CARDIOVASCULAR DISEASE

Healing hearts

Inhibitors of cytochrome P450 monooxygenases (CYPs) reduce infarct size and reperfusion injury in two animal models of heart attack, according to a new study published in the *Proceedings of the National Academy of Sciences*.

Restoration of blood flow to oxygen-deprived tissues is a priority following myocardial infarction. But this 'reperfusion' further damages ischaemic areas by inducing the production of reactive oxygen species (ROS). Recent data indicate that CYP2C9 — a member of the CYP family that modulates vascular homeostasis by converting arachidonic acid to vasoactive eicosanoids — is involved in the generation of ROS in human coronary arteries. As such, researchers from The Scripps Institute aimed to elucidate the role of CYPs in myocardial infarction.

The team's strategy capitalized on the CYP-inhibitory activity of the antibiotic chloramphenicol. In one experimental model, isolated rat hearts were deprived of blood and subsequently reperfused. Pretreatment with chloramphenicol reduced the amount of superoxide ROS produced by damaged heart tissue relative to drug-free controls. Infarct size was also significantly reduced. This result was duplicated in rabbits whose coronary arteries were occluded and subsequently reperfused in the presence of chloramphenicol. The cardioprotection conferred by the drug was apparent at the physiological level as improved post-ischaemic blood pressure.

In addition to its effect on CYPs, chloramphenicol inhibits mitochondrial protein synthesis. As mitochondria can generate ROS, the authors set out to determine whether the cardioprotective effect of the drug is mediated through its action on CYPs, on mitochondria or on both. Support for the former hypothesis included a 95% decrease in CYP activity in inhibitor-treated rat hearts, and unchanged levels of mitochondria-encoded proteins and respiratory-chain activity. The CYP inhibitors cimetidine and sulphaphenazole, which do not affect mitochondrial protein synthesis, also

GPCRS

Family traits

When searching for new ligands for a receptor drug target, having some idea of where to start, such as the structure of a natural ligand, can be very useful. But with 'orphan' G-protein-coupled receptors (GPCRs), such clues are, by definition, unavailable. Help could be at hand though in the structures of known ligands for other GPCRs, as highlighted in a recent study by Bondensgaard and colleagues reported in the *Journal of Medicinal Chemistry*.

The recurring presence of particular structural fragments in ligands for several GPCRs — so-called privileged structures — was first noted more than a decade ago, and has been widely used since to aid in the design of libraries for GPCR screening, with some notable successes in identifying high-affinity ligands. But why particular structural fragments can be the basis for ligands at different GPCRs is not certain, and it was this question that the authors set out to investigate.

As put forward by the authors, one potential explanation for the presence of a privileged structure in ligands for a range of GPCRs is that each of those GPCRs has a region in its ligand-binding pocket that is complementary to the privileged structure. To test this idea, they took three pairs of ligands for widely differing class A GPCRs — with each ligand pair sharing a particular privileged structure — and computationally docked them into models of the GPCRs they bind to. For each pair of ligands, analysis of the ligand–receptor complexes revealed that the nature of the part of the ligand-binding pocket that interacts with the privileged structure was conserved between the two complexes, confirming the authors' hypothesis. Consideration of the receptor interactions made by the 'nonprivileged' parts of the ligands suggested that these were responsible for receptor selectivity towards the ligands.

So, one approach to ligand discovery for other GPCRs in class A (the largest GPCR class, members of which are thought to share the same binding-pocket position) could be to analyse the receptor binding pocket to see whether a particular privileged-structure-binding region is present and, if so, to design a screening library incorporating this privileged structure. This approach could clearly be particularly valuable for accelerating the discovery of modulators of orphan GPCRs. One problem, however, is that the limited number of privileged structures known at present means that there could be intellectual property issues related to their use, so there is a need to identify new such fragments.

Peter Kirkpatrick

References and links

ORIGINAL RESEARCH PAPER Bondensgaard, K. *et al.* Recognition of privileged structures by G-protein-coupled receptors. *J. Med. Chem.* **47**, 888–899 (2004)
FURTHER READING Bleicher, K. H. *et al.* Hit and lead generation: beyond high-throughput screening. *Nature Rev. Drug Discov.* **2**, 369–378 (2003) | Klabunde, T. & Hessler, G. Drug design strategies for targeting G-protein-coupled receptors. *Chembiochem* **4**, 928–944 (2002)

reduced infarct size in ischaemic rat hearts, confirming that chloramphenicol acts predominantly through CYPs in this model.

