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Aspirin, prostacyclin and nitric oxide

The final day of the Eighth World Congress on Inflammation began with the awarding of the IAIS Lifetime Achievement Award to Salvador Moncada (Director of the Wolfson Institute for Biomedical Research at University College London), recognizing his major and sustained contribution to inflammation research over the continuing course of his distinguished career.

In his speech, Professor Moncada charted the course of his career, which includes the determination of aspirin's mechanism of action, the discovery of prostacyclin, and the discovery of NO in the vasculature and the elucidation of its role, the work of which is still ongoing. From its discovery in 1845 up until 1960, the vascular endothelium was thought of merely as a structure, with no role of its own to play. When Professor Moncada came to the Royal College of Surgeons to work with John Vane and Sergio Ferreira all that was really known about the metabolism of arachidonic acid, which we now know produces fundamental vascular mediators such as prostaglandins (PG) and thomboxanes (TX) was that it produced PGF2-alpha and PGE2. Their work showed that prostaglandins infact enhanced the inflammatory reaction, rather than acting in the manner of direct effectors, such as bradykinin. They showed how aspirin blocked cyclooxygenase's conversion of arachidonic acid to PGG2 and PGH2 (the precursors of PGs and TXs), and demonstrated that the drug's gastric side effects were intrinsic to its inhibition of PG production. The enzyme that blocked the conversion of these precursors to TXs, such as TXA2, was identified as thromboxane synthase. It was also determined that PGG2 and TXA2 induced platelet aggregation, thus explaining why aspirin is anticoagulant.

In investigating what else the vasculature walls might make, a mediator, then dubbed PGX and now known as prostacyclin (PGI2), was discovered. In addition to acting as a vascular relaxant, studies demonstrated this agent to be a very potent anti-aggregating agent, which raised the question of why aspirin is an anticoagulant if it blocks this mediator as well as the pro-aggregatory, TXA2. Professor Moncada and colleagues have already shown that although the vessel wall makes PGI2, it does not make TXA2. Platelets make both; however aspirin's blockade of arachidonic acid metabolism lasts the whole of the platelet's lifetime, in contrast to its effect on the vascular wall, thus effectively inhibiting TXA2 while preserving PGI2. In the 1990s, the idea developed that the COX-2 isoform of cyclooxygenase was responsible for the pro-inflammatory effects, and thus COX-2-specific drugs were developed. However, unlike aspirin, COX-2-specific inhibition blocks PGI2 in the vessel wall as well as it does in the platelets. It was stressed that the cardiovascular risks of this remain unknown.

Professor Moncada has also investigated endothelium-derived relaxing factor (EDRF), which mediates the relaxant effect of ACh. In an elegant sequential bioassay, it was shown that EDRF had identical effects to, and was in fact, NO. Shortly after this discovery, his research laboratory successfully defined the L-arginine:NO pathway.

In the concluding section of his talk, Professor Moncada described how endothelial dysfunction, in which oxidative stress is implicated, is a cardiovascular disease predictor, for example in those individuals with atherosclerosis risk factors or with a family history of familial hypertension. High NO levels can lead to metabolic hypoxia, in which the use of oxygen by mitochondria is prevented by NO, thus flooding the cell with excess oxygen that is then metabolized by other enzymes in order to create reactive oxygen species (ROS). This excess oxygen at the end of the metabolic chain may also allow ROS to escape higher up the chain; this situation is seen in conditions such as septic shock, and may also be important in inflammation. Professor Moncada's current work includes the determination of the role that this pathway may play in metabolic syndrome and obesity.

This lecture was hailed as an "amazing presentation" by the session Chair, and was received with resounding applause and a standing ovation from the audience.

