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学位論文名	The unique target specificity of a non-peptide chemokine receptor antagonist : Selective blockade of two Th1 chemokine receptors CCR5 and CXCR3 （合成CCR5阻害剤TAK-779の標的ケモカイン受容体特異性:CCR5とCXCR3への選択的反応性）
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論文内容の要旨

Objective

CCR5 and CXCR3 are chemokine receptors expressed predominantly on Th1 cells and have been implicated in their migration to sites of inflammation. Because T cell migration to sites of inflammation is a key process of sequential inflammatory responses, this process could be a target for the control of various inflammatory diseases. In this context, our preceding study demonstrated that a non-peptide synthetic CCR5 antagonist TAK-779 inhibited the development of experimentally induced arthritis in the mouse model by down-regulating the migration of T cells to joints. However, since almost all of the joints-infiltrating T cells were found to express CXCR3 ($CXCR3^+$ or $CXCR3^+/CCR5^+$), a question remains to be answered is whether the down-regulation of the joint recruitment of T cells by TAK-779 is only via affecting CCR5 function. To address this question, we established a set of transfectants expressing mouse chemokine receptors CCR5, CXCR3, CCR4 or CXCR4 and investigated the target receptor specificity of TAK-779.

Methods

The stable transfectants expressing mouse chemokine receptors CCR5, CXCR3, CCR4 or CXCR4 were established by transfection of each relevant gene into 2B4 T cells, using retroviral expression vectors. The transfectants were then subjected to the following assays to investigate the target receptor specificity of TAK-779. First, the ligand binding to chemokine receptors was assayed by incubating transfectants with [125-I]-labeled relevant ligand followed by gamma counting of the cell-associated radioactivity, or by incubating the transfectants with the unlabeled relevant ligand followed by staining with anti-ligand Ab. Secondly, chemokine-induced LFA-1 activation was assayed by measuring the adhesion of cells to microculture plates coated with purified ICAM-1. Thirdly, chemokine-stimulated chemotaxis was assayed by observing the cell migration through tramswells.

Results

First, we established the stable transfectants expressing mouse CCR5, CXCR3, CCR4 or CXCR4, respectively. The results of FACS staining showed that these transfectants express the relevant chemokine receptors which function as the binding receptors of the corresponding chemokines. Secondly, we examined the functionality of the chemokine receptors expressed on these transfectants in cell adhesion and chemotaxis assays. CCR5 and CXCR3 transfectants exhibited similar dose responses in the adhesion to ICAM-1 when stimulated with the relevant chemokines (RANTES, MIP-1α and MIP-1β for CCR5 ; IP-10, MIG and I-TAC for CXCR3). In chemotaxis assays, both CCR5 and CXCR3 transfectants also displayed chemotactic responses when stimulated with relevant chemokines although the doses of chemokines required for the peak responses were different for CCR5 and CXCR3. CCR4 and CXCR4 transfectants responded similarly when stimulated with relevant chemokines. These results indicated that the transfectants prepared here express functional chemokine receptors. Thirdly, we examined the effect of TAK-779 on ligand-binding of CCR5, CXCR3, CCR4 and CXCR4. Preincubation with TAK-779 efficiently blocked the ligand-binding of CCR5 and CXCR3, with the comparable IC_{50} of 236 nM for CCR5 and 369 nM for CXCR3. However, preincubation with TAK-779 did not exert inhibitory effects on the ligand-binding of CCR4 and CXCR4. Fourthly, we examined the effect of TAK-779 on the function of CCR5, CXCR3, CCR4 and CXCR4 as assessed by the binding to ICAM-1 and chemotaxis. The results demonstrated that preincubation of CCR5 or CXCR3 transfectants with TAK-779 resulted in a dose-dependent inhibition of the relevant chemokine-mediated cell-adhesion and chemotaxis. In contrast, this antagonist induced only slight or marginal inhibition of CCR4- or CXCR4-mediated cell adhesion and chemotaxis.

Conclusion

The present study demonstrated that TAK-779 blocks the function of CXCR3 and CCR5 as assessed by the ligand binding assays, cell adhesion assays and chemotaxis. In contrast, it does not elicit biologically significant inhibition on those of CCR4 and CXCR4. These observations indicate the unique targrt specificity of TAK-779 and provide a mechanistic explanation for its efficient blockade of Th1 cell migration to inflammatory lesions.

論文審査の結果の要旨

ケモカイン受容体 CCR5 と CXCR3 は、Th1 細胞の炎症局所への浸潤に重要な役割を果たすことが示唆されてきた。当教室では、CCR5 特異的阻害剤として開発された TAK-779 の投与により、腫瘍局所への T 細胞浸潤が抑制され、腫瘍拒絶が阻止されることを報告してきた。この抑制効果が CCR5 のみの阻害に基づくものか否かを検討する為に、本研究では、TAK-779 の標的受容体特異性を調べ、以下の結果を得た。

(1)マウス 2B4T 細胞株において CCR5、CCR3、CCR4 及び CXCR4 遺伝子を導入し、transfectants を作成した。(2)ligand binding assay, cell adhesion assay, chemotaxis assay により TAK-779 の阻害効果を調べた。その結果、TAK-779 は CCR5 と CXCR3 に対してほぼ同等の抑制効果を示した。一方、対照としての CCR4 及び CXCR4 にはほとんど有意な抑制を示さなかった。

以上より本研究は、TAK-779 が Th1 細胞に発現されるケモカイン受容体（CCR5 と CXCR3）に特異的な抑制効果を示すという興味深い知見を提供すると共に、T 細胞の炎症局所浸潤を効果的に抑制する機構を説明しうるため、学位論文に値するものと考える。