본 발명은 히스티딘이 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<22> 발명의 다른 양태에서, 본 발명은 에타너셉트가 1 내지 100 mg/ml의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<23> 발명의 다른 양태에서, 본 발명은 완충제, 등장화제, 부형제 또는 보존제를 추가로 포함하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<24> 발명의 다른 양태에서, 본 발명은 완충제가 0.1 내지 100 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<25> 발명의 다른 양태에서, 본 발명은 등장화제가 1 내지 1000 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<26> 발명의 다른 양태에서, 본 발명은 1 내지 100 mg/ml의 에타너셉트, 0.1 내지 250 mM의 메티오닌, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<27> 발명의 다른 양태에서, 본 발명은 1 내지 100 mg/ml의 에타너셉트, 0.1 내지 250 mM의 라이신, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<28> 발명의 다른 양태에서, 본 발명은 1 내지 100 mg/ml의 에타너셉트, 0.1 내지

250 mM의 히스티딘, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형을 제공한다.

<29> 이하에서는, 본 발명의 용액 제형에 대해 구성 요인별로 상세히 설명하고자 한다.

<30> 본 발명은 메티오닌, 라이신, 히스티딘 및 이의 유사체가 수용액 상에서 에타너셉트의 부산물 형성을 감소시켜 이의 활성을 안정하게 장기간 유지하는데 효과가 있음을 최초로 밝히고, 이들을 약리학적 유효량의 에타너셉트의 안정화제로서 사용하는 신규한 용도를 제안한데 발명의 특징이 있다.

<31> 본 발명에서 용어 "에타너셉트(Etanercept, recombinant p75 sTNFR:Fc fusion protein)"는 상기에서 설명한 바와 같이, 수용성의 p75 TNF 리셉터와 인간 재조합 면역글로불린 시프틀레스 1의 Fc가 결합된 Fc 융합 단백질 2개가 이황화 결합으로 연결된 동형이량체의 형태를 갖는 분자량 150 kDa의 거대분자를 지칭한다. 본 발명의 에타너셉트는 체내에서 TNF-α 리셉터의 세포 표면과 TNF-α가 결합하는 것을 경쟁적으로 저해하여, TNF-α와 관계된 면역반응을 억제하는 역할을 하는 생물학적 염증 조절제로서, 류마티스 관절염, 건선, 강직성 척추염 등에 사용되고 있으며, 혈관염, 알츠하이머병 및 크론병에 적용하기 위한 임상 연구가 현재 진행 중에 있다.

<32> 본 발명에서 용어 "안정화한 용액 제형" 및 "안정한 용액 제형"은 제형 중의 치료학적 유효성분인 에타너셉트가 저장시에 본질적으로 이의 물리적 및 화학적 동일성 및 완전성을 보유하는 제형을 지칭한다. 에타너셉트의 안정성을 측정하기 위한 각종 분석 기술은 당해 분야에서 널리 알려진 단백질 안정성 분석법이 이용될 수 있다. 안정성은 선택된 시간 동안에 선택된 온도에서 측정할 수 있다. 신속한 시험을 위해, 제형은 보다 높은 또는 "가속화된" 온도, 예를 들면, 2주 내지 1개월 이상 동안 40℃에서 보관할 수 있으며, 이 시점에서 시간 안정성을 측정한다.

<33> 본 발명에서 용어 "안정화제"는 제형 중의 생물학적 분자 및/또는 일반적인 약학적 부형제와 상호작용하여 안정화시키는 특이적 화학적 화합물을 지칭한다. 안정화제는 일반적으로 단백질 응집을 초래하는 공기/용액 계면 유도된 스트레스 및 용액/표면 유도된 스트레스로부터 단백질을 보호한다. 본 발명에서 안정화제는 저장동안에 에타너셉트의 부산물 형성을 감소시켜 이의 활성을 장기간 유지하게 만드는 성분으로, 바람직하게는 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 군으로부터 선택되는 하나 이상을 사용할 수 있다.

<34> 본 발명에서 용어 "유사체"는 본 발명의 안정화제로 사용되어지는 메티오닌, 라이신, 히스티딘과 같은 아미노산이 그 기능이 유지될 수 있는 범위 내에서 염의 형태로 제조되어진 공지된 화합물들을 지칭한다. 구체적으로 산이 첨가된 염의 형태일 수 있고, 예를 들어 무기산(예: 염산, 하드로브롬산, 인산, 질산, 황산 등), 유기 카르복실산(예: 아세트산, 트리플루오로아세트산과 같은 할로 아세트산, 프로피온산, 말레산, 숙신산, 말산, 시트르산, 타르타르산, 살리실산), 산성 당(글루쿠

론산, 갈락투론산, 글루콘산, 아스코르브산), 산성 폴리사카리드(예: 히알우론산, 콘드로이틴 술페이트, 아르기닌산), 콘드로이틴 술페이트와 같은 술폰산 당 에스테르를 포함하는 유기 술폰산(예: 메탄, 술폰산, p-톨루엔 술폰산) 등이 첨가된 염의 형태 일 수 있다. 본 발명에서 유사체의 일례로는 메티오닌의 유사체인 셀레노메티오닌, 하이드록시 메틸 부탄산, 에티오닌 또는 프리플루오로메티오닌 등을 들 수 있으나, 이에 한정되지는 않는다.

<35> 본 발명에서 용어 "부산물"은 제형 중의 치료학적 유효성분인 에타너셉트의 비율을 저하시키거나 감소시키는 목적하지 않는 산물을 의미한다. 전형적 부산물은 에타너셉트가 탈아미드화 또는 가수분해 등에 의해 변성되어 생성된 "저분자 생성물", 올리고머, 응집체 등의 "고분자 생성물" 또는 이들의 혼합물을 포함한다.

<36> 본 발명에서 용어 "고분자 생성물"은 변성 등에 의해 후속적으로 응집되는 에타너셉트의 단편(예를 들면, 탈아미드화 또는 가수분해에 의한 폴리펩타이드의 분해에 의해 생산됨) 또는 이들의 혼합물을 포함한다. 전형적으로, 고분자 생성물은 치료학적 단량체 형태의 에타너셉트보다 큰 분자량을 갖는 복합체로, 이들은 약 150 kDa을 초과하는 분자량을 가질 것이다.

<37> 본 발명에서 용어 "저분자 생성물"은 예를 들면, 탈아미드화 또는 가수분해에 의해 생성된 치료학적 폴리펩타이드, 즉 에타너셉트의 단편을 포함한다. 전형적으로, 저분자 생성물은 치료학적 단량체 형태의 에타너셉트보다 적은 분자량을 갖는 복합체로, 이들은 약 150 kDa 미만의 분자량을 가질 것이다.

<38> 본 발명의 용액 제형은 안정화제로서 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상을 포함한다. 바람직하게는 안정화제로서 메티오닌을 포함한다.

<39> 메티오닌은 필수 아미노산으로서 화학식은 $C_5H_{11}NO_2S$이며, 분자량은 149.21이다. 메티오닌은 수용액상에 존재하는 항체가 가진 메티오닌 잔기와 자유 하이드록시 라디칼과 반응하기 위해 경쟁적으로 작용하여 항체가 산화되는 것을 방지하여 항체를 안정화시키는 효과가 있다(Lam et al., *J. Pharm. Sci.*, 86(11): 1250-1255, 1997; Wang, *Int. J. Pharm.*, 185: 129-188, 1999). 상기 메티오닌의 함량은 0.1 내지 250 mM, 바람직하게는 1 내지 100 mM, 더욱 바람직하게는 5 내지 50 mM이다.

<40> 또한, 본 발명의 용액 제형에는 안정화제로서 라이신, 히스티딘 등을 포함할 수 있다. 상기 라이신, 히스티딘의 함량은 0.1 내지 250 mM, 바람직하게는 1 내지 100 mM, 더욱 바람직하게는 5 내지 50 mM이다.

<41> 본 발명의 바람직한 실시양태에서는, 에타너셉트 안정화 용액 제형의 제조에 사용하기 위한 최적 안정화제를 확인하기 위해, 다양한 아미노산, 계면활성제 및 고분자 등을 첨가하여 그들의 안정화 효과를 확인한 결과, 라이신, 히스티딘, 메티오닌이 공지된 안정화제인 L-아르기닌과 동등 또는 그 이상으로 에타너셉트의 변성을 방지하여 안정화하는 효과가 있음을 확인하였고, 특히 메티오닌이 L-아르기닌보다 에타너셉트를 안정화하는데 탁월한 효과가 있음을 입증하였다.

<42> 구체적으로, SE-HPLC를 이용하여 보관 중에 형성되는 에타너셉트의 부산물을 확인한 결과, 미국 등록특허 제7,628,702호에 게시된 L-아르기닌을 안정화제로 사용한 에타너셉트 제형의 총 불순물 양과 비교하였을 때, 안정화제로 라이신을 사용한 경우는 불순물 생성량이 유사하고, 히스티딘 또는 메티오닌을 사용한 경우는 불순물 생성량이 감소되는 것을 확인하였다(표 2 참조). 또한, HI-HPLC 분석에서도 상기와 유사한 결과를 나타내어 히스티딘, 라이신, 메티오닌이 에타너셉트의 변성을 방지하여 이의 부산물 형성을 억제하는 효과를 나타냄을 확인하였다(표 3 참조).

