Isoform-selective PI3K inhibitors as novel therapeutics for the treatment of acute myocardial infarction

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Abstract
In the present paper, we review the preclinical development of TG100-115, a PI3K (phosphoinositide 3-kinase) γ/δ isofrom-specific inhibitor currently in clinical trials for the reduction of acute MI (myocardial infarction). An overview is presented outlining the pathogenesis of acute MI and the rationale for clinical use of PI3K γ/δ-specific inhibitors in this indication. TG100-115’s broad anti-inflammatory activities are described, as well as its ability to discriminate between cellular signalling pathways downstream of receptor tyrosine kinase ligands such as vascular endothelial growth factor. Finally, we review TG100-115’s potent cardioprotective activities as revealed in rigorous animal models of acute MI, and, based on these data, this compound’s potential for clinical utility.

MI (myocardial infarction) represents the heart’s attempt to manage an I/R (ischaemia/reperfusion) injury. The acute phase of infarct development initiates with an ischaemic event, the loss of regional blood flow resulting from occlusion of a major coronary vessel, which then directly induces cardiomyocyte apoptosis as well as the up-regulation of pro-inflammatory mediators such as VEGF (vascular endothelial growth factor) and PAF (platelet-activating factor) [1,2]. Of immediate concern is ischaemia-induced cardiomyocyte apoptosis and necrosis, both of which unfold within minutes [3]. While considerable information has been gathered on these events, such data have not translated into effective MI therapies. This was to some degree predictable, as ischaemic injury largely evolves prior to presentation at an interventional setting, rendering the patient inaccessible during times when an anti-ischaemia therapy would be most effective [4].

Reperfusion injury, by contrast, unfolds in the clinic after re-establishment of macrovascular flow (via angioplasty and/or thrombolysis). Reperfusion generates damage in large part by fostering myocardial inflammation [5]. Its mode of action therefore centres on the vascular compartment (leucocytes and endothelium) as opposed to the cardiomyocyte (i.e. on the micro- rather than macro-vasculature). Once again, although therapeutic attempts have been made to limit reperfusion injury (e.g. with leucocyte adhesion molecule antagonists), these too have met with limited clinical success [6].

One issue with such therapies may have been a too narrow focus. While leucocyte recruitment is a key component of nascent inflammatory reactions, so too are leucocyte activation and vascular oedema. Furthermore, these events are closely interrelated, with activated leucocytes releasing factors that contribute to the compromise of the endothelial barrier. Oedema, in consequence, not only directly damages myocardium (disrupting tissue homeostasis and spreading cytotoxic factors), but also exacerbates tissue ischaemia by collapsing microvascular blood flow (the ‘no-reflow phenomenon’) [7]. Indeed, we previously implicated VEGF-induced oedema as a major contributor to infarct development [8]. We reasoned, then, that a therapeutic approach that sought to more broadly block inflammation might prove more suited to reducing MI pathogenesis. The challenge of such an approach, however, lies in the wide number of pro-inflammatory, pro-oedema agents generated during I/R injury. These include not only VEGF and PAF [1,2], but also various cytokines and eicosanoids [9–11], histamine [12], thrombin [13], and complement factors [14]. Clearly this diversity makes blockade at the receptor level unfeasible. Inhibition at the subreceptor level, however, might be reasonable if a common signalling element were identifiable.

PI3K (phosphoinositide 3-kinase) could represent this gatekeeper, lying downstream of both receptor tyrosine kinases and GPCRs (G-protein-coupled receptors), two receptor classes encompassing the ligands listed above. The γ and δ isoforms in particular would appear promising targets, as genetic deletion studies establish their roles in both oedema and inflammatory responses [15–20]. By contrast, PI3K α and β, two broadly expressed isoforms, apparently play more fundamental biological roles as genetic deletion of either is lethal [21], and therefore potential anti-inflammatory therapies would do best to avoid disruption of these two kinases.

Key words: inflammation, ischaemia, myocardial infarction, oedema, phosphoinositide 3-kinase (PI3K).
Abbreviations used: EC, endothelial cell; GPCR, G-protein-coupled receptor; I/R, ischaemia/reperfusion; LAD, left anterior descending coronary artery; MI, myocardial infarction; PAF, platelet-activating factor; PEG, poly(ethylene glycol); PI3K, phosphoinositide 3-kinase; VEGF, vascular endothelial growth factor.

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There are considerable data, however, that could lead one to argue against proposing even isoform-specific PI3K inhibition as a cardioprotective therapy. Foremost are numerous studies attributing a pro-survival activity to PI3K (or its downstream target Akt) during MI development [22–24]. Studies utilizing pharmacological inhibitors of PI3K also provide conflicting signals. For example, the PI3K inhibitor wortmannin reduced neutrophil infiltration in isolated hearts exposed to I/R injury [25] but failed to reduce infarct size when delivered post-reperfusion, as did LY294002, another compound commonly cited as a PI3K inhibitor [26]. Whether these results are predictive of all PI3K inhibitors, of panisoform inhibitors, or just these particular compounds is unclear, and it therefore remains uncertain whether inhibition of PI3K signalling would prove beneficial, detrimental or inconsequential to infarct development.

