Non-electrophilic activation of the Nrf2 pathway ameliorated experimental Nonalcoholic Steatohepatitis

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INTRODUCTION

Oxidative stress and inflammation play a key role in a wide range of diseases. The transcription factor Nrf2 mediates adaptation to oxidative stress by inducing antioxidant and cytoprotecting genes. Recent studies show Nrf2 activation has multiple benefits including decreased oxidative stress, enhanced redox capacity, anti-inflammation, modulation of lipogenesis and gluconeogenesis, regulation of autophagy, proteostasis and mitochondrial biogenesis and energetics. Pharmacological activation of the Nrf2 pathway has been recognized as a potential strategy to reduce oxidative stress and resolve inflammation associated with acute and chronic illnesses[1].

vTv Therapeutics has developed non-electrophilic, orally bioavailable molecules that activate the Nrf2 pathway via the inhibition of Bach1 transcriptional repression and the stabilization of Nrf2. Numerous *in vitro* and *in vivo* studies have demonstrated that activation of the Nrf2 pathway by these compounds results in the reduction of both oxidative stress and inflammation. However, unlike electrophiles, these compounds are not reactive, their effect is not suppressed by N-acetyl cysteine, and they do not perturb either ROS or cellular glutathione levels[3,4,5,6].

Non-alcoholic steatohepatitis (NASH) is a progressive liver disease highly associated with oxidative stress, inflammation and lipid metabolism. Therefore, activation of the Nrf2 pathway could be a potential therapeutic target to treat chronic liver disease such as NASH.

AIM

To evaluate efficacy of the non-electrophilic Nrf2 pathway activator HPP3033 on diet induced NASH in a methionine/choline deficient diet (MCD) model.

METHODS

METHODS

in vitro:

HMOX1, NQO1, GCLC, and TXRND1 gene expression were measured in HepG2 cells *in vitro*. Normal human lung fibroblast were treated with HPP3033 for 0.5, 1, 2 and 4 hours for reduced glutathione (GSH). HepG2 cells were loaded using 2,7 dihydrodichlorofluorescin diacetate (H_2DCF-DA) prior to treatment with DMSO, H_2O_2, Curcumin or HPP3033 compounds for 1 hour. Increase in reactive oxygen species (ROS) was detected by measuring the fluorescence of H_2DCF-DA.

in vivo:

Mice were fed a choline/methionine deficient diet for 4 weeks. After 1 week of diet, mice were randomized into 3 treatment groups balanced for: 1) ALT, 2) AST and 3) body weight. After randomization, mice were treated QD for 3 weeks with vehicle (n=10), HPP3033 30 mg/kg (n=22) or FXR agonist WAY-362450 30 mg/kg (n=10). At the end of the treatment period, blood and liver samples were collected for histology and biomarker quantification. Data are shown as mean ± SEM. Statistical analysis was performed using a 1-way or 2-way ANOVA+Dunnet or Bonferroni post-test, respectively. When relevant and indicated, a Kruskal Wallis+Dunns test was performed.

RESULTS

in vitro:

- HPP3033 activates the Nrf2 pathway and increases expression of ARE regulated genes.
- Non-electrophilic activation of the Nrf2 pathway is not mediated via glutathione depletion or increase in intracellular reactive oxygen species.
- HPP3033 causes an increase in intracellular GSH levels 2-4 hrs after treatment of cells.

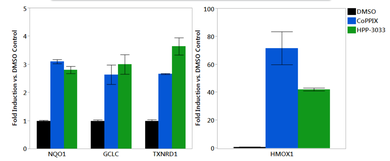
in vivo:

- Oral administration of HPP3033 activated the Nrf2 pathway leading to an increase in hepatic HMOX1 gene expression.
- ALT and AST levels were increased without increase in liver weight, suggesting the absence of signs of liver injury, which is an expected result of Nrf2 activation[2].
- Trend towards lowering liver lipids with HPP3033 treatment.
- Trend towards lowering markers of inflammation with HPP3033 treatment.
- Hepatocyte ballooning was significantly reduced with HPP3033 treatment.
- Significant reduction in total NAS score.

in vitro

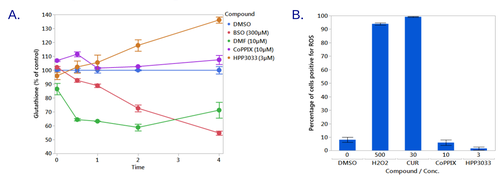
HPP3033 activates Nrf2/Bach1/ARE regulated genes

Compound	Conc. (μM)	HMOX1	NQO1	GCLC	TXNRD1
CoPPIX	50	71.7	3.1	2.6	2.7
HPP3033	3	42.2	2.8	3.0	3.6



Fold mRNA expression of HMOX1, NQO1, GCLC and TXRD1 in HepG2 cells.