<43> 본 발명에 따른 용액 제형은 상기 안정화제에 추가로 완충제를 포함할 수 있다. 본 발명에서 용어 "완충제"는 제형의 등장성 및 화학적 안정성을 증진시키는 성분으로, 생리학적으로 적합한 pH를 유지하는 작용을 한다. 상기 완충제는 에타너셉트의 안정화를 위해 용액 제형의 pH가 급격히 변화하는 것을 방지하며, 용액의 pH를 유지시키는 역할을 하고, 그러한 완충제의 바람직한 예로는 시트레이트, 포스페이트, 숙시네이트, 타르트레이트, 푸마레이트, 글루코네이트, 옥살레이트, 락테이트, 아세테이트, 히스티딘, 트리스(Tris) 등을 포함하나 이들로 제한되지 않는다. 특정한 양태에서, 완충제는 인산염 완충액이다. 이러한 완충제는 1종 단독으로 사용하거나 2종 이상을 임의로 조합하여 사용할 수도 있다.

<44> 본 발명의 다양한 양태에서, 제형은 약 5 내지 약 7의 pH 또는 약 5.5 내지 약 6.5의 pH를 가지야 한다. 특정한 양태에서, 제형은 약 6의 pH를 갖는다. 중간

값부터 상기 언급한 pH 수준에 이르는 범위, 예를 들면, 약 pH 5.2 내지 약 pH 6.3 (예: pH 6.2)도 또한 본 발명의 일부분이다. 예를 들면, 상한치 및/또는 하한치로서 상기 언급한 값 중 임의의 값의 조합을 사용한 값의 범위가 포함된다. pH는 필요에 따라 당해 분야에 공지된 기술에 의해 조절할 수 있다. 예를 들면, 필요에 따라서 HCl을 부가하여 pH를 목적 수준으로 조절할 수 있거나, 필요에 따라 히스티딘을 부가하여 pH를 목적 수준으로 조절할 수 있다.

<45> 하나의 양태에서, 완충제는 0.1 내지 100 mM, 바람직하게는 1 내지 50 mM, 더욱 바람직하게는 5 내지 25 mM로 존재한다. 중간 값부터 상기 언급한 농도에 이르는 범위도 또한 본 발명의 부분이다. 예를 들면, 상한치 및/또는 하한치로서 상기 언급한 값 중 임의의 값의 조합을 사용한 값의 범위가 포함된다. 특정 양태에서, 완충제는 생리학적으로 적합한 pH를 유지하기에 충분한 양으로 존재해야 한다.

<46> 본 발명에 따른 용액 제형은 상기 안정화제에 추가로 등장화제를 포함할 수 있다. 본 발명에서 용어 "등장화제"는 부분적으로, 제형의 등장성을 유지하고 단백질 수준을 유지시키는데 기여하고, 부분적으로, 제형 중에 존재하는 치료학적 활성 폴리펩타이드의 수준, 비 또는 비율을 보존하는데 기여하는 성분을 지칭한다. 등장화제 용액은 혈중 혈장과 동일한 삼투압을 지니고, 따라서 피험체의 혈중 혈장의 삼투압을 변화시키지 않으면서 피험체에게 정맥 내 주입될 수 있다. 또한, 본 발명에 따른 하나의 양태에서, 삼투압은 당해 제형이 정맥 내 주입에 적합하도록 제조

된다. 흔히, 등장화제는 벌킹제(bulking agent)로서도 작용한다. 이와 같이, 등장화는 단백질이 동결 및 진단력과 같은 다양한 스트레스를 극복할 수 있게 한다.

<47> 상기 등장화제는 에타너셉트를 수용액상으로 체내에 투여할 때 체내에서 적절하게 삼투압을 유지하는 역할을 한다. 그러한 등장화제의 예로는 통상적으로 사용되는, 염화나트륨, 염화칼륨, 붕산, 붕산나트륨, 만니톨, 글리세린, 프로필렌글리콜, 폴리에틸렌글리콜, 말토스, 자당, 에리쓰리톨, 아라비톨, 자일리톨, 소르비톨, 포도당 등을 사용할 수 있지만, 이에 제한되는 것은 아니다. 특정 양태에서, 등장화제는 NaCl이다. 이들 등장화제는 1종 단독으로 사용하거나 2종 이상을 임의로 조합하여 사용할 수도 있다.

<48> 하나의 양태에서, 등장화제(예: NaCl)는 1 내지 1000 mM 바람직하게는 10 내지 500 mM, 더욱 바람직하게는 50 내지 250 mM로 존재한다. 중간값부터 상기 언급한 농도에 이르는 범위도 또한 본 발명의 일부이다. 예를 들면, 상한치 및/또는 하한치로서 상기 언급한 값 중 임의의 값의 조합을 사용한 값의 범위가 포함된다. 등장화제는 제형의 삼투성을 유지하기에 충분한 양으로 존재해야 한다.

<49> 본 발명에 따른 용액 제형은 추가로 약제학적으로 허용가능한 부형제를 포함할 수 있으며, 부형제로서 당알콜류(Sugars and Polyols), 계면활성제(Surfactants), 고분자(Polymes) 등을 포함할 수 있다. 당알콜류는 수크로스, 트레알로스, 락토스, 말토스, 갈락토스, 만니톨, 솔비톨, 글리세롤 등이 사용될 수 있으며, 계면활성제로는 폴리솔베이트 20, 폴리솔베이트 80, 폴록사머 등의 비이온

계면활성제가 사용될 수 있고, 고분자로는 덱스트란, 폴리에틸렌글리콜, 카르복실 메틸셀룰로스, 히아루론산, 사이클로덱스트린 등이 사용될 수 있다.

<50> 본 발명에 따른 용액 제형은 추가로 보존제를 포함할 수 있다. 상기 보존제는 항균제로서 작용하기 위해 제약 제제에 첨가되는 화합물을 의미하는데, 이러한 보존제로 벤즈알코늄 클로라이드, 벤즈에토늄, 클로로헥시딘, 페놀, m-크레졸, 벤질 알코올, 메틸파라벤, 프로필파라벤, 클로로부탄올, o-크레졸, p-크레졸, 클로로크레졸, 페닐수은산 질산염, 티메로살, 벤조산 등을 사용할 수 있지만, 이에 제한되는 것은 아니다. 이들 보존제는 1종 단독으로 사용하거나 2종 이상을 임의로 조합하여 사용할 수도 있다.

【발명의 효과】

<51> 본 발명에 따른 용액 제형을 안정화제로서 에타너셉트의 부산물 생성을 효과적으로 방지할 수 있는 메티오닌, 라이신 및 히스티딘을 포함하여 에타너셉트의 약리 효능을 장기간 안정하게 보존할 수 있으므로, 환자에게 투여 시 제구성해야할 필요성이 줄어들며, 무균된 상태로 환자에게 공급할 수 있어서 환자의 안전을 확보할 수 있고, 향후 에타너셉트의 약리 효능에 관련된 치료 분야에 효과적으로 응용될 수 있을 것이다.

【발명을 실시하기 위한 구체적인 내용】

<52> 이하, 본 발명을 실시예를 통하여 더욱 상세하게 설명한다. 그러나 이들 실시예는 본 발명을 예시적으로 설명하기 위한 것으로, 본 발명의 범위가 이들 실시예에 한정되는 것은 아니다.

<53> <실시예 1> 안정화제 첨가에 따른 에타너셉트 용액의 안정성 조사

<54> 에타너셉트 안정화 용액 제형의 제조에 사용하기 위한 최적 안정화제를 확인하기 위해, 5 mM의 인산염 용액에 안정화제로 히스티딘과 에타너셉트의 농도가 50 mg/ml이 되도록 첨가하고, 등장화제로 염화나트륨을 첨가하여 제형 1을 제조하였다. 또한, 10 mM 인산염 용액에 에타너셉트의 농도가 50 mg/ml이 되도록 첨가하고, 등장화제로 염화나트륨을 첨가한 후, 안정화제로 각각 라이신, 아르기닌, 메티오닌, 글리신, 폴리소르베이트 20, 폴리소르베이트 80, 폴록사머 188, 프로필렌 글리콜, 황산 프로타민, 수크로오스, 염산 구아니딘을 첨가하여 제형 2 내지 12를 제조하였다. 마지막으로, 10 mM 인산염 용액에 등장화제로 염화나트륨과 에타너셉트의 농도가 50 mg/ml이 되도록 첨가하여 안정화제 무첨가 제형을 제조하였다. 상기 제형들에 첨가된 안정화제의 조성을 하기 표 1에 나타내었다. 각각의 제형을 약 1.0 mL 용량의 유리 주사기에 0.5 mL씩 담은 후에 밀봉하여, 50 ℃에서 보관하였다.

