540 Determination of binding sites of a unique CCR5 inhibitor AK602 / ONO-4128/ GW873140 on human CCR5

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Introduction

A novel spirodiketopiperazine (SDP) derivative, AK602/ONO-4128/ GW873140, exerts potent activity against R5 HIV-1 *in vitro*. We characterized the CCR5 binding profile of AK602 and its interactions with HIV-1 gp120, CC-chemokines, and CCR5 in comparison with those of previously published CCR5 inhibitors.

Methods

A variety of mutant CCR5s were expressed on various cell lines and assays for CC-chemokine binding to CCR5, CC-chemokine- elicited cytosolic Ca^{2+} mobilization, and susceptibility to HIV-1 were conducted. Effects of various CCR5 inhibitors on CCR5/gp120 interactions were also examined. Using selected ^{3}H-labeled CCR5 inhibitors and cells expressing a variety of mutant CCR5s, the binding sites of CCR5 inhibitors were determined.

Figure 1.

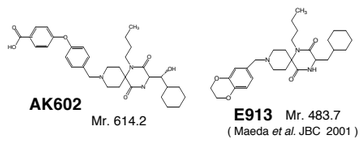


Table 1.

Anti HIV-1 activity of novel SDP derivatives in PBMs

	IC_{50} value (nM)				
	Ba-L (R5)	MOKW (R5)	MM ($R5_{MDR}$)	JSL ($R5_{MDR}$)	NL4-3 (X4)
AK602	**0.4**	**0.2**	**0.6**	**0.4**	**>1000**
TAK779	28	11	14	7	>1000
SCH-C	4	2	2	2	>1000
ZDV	7	6	250	70	8
NFV	12	14	>1000	>1000	20
SQV	11	5	300	350	10

* $HIV\text{-}1_{MOKW}$ was isolated from a drug-naive AIDS patient, while $HIV\text{-}1_{MM}$ and $HIV\text{-}1_{JSL}$ from patients who received anti-retroviral therapy for long periods of time and whose virus acquired a number of mutations in the RT- and PR- encoding HIV-1 genes.
* n.d., not determined.

Figure 2.

CCR5 binding profile of AK602

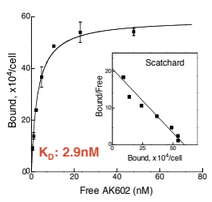


Table 2.

Binding Affinity to CCR5

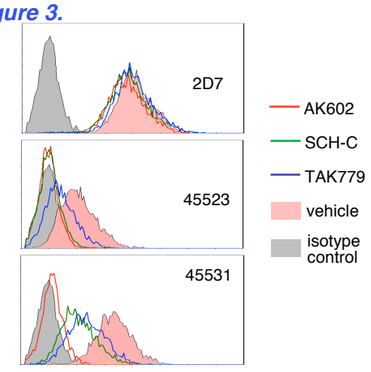
CCR5 inhibitor	K_D value (nM)
AK602	**2.9**
TAK779	32.2
SCH-C	16.0
E913	117

CCR5* CHO cells were incubated with each of ^{3}H-labeled CCR5 inhibitors (AK602, E913, TAK779 and SCH-C) for 1 hr.□Following thorough washing, the cells were lysed and the radioactivity in the lysates was determined and B_{max} and K_D values calculated using PRISM software (Intuitive Software for Science).

Results 1

Three selected CCR5 inhibitors (AK602, SCH-C, and TAK-779) exerted potent activity against R5 HIV (Table 1) and had high affinity to CCR5 with K_D values of 3, 16, and 30 nM, respectively (Table 2). AK602, unlike SCH-C and TAK-779, completely blocked the binding of an extracellular loop (ECL) 2-specific mAb (45531), suggesting AK602 interacts with ECL2 (Figure 3).

Figure 3.



AK602 binding to the second extracellular loop of CCR5 or its proximity.
AK602 at 100 nM virtually completely inhibited the binding of two monoclonal antibodies, 45523 directed against multidomain epitopes of CCR5 and 45531 which recognizes the second extracellular loop (ECL2B) of CCR5. In contrast, TAK779 and SCH-C only moderately blocked the binding of 45523 and 45531. Note that there was no AK602 inhibition of the binding of a monoclonal antibody 2D7 which is known to bind to the domain A (N-terminus) of the second extracellular loop (ECL2A) of CCR5.

