Pharmacological Agents in the Clinic: Trial and Error

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ABSTRACT

Following an explosion of molecular and cellular research in the last 50 years, the study of specific molecular targets in the context of heart failure has generated much excitement. With the incidence of heart failure rising worldwide, new pharmacological agents are eagerly anticipated as a key tool with the potential for significant impact. In this regard, the results of the keenly awaited Does Cyclosporine Improve Clinical Outcome in ST Elevation Myocardial Infarction Patients (CIRCUS) trial have just been made available. Despite a substantial programme of supporting experimental and preclinical evidence however, the results have proved negative. This is of great disappointment to the clinical and scientific community. Here, this is discussed in the context of previous studies and future directions explored.

TARGETING MYOCARDIAL REPERFUSION INJURY TO MITIGATE HEART FAILURE

Molecular treatment regimens were first introduced into standard clinical practice in the 1980’s but have not changed substantially since. Myocardial infarction is one of the most widespread causes of secondary events, heart failure and death, and patients with ST-segment Elevation Myocardial Infarction (STEMI) are a particularly high risk group. The extent of cell death in infarction i.e. infarct size, is suggested to be a major determinant in the likelihood of survival. Since myocardial reperfusion injury contributes greatly to the final infarct size, treatments that mitigate this have garnered significant interest.

The trial discussed herein focuses on the use of cyclosporine, a pharmacologic inhibitor of cyclophilin D that is a major component of the mitochondrial Permeability Transition Pore (mPTP). In ischemic tissue, Adenosine triphosphate (ATP) is depleted and cell death ensues. Upon reperfusion, further injury occurs due to generation of reactive oxygen species and calcium cation overload in mitochondria. This results in opening of the mPTP and mitochondria dependent cell death. Inhibition of cyclophilin D by cyclosporine and subsequently mPTP opening reduces the severity of myocardial reperfusion injury in animal models. Cyclosporine is in fact already in use as an immunosuppressant in organ transplantation and autoimmune disorders, though associated with toxicity in chronic administration. Of further concern, it has also been shown to impede the compensatory hypertrophy of the remote noninfarcted myocardium by additionally inhibiting cyclophilin A and calcineurin. Thus cardiac studies have used single, acute doses to minimise off-target effects while attempting to retain benefits.

CYCLOSPORINE PHASE II TRIALS

Initial results in a small phase II trial indicated a reduction in infarct size in STEMI patients when cyclosporine was administered immediately before reperfusion (primary Percutaneous Coronary Intervention; PCI), n=30 treated with cyclosporine, n=28 controls. The primary endpoint, infarct size, was assessed by measuring the cardiac injury biomarkers creatine kinase...
and troponin I and by Magnetic Resonance Imaging (MRI) in 11 control and 16 cyclosporine treated patients. While creatine kinase levels were significantly reduced by cyclosporine treatment, troponin I was not. MRI showed a significant reduction in infarct size, but this did not translate into any improvement in left ventricular (LV) ejection fraction three months later. A follow-up report on the same patient cohort at 6 months, showed a persistent reduction in infarct size in the cyclosporine treated group, with a minor reduction in diastolic LV volume and a slight, though non-significant trend for reduced wall thickness. Similar to 3 months, cardiac function (ejection fraction) was not improved. However in another trial, in conjunction with thrombolytic therapy in STEMI patients cyclosporine addition did not improve any outcomes tested up to 6 months (n=50, n=51 controls), nor was infarct size affected though this was measured only by creatine kinase release.

**CYCLOSPORINE PHASE III TRIAL: CIRCUS**

A phase III trial was commenced with larger numbers; 395 patients in the cyclosporine group and 396 controls, to assess whether clinical outcomes were improved at 1 year. The primary outcome was a combination of death, worsening of heart failure during the first admittance, rehospitalization and an increase of 15% or more in LV end diastolic volume. However, cyclosporine showed no benefit on these outcomes and cardiac parameters such as infarct size and LV wall thickness that were improved. The authors state that a sub-population of phase III patients were selected to evaluate infarct size and LV remodelling in a more stringent manner and publication of the results of these will permit more direct comparison and dissection of the reasons for failure in moving to phase III.

The formulation of cyclosporine used is also given weight as a confounding factor; previously being Sandimmune (Novartis, which uses Cremophor EL, a polyoxyethylated castor oil as a vehicle) and currently, CicloMulsion (NeuroVive Pharmaceutical, where the vehicle is a lipid emulsion). However, in contrast to the preliminary positive study, Sandimmune was shown to decrease maximal respiration in muscle, though was an effect of the vehicle control itself rather than cyclosporine. In addition, it was not found to be cardioprotective or to reduce infarct size in humans or pigs, although it did reduce infarct size in rabbits. With regards to CicloMulsion used in the recently reported negative study, lipid emulsion itself reduces infarct size and improves functional recovery to a greater extent than cyclosporine through inhibition of cyclophilin D, although downstream signalling events indicate divergent mechanisms in cardioprotection. This would rather indicate that an improvement might be expected whether from cyclosporine, the vehicle itself or both, but this simply did not translate into the clinic. In addition, further confounding factors might also exist, for example, the effects of cyclosporine, cardioprotective or otherwise appear to be influenced by anaesthesia protocols.