<55> 【표 1】

	안정화제 조성
제형 1	히스티딘 10 mM
제형 2	라이신 25 mM
제형 3	아르기닌 25 mM
제형 4	메티오닌 25 mM
제형 5	글리신 1%
제형 6	트윈 20 0.1%
제형 7	트윈 80 0.1%
제형 8	F68 0.1%
제형 9	프로필렌글리콜 0.1%
제형 10	프로타민 황산염 0.03%
제형 11	수크로스 2%
제형 12	구아니딘 HCl 0.2%
무첨가 제형	-

<56> 상기 표 1의 제형 1 내지 12와 무첨가 제형을 50℃ 보관 7일 시점에서 SE-HPLC(Size Exclusion HPLC)로 에타너셉트가 변성되어 생성된 저분자 생성물의 종류와 올리고머, 응집체 등의 고분자 생성물의 종류를 측정한 후, 이들의 양을 더하여 총 불순물(Total Impurity)로 나타내었다. 또한, HI-HPLC(Hydrophobic Interaction-HPLC)로 에타너셉트의 구조적 변화 정도를 확인하였다. 에타너셉트 관련 물질은 HI-HPLC에서 pre-Peak, Peak 1, Peak 2, Peak 3 등 4개의 피크로 분리되며, pre Peak 과 Peak 1은 저분자 생성물이고, Peak 2가 에타너셉트이며, Peak 3은 응집이나 활성이 낮은 이량체 종류를 나타낸다. 따라서, pre-Peak, Peak 1, Peak 3의 양을 더하여 총 불순물로 표기하였다. 상기 분석 결과를 표 2 및 3에 각각 나타내었다.

<57> 【표 2】

	SE-HPLC 후 불순물 (%)	
	0 일째	7 일째

제형 1	4.6	22.2
제형 2	4.7	24.8
제형 3	4.6	24.2
제형 4	4.6	20.8
제형 5	4.6	28.5
제형 6	4.7	29.4
제형 7	4.6	29.0
제형 8	4.6	28.8
제형 9	4.7	27.8
제형 10	4.6	29.4
제형 11	4.8	28.4
제형 12	4.7	27.5
무첨가 제형	4.7	30.2

<58> 【표 3】

	HI-HPLC 총 불순물 (%)	
	0 일째	7 일째
제형 1	14.7	33.7
제형2	14.6	34.9
제형3	15.0	34.3
제형4	15.2	30.3
제형5	14.8	36.6
제형6	15.2	35.5
제형7	15.2	36.2
제형8	15.4	35.5
제형9	15.2	35.1
제형10	15.0	36.9
제형11	14.9	35.1
제형 12	15.1	34.8
무첨가 제형	14.1	41.9

<59> 상기 표 2의 SEC-HPLC 결과를 보면, 50℃에서 7일간 보관 시에 안정화제로 히스티딘을 포함한 제형 1의 총 불순물의 양은 22.2%, 리이신을 포함한 제형 2는 24.8%, 아르기닌을 포함한 제형 3은 24.2%, 메티오닌을 포함한 제형 4는 20.8%로 안정화제를 넣지 않은 무첨가 제형의 불순물량 30.2% 보다 총 불순물의 양이 현저히 감소되는 것을 확인하였다. 상기 제형들을 L-아르기닌을 안정화제로 사용한 제

형 3(미국 등록특허 제7,628,702호)의 총 불순물의 양과 비교한 결과, 라이신을 포함한 제형 2는 24.8%로서 유사한 결과를 나타내었고, 히스티딘을 포함한 제형 1은 22.2%, 메티오닌을 포함한 제형 4는 20.4%로서 불순물의 생성이 감소되는 것을 확인하였다.

<60> 상기 표 3의 IE-HPLC 결과는 SE-HPLC의 결과와 유사하였다. 무첨가 제형은 41.9%의 불순물이 생성되었으나, 제형 1은 33.7%, 제형 2는 34.9%, 제형 3은 34.3%, 제형 4는 30.3%로 각 제형에 포함된 히스티딘, 라이신, L-아르기닌, 메티오닌이 에타너셉트의 변성을 방지하는 효과를 갖음을 확인하였다. 특히, 그 중에서도 메티오닌을 포함한 제형 4에서 불순물이 가장 적게 생성되어 에타너셉트의 안정화 효과가 가장 뛰어남을 알 수 있었다.

<61> 이로써 라이신, 히스티딘, 메티오닌이 L-아르기닌과 동등 또는 그 이상으로 에타너셉트의 변성을 방지하여 안정화하는 효과가 있음을 확인하였고, 특히 메티오닌이 L-아르기닌보다 에타너셉트를 안정화하는 탁월한 효과가 있음을 본 발명에서 입증하였다.

<62>

<63> <실시예 2> 메티오닌 농도에 따른 에타너셉트 용액의 안정성 조사

<64> 에타너셉트를 안정화할 수 있는 메티오닌의 최적 농도를 확인하기 위해, 10 mM 인산염 용액에 120 mM의 염화나트륨을 넣고, 메티오닌을 각각 25 및 12.5 mM을

첨가하고 에타너셉트의 농도가 50 mg/ml이 되도록 넣어 각각 제형 13 및 14를 제조하였다. 또한, 시판 중인 에타너셉트 용액 제형을 제조하는 방법에 따라 10 mM 인산염 용액에 100 mM의 염화나트륨, 1%의 수크로오스를 첨가한 용액에 L-아르기닌을 25 mM 첨가하고, 에타너셉트의 농도가 50 mg/ml이 되도록 제조하여 대조군으로 사용하였다. 각각 제조된 용액을 약 1.0 mL 용량의 유리 주사기에 0.5 mL씩 담은 후에 밀봉하여, 40℃, 25℃ 및 4℃에 보관하였다. 각각의 제조된 용액 제형의 조성을 표 4에 나타내었다.

<65> 【표 4】

	조성
제형 13	에타너셉트 50mg/ml, 인산염 용액 10mM, NaCl 120mM, 메티오닌 25mM (pH 6.3)
제형 14	에타너셉트 50mg/ml, 인산염 용액 10mM, NaCl 120mM, 메티오닌 12.5mM (pH 6.3)
대조군	에타너셉트 50mg/ml, 인산염 용액 25mM, NaCl 100mM, 수크로오스 1%, L-아르기닌 25mM (pH 6.3)

<66> 상기 제형 13, 14 및 대조군을 40℃에서 1 및 3주 보관하고, 25℃ 및 4℃에서 4 및 8주 보관한 후, 상기 실시예 1에서와 같이 SE-HPLC 및 HI-HPLC로 총 불순물의 양을 측정하였다. 상기 분석 결과를 표 5 및 6에 각각 나타내었다.

<67> 【표 5】

	SE-HPLC 총 불순물 (%)								
	40℃			25℃			4℃		
	0주	1주	3주	0주	4주	8주	0주	4주	8주
제형 13	5.5	11.5	20.5	5.5	14.8	18.8	5.5	8.8	10.2
제형 14	5.5	12.5	22.0	5.5	16.0	19.9	5.5	8.9	10.5
대조군	5.5	13.4	23.3	5.5	16.2	20.4	5.5	8.8	10.3

<68> 【표 6】

	III-HPLC 총 불순물 (%)								
	40℃			25℃			4℃		
	0주	1주	3주	0주	4주	8주	0주	4주	8주
제형 13	10.0	15.9	21.6	10.0	15.3	18.5	10.0	11.0	11.9
제형 14	9.9	17.0	23.3	9.9	16.7	19.8	9.9	11.3	11.6
대조군	10.2	18.0	25.5	10.2	17.4	20.6	10.2	10.0	11.9

<69> 상기 표 5와 6의 결과에서 알 수 있듯이, 메티오닌을 안정화제로 포함한 제형 13 및 14가 시판중인 에타너셉트 용액 제형대로 제조한 대조군보다 40℃, 25℃ 및 4℃의 모든 보관 조건에서 불순물의 생성이 적은 것을 확인하였다. 구체적으로, 메티오닌 25 mM을 포함한 제형 13과 메티오닌 12.5 mM을 포함한 제형 14의 SE-HPLC와 III-HPLC 결과를 보면, 40℃ 3주 및 25℃ 8주에서 대조군보다 에타너셉트의 불순물량이 적게 검출되었으며, 4℃ 8주에서는 유사하게 나타났다. 이는 본 발명의 메티오닌 함유 제형이 현재 시판 중인 에타너셉트 제형보다 장기적으로 에타너셉트의 활성을 유지하는데 더 안정한 제형임을 나타내는 것이다.

<70> 이로써, 본 발명은 메티오닌이 포함된 에타너셉트 용액 제형이 에타너셉트의 변성을 방지하여 장기적으로 활성을 유지할 수 있음을 발견하였으며, 메티오닌이 에타너셉트 용액에서 안정화제로 탁월한 효과가 있음을 입증하였다.

2011-06-03

【특허청구범위】

【청구항 1】

메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상을 안정화제로 포함하는 에타너셉트(etanercept)의 안정화 용액 제형.

【청구항 2】

제1항에 있어서, 안정화제가 메티오닌 및 이의 유사체인 것인 에타너셉트의 안정화 용액 제형.

【청구항 3】

제1항에 있어서, 메티오닌 및 이의 유사체가 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 4】

제1항에 있어서, 라이신 및 이의 유사체가 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 5】

제1항에 있어서, 히스티딘 및 이의 유사체가 0.1 내지 250 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 6】

제1항에 있어서, 에타너셉트가 1 내지 100 mg/ml의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 7】

제1항에 있어서, 저장 보관 시 변성에 의한 에타너셉트 부산물의 생성 감소를 통해 저장 안정성을 나타내는 것인 에타너셉트의 안정화 용액 제형.

【청구항 8】

제1항에 있어서, 완충제, 등장화제, 부형제 또는 보존제를 추가로 포함하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 9】

제8항에 있어서, 완충제가 0.1 내지 100 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 10】

제8항에 있어서, 등장화제가 1 내지 1000 mM의 양으로 존재하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 11】

제8항에 있어서, 완충제가 시트레이트, 포스페이트, 숙시네이트, 타르트레이트, 푸마레이트, 글루코네이트, 옥살레이트, 락테이트, 아세테이트, 히스티딘 및 트리스(Tris)로 이루어진 그룹으로부터 선택되는 것인 에타너셉트의 안정화 용액 제형.