Results 2

When mutations were introduced in the interface of ECL2 (G163A/R and K191A), only the binding of AK602 to CCR5 was totally nullified. However, several mutations (*e.g.*, Y108A and E283A) decreased the binding to CCR5 of AK602, TAK-779 and/or SCH-C (Table 3).

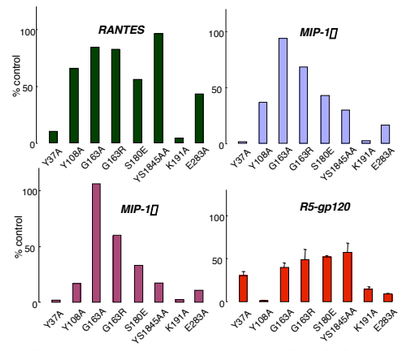
Table 3.

Binding Affinity to mutant CCR5s

Mutant CCR5 on CHO cells		K_D (nM) AK602	SCH-C	TAK779
Wild Type		2.9	16.0	32.2
D11A	*NH_2 terminus*	3.0	12.4	n.d.
Y37A	*TM1*	7.9	**>200**	98.9
Y108A		**19.8**	11.8	**>200**
F112L	*TM3*	4.0	30.0	33.0
Y113F		8.6	39.0	32.4
G163A	*TM4~ECL2*	9.9	25.0	24.0
G163R		**>200**	52.3	88.3
KE171AA		2.8	45.0	30.5
S180A		5.7	42.0	18.2
S180T		1.5	25.4	41.0
S180E		13.9	16.5	25.0
YS184AA	*ECL2*	2.0	21.3	28.0
YSQY184AAAA		2.0	n.d.	n.d.
QY186AA		2.8	14.7	35.0
Q188A		6.6	23.8	38.0
WKNF190del		**>200**	49.2	80.1
K191A		**>200**	27.1	34.5
K191R	*ECL2~TM5*	9.0	34.1	47.0
K191N		14.2	35.8	35.0
K197A	*TM5*	9.2	16.8	14.7
E283A	*TM6*	**>200**	**>200**	**>200**
				(x3~5, >5)

The K_D values against wild type / mutant CCR5 were determined as shown in Table 2.

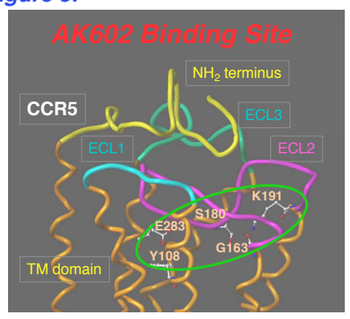
Figure 4.



CC-chemokines and HIV-1 gp120 binding to mutant CCR5s.
The expression levels of CCR5 mutants on CHO cells vary (30-150% of wild type). The amount of ^{125}I-RANTES which binds to each CCR5 is expressed as % control using the following formula:

$$\%\ \text{Control} = 100 \times \frac{(\text{Count of Bound } CCR5_{MT} \,/\, \text{Count of Bound } CCR5_{WT})}{(\#\ \text{of } CCR5_{MT} \text{ expressed} \,/\, \#\ \text{of } CCR5_{WT} \text{ expressed})}$$

Figure 5.



CCR5 model and AK602 binding site.

Results 3

We also determined the effects of the introduction of mutations to CCR5 on CC-chemokine binding/ functions and gp120 binding to CCR5. A variety of mutations in the transmembrane domain of CCR5 and the K191A mutation decreased the affinity of CC-chemokines and/or gp120 binding (Figure 4), suggesting that these mutations induce structural/conformational changes in the helices and/or ECL domains. The data suggest that unlike SCH-C and TAK-779 whose binding sites are reportedly located in the transmembrane domain, the binding sites of AK602 are clustered around the interface of ECL2 (Figure 5).

Conclusions

The data suggest that the binding profile of AK602 on CCR5 substantially differs from those of previously published CCR5 inhibitors. The data also should help to design more potent and biologically favorable CCR5 inhibitors and to develop combination regimens using SDP derivatives and other CCR5 inhibitors.