Editors' view

Novel targets and regulatory ordeal by QT interval

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The PAF pathway as a potential therapeutic target

Platelet-activating factor (PAF), also known as PAF-acether and AGEPC (acetyl-glycerol-ether-phosphorylcholine), is a lipid-derived biological messenger active at sub-nanomolar concentrations [1]. 'PAF' is something of a misnomer as it acts on a variety of cells, not only on platelets, with possible roles in allergy and inflammation as well as haemostasis/ thrombosis. PAF is made from acyl-PAF in a two-step process summarized in Figure 1: a highly-regulated cytosolic enzyme phospholipase A₂ (PLA₂) acts on acyl-PAF to produce lyso-PAF, which is then acetylated to give PAF. PLA₂ simultaneously liberates free arachidonic acid from the 2-position of glycerol in cell membranes initiating the production of eicosanoids (prostaglandins, leukotrienes, and lipoxins) in parallel with PAF. PAF is inactivated by deacetylation which regenerates the inactive lyso-PAF precursor. PAF is produced by activated inflammatory cells and acts via specific G-protein-coupled PAF-receptors. Injected subcutaneously it produces many of the signs and symptoms of inflammation, including vasodilatation and redness, increased vascular permeability and wheal formation, and, at higher concentrations, hyperalgesia. It is a potent chemotaxin for neutrophils and monocytes, and recruits eosinophils into the bronchial mucosa in the late phase of asthma. It can itself activate PLA₂, reinforcing eicosanoid synthesis by positive feedback. It has spasmogenic effects on both bronchial and ileal smooth muscle and stimulates arachidonate turnover and TXA₂ generation by platelets, producing shape change and releasing platelet granule contents.

The PAF pathway (of biosynthesis, receptors, and downstream transduction events) is thus an attractive therapeutic target, but it is not yet clear whether inhibiting it will prove therapeutically advantageous in thrombotic or inflammatory diseases. There was great excitement follow-

ing the discovery of PAF (by Jacques Benveniste, a French immunologist) in the early 1970s, but sadly no drugs have really made it to market. However, the anti-inflammatory actions of the glucocorticoids may partly be caused by inhibition of PAF synthesis [2], so we should not give up hope. Competitive antagonists of PAF and/or specific inhibitors of lyso-PAF acetyltransferase could well be useful anti-inflammatory and/or anti-asthmatic drugs. The PAF antagonist lexipafant is effective in a rat model of acute pancreatitis [3], but several small human studies have been negative or inconclusive. Icatibant, an antagonist at bradykinin B2 receptors, was also effective in animal models of pancreatitis but not in the human disease. It has since been licensed in Europe for use in hereditary angio-oedema in patients with C1-esterase deficiency, exemplifying how difficult it can be to find the optimum match between an inhibitor or antagonist of a mediator and disease target: one of many challenges for translational medicine [4]. The same message is provided by a complement inhibitor, eculizumab, also recently licensed in Europe for the rare and previously almost completely untreatable disease paroxysmal nocturnal haemoglobinuria – see articles in our new drugs' mechanisms feature [5, 6].

Rupatidine is a combined H₁ and PAF antagonist [7], licensed in several parts of the world for treating allergic rhinitis and for chronic urticaria, but it is not clear what (if anything) its action on PAF receptors adds to its therapeutic efficacy over and above its antihistamine action [8]. The withdrawal from the market of another less-sedating antihistamine, terfenadine, ushered in the modern era of regulatory concern over cardiac safety of non-cardiac drugs. Astemizole, also a less-sedating antihistamine, had similar effects and was also withdrawn, raising the spectre of a drug-class effect. This has not proved to be the case: many non-cardiac drugs with quite different primary pharmacology have been implicated [9] and several antihistamines

Figure 1

Biosynthesis of chemical mediators (PAF and eicosanoids) from acyl-PAF (embedded in phospholipid in the cell membrane) via the intermediates lyso-PAF and arachidonic acid, and regeneration of acyl-PAF. Enzymes are shown in green. PLA₂ = phospholipase A₂, the highly regulated cytosolic form of which initiates both pathways by cleaving a fatty acid (eg arachidonic acid) from the sn 2 position of the 3 carbon atom glycerol backbone of acyl-PAF

exonerated, but, perhaps because of this early experience, antihistamines have been especially carefully scrutinized in this context. In the current issue of the *Journal* Ester Donaldo and her colleagues describe a 'thorough QT/QTc study' of rupatidine in 160 healthy volunteers [10]. The study had several interesting features, some of which we discuss below, and was convincingly negative. The cardiac safety of rupatidine had previously been extensively evaluated preclinically, clinically, and post registration, and one might question whether a perceived need to be ICH guideline-compliant led to a scientifically superfluous (albeit rigorously conceived and meticulously performed) study. Would the resources used in this way have been better spent in investigating the novel pharmacology of this PAF antagonist, and addressing whether this confers unique benefits in terms of unmet clinical need?

