

CARDIOVASCULAR DISEASE

Oestrogen activates COX2 pathway



Heart disease and atherosclerosis is more pronounced in men than in women, but this difference is reduced after menopause. Although some studies have shown that the female hormone oestrogen slows heart disease in mouse models, the underlying mechanism is largely unknown. In *Science Express*, FitzGerald and colleagues show that female mice are protected from hardening of the arteries as a result of oestrogen activation of the cyclooxygenase (COX)-2 enzyme, which causes an increase in the amount of the atheroprotective molecule prostacyclin, PGI₂.

During inflammation, phospholipase enzymes convert membrane phospholipids to arachidonic acid. Arachidonic acid is the precursor for prostaglandins, such as PGI₂, and thromboxanes, which are derived from the COX1 and COX2 pathways. The COX prostaglandin products are acted on by thromboxane synthase (in platelets) or prostacyclin synthase (in endothelium) to form thromboxanes or PGI₂, respectively. PGI₂ has an important and beneficial role in vascular function, as it inhibits platelet adhesion to the vascular endothelium and is a strong vasodilator. The COX pathway is inhibited by aspirin and

non-steroidal anti-inflammatory drugs (NSAIDs). Newer anti-inflammatory drugs selectively target the inducible isoform COX2 and have fewer gastrointestinal side effects than the non-selective COX inhibitors such as aspirin and ibuprofen.

Mice deficient in the low-density lipoprotein (LDL) receptor were used to investigate the role of PGI₂; LDL-deficient male mice develop atherosclerosis more rapidly than females. Knocking out the prostacyclin receptor (IP) in these female mice took away the atheroprotective effect of oestrogen. These mice also showed increased oxidative stress. By removing the ovaries of female LDL-deficient mice, the authors were able to identify the direct effects of oestrogen. Administration of oestrogen to ovariectomized mice increased PGI₂ synthesis. Furthermore, PGI₂ synthesis was also increased after administration of oestrogen to female mice deficient in the oestrogen receptor β , but not the α -receptor, showing that PGI₂ biosynthesis occurs by activation of the α -receptor. In LDL- and IP-deficient female mice, administration of oestrogen did not confer protection from atherosclerosis compared with LDL-deficient mice alone.

ANTIVIRAL DRUGS

Blocking the route to infection

A promising approach to developing agents that can prevent HIV transmission is described in a recent report in *Proceedings of the National Academy of Sciences*. Furthermore, the medicinal chemistry strategy used by Offord and co-workers to improve the pharmacological properties of a natural protein that blocks HIV entry could also be applicable to other therapeutic proteins.

The entry of HIV into target cells involves the CD4 protein, as well as the CC-chemokine receptor 5 (CCR5), which is especially important in the early stages of the disease. Natural ligands of CCR5 — such as RANTES, a small human protein of the chemokine family — can block the entry of HIV strains that require CCR5 to enter the target cells.

Optimizing such proteins to make them more potent inhibitors of HIV entry could lead to agents that effectively prevent HIV transmission. Small proteins have traditionally been modified using mutagenesis, but this approach is limited to natural amino acids; now, however, techniques are available for the rapid chemical synthesis of large numbers of analogues of small natural proteins, which allows non-natural amino acids to be incorporated that might improve the therapeutic properties of the analogues.

Previously, Offord and co-workers synthesized a RANTES analogue — AOP-RANTES — that was more effective at blocking HIV entry into target cells than natural RANTES. In AOP-RANTES, a serine residue was replaced with the non-natural amino acid aminooxypentane oxime on the amino terminus to increase its hydrophobicity. In the current study, the authors systematically determined the structure–activity relationships of analogues of AOP-RANTES, which led to the generation of several more potent inhibitors of HIV in mice. The analogues were further optimized by replacing the amino acids at the two

adjacent positions to the original amino acid that was modified. One derivative of AOP-RANTES, termed PSC-RANTES, was 50 times more potent than the starting molecule.

These RANTES analogues are thought to be successful at inhibiting HIV entry into target cells because they trigger the intracellular sequestration of CCR5. Offord and co-workers reported a correlation between potency and capacity to induce CCR5 sequestration.

However, contrary to earlier predictions that this capacity relates to increased affinity for the receptor, there was no correlation between affinity and potency. Future studies might shed light on the mechanism by which these modifications lead to intracellular sequestration of CCR5, and could lead to the development of clinically effective inhibitors of HIV transmission.

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References and links

ORIGINAL RESEARCH PAPER Hartley, O. *et al.* Medicinal chemistry applied to a synthetic protein: development of highly potent HIV entry inhibitors. *Proc. Natl Acad. Sci. USA* **101**, 16460–16465 (2004)

FURTHER READING Lederman, M. M. *et al.* Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* **306**, 485–487 (2004)