HPP3033 activates Nrf2 pathway without depletion of glutathione or increased ROS

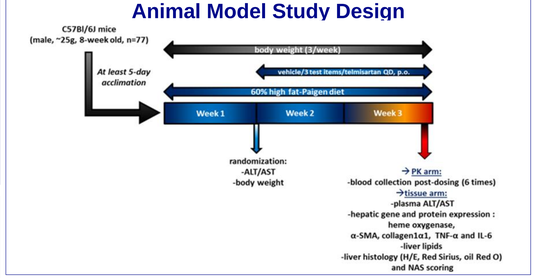


A) Measurement of NHLF GSH levels after treatment with HPP3033 compared to DMF and cobalt protoporphyrin (CoPPIX) for 0.5, 1, 2, and 4 hrs.

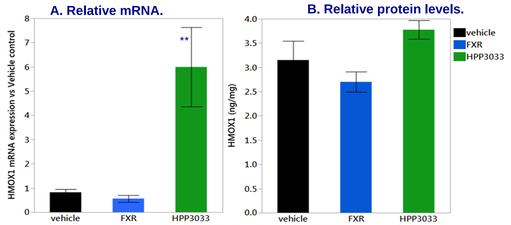
B) ROS levels measured in HepG2 cells after 1 h exposure.

in vivo

Animal Model Study Design



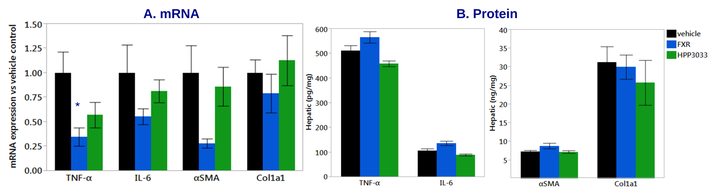
in vivo activation of Nrf2 pathway



Hepatic Nrf2 HMOX1 expression after 21 days of HPP3033 (30mg/kg).

**p<0.01 (Kruskal Wallis+Dunns) vs. vehicle

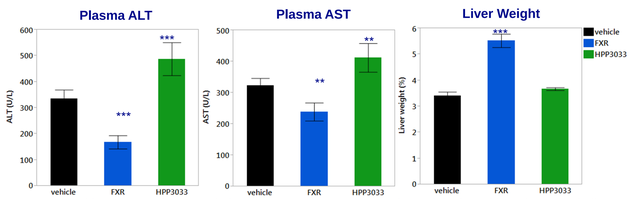
Hepatic gene and protein expression of inflammatory markers



Trends toward lowering hepatic expression of TNF-α, IL-6, alpha-SMA, and collagen1alpha1 (Col1a1) after 21 days dosing with HPP3033.

A) Relative mRNA (*p<0.05) (Kruskal Wallis+Dunns) vs. vehicle.
B) TNF-α, IL-6 relative, α-SMA and Col1a1 protein levels.

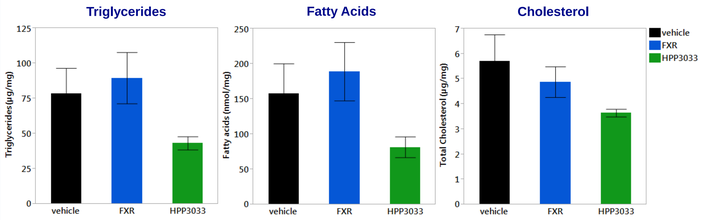
ALT, AST and Liver Weight



Treatment with HPP3033 increased plasma ALT and AST after 21 days of dosing without increase in liver weight.