As a step towards resolving this question, we have explored several novel chemistries for both anti-PI3K and anti-inflammatory activities, as preludes to their potential development as cardioprotective therapies. PI3K inhibitors of various specificities were identified, ranging from pan-active across class IA (α/β/γ) and Class IB (γ) isoforms to the more restricted. One compound in particular, TG100-115, provoked interest as a specific inhibitor of both PI3K γ and δ (while avoiding inhibition of PI3K α and β as well as a wide host of protein kinases) [27]. In vitro assays revealed intriguing activities suggestive of a selective rather than global inhibition of cell signalling. As expected, TG100-115 blocked the phosphorylation of kinases downstream of PI3K [e.g. Akt, mTOR (mammalian target of rapamycin) and p70S6K] by which this growth factor triggers disassembly of EC tight junctions, thereby enhancing vascular permeability [8]). In addition, TG100-115 antagonized leucocyte pro-inflammatory activities, for example reducing peripheral blood mononuclear cell adherence on collagen upon exposure to PAF.

Building on these cell-based data, in vivo modelling further confirmed the selective nature of PI3K γ/δ inhibition. For example, in agreement with its observed effects on cultured EC, TG100-115 strongly inhibited VEGF-induced oedema but not angiogenesis. In line with our hope that PI3K γ and/or δ inhibition would prove broadly anti-inflammatory, this compound also blocked oedema and inflammation induced by GPCR ligands (such as histamine and PAF) as well as general leucocyte activators (such as dextran). Therefore TG100-115 appears to display several properties that recommend its use in I/R injuries such as acute MI: differentiation between deleterious and beneficial cell responses (VEGF-induced oedema and angiogenesis respectively), broad anti-inflammatory activity (influencing both ECs and leucocytes to reduce both oedema and inflammation), and broad activity against diverse inflammatory mediators (including two of particular relevance, VEGF and PAF).

Following on to a rodent model of MI [in which temporary occlusion of the LAD (left anterior descending coronary artery) creates an I/R injury to the myocardium], administration of TG100-115 during the reperfusion phase reproducibly reduced the extent of infarction. For example, Table 1 presents data from a representative study in which three different TG100-115 formulations were compared for efficacy; all three decreased total infarct area by ≥47% as compared with animals subjected to myocardial I/R but dosed with a vehicle control (P < 0.001). Second-species validation was obtained using a porcine MI model, where TG100-115 delivered 2 h after the initiation of I/R injury (90 min of LAD occlusion followed by 30 min of reperfusion) decreased infarct area by approx. 40% compared with vehicle-treated controls [27].

Table 1 | PI3K γ/δ inhibition limits the extent of MI

<table>
<thead>
<tr>
<th>Test article</th>
<th>Infarct area (% ischaemic area)</th>
<th>Change compared with vehicle (%)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle alone</td>
<td>70 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>TG100-115 (8% PEG formulation)</td>
<td>32 ± 4</td>
<td>−54%</td>
</tr>
<tr>
<td>TG100-115 (15% cyclodextrin formulation)</td>
<td>37 ± 4</td>
<td>−47%</td>
</tr>
<tr>
<td>TG100-115 (4% cyclodextrin formulation)</td>
<td>36 ± 3</td>
<td>−49%</td>
</tr>
</tbody>
</table>

The most impressive aspect of these preclinical modelling data is that meaningful cardioprotection was achieved despite the fact that therapeutic intervention did not initiate until well after the onset of I/R injury (up to 4 h later in the rodent model). From a scientific standpoint, this validates our belief that concentrating on late reperfusion events, rather than early ischaemic injury, represents a logical approach to modulating infarct development. For example, VEGF production being under transcriptional control requires hours to develop [28], and therefore VEGF-mediated oedema represents a reasonable interventional target for I/R injury. The same is true for the numerous oedema and inflammatory
mediators (such as PAF, cytokines and eicosanoids) generated by activated leucocytes during reperfusion. This is not to deny that anti-apoptotic therapies could be of potential benefit, alone or in combination with an anti-inflammatory agent such as TG100-115. Rather, we suggest that focusing solely on a direct intervention at the level of cardiomyocyte apoptosis creates an insurmountable barrier to clinical application.

As outlined at the beginning of the present paper, the literature contains many reports of agents that successfully decrease infarct size. In the vast majority of studies, however, dosing was initiated after <60 min of ischaemic injury, generally during ischaemia or at reperfusion (even pre-ischaemia in many cases). Unfortunately, these scenarios have little relation to how therapies would be applied clinically, where the need is for interventions that can show efficacy despite the presence of pre-existing I/R injury [4]. In fact, for patients presenting at emergency centres, revascularization therapy does not initiate on average until more than 2 h after initial symptom onset [29]. By adopting rigorous standards in the preclinical work-up stage, it is our hope that additional import can be added to our observations of cardioprotection, and that TG100-115 can be presented as a logical candidate for bridging that gap between preclinical efficacy and true clinical utility. Indeed, this compound has been extended into the clinic, in a double-blinded dose-escalating trial delivering TG100-115 to patients shortly after angioplasty. To our knowledge, TG100-115, then, represents not only the first small-molecule kinase inhibitor to be tested as an MI therapy, but also the first isofrom-specific PI3K inhibitor to enter the clinic.

References


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