【청구항 12】

제8항에 있어서, 등장화제가 염화나트륨, 염화칼륨, 붕산, 붕산나트륨, 만니톨, 글리세린, 프로필렌글리콜, 폴리에틸렌, 글리콜, 말토스, 자당, 에리쓰리톨, 아라비톨, 자일리톨, 소르비톨 및 포도당으로 이루어진 그룹으로부터 선택되는 것인 에타너셉트의 안정화 용액 제형.

【청구항 13】

제1항에 있어서, 1 내지 100 mg/ml의 에타너셉트, 0.1 내지 250 mM의 메티오

닌, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 14】

제1항에 있어서, 1 내지 100 mg/ml의 에타너셉트, 0.1 내지 250 mM의 라이신, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형.

【청구항 15】

제1항에 있어서, 1 내지 100 mg/ml의 에타너셉트, 0.1 내지 250 mM의 히스티딘, 0.1 내지 100 mM의 인산염 완충액 및 1 내지 1000 mM의 염화나트륨을 포함하는 것인 에타너셉트의 안정화 용액 제형.

2011-06-03

【요약서】

【요약】

본 발명은 에타너셉트(Etanercept, recombinant p75 sTNFR:Fc fusion protein)의 안정한 용액 제형에 관한 것으로서, 보다 상세하게는 저장 동안에 에타너셉트의 부산물 형성을 감소시키기에 충분한 양의 메티오닌, 라이신, 히스티딘 및 이의 유사체로 이루어진 그룹으로부터 선택되는 하나 이상의 안정화제를 포함하는 용액 제형에 관한 것이다. 본 발명에 따른 용액 제형을 안정화제로서 에타너셉트의 부산물 생성을 효과적으로 방지할 수 있는 메티오닌, 라이신 및 히스티딘을 포함하여 에타너셉트의 약리 효능을 장기간 안정하게 보존할 수 있으므로, 환자에게 투여시 재구성해야할 필요성이 줄어들며, 무균된 상태로 환자에게 공급할 수 있어서 환자의 안전을 확보할 수 있고, 향후 에타너셉트의 약리 효능에 관련된 치료 분야에 효과적으로 응용될 수 있을 것이다.

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(54) **Title**: STABLE LIQUID FORMULATION OF ETANERCEPT

(57) **Abstract**: The present invention relates to a stable liquid formulation of etanercept (recombinant p75 sTNFR:Fc fusion protein), and more particularly, to a liquid formulation comprising one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof in an amount sufficient to reduce by-product formation of etanercept during storage. The liquid formulation according to the present invention effectively reduces production of etanercept by-products and to stably maintain its pharmaceutical efficacies for long-term storage. Therefore, the reconstitution procedure is not required before administration, and the sterile formulation can be administered to patients to ensure patient safety. Thus, it can be applied to the fields in need of etanercept treatment.

Description

Title of Invention: STABLE LIQUID FORMULATION OF ETANERCEPT

Technical Field

[1] The present invention relates to a liquid formulation of etanercept (recombinant p75 sTNFR:Fc fusion protein), and more particularly, to a liquid formulation comprising one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof in an amount sufficient to reduce by-products of etanercept during storage.

Background Art

[2] Etanercept is a biological inflammation modulator that functions as a competitive inhibitor of TNF-α, binding to cell surface TNF-α receptor, to inhibit TNF-α mediated immune responses. Etanercept is a macromolecule with a molecular weight of approximately 150 kDa, and is a homodimer of two Fc fusion proteins linked by a disulfide bond, each Fc fusion protein consisting of a human soluble p75 TNF receptor coupled to the Fc portion of human immunoglobulin G subclass 1 (Goldenberg, Clinical Therapeutics, 21(1): 75-87, 1999; Moreland et al., Ann. Intern. Med., 130(6): 478-486, 1999).

[3] This prototypic fusion protein was first synthesized in the early 1990s by Bruce A. Beutler at the University of Texas Southwestern Medical Center, and marketed by Amgen under the trade name of Enbrel in 2002. Etanercept is a TNF-α inhibitor used to treat rheumatoid arthritis, psoriasis, and ankylosing spondylitis, and under clinical trials for the treatment of vasculitis, Alzheimer's disease, and Crohn's disease.

[4] Like protein drugs, antibody drugs have a very short half life, and chemical and physical denaturation can be easily caused by unfavorable temperature, shear stress, vibration, freeze-thawing, UV exposure, excessive pH change, organic solvents, and microbial contamination. Chemical denaturation includes dimer dissociation, oxidation, deamidation, isomerization, and polymerization, which are influenced by the amino acids constituting the antibody and conditions of the solvent containing the antibody (salt, pH and temperature). Physical denaturation includes loss of tertiary structure, covalent/non-covalent aggregation and adhesion of monomers, which are influenced by hydrophobic patches on the protein surface changed by antibody-containing surrounding environments such as solvents, complex protein structures such as charge distribution, and thermal stability. The physical or chemical denaturation of an antibody causes loss of its physiological activities. Since the denaturation is an irreversible process, antibodies, once denatured, may not recover their native properties to

the initial state, leading to a reduction in their therapeutic efficacies. It has been also suggested that aggregation of monomers causes immune responses. Therefore, many studies have been conducted on antibody formulations containing a physiologically effective amount without aggregates (Ishikawa et al., Biol. Pharm. Bull., 33(8): 1413-1417, 2010; Levin et al., The Development of Therapeutic Monoclonal Antibody Products, editors. Boston (MA): BioProcess Technology Consultants, Inc, Chapter 9).

[5] There are many methods available for preventing protein denaturation in liquid formulations. In some protein drugs, the stability problems are addressed via lyophilization. For instance, U.S. Patent No. 7,592,004 discloses a lyophilized formulation of daclizumab that is stabilized using 5 to 25 mM of a histidine buffer solution (pH 5.5 to 6.5), 0.005 to 0.03% of a non-ionic surfactant, polysorbate and 100 to 300 mM of a non-reducing sugar, sucrose. U.S. Patent Publication No. 2010-0158925 discloses a lyophilized formulation of cetuximab that is stabilized using a histidine buffer solution and lactobionic acid. However, the lyophilization process generates freezing and drying stresses such as formation of ice crystals, pH change, and high concentration of solute, and these stresses may cause antibody denaturation. In addition, since a large-capacity lyophilizer is needed for the lyophilization process during the production, high production costs arise during a large scale of production. Dissolving the lyophilized product in sterile aqueous media for reconstitution before use also poses an inconvenience.

[6] As an alternative to solve these limitations, a stabilizer is added in liquid formulations for the improvement of antibody stability. Surfactants, serum albumins, polysaccharides, amino acids, polymers, salts or the like are known as stabilizers for proteins including antibodies (Wang, Int. J. Pharm., 185: 129-188, 1999; Wang et al., J. Pharm. Sci., 96(1): 1-26, 2007).

[7] US Patent Publication No. 2011-0070231 discloses a stable liquid composition of IgG antibody, in which the stable pharmaceutical liquid formulation includes 50 mg/mL or more of daclizumab in 20 to 60 mM of a succinate buffer solution (pH 5.5 to 6.5), 0.02 to 0.04% of polysorbate, and 75 to 150 mM of sodium chloride.

[8] US Patent Publication No. 2011-0020328 discloses a therapeutically effective amount of anti-CD20 antibody formulation, in which the pharmaceutically stable composition includes 10 to 100 mM of sodium acetate, 25 to 100 mM of sodium chloride, 0.5 to 5% of arginine free base, 0.02 to 0.2 mM of EDTA and 0.01 to 0.2% of polysorbate 80, and its pH is 5.0 to 7.0.

[9] US Patent No. 7,785,592 discloses a stable formulation, in which 75 mg/mL or more of palivizumab is stabilized by using a histidine buffer solution and glycine without ionic salts and a surfactant, and its stability is maintained at 2 to 8°C for at least 15 months.

[10] US Patent No. 6,991,790 discloses a stable aqueous pharmaceutical formulation, including a therapeutically effective amount of an antibody, an acetate buffer solution of approximately pH 4.8 to 5.5, a surfactant, and a polyol without an isotonic agent such as sodium chloride.

[11] US Patent No. 7,648,702 discloses a liquid formulation, in which a fusion protein of the human p75 tumor necrosis factor receptor linked to the Fc portion of the human immunoglobulin G1 (IgG1) is stabilized by using approximately 10 to 200 mM of L-arginine as an aggregation preventing agent. This patent is the only technique of using etanercept as an active ingredient.

[12] In order to prepare stable formulations, however, appropriate stabilizers should be used considering the physicochemical properties of each active ingredient. When the stabilizers are used in combination, competition therebetween and adverse effects may lead to undesirable effects. In addition, the concentrations of antibodies should be within the range suitable for the stabilization, and their concentrations are relatively higher than those of protein drugs. Thus, much effort and caution are required to stabilize antibodies in solutions (Shire et al., J. Pharm. Sci., 93(6): 1390-1402, 2004).

[13] There are few studies on the stable liquid formulations of etanercept, and the only method for stabilizing etanercept that the inventors are aware of is to use L-arginine in the liquid formulation. Therefore, there is an urgent need to develop a new liquid formulation which is able to stably maintain the activity of etanercept for a long period and which is more effective in etancercept stabilization than the known formulation comprising L-arginine.

