(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 315 966 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
03.10.2007 Bulletin 2007/40

(21) Application number: **01968093.3**

(22) Date of filing: **24.08.2001**

(51) Int Cl.:
G01N 33/535 *(2006.01)* ***G01N 33/545*** *(2006.01)*
C12Q 1/37 *(2006.01)* ***G01N 33/573*** *(2006.01)*

(86) International application number:
PCT/US2001/026373

(87) International publication number:
WO 2002/021129 (14.03.2002 Gazette 2002/11)

(54) **METHOD OF MONITORING THE EFFECT OF CATHEPSIN S INHIBITORS**

VERFAHREN ZUM ÜBERWACHEN DES EFFEKTS VON KATHEPSIN-S-INHIBITOREN

PROCEDE RELATIF AU CONTROLE DE L'EFFET D'INHIBITEURS DE CATHEPSINE S

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **06.09.2000 US 230484 P**

(43) Date of publication of application:
04.06.2003 Bulletin 2003/23

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to a method for monitoring the effect of in vivo administration of Cathepsin S inhibitors by measuring accumulation of an intermediate degradation product of invariant chain (Ii), in particular the p10 Ii fragment, in blood of dosed subjects.

BACKGROUND OF THE INVENTION

[0002] The recognition of antigen-presenting MHC class II molecules by $CD4^+$ T cells is a crucial component of the immunological response. Class II molecules, like other transmembrane proteins, are translocated into the endoplasmic reticulum after synthesis, where they associate with a third protein, the invariant chain (Ii): This molecule is a type II transmembrane protein that serves as a class II-specific chaperone which promotes the exit of class II-Ii complexes from the endoplasmic reticulum and prevents class II molecules from binding peptides and unfolded proteins in the endoplasmic reticulum and in the secretory pathway.

[0003] A targeting motif in the cytoplasmic tail of Ii directs the complexes from the secretory pathway into the endosomal system. Before the MHC class II molecules can present antigen the Ii must be removed. This is accomplished by a series of proteases that break Ii down into small peptides. However, an Ii fragment, called class II-associated invariant chain peptide (CLIP), which occupies the peptide-binding groove of the class II molecule, is in most cases not spontaneously released. The CLIP fragment serves as a substitute peptide that protects the class II binding pocket from collapsing both during intracellular transport and after Ii degradation in the endosomal system. Binding of antigenic peptides, generated from endocytosed proteins, requires an empty, yet open binding site, and therefore CLIP has to be released while the open binding site needs to be stabilized to allow the binding of other peptides. Human Leukocyte Antigen DM ('HLA-DM') has been well documented to mediate both of these functions, thus promoting the binding of antigenic peptides. After acquiring peptides, the class II molecules are transported to the cell surface via routes that are largely unknown.

[0004] Blocking the presentation of antigens is a promising way to inhibit the immune response. This could be done by disrupting the uptake, the proteolytic processing, or binding to MHC class II molecules. Blocking the uptake may be problematic since many different cell types require this function. Inhibition of the proteolytic processing of particular antigens may be of use since different proteases may be involved in cleaving different antigens, however these proteases are not specific and may lead to other side-effects. One way to specifically block the binding to the antigens to the MHC class II is to inhibit the proteolysis of the invariant chain. If this is not removed then the MHC class II molecules cannot be loaded with peptides, hence blocking Ii degradation would decrease antigen presentation to CD4+ T-cells and disrupt the normal immune response.

[0005] Cathepsin S (CatS) is a cysteine protease expressed in lymphatic tissues. It is has been identified as playing a major role in invariant chain proteolysis which is a prerequisite for peptide loading of MHC class II (Riese et al. (1996) Immunity 4:357). It has 50-60% identity to cathepsins L and K, but differs in that it has a broad pH optimum that extends to alkaline pH. Inhibitors have been shown in animal models to modulate antigen presentation and are effective in an asthma model (Riese et al., J. Clin. Invest. (1998) 101:2351). Mice deficient in cathepsin S have an impaired ability to present exogenous proteins by professional antigen presenting cells (Nakagawa et al., Immunity (1999) 10:207; Shi et al., Immunity (1999) 10:197).

[0006] Villadangos et al, J. Exp. Med. (1997) 186(4): 549 discloses the analysis of cathepsin S activity on MHC class II-associated Ii (invariant chain) degradation based on monitoring the effect of the cathepsin S inhibitor LVHS in which mouse splenocytes were pulsed-chased in the presence of LVHS and their MHC class II molecules were immunoprecipitated. Figures 1 and 2 show that the treatment with LVHS resulted in accumulation of LIP10.

[0007] The same method performed in spleen cells is described in Riese et al, J. Clin. Invest (1998) 101(11): 2351 which demonstrates that the LVHS cathepsin S inhibitor disrupts Ii processing.

[0008] Compounds that inhibit the proteolytic activity of human cathepsin S are expected to find utility in the treatment of chronic autoimmune diseases including, but not limited to, lupus, rheumatoid arthritis, and asthma; and have potential utility in modulating the immune response to tissue transplantation.

