BONE DISEASE

Shedding light on a silent pathway

A common misconception is that osteoporosis is a 'disease of old age'. In fact, the reduction in bone density that causes osteoporosis begins as early as 25, but because the loss is gradual and initially asymptomatic, this 'silent disease' often goes unnoticed until it reaches an advanced stage, when bones break or fracture. Lifestyle factors, such as poor diet, can contribute to the development of osteoporosis, but genetics also has an important role to play, and in a recent issue of *Science*, Robert Klein and colleagues describe how experiments in mice have uncovered a gene that could be relevant to human osteoporosis.

To identify genes that might regulate bone density, the researchers looked at a region of chromosome 11 that has been linked to peak bone mineral density (BMD) levels in mice. First, they bred two strains of mice (DBA/2 (D2) and C57BL/6 (B6)) that differ at the region of interest on chromosome 11, and found that B6 mice had higher peak BMD and increased bone strength than their D2 littermates. Microarray analysis was then used to compare gene expression in D2 and B6 mice, and the researchers found that *Alox15* was the only differentially expressed gene within the region of interest. In fact, *Alox15* expression was nearly 20 times higher in D2 mice compared with B6 mice.

Alox15 encodes the enzyme 12/15-lipoxygenase (12/15-LO), which converts fatty acids into ligands for the peroxisome proliferator-activated receptor-γ (PPAR-γ). This receptor is found on the surface of many cells, including pluripotent stem cells in the bone marrow that give rise to many cell types, such as adipocytes (fat cells) and osteoblasts (bone-forming cells). As activation of PPAR-γ had previously been shown to drive the differentiation of marrow stem cells towards fat deposition at the expense of bone formation, Klein and colleagues suggested that overexpression of 12/15-LO might limit BMD by suppressing bone formation through activation of PPAR-γ.

To confirm their hypothesis *in vivo*, the authors showed that bone density and strength were increased in B6 12/15-LO knockout mice compared with age-matched B6 controls. In addition, knocking out 12/15-LO rescued D2 mice from the low-bone-mass phenotype associated with the D2 strain.

To see if inhibition of 12/15-LO could rescue the low bone density associated with over-activity of the *Alox15* gene, the authors treated growing B6 mice overexpressing *Alox15* with the specific 12/15-LO inhibitor PD146176, which improved both bone density and strength compared with untreated controls. They then used a rat model of oestrogen

DRUG DELIVERY

Breaking the skin barrier

Skin patches are widely used for combating nicotine addiction or delivering contraceptive hormones, but few drugs are delivered in this way because not many approved chemicals are available to help therapeutics permeate skin. An approach for rapidly screening and identifying combinations of chemicals that can penetrate the skin could make medicine by patch, rather than needle or pill, more common. In the February issue of *Nature Biotechnology*, Samir Mitragotri and colleagues describe a large-scale screen that can find rare combinations of chemicals capable of transporting medicines across the skin, while minimizing irritation.

Skin, the largest organ of the human body, possesses very low permeability to the movement of foreign molecules across it, due to the hierarchical structure of the stratum corneum, a lipid-rich matrix with embedded corneocyte cells. Although the use of chemical penetration enhancers (CPEs) is a convenient way to overcome the stratum corneum barrier, only a few CPEs studied up to now induce therapeutic enhancement of drug transport; and when used at the concentrations necessary for penetration, the molecules are also potent irritants. However, two chemicals are better than one because each can act on a different layer of the skin, allowing smaller doses to be used.

The search for new families of potent and safe synergistic combinations of permeation enhancers (SCOPE) involved a number of individual enhancers chosen from older and newer CPEs. Eight distinct categories were represented, including anionic and cationic surfactants, fatty acids and esters, and azone-like compounds. SCOPE formulations are mixtures of known CPEs that show high potency on contact with the stratum corneum but a relative lack of irritation in the epidermis because of the differential retention of components in the stratum corneum. The discovery of these mixtures was enabled by an experimental tool that screens using measurements of skin conductivity — *in vitro* skin impedance guided high-throughput (INSIGHT) screening — which tested more than 5,000 putative synergistic mixtures using porcine skin as a model. INSIGHT is more than 100-fold more efficient than current tools. The leading hits from INSIGHT were evaluated *in vitro* for irritation potential; mixtures with high potency and low irritation potential were then tested for flux enhancement using candidate drugs, before *in vivo* testing for bioavailability and safety.

Two formulations were particularly good, specifically N-lauroyl sarcosine:sorbitan monolaurate 20 (NLS:S20) and sodium laureth sulphate:phenyl piperazine (SLA:PP). Both formulations increased the flux of macromolecules, such as leutinizing hormone releasing hormone and heparin, *in vitro* by 50- to 100-fold. The SLA:PP formulation also delivered leuprolide acetate to hairless rats without causing skin irritation, confirming the feasibility of using penetration enhancers for systemic delivery of macromolecules from a transdermal patch.

Melanie Brazil

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deficiency to show that 12/15-LO inhibitors could not only improve bone mass during development, but also offset the bone loss that is associated with oestrogen deficiency.