[14]

Disclosure of Invention

Technical Problem

[15] The present inventors have made many efforts to develop a method for preparing a liquid formulation capable of stably maintaining the activity of etanercept. As a result, they found that one or more stabilizers selected from the group consisting of methionine, lysine, and histidine show remarkable effects on the stabilization of etanercept in a solution, thereby completing the present invention.

Solution to Problem

[16] An object of the present invention is to provide an aqueous formulation, comprising stabilizers for reducing formation of etanercept by-products during storage in a solution and maintaining its activity for a long period of time.

Advantageous Effects of Invention

[17] The liquid formulation according to the present invention can effectively prevent formation of etanercept by-products and stably maintain its pharmaceutical efficacies

for long-term storage. Therefore, the reconstitution procedure is not required before administration, and the sterile formulation can be administered to patients to ensure patient safety. Thus, it is expected to be applied to the fields in need of etanercept treatment.

Best Mode for Carrying out the Invention

[18] In one aspect to achieve the above object, the present invention provides a liquid formulation, comprising one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof.

[19] The formulation of the present invention may be a liquid formulation of etanercept comprising, etanercept; and one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof.

[20] The formulation shows increased storage stability by reducing etanercept by-products that are produced due to denaturation during storage.

[21] As a stabilizer, methionine, lysine, histidine, or pharmaceutically acceptable salts thereof may be present in the formulation in an amount of 0.1 to 250 mM.

[22] Etanercept in the formulation may be present in an amount of 1 to 100 mg/mL.

[23] The formulation may further include one or more materials seleted from the group consisting of a buffer, an isotonic agent, an excipient, and a preservative.

[24] The buffer may be selected from the group consisting of citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate, histidine, and Tris, and may be present in an amount of 0.1 to 100 mM.

[25] The isotonic agent may be selected from the group consisting of sodium chloride, potassium chloride, boric acid, sodium borate, mannitol, glycerin, propylene glycol, polyethylene glycol, maltose, sucrose, erythritol, arabitol, xylitol, sorbitol, and glucose, and may be present in an amount of 1 to 1000 mM.

[26] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[27] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[28] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[29] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[30] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[31] The formulation of etanercept according to the present invention may be a liquid formulation, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[32]

[33] Hereinafter, the present invention will be described in detail.

[34] The present invention has demonstrated for the first time that methionine, lysine, histidine and pharmaceutically acceptable salts thereof have the effects of reducing formation of etanercept by-products in solution and stably maintaining its activity for long-term storage, and thus suggests a novel use thereof as stabilizers of a pharmaceutically effective amount of etanercept.

[35] In order to prepare stable formulations, appropriate stabilizers should be used considering the physicochemical properties of each active ingredient. According to the difference in types, concentration, and combinations of materials included in a formulation, competition between the materials in the formulation can lead to undesirable effects. Thus, it is difficult to prepare a stable drug specific formulation.

[36] In the light of the above background, the present inventors have found that the liquid formulation comprising one or more stabilizers selected from the group consisting of methionine, lysine, and histidine, and pharmaceutically acceptable salts thereof increases storage stability of etanercept compared to the formulation having no stabilizers, by reducing etanercept by-products during storage in a solution.

[37] Specifically, in one embodiment of the present invention, in order to investigate the optimal stabilizer for the preparation of the stable liquid formulation of etanercept, a variety of amino acids, surfactants, and polymers are added to examine their stabilization effects. As a result, lysine, histidine, methionine, or the combination thereof was found to show remarkable effects on stabilizing etanercept by preventing its denaturation during storage in a solution at high temperature.

[38] Further, the formulation according to the present invention was found to show etanercept stabilizing effects which were equivalent to or higher than that of the formulation comprising L-arginine which is the known stable formulation of etanercept (US Patent No. 7,648,702). In particular, methionine was found to show the very excellent effect of stabilizing etanercept better than L-arginine.

[39] Specifically, etanercept by-products formed during storage were examined by Size Exclusion-HPLC (SE-HPLC). As a result, compared to the total amount of impurities of the etanercept formulation that was prepared by using L-arginine as the stabilizer in US Patent No. 7,648,702, the formulation prepared using lysine as the stabilizer showed a similar amount of impurities, and the formulation prepared using histidine or methionine as the stabilizer showed a lower amount of impurities (see Table 2). Hydrophobic Interaction-HPLC (HI-HPLC) analysis also showed similar results. These results indicate that histidine, lysine, and methionine prevent etanercept denaturation to inhibit formation of by-products thereof (see Table 3).

[40] Therefore, the liquid formulation according to the present invention can stably maintain pharmaceutical efficacies of etanercept for a long period of time by effectively reducing formation of etanercept by-products.

[41]

[42] As used herein, the term "etanercept (recombinant p75 sTNFR:Fc fusion protein)" refers to a protein which is a homodimer form of two Fc fusion proteins linked by disulfide bonds, each Fc fusion protein consisting of a human soluble 75 kilodalton (p75) TNF(tumor necrosis factor) receptor coupled to the Fc portion of human IgG1.

[43] Specifically, etanercept is a homodimer form of two Fc fusion proteins linked by 3 disulfide bonds, each Fc fusion protein consisting of the extracellular ligand-binding portion of the human soluble p75 TNF receptor linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. The etanercept may have a molecular weight of approximately 150 kilodaltons(kDa). This etanercept may be currently sold under the trade name ENBREL® (Amgen Inc., Thousand Oaks, CA.), and have CAS number 185243-69-0.

[44] Etanercept may be produced by recombinant DNA technology, but is not limited thereto.

[45] The etanercept of the present invention is a biological inflammation modulator that functions as a competitive inhibitor of TNF-α binding to cell surface TNF-α receptor, to inhibit TNF-α mediated immune responses, and is used to treat rheumatoid arthritis, psoriasis, and ankylosing spondylitis, and is under clinical trials for the treatment of vasculitis, Alzheimer's disease, and Crohn's disease.

[46] In the liquid formulation according to the present invention, the etanercept is

contained in a therapeutically effective amount. Preferably, etanercept is present in an amount of 1 to 100 mg/ml.

[47] As used herein, the term "stabilized liquid formulation" or "stable liquid formulation" refers to a formulation that retains the physical and chemical identity and integrity of the therapeutically active ingredient, etanercept, during storage in a solution. An analytical measurement of the etanercep stability may be performed by protein stability assay widely known in the art. The stability may be measured at a predetermined temperature for a predetermined time. For rapid assay, the formulation may be stored at a higher or "elevated" temperature, for example, 40°C for 2 weeks to 1 month or longer, and its time-dependent stability measured at this time.

[48] As used herein, the term "stabilizer" refers to a specific chemical that interacts with a biological molecule and/or a general pharmaceutical excipient in a formulation to improve its stability. The stabilizer generally protects proteins from air/solution interface-induced stress and solution/surface-induced stress which cause protein aggregation. In the present invention, the stabilizer is a component that reduces formation of etanercept by-products during storage in a solution to maintain its activity for a long period of time, and is preferably one or more selected from the group consisting of methionine, lysine, histidine and pharmaceutically acceptable salts thereof.

[49] As for the amino acids, both L-forms and D-forms are included in the scope of the present invention. Not only the amino acids themselves such as methionine, lysine, and histidine, but also anlogues, solvates, hydrates and stereoisomers, and the pharmaceutically acceptable salts thereof are within the scope of the present invention as long as they show substantially the same effect.

[50] As used herein, the term "pharmaceutically acceptable salts" refers to compounds that are prepared in the form of salts of the amino acids, such as methionine, lysine, and histidine, within the range retaining their functions as the stabilizers of the present invention. Specifically, it may form a salt by acid addition, and for example, it may form a salt with an inorganic acid (e.g., hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid, sulfuric acid, etc.), organic carboxylic acid (e.g., acetic acid, halo acetic acid such as trifluoroacetic acid, propionic acid, maleic acid, succinic acid, malic acid, citric acid, tartaric acid, salicylic acid), sugar acid (glucuronic acid, galacturonic acid, gluconic acid, ascorbic acid), acidic polysaccharide (e.g., hyaluronic acid, chondroitin sulfate, arginine acid), organic sulfonic acid including sulfonic acid sugar ester such as chondroitin sulfate (e.g., methane sulfonic acid, p-toluene sulfonic acid).

[51] As used herein, the term "analogues" refers to compounds that show the same functions as the amino acids, such as methionine, lysine, and histidine, used as stabilizers of the present invention. In the present invention, the amino acids such as me-

thionine, lysine, and histidine include the analogues of thereof. In the present invention, examples of the analogues may include methionine analogues, namely, selenomethionine, hydroxy methyl butanoic acid, ethionine, and trifluoromethionine, but are not limited thereto.

[52] As used herein, the term "by-products" refers to the undesirable products that reduce the ratio of the therapeutically active ingredient, etanercept, in the formulation. The typical by-products include "low molecular weight products" resulting from etanercept denaturation by deamination or hydrolysis, "high molecular weight products" such as oligomers and aggregates, or mixtures thereof.

[53] As used herein, the term "high molecular weight products" includes etanercept fragments that are subsequently aggregated by denaturation (e.g., produced by polypeptide degradation due to deamination or hydrolysis) or mixtures thereof. Typically, the high molecular weight products are complexes having higher molecular weights than the therapeutic monomer etanercept, and may have a molecular weight of more than approximately 150 kDa.

