Change in Diet Reverses NASH in CDAA Diet Induced Mouse Model Along with Improvement of Overall Liver Function

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1 ABSTRACT

Non-alcoholic steatohepatitis (NASH) is associated with excess caloric intake and metabolic syndrome. Diet modification is front line treatment in the clinic, yet this has not been validated in animal models. In this work, we used the choline deficient, defined amino acid diet (CDAA) to induce NASH to test whether a change to regular diet can reverse the NASH phenotype. Fifty-five eight-week-old male C57BL/6J mice were used on study. Forty-seven were fed CDAA diet for 26 weeks. At 15 weeks, liver biopsies were obtained by laparotomy for NASH activity score (NAS) and blood samples collected for ALT and Procollagen Type III N-Terminal Propeptide (PIIINP) analysis. At 18 weeks, animals were distributed to treatment groups based upon ALT, PIIINP and NAS. Group 1 (vehicle control); Group 2 (Piogltazone (PGZ); 30mg/kg PO, QD for 8 weeks); Group 3 (returned to standard chow diet for 8 weeks (CDAA Δ Chow)); Group 4 (maintained on regular chow diet throughout the study). Blood was collected via submandibular vein on Day 1 (prior to treatment), Day 29 and Day 57 (terminal). Histopathology performed on biopsy tissue confirmed advanced steatosis and minimal fibrosis after 15 weeks of CDAA diet feeding, overall NAS score 3.9. ALT ($p<0.0003$) and PIIINP($p<0.02$) levels were significantly elevated compared to chow fed group. Diet change resulted in lowering body weight within first week and was accompanied by small increase in food intake. It also resulted in significant lowering of ALT ($p<2.2E-5$) and PIIINP ($p<0.0001$) accompanied by lowered liver weight, liver to body weight ratio, and liver cholesterol. The switch to chow diet significantly reduced liver triglycerides (TG) which was independently confirmed by histopathology. Pioglitazone lowered serum TG but did not impact the liver TG. Terminal histopathology NAS scores were as follows: vehicle 6.1, pioglitazone 4.5, diet switch 2.5, and chow control 0.1. Liver biopsy lowered the number of required animals and allowed the use of each animal as its own control. Our data showed that within 4 weeks of switching to standard chow, all liver function tests returned to normal and by 8 weeks the NASH phenotype was reversed, thus proving that diet change recommended in clinics is also effective in the CDAA diet induced model of NASH.

2 MATERIALS AND METHODS

Animals
55, 8-10 week old male C57BL/6J mice [JAX stock #000664, The Jackson Laboratory, Bar Harbor, ME] were used on study. Mice were singly housed in polycarbonate cages and maintained at 21 ± 2 °C with relative humidity of 30 - 70% and a 12:12 hour dark:light cycle. All conditions of animal preparation and use were in accordance with recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Liver Biopsy
Anesthesia/Analgesia: Buprenorphine (0.1 mg/kg) injected subcutaneously pre-operatively and continued BID for 3 additional days post operative, Isoflurane inhalant for induction and maintenance of anesthesia. Animals were allowed to recover in a heated cage.
Biopsy: A ventral midline incision was made just caudal to the xyphoid cartilage and extended to allow visualization of the liver. The left lateral lobe was exposed and a cone-shaped wedge biopsy obtained (~100 -150 mg). The tissue was collected in 10% NBF. Electrocautery was used as needed to manage hemostasis. The abdomen was lavaged with warm saline prior to closure. The abdominal muscle and skin incisions were closed in separate layers using monofilament suture.

Blood Collection
Venous blood samples were collected via submandibular route on Days -25, 1 (start of pioglitazone treatment), 29, and 43. The samples were processed to serum and used for ALT and PIIINP analysis. Blood glucose was measured at 15,18, 22, 24 weeks using AlphaTrack hand held glucometer (Abbott Labs). Terminal blood samples were collected on study day 57 via intracardiac puncture following euthanasia with carbon dioxide. Serum was aliquoted for ALT, PIIINP and insulin levels. The remaining was used for Free Fatty Acids (FFA), Triglycerides (TG) and Cholesterol (Chol) analysis.

