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Chemical Modification of Rapamycin: The Discovery of SDZ RAD

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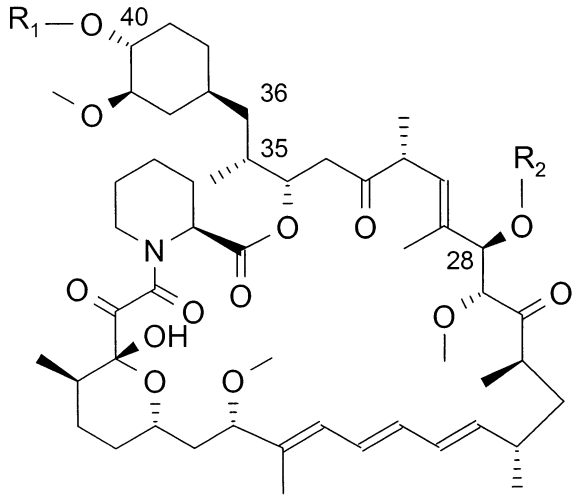
THE immunosuppressive macrolide rapamycin (RAP) **1** (Fig 1) has attracted interest in recent years because of its potential in the prevention of both allograft rejection[1] and the development of graft vessel disease (GVD).[2] This complex natural product, with its remarkable biological properties, unfortunately exhibits unfavorable physicochemical properties. As a consequence, the galenical formulation and oral administration of RAP has proven to be rather difficult. Until recently, the majority of published work demonstrating the immunosuppressive effect of RAP in vivo has dealt with parenteral administration of the compound (for references, see Granger et al[3]). We therefore embarked on a program aimed at overcoming these difficulties by chemical derivation of RAP. The nature of the chemical modification had to be carefully chosen. It had to allow for the introduction of a variety of chemical groups and functionalities.



1 RAP	R_1 = H	R_2 = H
2	R_1 = H	R_2 = Me
3	R_1 = Me	R_2 = H
4 SDZ RAD	R_1 = $HO(CH_2)_2$-	R_2 = H
5	R_1 = $HO(CH_2)_6$-	R_2 = H
6	R_1 = Ph	R_2 = H

Fig 1. Chemical structures of RAP **1**, 28-*O*-methylrapamycin **2** and 40-*O*-alkylated derivatives, including SDZ RAD **4**.

Another important criterion to be considered was the metabolic stability; the targeted RAP derivatives had to constitute the active principle, ie, they should not behave as prodrugs. Therefore, it was of utmost importance to find a position in RAP that could be chemically modified without resulting in loss of immunosuppressive activity. Alkylation of the hydroxyl (*O*-alkylation) in either position 28 or 40 (Fig 1) was envisaged as a type of modification that could be expected to correspond to our criteria. The data presented herein show that *O*-alkylation in position 40 can indeed lead to novel, potently immunosuppressive RAP-derivatives, provided that the newly introduced alkyl group is properly chosen.

O-alkylation in position 28 (eg, **2**, Fig 1), on the other hand, leads to loss of immunosuppressive activity, which can be explained on the basis of structural results obtained by X-ray crystallography. The efforts described herein ultimately resulted in the identification of a potent RAP derivative, SDZ RAD (40-*O*-(2-hydroxy)ethylrapamycin) **4** (Fig 1), which is currently undergoing clinical trials.

MATERIALS AND METHODS

Rapamycin and Analogs

RAP was obtained by fermentation of the actinomycete strain A91-259211. The analogs were prepared in our laboratories by chemical modification of RAP. The experimental details of the chemical syntheses will be reported elsewhere.

In Vitro Assays

FKBP12 Binding Assay. Binding to the FK 506 binding protein (FKBP12) was indirectly assessed by means of an ELISA-type competition assay. FK 506 was included in each individual experiment as a standard, and the inhibitory activity is expressed as relative IC_{50} compared to FK 506 (rIC_{50} = IC_{50} test compound/IC_{50} FK 506). Details regarding this assay have been reported.[4]

Mixed Lymphocyte Reaction (MLR). The immunosuppressive activities of RAP and its derivatives were assessed in a two-way MLR, using spleen cells of BALB/c and CBA mice. RAP was included in each individual experiment as a standard, and the inhibitory activity is expressed as relative

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0041-1345/98/$19.00
PII S0041-1345(98)00587-9

Table 1. In Vitro Activities of Rapamycin and *O*-Alkylated Derivatives

Compound	Substituent R_1	Substituent R_2	FKBP12 binding (rIC_{50})*	MLR (rIC_{50})†
1 RAP	H	H	0.6	1
2	H	Me	1.6	1300
3	Me	H	1.1	6.5
4 SDZ RAD	$HO(CH_2)_{2-}$	H	2.0	2.1
5	$HO(CH_2)_{6-}$	H	0.8	18
6	Ph−	H	23	>430

*The ability of the compounds to compete with immobilized FK 506 for binding to biotinylated FKBP12 was determined in a competitive binding assay. FK 506 was included as standard in each individual experiment. Results are expressed as means of the relative IC_{50} values (ie, IC_{50} test compound/IC_{50} FK 506). The range of absolute IC_{50} values for FK 506 was 0.8–1.2 nmol/L.

