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News & views

Pharmacology

Two-drug trick to block systemic toxicity

Matthias P. Wymann & Chiara Borsari

When combined, two drugs alter the activity of a protein complex called target of rapamycin complex 1 such that it is inhibited in the brain but not the body, enabling the treatment of brain tumours in mice without systemic toxicity.

Medicinal chemists and pharmacologists dream about how drugs might be directed specifically to selected organs. A particularly challenging target is the brain: many drugs do not pass easily through the blood–brain barrier (BBB), or are actively pumped out of the brain. The situation is complicated further when a drug to be delivered also shows body-wide (systemic) adverse effects. One such drug is rapamycin, which blocks tumour growth, but is also used as an immunosuppressant during organ transplantation[1]. Rapamycin and its semi-synthetic derivatives, dubbed rapalogs, inhibit a protein complex called target of rapamycin complex 1 (TORC 1). Writing in *Nature*, Zhang *et al.*[2] present an innovative chemical approach to confine the action of rapalogs to the brain, and thereby eliminate undesirable systemic effects such as immunosuppression. They combine a high-affinity rapalog (RapaLink-1) with a newly developed molecule (RapaBlock) that prevents TORC1 inhibition systemically, but cannot enter the brain.

TORC1 regulates many fundamental biological processes, including cell proliferation, autoimmunity, metabolism and cancer. Rapalogs have been used successfully to slow tumour growth[1], as well as to treat disorders of the central nervous system[3].

To stably inhibit TORC1, rapamycin must form a complex with a cellular protein called FKBP12 and the rapamycin-binding domain (the FRB domain) of a protein called mechanistic target of rapamycin (mTOR), which is part of TORC1. This happens through the initial formation of either an FKBP12–rapamycin or an FRB–rapamycin complex. The RapaBlock molecule developed by Zhang and colleagues is a high-affinity ligand (a binding molecule) for FKBP12, and blocks its association with rapamycin. Because the FKBP12–rapamycin complex is much more stable than the FRB–rapamycin complex[4] (with a dissociation constant (K_d) of about 0.2 nanomolar, as opposed to 26 micromolar), RapaBlock prevents the formation of a 'quasi-irreversible' tripartite FKBP12–rapamycin–FRB complex, and thus keeps TORC1 largely free to function (with some being temporarily bound and inactivated by RapaLink-1; Fig. 1).

How did Zhang *et al.* design the RapaBlock molecule? They built two structurally diverse chemical libraries of molecules based on a synthetic ligand of FKBP12 called SLF, and the higher-affinity natural ligand (FK506). The authors then tested the resulting molecules for their ability to block the inhibition of TORC1 either by rapamycin or by its derivative RapaLink-1. RapaLink-1 is composed of rapamycin linked to an mTOR kinase inhibitor (which binds to mTOR's catalytic ATP-binding pocket[5]). By engaging both the FRB domain and the ATP-binding site, RapaLink-1 binds to TORC1 exceptionally tightly.

Zhang *et al.* found that rapamycin could be impeded by the low-affinity SLF derivatives, but that RapaLink-1 was substantially intercepted only by the higher-affinity FK506 derivatives. They therefore selected an FK506 derivative as RapaBlock, which integrates three key properties: first, it has a high affinity for FKBP12 (a K_d of 1.6 nM); second, it cannot enter the brain; and third, it cannot bind to the calcineurin protein (which binds to the FK506–FKBP12 complex, triggering immunosuppression)[6,7]. These features allow RapaBlock to sequester systemic FKBP12 in the body, blocking the activity of RapaLink-1 and preventing its interference with immunity. Because RapaBlock does not penetrate the BBB, RapaLink-1 action is not suppressed in the central nervous system.

