CHEMICAL BIOLOGY

Modulating molecular gateways

Imagine if you no longer had to worry about whether your new drug was able to cross biological barriers, because you were able to transiently modify such barriers to allow compounds to pass through. New research recently published in *Nature Chemical Biology* could make this fantasy a reality.

Scientists working within the National Institutes of Health Molecular Libraries Screening Centers Network Initiative have identified an antagonist of a receptor that modulates barrier function and can be used together with a known agonist to finely regulate barrier activity. Being able to manipulate barriers in the brain, immune system, lung and skin could have wide-ranging applications for treating oedema, organ rejection and autoimmune diseases such as multiple sclerosis, and could also be used to deliver drugs to impermeable tissues.

Hugh Rosen and Chi-Huey Wong's groups at the Scripps Research Institute set out to use a pair of chemical 'probes' to study the sphingosine 1-phosphate (S1P)–S1P-receptor-1 ($S1P_1$) pathway, which is involved in the modulation and maintenance of biological barrier activity. Of the five S1P receptors, $S1P_1$ is known to be expressed predominantly on endothelial, cardiac and blood cells. They synthesized an antagonist of $S1P_1$ signalling that had improved potency and *in vivo* activity over previously described antagonists by replacing the phosphate ester with phosphonate to generate *R*- and *S*-enantiomers of 3-amino-4-(3-hexylphenylamino)-4-oxobutyl-phosphonic acid. The *R*-enantiomer was shown to be a full competitive antagonist of $S1P_1$, whereas the *S*-enantiomer was not, and so the *R*-enantiomer and the $S1P_1$-selective agonist SEW2871 were used as a pair of probes with which to study vascular and lymphoid responses regulated by SIP_1 activity.

The SIP_1 antagonist reversed the endothelial barrier protection conferred by endogenous and exogenous $S1P_1$ agonists against vascular endothelial growth factor (VEGF)-induced leakage of mouse vascular capillaries. However, when administered to mouse lung vasculature, the antagonist significantly enhanced pulmonary leakage in a dose-dependent manner in the absence of exogenous ligand. This suggests that there is variation in the level of basal receptor activity at barriers in different tissues, and that this could result in a tissue-specific effect of the exogenous antagonist.

The authors then studied the effect of the antagonist on lymphocyte egress — the process by which lymphocytes move from lymphoid tissue into the bloodstream. This was done by plotting the previously measured degree of vascular protection from VEGF-induced leakage against the induction of lymphopaenia, which revealed a positive correlation. SEW2871 induced lymphocyte sequestration from peripheral blood, whereas this was reversed in the presence of the $S1P_1$ antagonist. Moreover, the $S1P_1$ antagonist also inhibited the loss of expression of the lymphocyte activation marker CD69 on lymphocytes caused by $S1P_1$ agonists. Two-photon imaging of the effects of the antagonist in lymph nodes revealed that the actions of the exogenous agonist and antagonist pair were confined to the medullary region and had no effect on lymphocytes in other regions, showing that S1P-induced lymphocyte arrest is limited to medullary egress *in vivo*.

These findings provide valuable insights into the complexity of S1P–$S1P_1$ signalling but also cast a shadow on the usefulness of gene-deletion mutants studied alone for fully understanding the effects of modulating a single receptor. A comparison of these results with previous gene-deletion studies highlighted several areas of uncertainty regarding $S1P_1$ function. This suggests that a combined approach using knockouts and chemical probes could be more illuminating when studying receptor function *in vivo*.

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ORIGINAL RESEARCH PAPER Sanna, M. G. *et al.* Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral $S1P_1$ antagonist *in vivo*. *Nature Chem. Biol.* 9 July 2006 (doi: 10.1038/nchembio804)