[54] As used herein, the term "low molecular weight products" includes, for example, therapeutic polypeptides produced by deamination or hydrolysis, namely, etanercept fragments. Typically, the low molecular weight products are complexes having lower molecular weights than the therapeutic monomer etanercept, and may have a molecular weight of less than approximately 150 kDa.

[55]

[56] The liquid formulation of the present invention includes one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof. Specifically, the liquid formulation comprises methionine, lysine, or histidine as a stabilizer, or two stabilizers such as methionine and lysine, methionine and histidine, or lysine and histidine, or three stabilizers such as methionine, lysine, and histidine. In the above formulation comprising combinations of the amino acids, the pharmaceutically acceptable salts of methionine, lysine, histidine respectively may be added further, or methionine, lysine, and histidine may be the modified forms of the pharmaceutically acceptable salts respectively. The liquid formulation comprises preferably methionine, lysine, histidine, methionine and lysine, methionine and histidine, or lysine and histidine, and more preferably methionine as a stabilizer.

[57] Methionine is an essential amino acid, and has a chemical formula of $C_5H_{11}NO_2S$ and a molecular weight of 149.21. Methionine is a non-polar amino acid, and contains a thioether group(-S-CH_3) in its side chain. It has a pK_1 value of 2.28, pK_2 value of 9.21, and isoelectronic point (PI) value of 5.74. Methionine prevents oxidation of antibodies in liquid formulations to stabilize antibodies by competing with the methionine

residues in antibodies for reaction with the free hydroxyl radicals (Lam et al., J. Pharm. Sci., 86(11): 1250-1255, 1997; Wang, Int. J. Pharm., 185: 129-188, 1999).

[58] Lysine is an essential amino acid, and has a chemical formula of $C_6H_{14}N_2O_2$ and a molecular weight of 146.19. Lysine is a basic amino acid, and has a pK_1 value of 2.18, pK_2 value of 8.95, pK_3 value of 10.53 and PI value of 9.74.

[59] Histidine is an essential amino acid with an imidazole functional group having positive charge in its side chain, and has a chemical formula of $C_6H_9N_3O_2$ and a molecular weight of 155.15. Histidine is a basic amino acid, and has a pK_1 value of 1.82, pK_2 value of 9.17, pK_3 value of 6.0 and PI value of 7.59.

[60] In contrast, L-arginine contained in the known commercially available formulation of etanercept is an essential amino acid, and has a chemical formula of $C_6H_{14}N_4O_2$ and a molecular weight of 174.2. Arginine is a basic amino acid, and has a pK_1 value of 2.17, pK_2 value of 9.04, pK_3 value of 12.48 and PI value of 10.76. That is, methionine, lysine, and histidine used as a stabilizer in the present invention are totally different from arginine as a known stabilizer for etanercept in construct, chemical formula, physicochemical properties, and ionization tendency. The present invention has demonstrated for the first time that amino acids such methionine, lysine, and histidine are the appropriate stabilizers for etanercept.

[61] The content of the stabilizer in the liquid formulation of the present invention is 0.1 to 250 mM, preferably 1 to 100 mM, and more preferably 5 to 50 mM.

[62] The liquid formulation of the present invention may further include any material which is generally contained in formulations of protein drugs or antibody drugs to increase etanercept stability according to one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof except for those deteriorating the function of the present invention. For example, L-arginine known as a stabilizer for etanercept may be added to the liquid formulation of the present invention.

[63] The liquid formulation according to the present invention may further include a buffer in addition to the stabilizer. As used herein, the term "buffer" refers to the component that improves isotonicity and chemical stability of the formulation, and functions to maintain physiologically suitable pH. The buffer prevents a rapid pH change of the liquid formulation to maintain pH of the solution for the stabilization of etanercept. Preferred examples of the buffer include citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate, histidine, and Tris, but are not limited thereto. In the specific embodiment, the buffer is a phosphate buffer. The buffer may be used either alone or in combinations of two or more thereof.

[64] In the various embodiments of the present invention, the formulation should have pH of approximately 5 to 7.5 or pH of approximately 5.8 to 6.8. In the specific em-

bodiment, the formulation has pH of approximately 6.0 to 6.6. The ranges from the intermediate to the above mentioned pH levels, for example, approximately pH 5.2 to approximately pH 6.4 (e.g., pH 6.2) are also intended to be part of this invention. For example, ranges of values using a combination of any of the above mentioned values as upper and/or lower limits are intended to be included. If necessary, the pH may be adjusted by techniques known in the art. For example, the pH may be adjusted to any desirable range by addition of HCl or by addition of histidine, if necessary.

[65] In another embodiment, the buffer is present at a concentration of 0.1 to 100 mM, preferably 1 to 50 mM, and more preferably 5 to 25 mM. The ranges from the intermediate to the above mentioned concentrations are also intended to be part of this invention. For example, ranges of concentrations using a combination of any of the above mentioned concentrations as upper and/or lower limits are intended to be included. In the specific embodiment, the buffer should be present in an amount sufficient to maintain the physiologically sufficient pH.

[66] The liquid formulation according to the present invention may further include an isotonic agent in addition to the stabilizer. As used herein, the term "isotonic agent" refers to a component that functions to partially maintain isotonicity of the formulation and the protein level, and partially maintain the level, ratio, or proportion of the therapeutically active polypeptide present in the formulation. The isotonic agent possesses the same osmotic pressure as blood plasma, and so can be intravenously infused into a subject without changing the osmotic pressure of the subject's blood plasma. Indeed, in one embodiment according to the present invention, isotonic agent is present in an amount sufficient to render the formulation suitable for intravenous infusion. Often, the isotonic agent serves as a bulking agent as well. As such, the isotonic agent may allow the protein to overcome various stresses such as freezing and shear.

[67] The isotonic agent serves to maintain the proper osmotic pressure in the body, when etanercept in the solution is administered into the body. Examples of the isotonic agent may include the commonly used sodium chloride, potassium chloride, boric acid, sodium borate, mannitol, glycerin, propylene glycol, polyethylene glycol, maltose, sucrose, erythritol, arabitol, xylitol, sorbitol, and glucose, but is not limited thereto. In the specific embodiment, the isotonic agent is NaCl. These isotonic agents may be used either alone or in combinations of two or more thereof.

[68] In still another embodiment, the isotonic agent (e.g., NaCl) is present at a concentration of 1 to 1000 mM, preferably 10 to 500 mM, and more preferably 50 to 250 mM. The ranges from the intermediate to the above mentioned concentrations are also intended to be part of this invention. For example, ranges of concentrations using a combination of any of the above mentioned concentrations as upper and/or lower limits are intended to be included. The isotonic agent should be present in an amount

sufficient to maintain osmosis of the formulation.

[69] The liquid formulation according to the present invention may further include a pharmaceutically acceptable excipient, and examples of the excipient may include sugars and polyols, surfactants, polymers or the like. Examples of the sugars and polyols may include sucrose, trehalose, lactose, maltose, galactose, mannitol, sorbitol, glycerol, examples of the surfactants may include non-ionic surfactants such as polysorbate 20, polysorbate 80, and poloxamer, and examples of the polymers may include dextran, polyethylene glycol, carboxyl methylcellulose, hyaluronic acid, and cyclodextrin.

[70] The liquid formulation according to the present invention may further include a preservative. The preservative means a chemical that is added to pharmaceutical formulations as an antimicrobial agent. Examples of the preservative may include benzalkonium chloride, benzethonium, chlorhexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chloro-cresol, phenylmercuric nitrate, thimerosal, and benzoic acid, but are not limited thereto. These preservatives may be used either alone or in combinations of two or more thereof.

[71] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 100 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[72] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 100 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[73] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 100 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[74] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or

pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 1 to 50 mM of histidine or pharmaceutically acceptable salts thereof, 1 to 100 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[75] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 1 to 50 mM of histidine or pharmaceutically acceptable salts thereof, 1 to 100 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[76] In the specific embodiment, the formulation of the present invention is a stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride. More specifically, the formulation of the present invention is the stable liquid formulation comprising 1 to 100 mg/mL of etanercept, 1 to 50 mM of methionine or pharmaceutically acceptable salts thereof, 1 to 50 mM of lysinee or pharmaceutically acceptable salts thereof, 0.1 to 50 mM of phosphate buffer, and 1 to 500 mM of sodium chloride, at pH 6.0 to 6.6.

[77] The formulation of the present invention can be used for treatment of a disease in which etanercept is therapeutically effective. Etanercept is a biological inflammation modulator to inhibit TNF-α mediated immune responses, and the formulation of the present invention is used to treat rheumatoid arthritis, psoriasis, ankylosing spondylitis, vasculitis, Alzheimer's disease, or Crohn's disease, not limited thereto. The formulation of the present invention may be administered by oral or parenteral, i.e., subcutanelusly, intramuscularly, ntraperitoneail, intra-abdominal, transdermal route, and/or intravenously, but is not limited thereto.

[78]

[79] In another aspect, the present invention provides a method for incresing stability of etanercept using a liquid formulation comprising one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof.

[80] The liquid formulation can increase storage stability of etanercept by reducing etanercept by-products that are produced due to denaturation during storage.

[81]

Mode for the Invention

[82] Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are for illustrative purposes only, and the invention is not intended to be limited by these Examples.

