Oral pentamidine (VLX103) is an effective treatment for high fat diet-induced NASH in mice

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1. ABSTRACT

In nonalcoholic steatohepatitis (NASH) excessive hepatic lipid accumulation and hepatocyte injury are driven in part by an overactive innate immune response generated by stimulation from intestinal bacterial products such as lipopolysaccharide (LPS).[1,2] Previously we have demonstrated that a novel form of oral pentamidine, VLX103, significantly reduces hepatotoxic liver injury induced by galactosamine/LPS or alcohol.[3,4] The therapeutic efficacy of VLX103 in these models, together with the known anti-inflammatory and LPS-binding properties of pentamidine, suggested that VLX103 may be an effective treatment of NASH. We therefore tested the hypothesis that oral VLX103 would reduce liver injury in the high-fat diet (HFD) mouse model of NASH. **Methods:** Three independent experiments were conducted in male C57BL/6 mice fed a chow diet (CD; 5% fat; LabDiet, PicoLab Rodent Diet 20, 5053) or HFD (60% fat; Research Diets, D12492) for 27 to 36 weeks and treated by daily gavage of normal saline (NS) vehicle control or VLX103 (2.25 mg/day) for additional two to three weeks of CD or HFD feeding (total of 30-39 weeks). **Results**: Treatment with VLX103 led to a significant 20% decrease (P<0.000001) in body weight in HFD-fed mice as compared to a 6% reduction in weight in the three other treatment groups. Weight reduction correlated with a significant decrease in food intake in the VLX103-treated versus NS-treated, HFD-fed groups (daily average of 1.8 vs 2.7 g/day, P<0.000001). VLX103 significantly reduced the degree of HFD-induced steatosis and liver injury as demonstrated by decreases of 40% in hepatic triglyceride content (259 vs 436 µg/mg, P<0.003), 54% in serum ALT (95 vs 204 IU/L, P<0.001) and 17% in serum AST (162 vs 194 IU/L, P<0.04). **Conclusions**: VLX103, an oral form of pentamidine, significantly decreased hepatic triglyceride content and liver injury in HFD-induced NASH in mice after only two to three weeks of treatment. These findings indicate that VLX103 may be novel therapy for human NASH.

2. METHODS

Male C57BL/6 mice were started on CD or HFD after weaning (Fig. 1). After 27 to 36 weeks of diet, mice were randomized to 2 or 3 weeks of daily, saline vehicle or VLX103 treatment. The VLX103 dose was set during Part 1 based on the mean body weight of CD-fed mice to deliver a daily dose of 75 mg/kg. A dose of 2.25 mg/day (VLX75) was given by oral gavage to all VLX103-treated animals.

3. RESULTS

Figure 1. Experimental design of the three independent experiments.

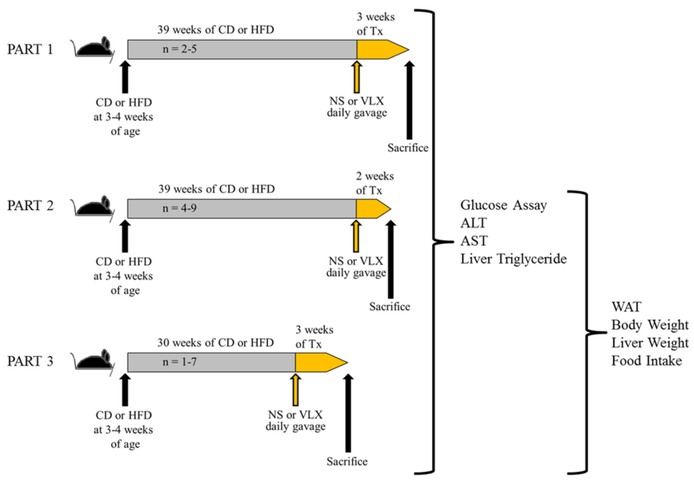
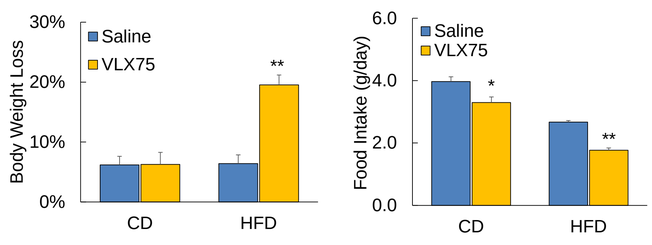
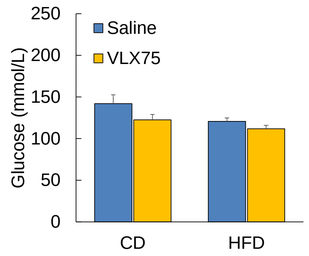


