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STRUCTURAL ALTERATIONS IN LIVER ENDOTHELIUM OF RATS WITH DEXAMETHASONE-INDUCED INSULIN RESISTANCE

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ABSTRACT

Liver sinusoidal endothelial cells (LSECs) are perforated with transcellular fenestrations that provide unimpeded access of substrates between sinusoidal blood and hepatocytes. Defenestration refers to the loss of fenestration number and/or decreasing in fenestration diameter which can alter metabolic homeostasis. Insulin resistance has been reported to promote fat accumulation in the liver leading to fatty liver disease. However, the effect of insulin resistance specifically on fenestrations is yet to be investigated. This study was conducted to observe changes in fenestrations of LSEC in response to insulin resistance. Adult male Sprague-Dawley rats were divided into two groups (n=8) where the control group received 0.9% NaCl and the treatment group received dexamethasone injection (1mg/kg) i.p once daily for ten days. At day 11, all rats were anaesthetised using ketamine/xylazine followed by cardiac puncture. Rats were dissected and livers were perfusion-fixed for electron microscopy. Fenestrations were examined using a Quanta FEG450 Scanning electron microscope at 15000x magnification. Ten random images per sample were taken for fenestrations diameter and porosity analysis using ImageJ software. Data was analysed using SPSS version 23.0. Results showed that dexamethasone has induced insulin resistance by a significant reduction of body weight (D=276.84 ±7.87 vs C=393.84±12.47g; p=0.00), increased fasting blood glucose (D=5.57 ±1.30 vs C=3.97±0.55mg/dl; p=0.02) and higher HOMA-IR value (D=1.37±0.52 vs C=0.85±0.22; p=0.00) in treatment group compared to the control. Analysis of the liver images has shown that insulin resistance causes defenestration of LSEC where there is a significant decrease in fenestration frequency (D=3.202±1.16 vs C=2.656±1.044; p=0.04) and endothelial porosity (D=2.17±0.74 vs C=1.77±0.9; p=0.049) but not fenestration diameter. In conclusion, this finding shows that insulin resistance can affect the integrity of liver endothelium specifically on fenestration frequency and liver porosity which will consequently lead to serious implications on liver function as the main site for metabolism.

Keywords: Insulin resistance, dexamethasone, liver endothelium, fenestrations, electron microscopy.

INTRODUCTION

The liver plays a major role in the glucose homeostasis regulation, which is tightly regulated by insulin. Liver sinusoidal endothelial cells (LSEC) are perforated with transcellular fenestrations that provide permeability and access of substrates between sinusoidal blood and hepatocytes. During ageing and in liver diseases, LSECs undergo structural changes ranging from decreased fenestration number (defenestration) and/or diameter known as capillarisation (1). Insulin resistance is defined as the inability of tissues to respond to normal circulating levels of insulin and has been reported to promote non-alcoholic fatty liver disease (NAFLD) (2). However, the association between insulin resistance and fenestrations is yet to be investigated. Hence, this study aimed to analyse changes in fenestrations of LSEC in rat model models of insulin resistance. We hypothesised that LSEC ultrastructure is a contributing factor towards the clinical manifestations of insulin resistance.

MATERIAL AND METHOD

Adult male Sprague-Dawley rats were divided into two groups where the control group (n=8) received 0.9% NaCl and the treatment group (n=8) received dexamethasone injection (1mg/kg) i.p once daily for ten days (3). Body weight and fasting blood glucose were recorded daily. At day 11, all rats were sacrificed using ketamine/xylazine followed by cardiac puncture. Rats were dissected and livers were perfusion-fixed for electron microscopy. Fenestrations were examined using a Quanta FEG450 Scanning electron microscope at 15000x magnification. Ten random images per sample were taken for

fenestration frequency, diameter and liver porosity analysis using ImageJ software (4). All data were analysed using SPSS Version 23.0. Values for measurements were presented as mean ± SD.

RESULTS AND DISCUSSION

Dexamethasone has caused a significant decreasing daily body weight starting at day 3 of treatment and subsequently mean body weight changes compared to control (5.38 vs -22.92%) throughout the experiment. There is no significant difference in liver-to-body-weight ratio although the treated liver showed a higher value as compared to the control. Fasting plasma insulin concentrations were significantly elevated in the treatment group compared to control (Figure 1A). As hyperinsulinemia occurs, the homeostatic model assessment index for insulin resistance (HOMA-IR), calculated from the fasting glucose-insulin product, was significantly increased by more than two-fold in the treatment group, indicating insulin resistance (Figure 1B). Dexamethasone harms pancreatic beta cell function by reducing insulin receptor sensitivity, leading to decreased glucose uptake, tissue starvation, proteolysis and consequently weight reduction (3).

