Derivation of the Immunosuppressive Macrolide Rapamycin: Chemical, Structural and Biological Aspects

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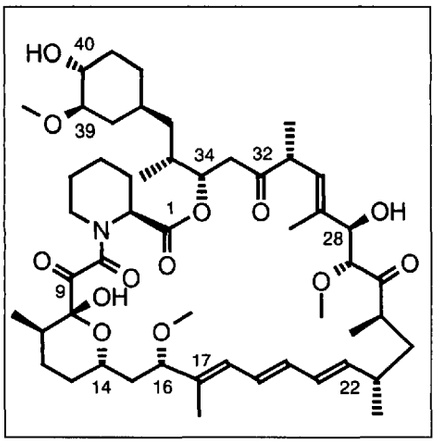
The macrolide rapamycin was discovered in 1975 at *Ayerst* in a screen for novel antifungal agents. It has attracted interest since the beginning of the nineties because of its remarkable immunosuppressive properties and is presently undergoing clinical trials for the prevention of allograft rejection.



Rapamycin exerts its immunosupressive activity by forming, in a first step, a complex with its intracellular receptor FKBP12. This complex formation involves the 'binding domain'. The latter consists mainly of the C(1)–C(14) subunit, but also includes the C(40) hydroxy group, which makes a hydrogen bond with FKBP12. Binding of rapamycin to FKBP12 is necessary, but not sufficient, for immunosupressive activity. Indeed, in a second step, the FKBP12/rapamycin complex binds to a protein termed FRAP, and it is this second interaction which is ultimately responsible for immunosuppression. The interaction of the FKBP12/rapamycin complex with FRAP is mediated by the 'effector domain' which comprises the region between C(14) and C(28) and, thus, includes the conjugated triene subunit.

Through chemical derivation of the macrolide, we could show that the triene moiety is indeed critical: partial or complete hydrogenation and dihydroxylation drastically decreased or even abrogated the immunosuppressive activity. Modification of the allylic C(16) methoxy group, on the other hand, led to potent derivatives. While replacement of this group by larger alkoxy moities resulted in loss of activity, the introduction of propargylic ethers led to derivatives which were up to threefold more potent than rapamycin.

Methylation of the C(28) hydroxy function resulted in a thousandfold decrease in activity, though binding to FKBP12 was not affected. The X-ray crystal structure of 28-*O*-methylrapamycin complexed with FKBP12 showed that the cyclohexyl ring of this derivative has a completely different orientation compared to the one found for bound rapamycin. Structural evidence indicated that the cyclohexyl ring in its new position impedes the second complexation step with the target protein FRAP, apparently mainly for steric reasons. These results prompted us to further investigate the influence of the cyclohexyl moiety on the immunosuppressive activity. We found that cleavage of the C(39),C(40) bond, a modification which does not add steric bulk to the molecule, abrogates the activity. This finding indicated that part of the cyclohexyl subunit, which until now was thought to be only involved in binding to FKBP12, might also be important for interaction with FRAP, and thus might also belong to the effector domain of rapamycin.

Somatic Cell and Gene Therapy: Perspectives in Oncology

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The *ex vivo* expansion of primitive haematopoietic cell harvests is of interest for gene therapy and transplantation applications. The possibility of maintaining, manipulating and expanding harvested human haematopoietic stem cells in *ex vivo* culture could help to provide autologous and allogeneic transplants of improved purity and performance. Such improved cell yields may be of clinical benefit to poor-mobilising patients and those requiring multiple transplantation, and enable access to gene therapy of the haematopoietic system. Efficient gene transfer into haematopoietic stem cells could improve the treatment of inherited diseases, viral infections and cancer, and aid the therapeutic evaluation of tumor-cell purging in autologous stem cell transplantation. Successful gene transfer into haematopoietic repopulating cells has been reported at low levels, but improvements are needed for clinical application.

Serum-free GMP culture conditions have been developed for the expansion, differentiation, purging and gene transfer of haematopoietic stem cells. Such serum-free conditions have theoretical advantages for the biological safety and standardisation of clinical gene transfer into haematopoietic cells and have been shown to allow greater cell-culture expansion than serum-containing conditions. Under these conditions, efficient purging (>3 logs) with recombinant immunotoxins or dendritic cell differentiation can readily be obtained. The same GMP medium (CellGro = AE) also allows serum-free production of retroviral vector-containing medium using the gibbon ape leukaemia virus (GALV) pseudotyped packaging cell line PGT13/LN. The GALV envelope binds to a spe-