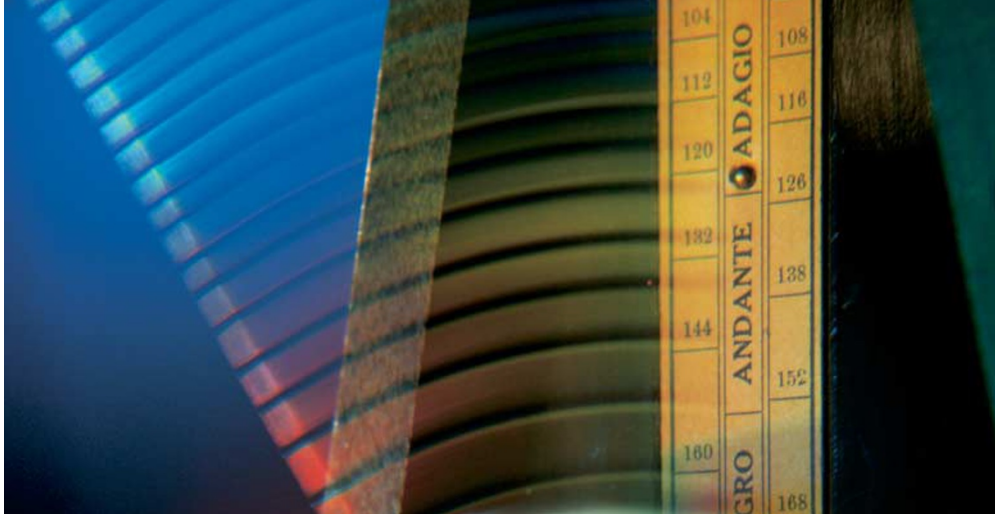
Perhaps the most significant finding of the study was that both chloramphenicol and sulphaphenazole protect heart tissue when administered after — as well as before — blood flow is interrupted. This experimental paradigm more accurately reflects the clinical situation of patients presenting to hospital after suffering a heart attack. As such, these data provide hope that exploitation of the CYP-inhibitory activity of various approved agents — such as some members of the statin family of cholesterol-lowering drugs — might improve treatment outcome for the millions of people that live through this devastating experience each year.

Suzanne Farley

References and links

ORIGINAL RESEARCH PAPER Granville, D. J. *et al.* Reduction of ischemia and reperfusion-induced myocardial damage by cytochrome P450 inhibitors. *Proc. Natl Acad. Sci.* USA **101**, 1321–1326 (2004)

FURTHER READING Gottlieb, R. A. Cytochrome P450: major player in reperfusion injury. *Arch. Biochem. Biophys.* **420**, 262–267 (2003)



TARGET VALIDATION

Regulating the beat

Heart disease is the leading cause of death in industrialized nations and is characterized by diverse cellular abnormalities associated with decreased ventricular function. In the March issue of *Nature Medicine*, Jeffery Molkentin and colleagues report a new strategy to treat heart disease by inhibiting the enzyme protein kinase C-α (PKC-α). The authors' findings help to explain the mechanisms behind heart muscle contraction, and indicate that PKC-α could be a pharmacological target for treating human heart failure.

At the onset of many forms of heart disease, cardiac hypertrophy (excessive cellular growth) and ventricular remodelling (changes in wall thickness and/or chamber volume) occur as a compensatory response to maintain cardiac output. These changes eventually lead to increases in oxygen consumption, greater vascular resistance, chamber dilation, wall stiffening and fibrosis, which ultimately impair the ability of the ventricles to pump blood and lead to overt failure. The hypertrophic response results from growth of the contractile cells, called cardiomyocytes, and is associated with changes in gene expression, including elevated PKC-α expression. During heart failure, contractility is also compromised by a deficiency in the release and uptake of calcium ions within the myocytes themselves. The PKC family comprises at least twelve serine/threonine kinases that form three sub-groups on the basis of sensitivity to calcium and lipids. The functions of many individual PKC isoforms are still obscure, especially that of PKC-α, which is the dominant isoform expressed in the heart.

Myocyte contraction and relaxation are directly regulated by intracellular calcium cycling. Calcium ions enter through the voltage-dependent sarcolemma membrane, which induces the release of a large amount of calcium from the sarcoplasmic reticulum (SR) storage compartment through the ryanodine receptor. Myocyte relaxation is initiated by sequestration of calcium back into the SR through the activity of the SR/ER calcium pump (SERCA2). Signalling from β-adrenoceptors controls the magnitude and timing of calcium release through effects that impact SERCA2 function, as well as other calcium-handling proteins.

Molkentin and colleagues show that PKC-α directly controls the activity of those key enzymes that regulate heart muscle contraction. Mechanistically, modulation of PKC-α activity affects dephosphorylation of the SERCA2 inhibitory protein phospholamban and affects both SR calcium loading and the magnitude of subsequent calcium release. The authors used three mouse models of heart disease to show that deleting the gene that encodes PKC-α from the diseased heart improves its function. In two of the models, the technique helped the mice survive longer.

Antagonizing PKC-α should enhance the calcium signalling response and therefore increase the force of contraction of the myocardium. However, current classes of inotropic drugs that augment calcium cycling, such as β-adrenoceptor antagonists or phosphodiesterase inhibitors, have shown adverse outcomes in clinical trials. However, PKC-α functions downstream from the action of classic inotropic agents, and might therefore be less problematic and offer new hope for treating heart failure; kinases are attractive therapeutic targets because of their central role in cellular signalling and have now become the second most important group of drug targets, after G-protein-coupled receptors.

Melanie Brazil

References and links

ORIGINAL RESEARCH PAPER Braz, J. C. *et al.* PKC-α regulates cardiac contractility and propensity towards heart failure. *Nature Med.* (15 March 2004) (doi:10.1038/nm1000)

FURTHER READING Vlahos, C. J., McDowell, S. A. & Clerk, A. Kinases as therapeutic targets for heart failure. *Nature Rev. Drug Discov.* **2**, 99–113 (2003) | Cohen, P. Protein kinases — the major drug targets of the twenty-first century? *Nature Rev. Drug Discov.* **1**, 309–315 (2002)