The liver endothelium observed via SEM showed a normal fenestration morphology in control rats (Figure 2A) while a marked defenestration is present in insulin resistance rats (Figure 2B). Quantification analysis of LSECs fenestrations showed a significant decrease in fenestration frequency and liver porosity, but not fenestration diameter for the insulin resistance liver (Table I). Although the mechanism of defenestration is still under investigation, these liver alterations are probably due to the dexamethasone effect on the mitochondrial energy production at the LSEC that causes depletion of ATP that is needed to support the cytoskeleton rings for the maintenance of fenestrae patency (5).

TABLE, IMAGE AND FIGURE

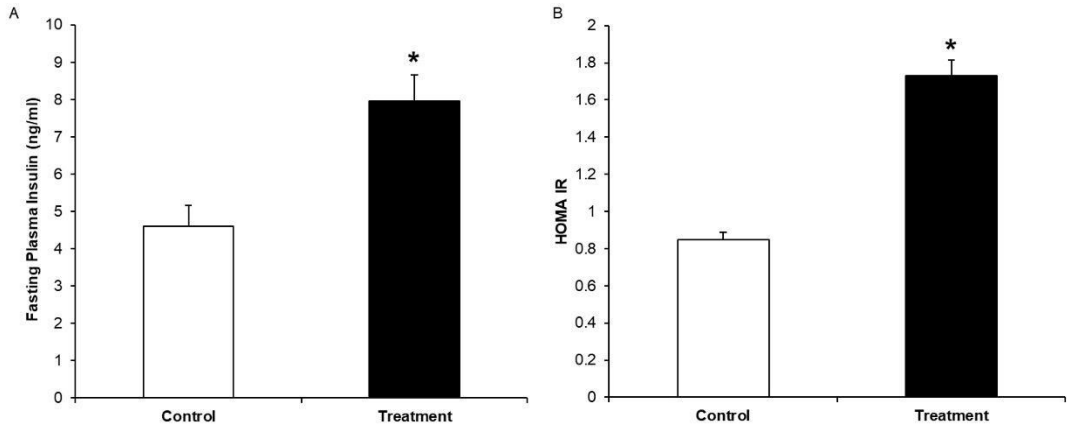

Fig. 1: Insulin resistance parameters in control and treatment group. (A) Fasting plasma insulin levels, (B) HOMA-IR. *significant at p<0.05

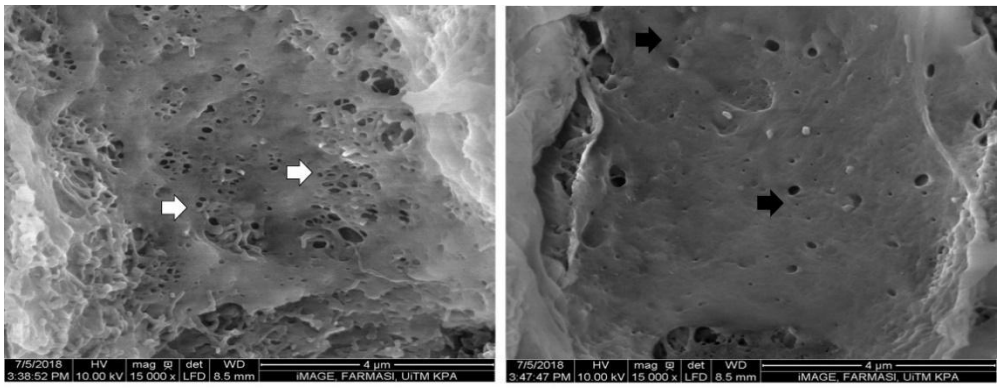

Fig. 2: Representative scanning electron micrograph of liver sinusoids. (A) Control liver showing fenestrations clustered into sieve plates (white arrows), (B) Marked defenestration (black arrows) is observed in insulin resistance rats (Mag 15000x)

Table 1: Quantification analysis of LSEC fenestrations

	Control (n=6)	Treatment (n=6)
Frequency	3.21 ± 1.2	2.65 ± 1.0*

Diameter	0.08 ± 0.1	0.07 ± 0.2
Porosity	2.17 ± 0.7	1.77 ± 0.9*

CONCLUSION

Insulin resistance has the ability to affect the integrity of liver endothelium specifically on fenestration frequency and liver porosity. These findings affirm the important role of liver ultrastructure in hepatic metabolic processes and highlight the LSECs as potential therapeutic targets for metabolic diseases such as diabetes and metabolic syndrome.

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