246 **CHARACTERISATION OF ILOMASTAT-MEDIATED PROTECTION**

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We have recently demonstrated that broad-spectrum matrix metalloproteinase (MMP) inhibition with ilomastat significantly attenuates infarct size when administered upon reperfusion following injurious ischaemia. Unlike pre- or post-conditioning that are thought to be reliant upon inhibition of mitochondrial permeability transition pore (mPTP), ilomastat protection occurs even in the absence of the critical mPTP regulatory protein, cyclophilin-D. MMP-inhibited cardioprotection remains incompletely characterised; we hypothesised that conditioning plus MMP inhibition would lead to summative infarct attenuation.

In 5–6 week old C57Bl6, Langendorff-perfused mouse hearts, ilomastat (250 nmol/L) was administered for 15 min after 35 min of global ischaemia prior to assessment of infarct size by triphenyl tetrazolium chloride staining. As found previously, ilomastat administered from the onset of reperfusion significantly attenuated infarct size (from 34±2.2% to 21±3.5% $p<0.05$) equivalent to that seen following ischaemic postconditioning (iPost: 6 cycles 10 sec reperfusion/10 sec ischaemia, 17±2.3%). However, delaying the administration of ilomastat for 15 min after the onset of reperfusion, protection was lost (37±3.7%).

To study the efficacy of ilomastat protection, we undertook a dose-response curve of increasing durations of injurious ischaemia in 10min intervals from 30 to 60 min. Interestingly, while iPost became ineffective against 50 min of injurious ischaemia, ilomastat remained protective (48±5.6%, 44±3.6% and 32±2.4% respectively, $p<0.01$ ilomastat versus control). Supplementing ilomastat with ischaemic postconditioning did not add further protection (33±2.1%).

Curiously, neither pre- nor postconditioning were able to augment the protection seen with ilomastat after 35min ischaemia. Pharmacologically targeting cyclophilin-D at reperfusion with cyclosporine A (CsA, 200 nmol/L) resulted in significant attenuation of infarct size when compared to control (24±5% versus 34±2.2%, $p<0.05$), and offered comparable protection to that seen with ilomastat (23±2.8%, $p<0.05$). Combination of ilomastat and CsA however failed to result in significant attenuation of infarction; paradoxically the protection observed with either drug on its own was lost when administered together at reperfusion (31±3.8%, p=NS).

Ilomastat offers robust protection against injurious ischaemia/reperfusion injury when administered during the first minutes of reperfusion and has greater efficacy than iPost against longer ischaemic insults. However, the robust summative protection seen previously with ilomastat in cyclophilin-D knockout mice does not translate to additive protection with either ischaemic pre- or postconditioning, or with pharmacological inhibition of cyclophilin using CsA. Therefore MMP inhibition still offers a novel approach to infarct size reduction, but there appears no benefit in combining this therapy with other cardioprotective modalities.