G2's anti-C5aR antibodies prove effective in arthritis models

Peter Whitfeld (G2 Therapies) described the generation of anti-human-C5a receptor (C5aR) mAbs in wild-type mice that have been immunized with neutrophils from human-C5aR knock-in mice. This antibody binds a region of the second extracellular loop of C5aR, thus inhibiting C5a-induced neutrophil migration and calcium-flux. In human-C5aR knock-in mice, dosing with the antibody (1 to 10 mg/kg, administered intraperitoneally) 1-day prior to K/BxN serum-

transfer-induced inflammatory arthritis, dose-dependently inhibited swelling, leukocyte infiltration and cartilage erosion when compared with the control. The antibody was also shown to reverse inflammation in this model when given 5-days after serum transfer, and also to reverse collagen-induced arthritis (CIA) when dosed after the onset of inflammation. In February 2006, G2 Therapies signed a deal worth $108 million to codevelop the antibodies with Novo Nordisk.

Migration inhibitory factor antagonists

The creation of mouse anti-human macrophage migration inhibitory factor (MIF) antagonists was described by Jie Tang (Chinese Academy of Sciences), whose research group has developed a method for breaking the immune tolerance that is due to the evolutionary conservation between mouse and human MIF, and which hampers the ordinary mechanisms of murine antibody production. Of three high-affinity clones, 10C3 was found to inhibit MIF-induced NO and TNF-alpha secretion via a macrophage cell line. In a murine LPS-induced sepsis model, the drug increased survival when used alone and when synergized with dexamethasone. The academy is currently investigating the development of a mouse-human chimeric version of this antibody, for which it has a high-producing cell line and is now ready to scale-up production. Dr Tang noted that preclinical and clinical studies are planned for the future.

Eric Morand of Cortical described the suppression of CIA by his company's MIF inhibitor, COR-100140. Injection of bovine collagen on days 0 and 21 was used to induce severe CIA in DBA-1 mice that then received a daily oral, gavage dose of 15 or 60 mg/kg COR-100140, or an intraperitoneal 8 mg/kg dose of etanercept, every other day from day-21 onwards. Disease initiation for the two treatments was offset in order to compensate for the worsening of arthritis observed with the gavage-dosing regime. Although the 15 mg/kg dose was not effective, 60 mg/kg of COR-100140 reduced disease severity comparably to etanercept, which had a maximum suppression of approximately 30%. COR-100140 achieved maximum suppression by day 36, compared with day 48 for etanercept, and halved serum MCP-1 and significantly decreased TNF on day 50. No histopathological data were gathered. Dr Morand concluded his talk by noting that Cortical currently has additional compounds in this series that have demonstrated greater water-solubility than COR-100140.

LEO-15520 reduces arthritis in preclinical models

LEO Pharma's selective p38 MAP kinase inhibitor, LEO-15520, has shown efficacy in various inflammatory disease models. Data from such studies were presented by Lene Jensen (LEO Pharma). This oral drug, which is being investigated for inflammatory conditions such as psoriasis, psoriatic arthritis and rheumatoid arthritis, has been shown to inhibit MKK6, p38 alpha and p38 beta by 99%, with an IC50 value of 1.6 nM against p38 alpha, while not inhibiting any of the other 77 kinases screened. It inhibited the production of TNF-alpha, IL-1, IL-6 and IL-8 by human activated monocytes in vitro with IC50 values of 0.3, 2.3, 5.2 and 0.5 nM, respectively. In vitro, the drug inhibited proliferation of activated human T-cells, with an IC50 value of 2.5 nm, and inhibited IL-8 release from human primary keratinocytes. In vivo, LEO-15520 inhibited CD3-induced IL-2 release with an ED50 value of 0.3 mg/kg, and dose-dependently inhibited lymphocyte activation in an oral GvH rat model. A 10 mg/kg oral dose of the drug reduced CIA by 65 and 77% in mice and rats, respectively. In the mouse model, its effect was comparable to 1 mg/kg of prednisolone, and it reduced arthritis and joint and bone destruction scores to 0.7 and 0.35, respectively, compared with 1.7 and 1.1 for vehicle, and 0.4 and 0.2 for prednisolone. The drug was found to be effective at 3 and 10 mg/kg in a guinea pig UVB-induced skin inflammation model. In a humanized psoriasis model, 20 mg/kg of LEO-15520 reduced epidermal thickness by 41% and was comparable to 1 mg/kg dexamethasone.