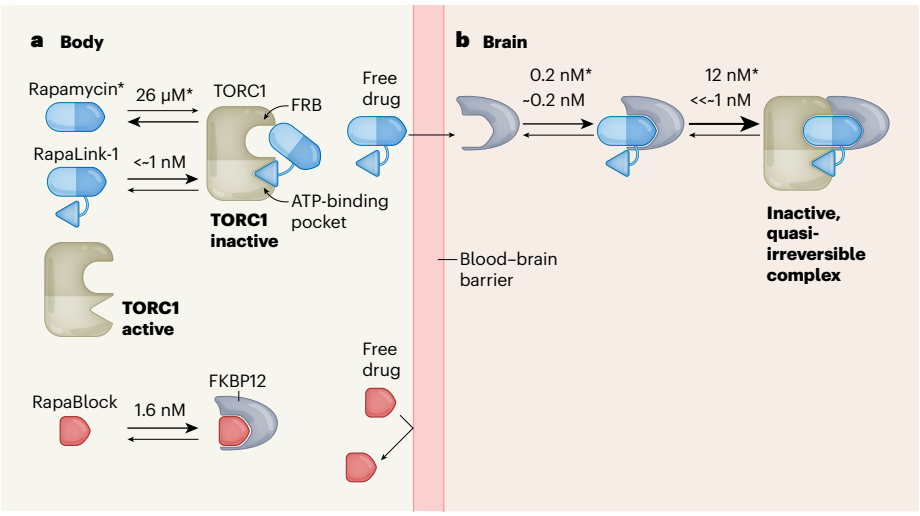

Figure 1 | Drug targeting to the brain. a, The drug rapamycin is used to inactivate signalling of the TORC1 enzyme complex (shown without accessory proteins). When used to treat brain diseases, rapamycin and its synthetic derivatives, such as RapaLink-1, can have adverse systemic effects. RapaLink-1 contains two motifs that bind to TORC1: one, shared with rapamycin, binds to the enzyme's FRB domain; the other binds to its ATP-binding pocket (numbers above the arrows reflect dissociation constants, with asterisks denoting the values for rapamycin. Arrow thickness indicates the ligand's propensity for binding or dissociation.) This interaction can temporarily inhibit TORC1, but to stably inhibit the complex, RapaLink-1 must also bind to the protein FKBP12. Zhang *et al.*[2] have developed a molecule called RapaBlock that binds tightly to FKBP12 in the body, preventing its association with the TORC1–RapaLink-1 complex. **b**, RapaBlock cannot cross the blood–brain barrier, so a quasi-irreversible TORC1–RapaLink-1–FKBP12 complex forms in the brain, inactivating TORC1.

Delivering compounds to the brain is demanding, and a number of tools have been developed to predict whether a chemical will be able to penetrate the BBB. One widely used example is the multiparameter optimization (MPO) algorithm, which incorporates six key physico-chemical properties (including a molecule's topological polar surface area, lipophilicity and molecular weight). An MPO score of 4 or higher predicts a good equilibration of drug levels in the blood and brain[8]. Newer versions of mTOR inhibitors (such as mTOR kinase inhibitors, which are structurally and functionally unrelated to rapamycin) have been optimized for access to the brain: PQR620, for instance, has an MPO of 3.8, and reaches brain–blood ratios of 1–1.5, depending on the dose[9,10].

The MPO scores of rapamycin (1.25), RapaLink-1 (1.0) and RapaBlock (1.25) predict very low brain penetration[8]. This has been confirmed for rapamycin and its derivative, everolimus, which reach maximum brain–blood ratios of around 1:100 (ref. 10). Nonetheless, rapamycin and everolimus can reduce epileptic seizures in individuals with tuberous sclerosis complex (a condition that is caused by hyperactivation of TORC1 in the brain). The actions of rapamycin, its derivatives and RapaLink-1 therefore seem to be dominated by a sink effect that traps the small amount of compound reaching the brain in a quasi-irreversible tripartite FKBP12–rapamycin–TORC1 complex.

In keeping with this model, Zhang *et al.* found that combining RapaLink-1 and RapaBlock prolonged survival in two mouse models of glioblastoma brain tumours, and also reduced tumour growth in one of the models. Moreover, a previous study[11] showed that the combination of RapaLink-1 and RapaBlock could also be successfully applied in a mouse model of alcohol abuse. Co-administering the two drugs prevented TORC1 signalling in the brain's nucleus accumbens, reduced alcohol craving and consumption and protected against RapaLink-1-induced liver toxicity, glucose intolerance and loss of body weight[11].

Zhang *et al.* have also modelled the RapaLink-1–RapaBlock and rapamycin–RapaBlock interactions and brain penetration. They predict a need for a blood-plasma concentration of around 1 μM of RapaBlock to intercept rapamycin, and roughly 10 μM of RapaBlock to substantially protect TORC1 signalling from inhibition by RapaLink-1. These concentrations will be difficult to reach in therapeutic settings, so properties such as pharmacological kinetics and the affinity of RapaBlock for FKBP12 will need improvement.

Finally, the authors claim that combining drugs that act intracellularly (such as protein kinase inhibitors) with an FK506-derived FKBP12-binding module would yield other programmable inhibitors that could be directed to the brain rather than to the body by means of RapaBlock. The molecules used by the authors do accumulate intracellularly, but their penetration of the BBB still needs to be demonstrated. In general, there is no 'on-target trap' akin to the tripartite FKBP12–rapamycin–TORC1 complex for the proposed programmable drugs. But, with further studies and refinements, Zhang and colleagues' impressive chemical strategy should open up new routes to organ-specific drug targeting.

Matthias P. Wymann and **Chiara Borsari** are in the Department of Biomedicine, University of Basel, CH-4058 Basel, Switzerland.
e-mail: matthias.wymann@unibas.ch

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