Figure 2. VLX103 decreases body weight and daily food intake.



In each experiment weight loss occurred with VLX103 treatment that was not associated with drug toxicity (** P<0.000001, n=3-18). All mice were observed to have normal levels of activity. Weight loss of 6% in the VLX103-treated CD-fed group is identical to that in the saline-treated CD and HFD groups, indicating that decreased weight was not secondary to VLX103 toxicity. Over the entire treatment period, VLX103-treated animals ingested 17% and 34% less food daily in the CD- and HFD-fed groups, respectively (*P<0.01, **P<0.00001; n=5-16).

Figure 3. Blood glucose levels.



The HFD induced a fatty liver, with elevated liver triglyceride content, as well as elevations of alanine and aspartate transaminases (ALT and AST). The HFD did not cause fibrosis or hyperglycemia in mice over the period tested (n=3-18).

Figure 4. Liver triglycerides.

The effects of VLX103 on steatosis were assessed by the quantitative measure of liver triglyceride content. Hepatic triglycerides were markedly increased by HFD feeding in all experiments. Liver triglycerides were significantly decreased by 40% with VLX103 treatment (**P<0.005; n=3-18) .

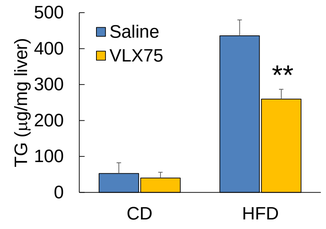
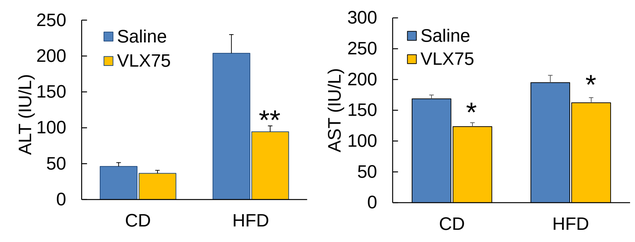


Figure 5. Serum ALT and AST levels.

The HFD induced ALT elevations, but only a minimal increase in AST was observed. VLX103-treated HFD mice had significant ALT and AST reductions of 54% and 17%, respectively (*P<0.05, **P<0.001; n=3-18).



4. SUMMARY

1. **VLX103 is hepatoprotective in the high-fat diet (HFD) murine model of NASH, even with a short period of treatment (2-3 weeks) at a late stage of the disease (27-36 weeks of HFD feeding).**
2. **VLX103 reduced body weight, steatosis (liver triglycerides) and hepatic injury (ALT and AST) in the HFD model of NASH, all important parameters of disease progression and pathology.**
3. **We speculate that a second pharmacological effect on appetite is a partial mechanism of action of VLX103 contributing to the beneficial effects of VLX103 in NASH.**
4. **These results suggest that VLX103's pharmacological profile merits therapeutic exploration in human NASH.**

5. REFERENCES

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3. Ilyas, G *et al.*, Oral pentamidine (VLX103) prevents the development of alcoholic liver disease in mice. Hepatology 62, 865A-865A.
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6. DISCLOSURES

- François Ravenelle is an employee of Verlyx Pharma Inc.
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