Efficacy of DRX-065, the stabilized *R*-enantiomer of pioglitazone (pio), in choline-deficient (CD) and methionine/choline-deficient (MCD) diet mouse models of nonalcoholic steatohepatitis (NASH)

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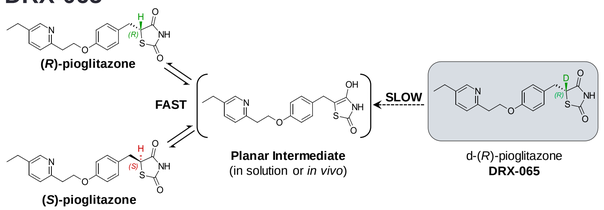
BACKGROUND

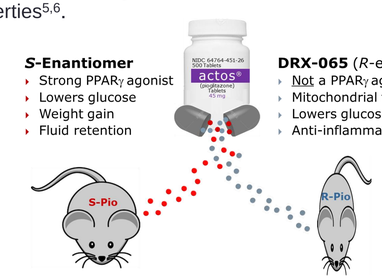
Pio for NASH

Pio is the most extensively studied drug for NASH[1] and is also the only drug recommended off-label for the treatment of NASH[2,3]. Most recently, Cusi *et al* demonstrated benefits of pio for prediabetic and diabetic NASH patients[4], where 58% achieved the primary outcome (2 point improvement of NAFLD activity score, NAS without worsening of fibrosis) and 51% had resolution of NASH. All improvements in metabolic and histologic scores (e.g. fibrosis) persisted over 36 months. However, weight gain was observed despite a reduced calorie diet throughout the study. Weight gain and edema are PPARγ agonist-related side effects of that limit the suitability of pio for the treatment of NASH.

DRX-065



Pio is a mixture of two interconverting enantiomers. By stabilizing each enantiomer with deuterium, DeuteRx discovered that pio is a mixture of two drugs with two discrete mechanistic and functional properties[5,6].

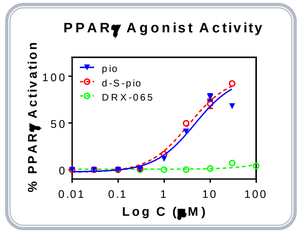


We show herein that DRX-065 possesses the pharmacological properties of pio required for the treatment of NASH by demonstrating its efficacy in two mouse models of NASH. The MCD diet model was selected because pio has previously demonstrated activity in this model that recapitulates the activity seen in NASH patients[7]. The CD diet model[8] was an exploratory study and a possible alternative to the MCD model given the less severe weight loss (MCD model leads to weight loss of up to 40% during a 6 week study).

DRX-065 is currently in Phase 1 clinical trials.

DRX-065: No PPARγ or Weight Gain

- DRX-065, the deuterated (*S*)-enantiomer of pio (d-S-pio), and pio have been evaluated by DeuteRx in several *in vitro* and *in vivo* studies[5,6].
- DeuteRx discovered that DRX-065 has no PPARγ agonist activity. PPARγ agonist activity is due exclusively to the S-enantiomer.
- DeuteRx demonstrated that DRX-065 does not cause PPARγ-related weight gain in a rodent model (C57BL/6J mice, oral doses of pio at 30 mg/kg/day, DRX-065 and d-S-pio at 15 mg/kg/day). Weight gain of pio is due to the S-enantiomer.

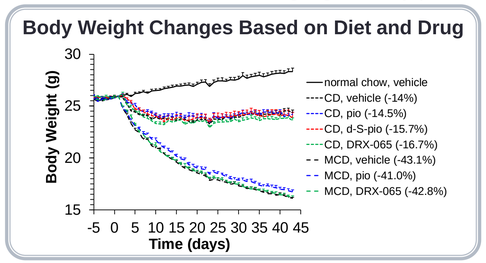


METHODS – NASH Mouse Models

The efficacy of DRX-065 and d-S-pio were evaluated against pio in the CD (DRX-065 and d-S-pio) and MCD (DRX-065) diet mouse models of NASH with male C57BL/6J mice on normal chow as controls. Due to the large number of animals needed and concerns about weight loss in the MCD model, only pio and DRX-065 were tested in the MCD model. All drugs were administered orally, twice daily for 6 weeks at 30, 15, and 15 mg/kg/day for pio, DRX-065, and d-S-pio, respectively. At the end of the study, all animals were sacrificed (n = 11-12 per group). Plasma ALT, AST, ALP, triglycerides (TG), free fatty acids (FFA), serum amyloid A, and adiponectin were determined using standard methods. Livers were harvested and evaluated for measures of TG, FFA, and cholesterol as well as for histopathology. Comparisons to the normal chow and the appropriate CD or MCD diet group were by the multiple t-test.