†The inhibitory effect on a two-way MLR performed with spleen cells from BALB/c and CBA mice was tested. RAP was included as standard in each individual experiment. Results are expressed as means of the relative IC_{50} values (ie, IC_{50} test compound/IC_{50} RAP). The range of absolute IC_{50} values for RAP was 0.06–0.9 nmol/L.

See Fig 1.

IC_{50} compared to RAP (rIC_{50} = IC_{50} test compound/IC_{50} RAP). Details regarding this assay have been reported.[4]

RESULTS AND DISCUSSION

As can be seen from the data shown in Table 1, alkylation in either position 28 (compound **2**) or position 40 (compounds **3–5**) did not greatly affect binding to FKBP12. A significant loss in affinity was only observed when the rather bulky phenyl group was added to the C40-hydroxyl (compound **6**). Methylation of the C28-hydroxyl (compound **2**) resulted in a 1000-fold loss of activity in the MLR. When the same modification was carried out in position 40 (compound **3**), the immunosuppressive activity was only reduced by a factor of 6.5 as compared to RAP. This clearly indicated that, in contrast to the C28-hydroxyl, the C40-hydroxyl was amenable to chemical modifications without resulting in a prohibitive loss of potency, and that further optimization could be envisaged. Indeed, introduction of a 2-hydroxyethyl group in that same position, leading to SDZ RAD **4**, enhanced the potency with respect to the methyl-derivative **3**; the immunosuppressive activity of SDZ RAD in vitro was found to be comparable to that of RAP. When the hydroxyalkyl chain was extended, as in derivative **5**, the trend was reversed, and a reduction of activity was observed.

The results reported herein can be explained on the basis of structural results obtained by X-ray crystallography. RAP exerts its immunosuppressive activity by first binding to FKBP12. This binding is necessary, but not sufficient, as was recognized earlier (see Brown et al[5] and references cited therein), and as can be seen from our data in Table 1 (ie, compounds **2** and **6**). In the case of compound **6** the chemical modification results in decreased affinity for FKBP12 and, as a consequence, in a significant loss of immunosuppressive potency. Compound **2**, on the other hand, exhibits a 1300-fold loss in activity despite its high affinity for FKBP12. The active principle is actually rather the FKBP12/RAP complex, which binds to the target protein mTOR[6] (also termed FRAP[5] or RAFT[7].

As far as **2** is concerned, we have previously shown that in the complex with FKBP12, the newly introduced *O*-methyl group in position 28 causes a 120° rotation around the C35–C36 bond (Fig 1), considerably shifting the cyclohexyl subunit.[8] The macrocyclic part of **2** remains virtually unchanged with respect to FKBP12-bound RAP and can be superimposed with the macrocyclic part of RAP in the X-ray crystal structure of the FKBP12-RAP-FRB complex[9] (FRB = FKBP12-rapamycin-binding domain of mTOR). This analysis shows that the cyclohexyl subunit of **2**, in its new position, collides with a loop of the mTOR fragment. This actually prevents binding of **2** in complex with FKBP12 to mTOR, thus explaining the lack of activity in the MLR. The X-ray crystal structure of the FKBP12/SDZ RAD **4** complex, on the other hand, reveals a three-dimensional structure for bound SDZ RAD, which very closely resembles that of RAP (details concerning the structure will be published elsewhere). From a structural point of view, there is no impediment to complex formation between FKBP12/SDZ RAD and the FRB fragment of mTOR, thus explaining its high potency. The loss of activity observed when going from SDZ RAD **4** to **5** is probably due to the larger size of the modification at the C40-hydroxyl in the latter. This can conceivably result in steric hindrance with, and reduced affinity for, mTOR. The modification in **6**, finally, clearly affects binding to FKBP12, a prerequisite for activity, and probably also, because of its size, binding of the FKBP12/**6** complex to mTOR.

In conclusion, we have shown that *O*-alkylation of RAP can lead to highly potent derivatives, provided that a suitable alkyl group is introduced into the appropriate position and that the structure-activity relationship of *O*-alkylated RAP derivatives can be explained by X-ray structural results. The 40-*O*-(2-hydroxyethyl) derivative SDZ RAD **4** is presently undergoing clinical trials for use in combination with cyclosporine A to prevent acute and chronic rejection after solid-organ allotransplantation.[4,10]

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