According to a poster presented by LEO Pharma's Katherine Abell, LEO-15520 demonstrated no effect on dextran sodium sulfate-induced colitis in mice, a model of inflammatory bowel disease (IBD). However, as p38 was shown not to be involved in the pathology of this model, it has been suggested that it may also have no involvement in IBD either.

LEO Pharma also presented two posters on its multikinase inhibitor, LEO-A, which inhibited 26 inflammation/immunity-related kinases out of 207 tested, with IC50 values of: VEGFR-1 = less than 3 nM; VEGFR-2 = 10 nM; VEGFR-3 = less than 3 nM; PDGFR alpha = 91 nM; PDGFR beta = 129 nM; cKit = 23 nM; c-RAF = 14 nM; cSRC = 65 nM; and Fms = 12 nM. It also inhibited VEGF-induced proliferation in huvec cells, with an IC50 value of 2 nM, and did not affect MRC-5 or A431 cell proliferation (IC50 values were 10,000 and >10,000 nM, respectively). In vivo studies in a human epithelial carcinoma xenograft model found that LEO-A was orally available and well tolerated. An oral dose of 75 mg/kg/day inhibited in-vivo tumor growth of colon, renal and prostate cancers.

The second poster described the anti-inflammatory effect of LEO-A, which dose-dependently (3 to 30 mg/kg) inhibited LPS-induced TNF-alpha release in rats and mice, and anti-CD3-induced IL-2 release in mice. Lymph node size was reduced by the drug following allogeneic stimulation in a GvH model. In rats with CIA, LEO-A reduced clinical score when administered intraperitoneally with doses of 5, 10 or 25 mg/kg before onset of the disease, and with 25 mg/kg after disease onset. However, this effect was less than that observed with the earlier dosing regime. In CIA mice, intraperitoneal administration of the drug at 25 mg/kg was effective when dosed before disease onset, but not when dosed later. In a choroidal neovascularization model, intraperitoneal doses of 25 mg/kg, or oral doses of 50 or 100

mg/kg were found to inhibit choroidal lesions. A trend towards lesion reduction was seen when 2.5 microg of LEO was dosed locally.

Surface Logix's SLx-2119 reduces septic liver injury

James Ellis (Surface Logix) presented preclinical septic liver injury data on the company's oral selective ROCK-2 inhibitor, SLx-2119. Liver injury was induced in mice by LPS and D-galactosamine challenge, resulting in a ten-fold increase in ALT- and AST-serum levels. Oral pretreatment with 100 mg/kg SLx-2119 at -20 and -2 h, or intraperitoneal pretreatment of 10 to 100 mg/kg at -15 min, reduced both ALT and AST elevation by 90%, and TNF-alpha elevation by 50%. When administered intraperitoneally at 100 mg/kg, the drug was also effective when dosed 15 or 60 min post injury: the 25 h immortality rate was 50 and 70% for the two timepoints respectively, compared with 100% for vehicle control, and no mortality observed at 48 h following dosing of the drug 15 min before induction of liver injury. SLx-2119 reduced mortality in a survival study, with only one in ten animals dying by 48 h, compared with nine deaths out of ten mice in the control group by the 8 h timepoint. In vitro, 25 h culture with 10 microM of the drug protected huvec monolayers from damage caused by 24-h incubation with TNF-alpha, starting 1-h after the addition of SLx-2119.

PKC-theta inhibitors

Boehringer Ingelheim's small-molecule PKC-theta inhibitor program was showcased in a talk given by the company's Rene M Lemieux. Compound 1 in this program has shown to binds the target with high affinity and to be ATP-competitive. It was also shown to bind PKC-delta, but not any other kinases tested. In vitro, compound 1 demonstrated inhibition of IL-2 production by anti-CD3 and anti-CD28-stimulated, or SEB-stimulated, human T-cells, with IC50 values of 12 and 414 nM, respectively; this potency is similar to that of cyclosporin. The drug had sustained oral exposure across different species, with half-life values of 10, 2.6 and 3.8 h in mice, rats and dogs, respectively. In the murine delayed-type hypersensitivity model, inflammatory-cell recruitment was inhibited at 0.03, 0.3 and 3 mg/kg doses. Dr Lemieux noted that the safety profile of compound 1 is yet to be assessed.