[0009] Methods of modulating autoimmunity with an agent that modulates cathepsin S activity, e.g. proteolysis of the Ii chain, as well as methods of treating a subject having an autoimmune disorder, methods of evaluating a treatment for its ability to modulate an immune response are described in WO 99/58153.

SUMMARY OF THE INVENTION

[0010] The present invention concerns a method of or an assay for monitoring the effect of in vivo administration of a cathepsin S inhibitor, said method or assay comprising:

(a) taking a blood sample of the subject treated;
(b) purifying the white blood cells from said sample;
(c) making whole cell lysates of the purified white blood cells;
(d) analyzing the lysates for presence of an intermediate degradation product of invariant chain (Ii) p10I ; by a suitable assay method.

[0011] Suitable assay methods comprise for example Western blot assay or ELISA assay methods.

DETAILED DESCRIPTION OF THE INVENTION

[0012] Thus the present invention provides a method for monitoring the effect of in vivo administration of Cathepsin S inhibitors, by measuring accumulation of an intermediate degradation product of invariant chain (Ii), i.e. the p10Ii fragment, in blood of dosed subjects.

[0013] The said assay of the present invention is particularly useful in a clinical trial setting. However it canalso be applied to monitor the effect of Cathepsin S inhibitors in vivo in animal studies, which include but are not limited to monkey, dog, pig, rabbit, guinea pig, and rodents.

[0014] The effect of in vivo administration of Cathepsin S inhibitors, in a clinical trial setting, can be monitored by measuring accumulation of an intermediate degradation product of invariant chain (Ii), i.e. the p10Ii fragment, in blood of dosed subjects.

[0015] Briefly, after administration of Cathepsin inhibitors for a certain period of time, preferably 16-30hr, blood is drawn and white blood cells are purified, e.g. either by lysis of red blood cells or by a FICOLL gradient centrifugation. Whole cell lysates of WBC are then made and then analyzed by either a Western blot assay or a ELISA assay. For Western assay, cell lysates are first resolved on PAGE gels. After transferring to nitrocellulose membrane, Ii and its intermediate degradation products, including the p10Ii, can then be detected using a mouse mAb against Ii, e.g. Pin1.1, or a rabbit polyclonal antibody against the entire Ii or a peptide fragment. For ELISA assay, a pair of antibodies against Ii, including Pin1.1 and a rabbit polyclonal antibody against C-terminal of p10Ii, can be used.

[0016] The said assay can also be applied to monitor effect of Cathepsin S inhibitors in vivo in animal studies, which include but not limit to monkey, dog, pig, rabbit, guinea pig and rodents.

[0017] The advantage of the invention is that the method is simple and considered more reliable in determining in vivo efficacy of Cathepsin inhibitor compounds.

[0018] The method can be used to assay the efficacy of Cathepsin inhibitory compounds not only in an experimental screening or clinical setting. It is also useful to monitor patients who have been treated with Cathepsin inhibitors to check efficacy of their treatment and to adjust dosing where necessary. This will allow the prescribing or supervising physician to more precisely and effectively dose the desired cathepsin S inhibitory drug regimen.

EXAMPLE 1.

MONITORING CATHEPSIN S INHIBITION IN HUMAN BLOOD

[0019] The effect of in vivo administration of Cathepsin S inhibitors, in a clinical trial setting, can be monitored by measuring accumulation of an intermediate degradation product of invariant chain (Ii), i.e. the p10Ii fragment, in blood of dosed subjects. Briefly, after administration of Cathepsin inhibitors for a certain period of time, preferably 16-30hr, blood is drawn and white blood cells are purified, e.g. either by lysis of red blood cells or by a FICOLL gradient centrifugation. Whole cell lysates of WBC are then made and analyzed by either a Western blot assay or an ELISA assay. For Western assay, cell lysates are first resolved on SDS-PAGE gels. After transferring to nitrocellulose membrane, Ii and its intermediate degradation products, including the p10Ii, can then be detected using a mouse mAb against Ii , e.g. Pin1.1 or rabbit polyclonal antibodies specific for the C-terminal of the p10Ii fragment. For ELISA assay, a pair of antibodies against Ii, including Pin1.1 and a rabbit polyclonal antibody against C-terminal of p10Ii, can be used. The same assay can also be applied to monitor effect of Cathepsin S inhibitors in vivo in animal studies, for example in monkeys, dogs, pigs, rabbits, guinea pigs, and rodents. In the present example purified peripheral blood mononuclear cells from human blood were incubated with the cathepsin S inhibitor, LHVS (morpholinurea-leucine-homophenylalanine-vinylsulfonephenyl, also referred to as 4-morpholinecarboxamide, N-[(1S)-3-methyl-1-[[[(1S,2E)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propenyl]amino] carbonyl]butyl]- (9CI)). This compound has been described in US patent no. 5,976,858 and in Palmer et al. (1995) J. Med. Chem. 38:3193 and Riese et al. (1996) Immunity 4:357. After incubation for 24 hours, the samples were run using standard SDS-PAGE protocols, transferred to nitrocellulose membranes and probed with an antibody which recognizes the invariant chain including the p10Ii fragment. In the presence of LHVS the p10Ii fragment can easily be seen (**Figure 1**). This represents a block in the degradation of Ii due to inhibition of cathepsin S.