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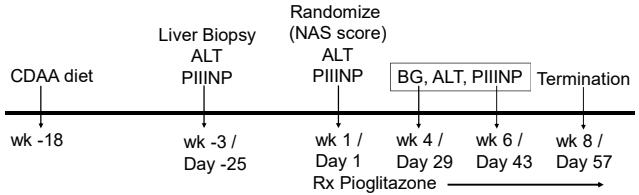
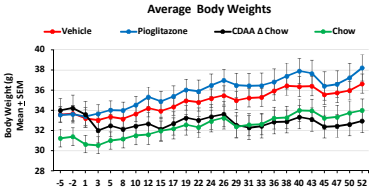
3 EXPERIMENTAL DESIGN

47 animals were fed a choline deficient defined amino acid diet (CDAA, Research Diets # D518753) for a total of 26 weeks, starting at week -18 from day 1 of dosing. At -3 weeks (15 weeks of CDAA diet feeding), a liver biopsy was obtained for NAS scoring and blood sample was collected via the submandibular vein for ALT and PIIINP analysis. On Day 1 animals were randomized into 4 treatment groups based upon ALT, PIIINP and NAS score. Group 1 (vehicle control); Group 2 Pioglitazone (30mg/kg PO, QD for 8 weeks); Group 3 (switch from CDAA to standard chow diet for 8 weeks); Group 4 (maintained on regular chow diet throughout the study). Body weights were collected 3 times a week for all animals for the duration of the study.

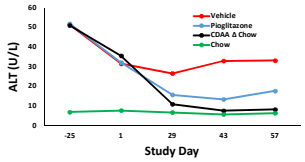


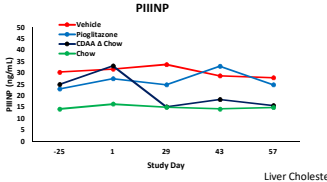
4 RESULTS



Consistent with the published literature, PGZ treatment resulted in continued weight gain. The animals that were returned to regular chow diet after 18 weeks of CDAA diet feeding, demonstrated weight loss within one week that continued over the course of the study. These animals also had lower terminal body weights than animals fed chow throughout the study period.

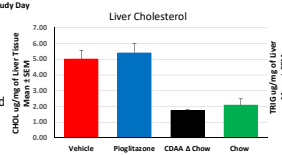
PGZ treatment lowered serum ALT consistent with improvement in liver function. Returning animals to a chow diet resulted in a much more pronounced decrease in serum ALT, bringing values to within normal limits.

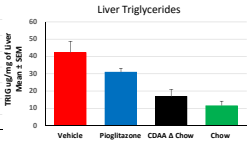


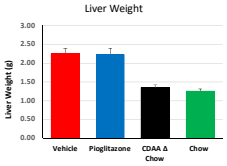


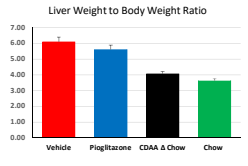
Return to a standard chow brought serum PIINP levels back to baseline within 30 days.

The switch to chow diet significantly reduced liver lipids (triglycerides and cholesterol), consistent with histopathologic assessment by the end of the study.



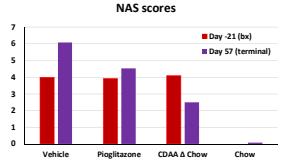






Liver weights and Liver to BW ratio normalized with diet alteration.

NAS scores were standardized across groups prior to dosing start. At study termination there was a reduction of NAS score in only in the animals returned to a standard laboratory rodent chow diet.



5 DISCUSSION

Liver biopsies taken 3 weeks prior to initiation of treatment confirmed the presence and magnitude of NASH in mice fed a CDAA diet. This enabled the rational distribution of animals to the various treatment groups and uses each animal as its own control to estimate response rates. This enriches the dataset, reduces variability (and the number of animals needed to power a study), and refines the overall model.

Frontline clinical therapy is dietary modification and exercise. We demonstrate that this approach is valid for assessing comparator therapies in the CDAA model of NASH. Reverting the diet to standard laboratory chow returned liver function to normal and decreased NASH disease activity scores significantly better than PGZ treatment alone. These data suggest that a combination of diet alteration and pharmacologic therapy might provide significantly better therapeutic benefit than either as monotherapy.