[83]

[84] **<Example 1> Stability test of etanercept aqueous formulation according to addition of stabilizer**

[85] In order to investigate the optimal stabilizer for the preparation of the stable aqueous formulation of etanercept, histidine as a stabilizer was added to a 5 mM phosphate solution to which etanercept was added to a concentration of 50 mg/mL, and sodium chloride was added thereto as an isotonic agentto prepare Formulation 1.

[86] In addition, etanercept was added to a 10 mM phosphate solution to a concentration of 50 mg/mL, and sodium chloride was added thereto as an isotonic agent. Then, each of lysine, arginine, methionine, glycine, polysorbate 20, polysorbate 80, poloxamer 188, propylene glycol, protamine sulfate, sucrose, and guanidine HCl was added to prepare Formulations 2 to 12.

[87] Finally, sodium chloride as the isotonic agent was added to a 10 mM phosphate solution to which etanercept was added to a concentration of 50 mg/mL to prepare a stabilizer-free formulation. The stabilizer compositions of the formulations are shown in the following Table 1. Each 0.5 mL of the prepared formulation was put in 1.0 mL glass syringes, sealed, and stored at 50°C.

[88] Table 1

[Table 1]

	Stabilizer composition
Formulation 1	Histidine 10 mM
Formulation 2	Lysine 25 mM
Formulation 3	Arginine 25 mM
Formulation 4	Methionine 25 mM
Formulation 5	Glycine 1%
Formulation 6	Tween 20 0.1%
Formulation 7	Tween 80 0.1%
Formulation 8	F68 0.1%
Formulation 9	Propylene glycol 0.1%
Formulation 10	Protamine sulfate 0.03%
Formulation 11	Sucrose 2%
Formulation 12	Guanidine HCl 0.2%
stabilizer-free formulation	-

[89]

[90] At 7 days after storage at 50°C, Formulations 1 to 12 and the stabilizer-free formulation of Table 1 were analyzed by SE-HPLC to examine the types of low molecular weight products and high molecular weight products such as oligomers and aggregates resulting from etanercept denaturation, and the total amount of these products was represented as Total Impurity. In addition, structural changes of etanercept were examined by HI-HPLC. In HI-HPLC, etanercept-related substances were separated into 4 peaks, including pre-Peak, Peak 1, Peak 2, and Peak 3. Pre-Peak and Peak 1 represent low molecular weight products, Peak 2 represents etanercept, and Peak 3 represents dimers with low aggregation or activity. Thus, the total amount of pre-Peak, Peak 1, and Peak 3 was represented as Total Impurity. The results are shown in Tables 2 and 3, respectively.

[91] Table 2

[Table 2]

	Total Impurity by SE-HPLC (%)	
	Day 0	Day 7
Formulation 1	4.6	22.2
Formulation 2	4.7	24.8
Formulation 3	4.6	24.2
Formulation 4	4.6	20.8
Formulation 5	4.6	28.5
Formulation 6	4.7	29.4
Formulation 7	4.6	29.0
Formulation 8	4.6	28.8
Formulation 9	4.7	27.8
Formulation 10	4.6	29.4
Formulation 11	4.8	28.4
Formulation 12	4.7	27.5
stabilizer-free formulation	4.7	30.2

[92] Table 3

[Table 3]

	Total Impurity by HI-HPLC (%)	
	Day 0	Day 7
Formulation 1	14.7	33.7
Formulation 2	14.6	34.9
Formulation 3	15.0	34.3
Formulation 4	15.2	30.3
Formulation 5	14.8	36.6
Formulation 6	15.2	35.5
Formulation 7	15.2	36.2
Formulation 8	15.4	35.5
Formulation 9	15.2	35.1
Formulation 10	15.0	36.9
Formulation 11	14.9	35.1
Formulation 12	15.1	34.8
stabilizer-free formulation	14.4	41.9

[93]

[94] As shown in the SE-HPLC results of Table 2, when stored at 50°C for 7 days, Formulation 1 containing histidine as a stabilizer showed total impurity of 22.2%, Formulation 2 containing lysine as a stabilizer showed total impurity of 24.8%, Formulation 3 containing arginine as a stabilizer showed total impurity of 24.2%, Formulation 4 containing methionine as a stabilizer showed total impurity of 20.8%. All of them showed remarkably low Total Impurity, compared to stabilizer-free formulation showing Total Impurity of 30.2%. When these formulations were compared to Formulation 3 using L-arginine as a stabilizer (US Patent No. 7,648,702), Formulation 2 containing lysine showed similar total impurity of 24.8%, and Formulation 1 containing histidine and Formulation 4 containing methionine showed reduced Total Impurity of 22.2% and 20.8%, respectively.

[95] The HI-HPLC results of Table 3 are similar to the SE-HPLC results. The stabilizer-free formulation showed Total Impurity of 41.9%. On the contrary, each of Formulations 1, 2, 3, and 4 showed Total Impurity of 33.7%, 34.9%, 34.3%, and 30.3% respectively, indicating that histidine, lysine, L-arginine, and methionine added in each formulation have the effects of preventing etanercept denaturation. In particular, Formulation 4 containing methionine showed the lowest Total Impurity, indicating that

methionine is most effective in etanercept stabilization.

[96] Consequently, the present invention demonstrated that lysine, histidine, and methionine have effects of stabilizing etanercept by preventing its denaturation, which are equivalent to or higher than that of L-arginine, and in particular, methionine is more effective in etanercept stabilization than L-arginine.

[97]

[98] **<Example 2> Stability test of etanercept aqueous formulation according to methionine concentration**

[99] In order to investigate the optimal concentration of methionine for the stabilization of etanercept, 120 mM sodium chloride was added to 10 mM phosphate solutions, and each of 25 mM and 12.5 mM methionine was added thereto, after which etanercept in a concentration of 50 mg/mL so was added as to prepare Formulations 13 and 14, respectively. According to the preparation of a commercially available etanercept aqueous formulation, 25 mM L-arginine was added to 100 mM sodium chloride and 1% sucrose in a 10 mM phosphate solution, and etanercept was added to a concentration of 50 mg/mL so as to prepare a control. Each 0.5 mL of the formulation was put in 1.0 mL glass syringes, sealed, and stored at 40°C, 25°C and 4°C. The compositions of the prepared aqueous formulations are shown in the following Table 4.

[100] Table 4

[Table 4]

	Composition
Formulation 13	Etanercept 50 mg/mL, phosphate solution 10 mM, NaCl 120 mM, methionine 25 mM (pH 6.3)
Formulation 14	Etanercept 50 mg/mL, phosphate solution 10 mM, NaCl 120 mM, methionine 12.5 mM (pH 6.3)
Control	Etanercept 50 mg/mL, phosphate solution 25 mM, NaCl 100 mM, sucrose 1%, L-arginine 25 mM (pH 6.3)

[101] After Formulation 13, Formulation 14, and the control were stored at 40°C for 1 and 3 weeks, and at 25°C and 4°C for 4 and 8 weeks each, their Total Impurity was measured by SE-HPLC and HI-HPLC, as in Example 1. The results are shown in Tables 5 and 6, respectively.

[102] Table 5

[Table 5]

	Total Impurity by SE-HPLC (%)								
	40℃			25℃			4℃		
	0 week	1 week	3 weeks	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks
Formulation 13	5.5	11.5	20.5	5.5	14.8	18.8	5.5	8.8	10.2
Formulation 14	5.5	12.5	22.0	5.5	16.0	19.9	5.5	8.9	10.5
Control	5.5	13.4	23.3	5.5	16.2	20.4	5.5	8.8	10.3

[103] Table 6

[Table 6]

	Total Impurity by HI-HPLC (%)								
	40℃			25℃			4℃		
	0 week	1 week	3 weeks	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks
Formulation 13	10.0	15.9	21.6	10.0	15.3	18.5	10.0	11.0	11.9
Formulation 14	9.9	17.0	23.3	9.9	16.7	19.8	9.9	11.3	11.6
Control	10.2	18.0	25.5	10.2	17.4	20.6	10.2	10.0	11.9

[104]

[105] As shown in the results of Tables 5 and 6, Formulation 13 and Formulation 14 containing methionine as the stabilizer showed low impurity production under all of the storage conditions of 40℃, 25℃ and 4℃, compared to the control group prepared according to the preparation method of a commercially available etanercept aqueous formulation. Specifically, the SE-HPLC and HI-HPLC results showed that Formulation 13 containing 25 mM methionine and Formulation 14 containing 12.5 mM methionine showed lower impurity production at 40℃ and 3 weeks, and at 25℃ and 8 weeks, and similar impurity production at 4℃ and 8 weeks, compared to the control. These results indicate that the methionine-containing formulation of the present invention is a more stable formulation for maintaining the etanercept activity for a long

period of time than the commercially available formulation.

[106]

[107] In conclusion, the present invention demonstrated that the etanercept aqueous formulation containing methionine prevents denaturation of etanercept to maintain its activity for a long period of time, and methionine is very effective as a stabilizer in the etanercept aqueous formulation.

[108]

[109] **<Example 3> Stability test of etanercept aqueous formulation comprising histidine and lysine, or histidine and methionine**

[110] Histidine 5mM and lysine 25mM as a stabilizer were added to a 10 mM phosphate solution to which etanercept was added to a concentration of 50 mg/mL, and sodium chloride was added thereto as an isotonic agent to prepare Formulation 15.

[111] In addition, Histidine 5mM and methionine 25mM as a stabilizer were added to a 10 mM phosphate solution to which etanercept was added to a concentration of 50 mg/mL, and sodium chloride was added thereto as an isotonic agentto prepare Formulation 16.