QT interval change as a biomarker

Electrocardiographic QT interval prolongation caused one-third of drug withdrawals between 1990 and 2006. Torsade de pointes (TdP) is a serious, uncommon form of polymorphous ventricular tachycardia first described by François Dessertenne in 1966 [11]; abnormal cardiac repolarisation caused by an inherited mutation of the rapid delayed rectifier current channel (I_{Kr}) is a predisposing factor. Several drugs prolong the QT interval to a greater or lesser extent, and some of these (including the antihistamines men-

tioned above) also predispose to TdP. Drugs that inhibit the alpha subunit of the I_{Kr} potassium channel encoded by a gene termed *hERG* have particularly been implicated. This has led to a series of regulatory guidelines, culminating in the ICH E14 guideline of 2005, which defined the so-called 'thorough QT study'. Such studies are intended to identify confidently drugs that cause QT prolongation in a single definitive investigation in healthy volunteers. As well as a placebo (i.e. negative control) such studies incorporate a positive control (see below) and are stringently powered to exclude prolongation of QTc > 10 ms. Such 'thorough QT studies' are now an important part of the cardiac safety assessment of new drugs. Pre-human electrophysiological studies of new drugs on isolated cardiac myocytes, or studies of ligand displacement or rubidium efflux in Chinese hamster ovary cells expressing the *hERG*-encoded subunit of I_{Kr}, have aborted the development of large numbers of otherwise promising new drugs that consequently never reached the stage of a 'thorough QT study'. Still more potential new drugs do not get off the drawing board at all because the computational chemists calculate that their shared features with drugs that prolong QT make them a dangerous bet in this regard. This attrition is good only if it truly results in safer new drugs, and this is a negative that is hard to prove.

We have previously commented on drug-induced prolongation of the QT interval and drug development [12], including the risk of not developing good drugs, such as quinidine and doxorubicin (which prolong QT) or lamot-

rigine (which binds to the hERG-gene product but does not prolong QT [13]). Quinine has some cardiotoxicity, but its effectiveness in malaria is such that it would have been tragic if its therapeutic use had been curtailed because of concerns about its cardiac safety. Perceived hazards may lead to an excessively conservative approach; we shall not repeat all of the arguments here – the effects of this regulatory guidance have been reviewed in a themed section in our sister journal the *BJP* based on a symposium held at the European Pharmacological Societies congress held in Manchester (UK) in July 2008 [14]. Such guidance is certainly well-intentioned, but does have the potential for unintended consequences, some of which relate to imperfections of QT/QTc changes as biomarkers of TdP risk (rather horribly termed 'torsadogenicity' by the pundits). Direction of resources to a QT study of rupatadine, a drug that had already been extensively investigated in terms of cardiac safety, rather than to its novel pharmacodynamics might be one such, although it must be conceded that terfenadine had been used apparently safely for many years, including over the counter sales, before its cardiotoxicity was fully appreciated. Here, the problem was that terfenadine (which itself caused the toxicity) was acting therapeutically via its metabolite fexofenadine; bioavailability of the prodrug is less than 5% (very high pre-systemic hepatic metabolism), so very little of the toxic prodrug reaches the systemic circulation; consequently, it was only when interacting with CYP inhibitors such as erythromycin, and in the elderly, that its cardiac toxicity was manifested.

It may be that effects on QT interval are the best biomarker currently available as a surrogate for the risk of TdP, but regulators, industry scientists, and academics should not be blind to its limitations, such as that not all drugs that prolong QTc cause TdP, including even drugs such as alfuzosin, which has a large effect on QTc. Amiodarone markedly prolongs QTc, but while there have been reports of TdP in patients taking amiodarone this seems to occur less commonly than with other drugs that prolong the QT interval less profoundly. Other therapeutically useful drugs that prolong QTc without causing TdP include ranolazine, verapamil, and pentobarbital [15].

Another problem thrown up by thorough QT studies, which we have mentioned previously [12], is that by measuring the QTc effects of all new therapeutic drugs rigorously, some are discovered to *shorten* QTc. There are hereditary short QTc syndromes that are associated with a risk of ventricular fibrillation (although they are much rarer even than the long QT syndrome). Individuals with such syndromes should avoid drugs that shorten QT, which can be specified in product labelling, but should QT shortening *per se* be a more general regulatory concern? Cardiac glycosides shorten QT, perhaps by their well-known effect of increasing vagal tone and hence activating cardiac K_{ACh} channels. ATP-sensitive potassium channel activators, such as pinacidil and levcromokalm, also shorten QT and are

proarrhythmic in preclinical models, and Rashmi Shah (a senior regulatory scientist) argues the case for taking QT shortening seriously as a potential regulatory concern, using rufinamide (a recently licensed anticonvulsant) to illustrate one regulatory approach of approval followed by large-scale post-marketing studies focused on cardiac safety [16]. However, Marek Malik argues that intensive investigation of QT-shortening drugs is not warranted [17].

What is an appropriate positive control in a 'thorough QT study'?

We have commented previously on several technical and experimental challenges to the 'thorough QT study' [12] but here wish to comment only on one issue, that of an appropriate positive control. Fluoroquinolone antibiotics prolong QT in humans but are safe enough for use in volunteer studies. Moxifloxacin has a marked effect on QT and has been used for this purpose in many studies. In the current issue of the *Journal* we publish a paper from St George's Hospital, London (UK), which compares the effect of moxifloxacin with two doses of levofloxacin, which is less potent in prolonging QT [18]. The authors showed that either of these fluoroquinolones can fulfil the criteria for a positive comparator, but that levofloxacin has the potential to provide 'a more rigorous evaluation of the assay conditions used to detect clinically significant changes in QTc when evaluating new chemical entities'. This was because the two doses used (1 g and 1.5 g) caused mean QTc interval prolongations of 4.4 and 7.4 ms, in line with the ICH recommendation for a sensitivity target of a mean increase in QTc of approximately 5 ms. Moxifloxacin (400 mg), in contrast, prolonged mean QTc by 12 ms. An alternative to using levofloxacin would perhaps be to use a lower dose (e.g. 200 mg) of moxifloxacin.

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