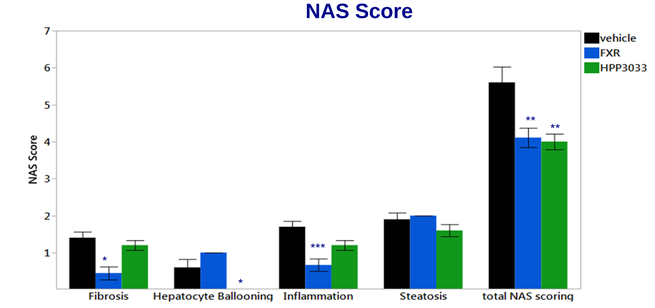
**p<0.01 and *** p<0.001 (Kruskal Wallis+Dunns) vs. vehicle

Hepatic Lipids



HPP3033 displayed trend toward lowering hepatic lipids after 21 days of dosing.

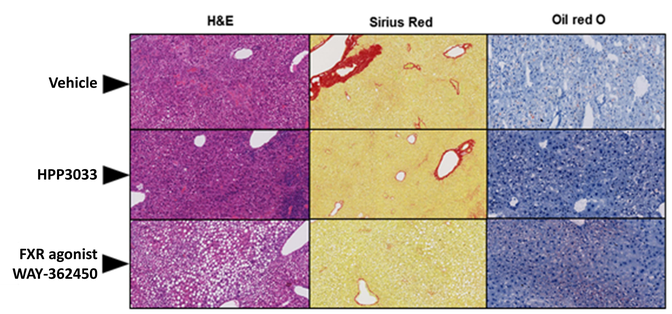
NAS Score

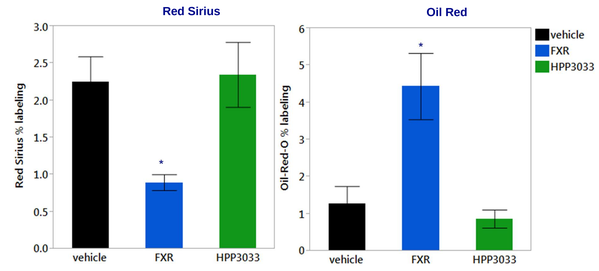


HPP3033 is hepatoprotective in MCD NASH model. Activation of the Nrf2 pathway by HPP3033 after 21 days of treatment prevented hepatocyte ballooning and displayed trends of lowering liver lipids and lowering inflammation. Total NAS scoring was significantly reduced by HPP3033 similar to FXR agonist WAY-362450.

*p<0.05, **p<0.01, ***p<0.001 vs. vehicle.

H7E Sirius Red Liver





*p<0.05 (Kruskal Wallis+Dunns) vs. vehicle

CONCLUSIONS

Treatment with HPP3033 leads to activation of the Nrf2 pathway, higher gene expression of heme oxygenase, and significant improvement of NAS score, confirming the potential of vTv's non-electrophilic Nrf2 activators to be developed as a treatment option for NASH.

ACKNOWLEDGEMENTS

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REFERENCES

1. Cuadrado, A et al. Therapeutic targeting of Nrf2 and Keap1 partnership in chronic diseases. Nature Reviews Drug Discovery 2018
2. Osburn WO et al. Genetic or Pharmacologic Amplification of Nrf2 Signaling Inhibits Acute Inflammatory Liver Injury in Mice. *Toxicol Sci*. 2008;104(1):218–227. doi:10.1093
3. Kostura M et al. Novel Bach1 modulators increase HMOX1 and suppress hypertension in the Goldblatt Model of Renovascular Hypertension. Best of AHA Specialty Conferences abstract. 2013
4. Kim SK et al. The novel Bach1 inhibitor HPP971 uniquely activates Nrf2 and reduces disease severity in a mouse model of experimental autoimmune encephalomyelitis. ACTRIMS-ECTRIMS Meeting 2014.
5. Attucks OC et al. Induction of Heme oxygenase-1 (HMOX1) by HPP-4382: a modulator of BACH1 activity. PLoS ONE 9(7): e101044
6. Kaidery AM et al. Preclinical validation of Bach1 inhibition for the development of Parkinson's disease therapy. Poster Neuroscience 2016; November 16, 2016; Published online: 2016 Neuroscience Meeting

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