RESULTS – Effect on Body Weight

- Both MCD and CD diets caused weight loss (compared to normal chow). Weight loss was much lower with the CD diet.
- MCD diet - Less weight loss with pio, DRX-065 weight loss same as vehicle. Consistent with earlier weight gain study (see above).

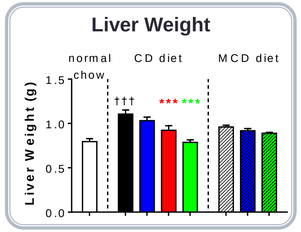


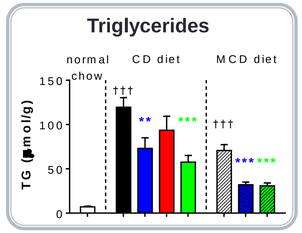
RESULTS – Plasma

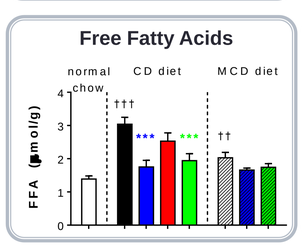
- ALT, AST, ALP – Increased by both diets. Modest, inconsistent effect of drug treatment.
- TG, FFA – Lowered by both diets. Reduced further by pio, DRX-065,and d-S-pio (CD diet).
- Serum amyloid A – Lowered by both diets. Reduced further by DRX-065 (CD diet).
- Adiponectin – Elevated by both diets. Increased further by all drugs, less by DRX-065.

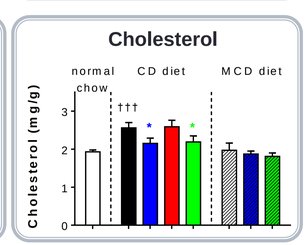
RESULTS – Liver

- Liver weight - Significantly increased by CD diet (relative to terminal body weight). Decreased by DRX-065 and d-S-pio.
- TG, FFA – Significantly increased by both diets, more profoundly by CD diet. Decreased by DRX-065 and pio only. DRX-065 reduced TG more robustly than pio (CD diet).
- Cholesterol – Significantly increased by CD diet. Decreased by DRX-065 and pio only (CD diet). No change in MCD diet.

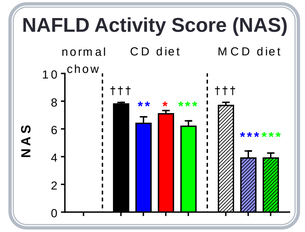


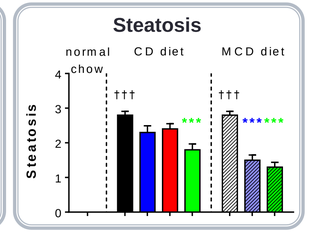


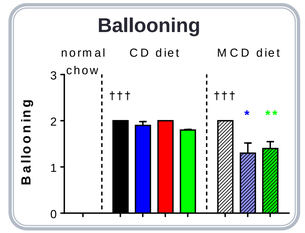


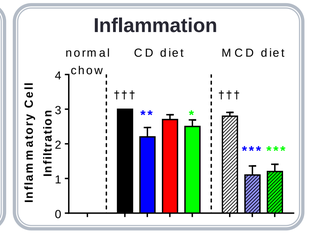


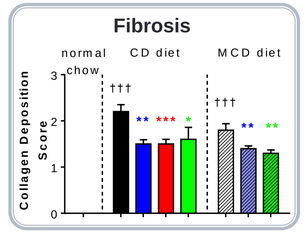
- NAS (sum of steatosis, ballooning & inflammation) – NAS and individual components increased by both diets. NAS reduced by all drugs, most robustly by DRX-065. Potency of d-S-pio marginal since it does not significantly reduce inflammation, steatosis, or ballooning.
- Steatosis - Only DRX-065 reduces steatosis in both models.
- Ballooning - Equally reduced by DRX-065 and pio (MCD diet).
- Inflammation - Equally reduced by DRX-065 and pio (both diets).
- Fibrosis - Equally reduced by all drugs with CD diet and by pio and DRX-065 with MCD diet.











LEGEND

- normal chow
- CD, vehicle
- CD, pio
- CD, d-S-pio
- CD, DRX-065
- MCD, vehicle
- MCD, pio
- MCD, DRX-065

††† *p<0.001, †† p<0.01 (compared to normal chow); *** p<0.001, ** p<0.01, * p<0.05 (compared to control on same diet)*

CONCLUSIONS

- DRX-065 is better than pio for reducing liver TG and steatosis.
- DRX-065 is equivalent to pio for reducing liver FFA, cholesterol, inflammation, ballooning, NAS, and fibrosis.
- DRX-065 is potentially more efficacious than pio for the treatment of NASH.

References

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