Low-Grade Systemic Inflammation Increases Endothelial Superoxide Levels and Reduces Nitric Oxide Availability

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Introduction / Statement of Purpose:

Low-grade systemic inflammation (LGSI) is a low-level system wide inflammatory response associated with obesity. It has been reported that Interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and leptin are elevated with LGSI[1-2], though not to the extent seen in classic, localized inflammation. In addition, LGSI is associated with obesity, pre-diabetes, and diabetes, which are major risk factors for cardiovascular disease and erectile dysfunction. The focus of this study is to probe the response of endothelial cells to elevated inflammatory mediators (TNF-α, IL-6, and leptin), modeling low-grade systemic inflammation.

Methods:

Cell Culture: Rat penile endothelial cells (RPEC) were harvested from whole rat penis and maintained in Medium 199 (Invitrogen, M199) supplemented with 10% Fetal Bovine Serum (Invitrogen), 1% Insulin-Transferrin-Selenium Supplement A (Invitrogen), 1% Penicillin-Streptomycin (Invitrogen), and 0.2% L-ascorbic acid (Invitrogen) on plates coated with 0.02% gelatin (Sigma-Aldrich). RPEC were used from passage 3-5 and maintained in a humidified environment (5% CO_2, 37ºC).

Determination of superoxide ($O_2^{\cdot-}$): $O_2^{\cdot-}$ was measured with enhanced chemi-luminescence. $O_2^{\cdot-}$ reacts with coelenterazine (Invitrogen) to generate light photons[3]. RPEC were exposed to control (0 pg/mL IL-6, 0 pg/mL TNF-α, and 0 ng/mL leptin), healthy (1.5 pg/mL IL-6, 1.0 pg/mL TNF-α, and 3.0 ng/mL leptin), or obese (3.0 pg/mL IL-6, 2.0 pg/mL TNF-α, and 15 ng/mL leptin) stages of the LGSI model for 2 hrs and then 5 μM coelenterazine was added. $O_2^{\cdot-}$ was then measured with an AutoLumat Plus Luminometer (Berthold). Data is expressed as relative chemi-luminescence units (RCLU) per cell. In addition, 1 mM Tiron was used as a positive control to verify that the chemi-luminescence was due to $O_2^{\cdot-}$.

Determination of nitric oxide ($NO^{\cdot}$): NO· availability was determined with DAF-FM diacetate (Molecular Probes). DAF-FM diacetate is a non-fluorescent, cell permeable probe that becomes green fluorescent upon cleavage by NO·[4]. RPEC were exposed to each LGSI model stage for 2 hrs. 5 μm DAF-FM diacetate was added 30 min prior to termination of LGSI agent incubation. RPEC were then washed, incubated an additional 15 min, and imaged via fluorescent microscopy. Data is expressed as relative fluorescent units (RFU) per cell. In addition, 0.5 mM L-NAME was used as a positive control to verify that DAF-FM diacetate fluorescence was produced by NO·.

Data analysis: Fluorescent images were analyzed with ImagePro Plus imaging software (Media Cybernetics).

Results / Discussion:

To probe the response of RPEC to modeled low-grade systemic inflammation, $O_2^{\cdot-}$ production was utilized as an indicator of cellular function. Elevated production of $O_2^{\cdot-}$ is associated with endothelial cell dysfunction and reduced $NO^{\cdot}$ availability.

RPEC exposed to obese conditions for 2 hrs exhibited 45647±2503 RCLU/cell, an 89% increase in $O_2^{\cdot-}$ production (figure 1) compared with control (26932±3974 RCLU/cell, n=4, $p<0.01$) and a 44% increase over healthy conditions (31778±2356 RCLU/cell, n=4, $p<0.05$). RPEC were also incubated under obese conditions with 1 mM Tiron, a $O_2^{\cdot-}$ scavenger, as a control ($O_2^{\cdot-}$ production was reduced compared to obese conditions, n=4, $p<0.05$).

RPEC incubated in simulated obese conditions for 2 hrs exhibited $1.70\pm0.19e^7$ RFU/cell, a 33% decrease in NO· availability from control ($2.54\pm0.09e^7$ RFU/cell, n=4, $p<0.05$) and a 26% reduction in NO· availability compared to healthy conditions ($2.29\pm0.26e^7$ RFU/cell, n=4, p~0.05). RPEC were also incubated under obese conditions with 1 mM Tiron, a $O_2^{\cdot-}$ scavenger, as a control ($NO^{\cdot}$ availability was increased compared to obese conditions, n=4, $p<0.05$). Future studies are planned to examine the role different material surfaces play on endothelial cell response in simulated LGSI.

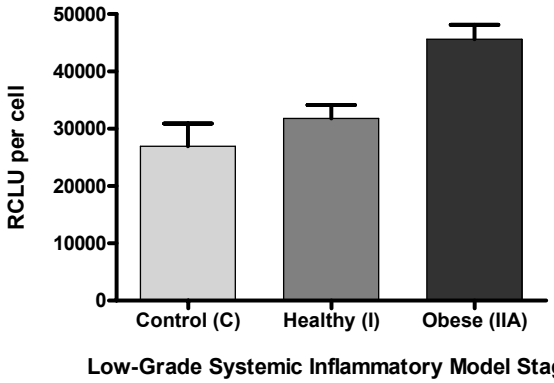

Figure 1: LGSI increases $O_2^{\cdot-}$ in RPEC in a dose-dependent manner. RPEC were exposed to each LGSI model stage for 2 hrs and $O_2^{\cdot-}$ was measured with coelenterazine. Data are expressed as mean±SEM.

Conclusions:

Increased levels of leptin, TNF-α, and IL-6 stimulate increased RPEC production of $O_2^{\cdot-}$ and reduced NO· availability, suggesting endothelial cell dysfunction. The increased production of $O_2^{\cdot-}$ may contribute to the loss of NO·, as $O_2^{\cdot-}$ reacts with NO· to form peroxynitrite ($ONOO^-$). The decrease in NO· may affect NO· signaling, allowing for disease development or erectile dysfunction.

References:

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