Claims

1. A method of monitoring the effect of *in vivo* administration of a cathepsin S inhibitor to a subject, said method comprising the steps of:

   a) purifying white blood cells from a blood sample of the subject;
   b) making whole cell lysates of the purified white blood cells; and
   c) analyzing the lysates for presence of a p10Ii fragment of invariant chain (Ii).

2. A method according to claim 1 wherein the analyzing in step c) is by Western blotting or ELISA.

3. A method according to claim 1 wherein the subject is a human.

Patentansprüche

1. Verfahren zum Überwachen des Effektes einer in-vivo-Verabreichung eines Kathepsin S-Inhibitors an ein Subjekt, wobei das Verfahren die Schritte umfaßt:

   a) Reinigen weißer Blutzellen aus einer Blutprobe des Subjekts;
   b) Herstellen von Vollzelllysaten der gereinigten weißen Blutzellen; und
   c) Analysieren der Lysate bezüglich der Gegenwart eines p10li-Fragments einer unveränderlichen Kette (li).

2. Verfahren nach Anspruch 1, wobei das Analysieren in Schritt c) durch Westem-Blotting oder ELISA ist.

3. Verfahren nach Anspruch 1, wobei das Subjekt ein Mensch ist.

Revendications

1. Méthode de surveillance de l'effet de l'administration *in vivo* d'un inhibiteur de la cathepsine S à un sujet, ladite méthode comprenant les étapes de :

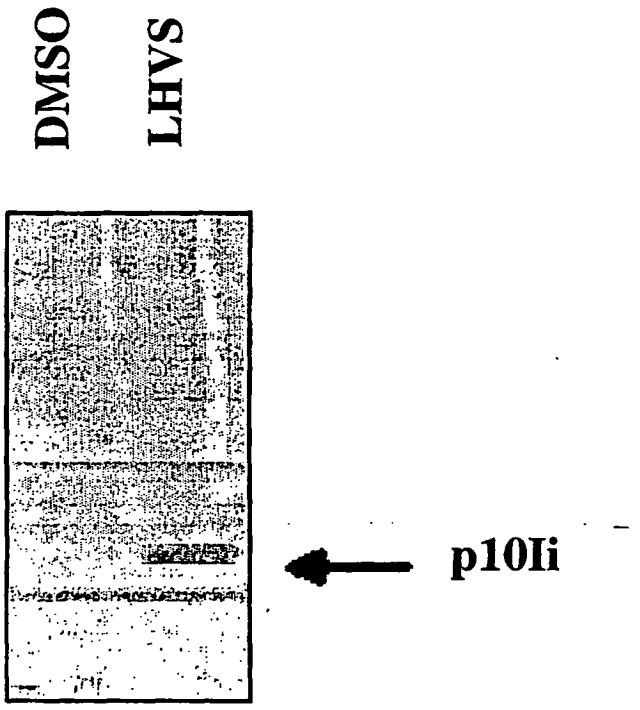
   a) purifier des globules blancs d'un échantillon de sang du sujet;
   b) produire des lysats de cellules entières des globules blancs purifiés; et
   c) analyser les lysats pour la présence d'un fragment p10li de chaîne invariante (li).

2. Méthode selon la revendication 1 où l'analyse à l'étape c) est par Western blot ou ELISA.

3. Méthode selon la revendication 1 où le sujet est un humain.

Figure 1

Invariant Chain Degradation in PCBMs



REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	监测组织蛋白酶抑制剂作用的方法		
公开(公告)号	EP1315966B1	公开(公告)日	2007-10-03
申请号	EP2001968093	申请日	2001-08-24
[标]申请(专利权)人(译)	奥索 - 麦克尼尔药品公司		
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[标]发明人	THURMOND ROBIN SUN SIQUAN KARLSSON LARS		
发明人	THURMOND, ROBIN SUN, SIQUAN KARLSSON, LARS		
IPC分类号	G01N33/535 G01N33/545 C12Q1/37 G01N33/573 G01N33/53		
CPC分类号	G01N33/573 C12Q1/37 G01N2333/8139 G01N2333/96466 Y10S435/962 Y10S436/811 Y10S436/813 Y10S436/825 Y10T436/25125 Y10T436/25375		
代理机构(译)	FISHER，ADRIAN JOHN		
优先权	60/230484 2000-09-06 US		
其他公开文献	EP1315966A4 EP1315966A1		
外部链接	Espacenet		

摘要(译)

本发明涉及通过测量给药受试者血液中恒定链（Ii），特别是p10 Ii片段的中间降解产物的积累来监测组织蛋白酶S抑制剂的体内施用效果的方法。

Figure 1

Invariant Chain Degradation in PCBMs