**CLINICAL TRIALS: LOST IN TRANSLATION**

The disappointing results of this trial also call into question the translational abilities of current pre-clinical protocols. Such testing rarely includes prevalent comorbidities and risk factors or existing clinical treatments; without this, the interactions and outcomes in humans cannot be predicted. Consideration of these interacting factors could in fact reveal a sub-population of patients that might benefit greatly from this treatment. In addition, drug delivery methods and formulations that are clinically the most practical and likely to be used should be closely recapitulated in animal models. Further, many of the primary and secondary outcomes measured in trials do not form part of pre-clinical studies. For reasons of cost, survival up to one year for example might not be preferred but how can mouse to man possibly translate when different end-points are measured?

Clinical trial design itself is a crucial factor and must be reflected in preclinical animal models. Selection of inclusion and exclusion criteria, along with primary and secondary outcome measurements can significantly alter trial results and outcomes must be used that are sufficiently powered by the sample size. In addition, transparent and full reporting of results from animal studies are key to improving translation. The recently published Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for improving study design and reporting of animal studies are intended to do just this.
More substantial target validation and mechanistic information across multiple model systems more relevant to the human heart failure and clinical environment is also required. A carefully selected panel of heart failure mouse models that incorporate common co-morbidities along with commonly used clinical measurements and outcomes must be used to provide a more reliable basis for further drug development. In parallel, use of human stem cell-derived cardiomyocytes can address the concern of translatability from mouse to man. Although a drawback of this is cellular immaturity, efforts are underway to better recapitulate more mature human myocardium and a number of usable models now exist such as Engineered Heart Tissue (EHTs) that better model many of the key characteristics of the intact human heart.25,26

A unified workflow prior to clinical trials with worldwide input and adherence would also provide a much improved and clear pathway from target identification to the patient. Indeed, such approaches now exist in the US and recommendations for generating similar in Europe have been recently proposed.27,28 Computational tools also provide an unprecedented resource that should be fully utilised.

Finally, an important point to consider is simply that targeting opening of the mPTP alone is not sufficient to prevent progression to heart failure in some settings, discussed below.

**TARGETING CELL DEATH TO MITIGATE HEART FAILURE**

It has been noted that the results of the recently completed Cyclosporine A in Reperfused Acute Myocardial Infarction (CYCLE) study using Sandimmune are expected immi-

ently although the primary end point is ST-segment resolution rather than clinical outcomes.29 These authors also propose that discovery of more specific inhibitors of mPTP opening might yield more promising results.30 Perhaps however, the opposite might be required. In considering cell death, cardiomyocyte loss is a defining feature not only of infarction (acute, high levels of cell death) but of heart failure in general (chronic, low levels of cell death).30 ‘Apoptotic’ cell death, measured by Deoxiribonucleic acid (DNA) fragmentation and caspase activity is prevalent in the failing heart31-33 in addition to ‘necrotic’ cell death, inferred on the basis of reparative fibrosis and a diffuse smearing pattern of DNA, suggesting the contributions of multiple mechanisms for cell death in end-stage heart failure.33 Further, it has been suggested that chronic, low levels of cardiomyocyte death are equally, if not more so, deleterious to heart function than acute high levels of cell death associated with myocardial ischemia and reperfusion injury. Up to 50% of LV cardiomyocytes can be lost in ischemia before heart failure occurs, while only up to 20% of sporadic myocyte loss/dropout is required in the setting of pressure overload.34 In addition, chronic low levels of sporadic cardiac muscle cell death are sufficient to cause heart failure in mice, and inhibition of caspase-dependent cell death in this model was protective.35 Diverse gain- or loss-of-function mutations and pharmacological interventions in murine models directly implicate cell-death signaling pathways as relevant therapeutic targets in reducing the inexorable progression to heart failure.36

In conclusion, in the setting of acute infarction, a pharmacological agent that inhibits cell death, salvages jeopardized myocardium and reduces infarct size would be expected to prove highly effective. However, the outcome of the recent cyclosporine trial would indicate otherwise.17 It should be noted that cyclosporine only targets one aspect of cell death37 and that heart failure incorporates multiple aspects.38 A dual inhibitor or combined inhibitors targeting both mitochondria dependent and independent cell death might provide a more productive method than either alone to significantly impact on heart failure progression. Even if early, acute cell death is limited during reperfusion, subsequent chronic, low levels of cell death will still contribute significantly to ensuing heart failure and death. Inhibition of both acute and chronic, ongoing sporadic cell death should therefore be targeted.

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