[112] Finally, sodium chloride as the isotonic agent was added to a 10 mM phosphate solution and etanerceptwas added thereto to a concentration of 50 mg/mL to prepare a stabilizer-free formulation.

[113] Each 0.5 mL of the prepared formulation was put in 1.0 mL glass syringes, sealed, and stored at 50°C.

[114] The stabilizer compositions of the formulations are shown in the following Table 7.

[115] Table 7

[Table 7]

	Stabilizer composition
Formulation 15	Histidine 5mM + Lysine 25mM
Formulation 16	Histidine 5mM + Methionine 25mM
stabilizer-free formulation	-

[116]

[117] At 7 days after storage at 50°C, Formulations 15, 16 and stabilizer-free formulation of Table 7 were analyzed by SE-HPLC as example 1 to measure their Total Impurity. The results are shown in Tables 8.

[118] Table 8

[Table 8]

	SE-HPLC Total Impurity (%)	
	0 day	7 days
Formulation 15	4.7	21.5
Formulation 16	4.7	20.9
stabilizer-free formulation	4.7	30.2

[119]

[120] As shown in the SE-HPLC results of Table 8, when stored at 50℃ for 7 days, Formulation 16 containing histidine as a stabilizer showed total impurity of 21.5%, and showed remarkably low Total Impurity, compared to the stabilizer-free formulation showing Total Impurity of 30.2%. And Formulation 16 showed remarkably low Total Impurity, compared to Formulation 1 containing 10mM histidine showing Total Impurity of 22.2%, and Formulation 2 containing lysine 25mM showing Total Impurity of 24.8%.

[121] That is, the present invention demonstrated that the formulation comprising histidine and lysine has the remarkably excellent effects of preventing etanercept denaturation by reducing the amount of total impurity for storage, compared to the stabilizer-free formulation and each formulation comprising histidine, lysine, and methionine respectively.

[122] In addition, as shown in the SE-HPLC results of Table 8, when stored at 50℃ for 7 days, Formulation 16 containing histidine as a stabilizer showed Total Impurity of 20.9%, which is remarkably low, compared to the stabilizer-free formulation showing Total Impurity of 30.2%. In particular, Formulation 16 showed low Total Impurity compared to Formulation 15 containing histidine and lysine as a stabilizer.

[123] That is, the present invention demonstrated that the formulation comprising histidine and methionine has remarkably effects on the preventing of etanercept denaturation by reducing the amount of Total Impurity for storage, compared to the stabilizer-free formulation.

[124]

[125] **<Example 4> Stability test of etanercept aqueous formulation comprising methionine and lysine**

[126] Methionine 12.5mM and lysine 12.5mM as a stabilizer were added to a 10 mM phosphate solution to which etanercept was added to a concentration of 50 mg/mL, and sodium chloride was added thereto as an isotonic agent to prepare Formulation 17.

[127] In addition, sodium chloride as the isotonic agent was added to a 10 mM phosphate solution and etanercept was added thereto to a concentration of 50 mg/mL to prepare a

stabilizer-free formulation.

[128] Each 0.5 mL of the prepared formulation was put in 1.0 mL glass syringes, sealed, and stored at 50℃. The stabilizer compositions of the formulations are shown in the following Table 9.

[129] Table 9

[Table 9]

	Stabilizer composition
Formulation 17	Methionine 12.5mM +Lysine 12.5mM
stabilizer-free formulation	-

[130]

[131] At 7 days after storage at 50℃, Formulations 17, and stabilizer-free formulation of Table 9 were analyzed by SE-HPLC as example 1 to measure their Total Impurity. The results are shown in Table 10.

[132] Table 10

[Table 10]

	SE-HPLC Total Impurity (%)	
	0 day	7 dyas
Formulation 17	5.2	27.0
stabilizer-free formulation	5.2	32.8

[133]

[134] As shown in the SE-HPLC results of Table 10, when stored at 50℃ for 7 days, Formulation 17 containing methionine and lysine as a stabilizer showed Total Impurity of 27%, which is remarkably low, compared to the stabilizer-free formulation showing Total Impurity of 32.8%.

[135] That is, the present invention demonstrated that the formulation comprising methionine and lysine has remarkably effects on the stabilizing of etanercept by reducing the amount of Total Impurity, compared to the stabilizer-free formulation.

[136]

Claims

[Claim 1] A liquid formulation of etanercept, comprising etanercept; and one or more stabilizers selected from the group consisting of methionine, lysine, histidine, and pharmaceutically acceptable salts thereof.

[Claim 2] The liquid formulation according to claim 1, wherein the stabilizer is methionine and/or pharmaceutically acceptable salts thereof.

[Claim 3] The liquid formulation according to claim 1, wherein the stabilizer is present in an amount of 0.1 to 250 mM.

[Claim 4] The liquid formulation according to claim 1, wherein the etanercept is present in an amount of 1 to 100 mg/mL.

[Claim 5] The liquid formulation according to claim 1, which have increased storage stability of etanercept compared to the formulation having no stabilizers, by reducing etanercept by-products that are produced due to denaturation during storage.

[Claim 6] The liquid formulation according to claim 1, which further comprises one or more materials selected from the group consisting of a buffer, an isotonic agent, an excipient, or a preservative.

[Claim 7] The liquid formulation according to claim 6, wherein the buffer is selected from the group consisting of citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate, histidine, and Tris.

[Claim 8] The liquid formulation according to claim 6, wherein the buffer is present in an amount of 0.1 to 100 mM.

[Claim 9] The liquid formulation according to claim 6, wherein the isotonic agent is selected from the group consisting of sodium chloride, potassium chloride, boric acid, sodium borate, mannitol, glycerin, propylene glycol, polyethylene glycol, maltose, sucrose, erythritol, arabitol, xylitol, sorbitol, and glucose.

[Claim 10] The liquid formulation according to claim 6, wherein the isotonic agent is present in an amount of 1 to 1000 mM.

[Claim 11] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[Claim 12] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to

1000 mM of sodium chloride.

[Claim 13] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[Claim 14] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[Claim 15] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of histidine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

[Claim 16] The liquid formulation according to claim 1, comprising 1 to 100 mg/mL of etanercept, 0.1 to 250 mM of methionine or pharmaceutically acceptable salts thereof, 0.1 to 250 mM of lysine or pharmaceutically acceptable salts thereof, 0.1 to 100 mM of phosphate buffer, and 1 to 1000 mM of sodium chloride.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 9/08; A61K 39/395; A61K 39/40; A61P 35/00; A61K 45/05; C07K 16/24

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: antibody, formulation, liquid, etanercept, methionine, stability, histidine, lysine, amino acid, buffer, isotonic agent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010-0285011 A1 (T. MOECHIHKA et al.) 11 November 2010 See abstract, paragraphs [0003], [0005], [0027], [0055], [0061]-[0063], [0070], [0075], [0076], examples 1, 3 and all claims.	1-16
Y	US 2006-0292148 A1 (T. MATSUMOTO) 28 December 2006 See abstract, paragraphs [0021], [0025], [0055], [0056] and all claims.	1-16
Y	US 7785592 B2 (C. N. OLIVER et al.) 31 August 2010 See abstract, line 59 in column 1-line 39 in column 5 and all claims.	1-16
A	US 2011-0070231 A1 (E. A. KAISHEVA et al.) 24 March 2011 See the whole document.	1-16
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A	US 05358708 A (S. T. PATEL) 25 October 1994 See the whole document.	1-16

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

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 OCTOBER 2012 (26.10.2012)	**29 OCTOBER 2012 (29.10.2012)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2012/004369

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International application No.
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99

PATENT COOPERATION TREATY PCT/KR2012/004369

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Date of mailing *(day/month/year)* 27 June 2012 (27.06.2012)	
Applicant's or agent's file reference OPA12055PCT	IMPORTANT NOTIFICATION
International application No. PCT/KR2012/004369	International filing date *(day/month/year)* 01 June 2012 (01.06.2012)
International publication date *(day/month/year)* **Not yet published**	Priority date *(day/month/year)* 03 June 2011 (03.06.2011)
Applicant LG LIFE SCIENCES LTD. et al	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
03 June 2011 (03.06.2011)	10-2011-0053890	KR	08 June 2012 (08.06.2012)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*). Where the applicant has failed to either submit, request to prepare and transmit or obtain and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

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RECIBO DE CA	999999999	122925	29/11/2013		4.000.00
CONSIGNACION	BANCO DE BOGOTA	062754387	599240972	28/11/2013	8.873.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCION	500.000.00	500.000.00
				$500.000.00

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SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*********-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-282255- -00000-0000

Fecha: 2013-12-02 15:16:57 Dep. 2020 DIR.NUEVASCREA(
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 101

101

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TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
RECIBO DE CA	999999999	122925	29/11/2013		4.000.00
CONSIGNACION	BANCO DE BOGOTA	062754387	599240972	28/11/2013	8.873.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
6	50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	30.000.00	180.000.00
				$180.000.00

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SON: **CIENTO OCHENTA MIL PESOS MONEDA CORRIENTE***

Responsable: _____
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PATENTE DE INVENCIÓN ☐ MODELO DE UTIL

- ☐ Indicación que se solicita una patente.
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- ☐ Descripción de la invención
- ☐ Dibujos de ser estos pertinentes
- ☐ Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)

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