The authors suggest that these findings in mice could have relevance to human osteoporosis, especially as a region of human chromosome 17 that contains the genes encoding 12-LO and 15-LO has already been linked to low bone density. And if a role for the 12/15-LO pathway in regulating human bone mass is confirmed, inhibitors of this enzyme that are already under development for other indications, including atherosclerosis, could provide a good starting point for preventing the silent progression of osteoporosis in humans.

Clare Ellis

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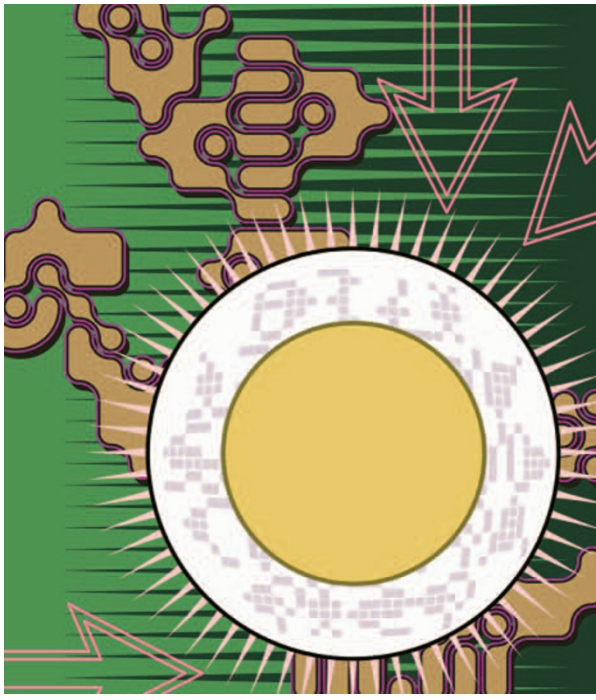
SEPSIS

New forms of attack



Treating sepsis — generally defined as the response of the host to microbial infection — is crucial in the clinical setting. Extreme forms of the condition, which usually arise as a result of complications from other diseases or treatments, has become the leading cause of death among patients in intensive care units. But developing therapies has proved difficult, as sepsis, although simple in definition, encompasses a wide range of inflammatory response mechanisms. Because of this complexity, two decades and billions of dollars invested in research on sepsis therapeutics has resulted in only one drug approval, activated protein C (Xigris), and an off-label indication for hydrocortisone.

Now, two studies have identified new targets that focus on, and reveal more about, different mechanisms in sepsis. The first, from Tracey and colleagues in *Proceedings of the National Academy of Sciences*, describes how antagonists of the transcription factor high-mobility group box 1 (HMGB1) can target mediators of the cytokine response of host cells to bacterial toxins. What makes this different from other early inflammatory mediator targets that have been identified, such as tumour-necrosis factor (TNF) and interleukin-1β (IL-1β), is that HMGB1 has a more delayed kinetic profile: the acute kinetics of TNF and IL-1β provide too narrow a therapeutic window for inhibitors of these cytokines to be clinically useful.

Using the cecal ligation and puncture (CLP) mouse model, regarded as the most clinically relevant model for sepsis, the authors showed that serum HMGB1 levels are increased significantly 18 hours post CLP, corresponding with the clinical development of the disorder. Antibodies to HMGB1 administered 24 hours after surgery increased survival significantly, which is striking as this is the first cytokine-based therapy that is effective when administered more than 8 hours after CLP, and antibodies to TNF worsened outcome in the CLP model. Structure–function studies showed that a domain called the HMGB1 A box inhibits HMGB1 activity, and animals treated with this HMGB1 antagonist were protected from organ injury and were conferred with lasting protection against lethality.

The second study, published in *Nature Medicine*, shows how the endogenous lysophospholipid lysophosphatidylcholine (LPC) treats sepsis in CLP, as well as in a model involving intraperitoneal injection of *Escherichia coli*, through a novel mode of enhancing bactericidal activity. The approach of Song and colleagues was predicated on the notion that defects in neutrophils, monocytes and other immune cells influence sepsis-associated mortality, and looked at LPC, a major component of oxidized low-density lipoprotein, which stimulates many immune cells *in vitro*.

They found that LPC was a potent antiseptic agent in preclinical models, and had a wide therapeutic window of 10–24 hours post-CLP surgery. This antiseptic effect seems to work through two mechanisms: enhancement of bacterial elimination and inhibition of the actions of the bacterial endotoxin lipopolysaccharide. *In vitro*, LPC increased the bactericidal activity by enhancing H_2O_2 production in neutrophils that ingested *E. coli*, but did not seem to work on any other immune cell. In the sepsis models, antibodies to the LPC receptor G2A inhibited these activities and increased mortality.

Given that so many potential sepsis therapeutics have shown only modest clinical efficacy despite the dramatic effects observed in preclinical models, the future of these targets is by no means certain. But both targets have the advantage of being complementary to current approaches and, in the case of HMGB1, this could allow the clinical subtyping of this complex and heterogeneous disorder.

Simon Frantz

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