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"Primary Rat Hepatocyte Spheroids as a Model of MetALD"

Objective: Metabolic and alcohol associated steatotic liver disease (MetALD) is a newly established diagnosis that considers both metabolic and alcohol associated factors contributing to liver injury. The objective of this study was to use a three-dimensional cell culture technique to model metabolic and inflammatory manifestations of MetALD in primary rat hepatocyte spheroids treated with high fats and sugars (HFS) plus ethanol (E) after chronic control- or alcohol-diet feeding.

Methods: Primary rat hepatocytes were isolated from Fischer 344 rats that were given either a Lieber-DeCarli control (n=1) or alcohol liquid diet (n=1) for 15-weeks. Hepatocytes were isolated by canulating the hepatic portal vein, perfusing the liver with a chelating buffer, and then digesting the liver with a collagenase IV solution. The liver was then resected and mechanically digested. Through a series of centrifugation steps and a 45% percoll isolation, viable rat hepatocytes were placed in ultra-low attachment plates to facilitate hepatocyte aggregation & spheroid formation. Five days later, the spheroids were changed to a serum free media, and treated with control (C; vehicle (1% BSA) + 5.5 mM glucose), ethanol (E; 50 mM ethanol), or high fat and sugar (HFS; 5:5:1 palmitic acid:oleic acid:BSA (400mM FAs), 1:1 glucose/fructose (11 mM sugars) media in combination (EHFS) or separately. After five days of incubation, supernatants from individual spheroids were collected. Using a sandwich ELISA assay, the concentration of IL-6 and C-reactive protein (CRP) in the supernatants from these spheroids were determined (n=3 technical replicates, n=2 spheroid supernatants from each group). The ATP and triglyceride (TG) contents of individual spheroids (n=6 each group) were also quantified using spheroid lysis bioluminescent assays.

Results: Spheroids incubated with the various conditions from both control- and alcohol-fed rats showed similar viability as indicated by ATP content relative to control media at endpoint ($p=0.479$). TG accumulation was significantly increased by in vitro HFS and EHFS 3.3-fold in control- and 5.5- and 5.3-fold, respectively, in alcohol-fed rat spheroids compared to control media treated spheroids ($p<0.0001$ for all comparisons). CRP secretion was 1.5-fold higher after in vitro ethanol treatment compared to control media-treated spheroids in the alcohol-fed rat spheroids ($p<0.0001$). IL6 secretion was below the limit of detection and could not be determined.

Conclusion: This study showed that viable primary rat hepatocyte spheroids from both control- and alcohol-diet fed rats could be maintained for 10 days in culture and model common manifestations of MetALD. Future studies will solidify these preliminary findings with increased sample sizes in both in vivo diet groups. Further, to better model the inflammatory environment encountered by hepatocytes, spheroids will be incubated with lipopolysaccharide to mimic components of alcohol- and metabolic-associated gut leak.