Title: Cardiac protection by butyric acid releasing prodrugs
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Background: Approximately half the mortality cases of cardiovascular disease result of myocardial infarction (MI) that leads to massive cell death. Promoting cell survival is the ultimate goal of heart protection. Histone acetylation status modulates gene expression and is maintained by histone acetyl transferases (HATs) and deacetylases (HDACs). HDAC inhibitors have become targets for disease therapy. Prodrugs releasing the HDAC inhibitor butyric acid (BA) improve its bioavailability and potency and have been shown to possess anti-cancer activity, protect against chemotherapy cardiotoxicity and minimal side effects.

Research Hypothesis: BA-releasing prodrugs protect the heart against ischemia.

Aims: To test whether AN-7 (BA/formaldehyde releasing) and AN-88 (BA/acetaldehyde releasing) BA-prodrugs confer tolerance to ischemia in hearts and heart cells, and examine possible mechanisms of their action.

Methods: Early in the study we found that the efficacy of AN-88 was significantly lower, therefore, we focused on the more promising prodrug, AN-7. Hearts of AN-7 treated rats were removed at 1.5h or 24h treatment and were challenged with ischemia (45 min) followed by reperfusion (20/60/90min) ex vivo. The mechanical performance was measured during heart perfusion, infarct size was evaluated (TTC staining) at 60/90 min reperfusion and protein profiles were assessed (Western blotting) at baseline and at the end of ischemia and reperfusion. Cardiac myocytes and fibroblasts, prepared from neonatal rat hearts, were subjected to ischemia (1% oxygen) in the presence or absence of AN-7 and cell damage was assessed by (1) JC-1 that indicates mitochondria membrane potential (ΔΨm) dissipation, a marker of cell damage; (2) Hoechst/propidium-iodide, an indicator of cell death. Proteins were analyzed by Western blotting and mRNAs by RT-qPCR.

Results: Hearts of rats treated with AN-7 for 1.5h and 24h showed improved recovery from I/R, smaller infarct size and higher levels of the cytoprotective heme oxygenase-1 (HO-1). In cultured cardiomyocytes, not cardiofibroblasts, AN-7 prevented hypoxia damage, indicating selective capability of cytoprotection. Differences in expression patterns of HO-1 and inducible NO syntase (iNOS) suggested that these enzymes may contribute to the opposite effects of AN-7 in the two cell types.

Discussion: In vivo, AN-7 endows early and late pharmacological preconditioning that affords the activation of cytoprotective enzymes upon ischemia injury. The selectivity of AN-7 for cardiomyocyte protection and cardiofibroblast killing bears the potential benefit of mitigating the loss in contractile tissue and preventing the accumulation of fibrotic tissue following myocardial injury.

Conclusions: BA-prodrugs emerge as a potential treatment modality for cardiac injury.

Key words: Histone deacetylase inhibitor; butyric acid; ischemia/reperfusion; hypoxia; cardioprotection;
Publications associated with the project: