A DRUG DISCOVERY PLATFORM FOR THE IDENTIFICATION OF NOVEL INFARCT SPARING AGENTS FOR TREATMENT OF ISCHEMIC HEART DIESASE

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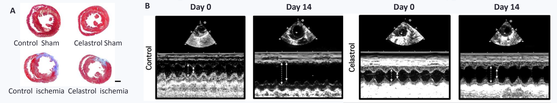
BACKGROUND

Myocardial infarction (MI) is characterized by the loss of cardiomyocytes that are replaced by non-contractile scar tissue.

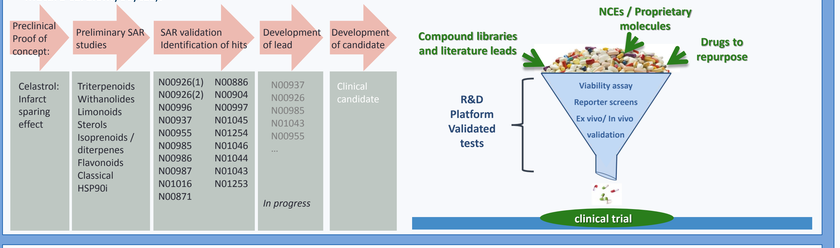
During reperfusion, cell damage is compounded by the increase in inflammation and free radicals, inducing cell death. Reducing cardiomyocyte death would reduce scar formation, improve cardiac function, and reduce the risk of heart failure.

We have previously shown that Celastrol, a compound isolated from an oriental medicinal plant and known as a modulator of HSP90 activity, activates HSF1, limits infarct size, and preserves heart function in a model of permanent ischemic myocardium. Celastrol induces overexpression of HSPs, including the potent cardiac antioxidant factor Heme-Oxygenase 1 (HO-1, or HSP32).



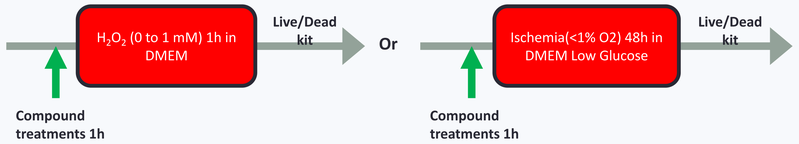
Animals where pre-treated with Celastrol or DMSO (Control), then subjected to permanent ischemia for 2 weeks.
A) Celastrol reduces fibrosis following 2 weeks of cardiac permanent ischemia (Masson's trichrome coloration).
B) Celastrol pre-treatment preserves cardiac function after 2weeks of permanent ischemia. (Short axis, 2D images taken before and after 2 weeks of cardiac ischemia).

Based on these findings, we set to validate Celastrol and expand our search for cardioprotective drugs in a clinically relevant model of ischemia/reperfusion (I/R) stress, using high throughput screening equipment and validation in appropriate cell lines (iPSC-derived human mature cardiomyocytes).

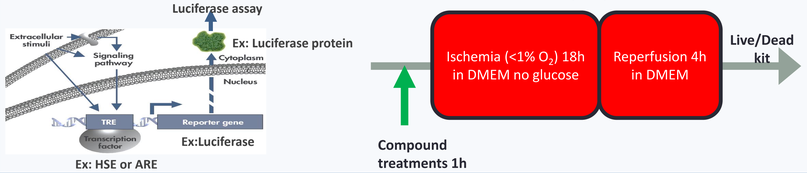


METHOD

Primary Screening in H9c2 cells (Viability following 2 components of I/R damage)

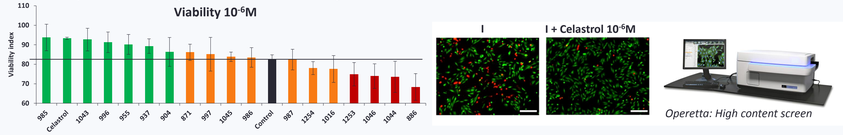


Validation in H9c2 and iPSC-derived human mature cardiomyocytes

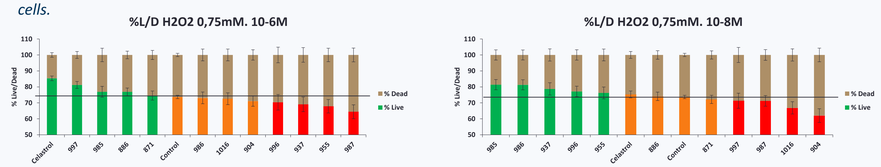


RESULTS

Primary screening: H9c2 cells subjected to 48h Ischemia or 1h oxidative stress

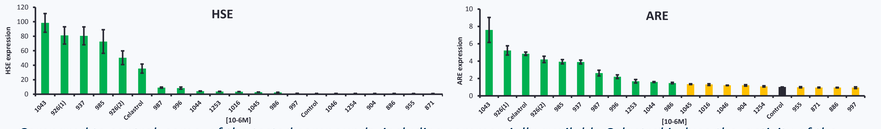


Compared to control, some compounds, including commercially available Celastrol protect form 48h ischemia (N=6) . In the microscopic image (Taken by the high content screen Operetta system) the green represent alive cells, and the red dead cells.

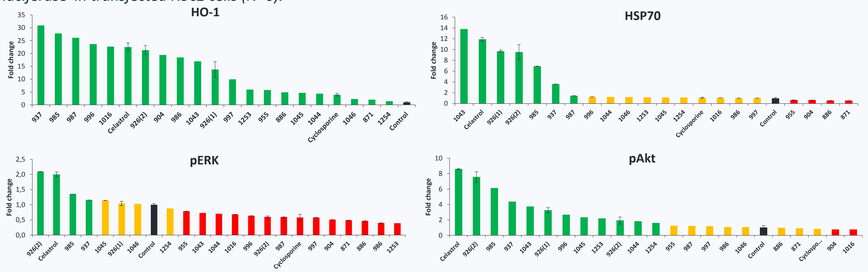


Compared to control, some compounds, including commercially available Celastrol protect form oxidative stress. Interestingly, some compounds are more potent than Celastrol. (n=6)

Reporter assay and western blot confirmation

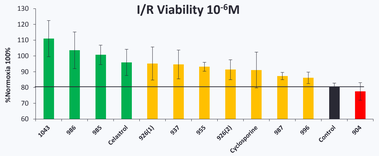


Compared to control, some of the tested compounds, including commercially available Celastrol induce the activity of the heat shock element (HSE) and the antioxidant response element (ARE), as measured by the expression of inducible luciferase in transfected H9c2 cells (N=6).

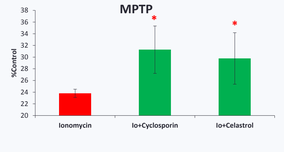


All of the compounds tested induce HO-1 expression. On the other hand, protective compounds also induce HSP70 expression and/or ERK or Akt activity.

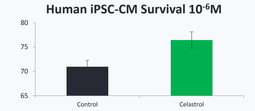
Validation:

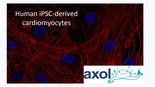


Compared to control, Celastrol and other of the selected compounds showed a protective effect on H9c2 cells subjected to I/R Stress (N=6)



*Compared to control, Celastrol pre-treatment induces resistance to MPTP opening in H9c2 cells following ionomycin 5nM challenge. *p<0,02.*

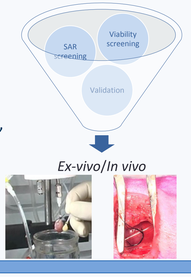




Preliminary results (N=2) of screening (I/R in vitro) using induced human induced pluripotent stem cell-derived mature beating cardiomyocytes showed that Celastrol is protective.

CONCLUSIONS

We have identified and validated Celastrol as a novel infarct sparing agent and identified as well other analog compounds with superior potency. These candidate compounds are tested and compared to Celastrol *ex vivo* (using a Langendorff preparation; see Can J Cardiol, Vol.32, Issue 10, S192-3) as a first step towards developing a novel drug designed as a first line medication for the treatment of MI and adjunct therapy to reperfusion procedures.



ACKNOWLEDGEMENTS

NEOMED; CIHR; MITACS; AXOL BIOSCIENCES.

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