Insulin Decreases Transcription of Three Proteins Associated with Lung Surfactant

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I. Introduction

The lungs are covered with a mixture of phospholipids called lung surfactant to lower the surface tension and to protect the organism from pathogens. There are four proteins associated with lung surfactant. Small and hydrophobic SP-B (SFTPB) and SP-C (SFTPC) help to spread and stabilize the surfactant layer, while large and hydrophilic SP-A (SFTPA1 and SFTPA2) and SP-D (SFTPD) mostly participate in immune responses [1,2]. Low quality or quantity of these proteins often causes severe symptoms in both developing and mature lungs [3].

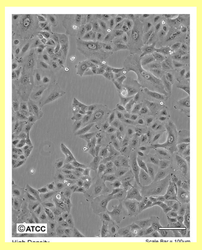
Our project is aimed to confirm the hypothesis that insulin intravenously supplied during lung resection affects diffusion capacity of lungs and levels of blood gases. The expected benefit is improved breathing, decreased number of post-operational complications, and shortened convalescence. The project is partially focused on insulin affecting four proteins mentioned above.

Stabilized human cell lines H441 and A549 were used in this experiment. H441 cells serve as a model of Clara cells and A549 cells represent type II pneumocytes. Main function of both Clara cells and type II pneumocytes is the production of lung surfactant. Clara cells are located in bronchi and type II pneumocytes in alveoli.

II. Methods

Stabilized Human Cell Lines

H441 - Clara cells
A549 – type II pneumocytes



Source: LGC/ATCC
http://www.lgcstandards-atcc.org/Attachments/1753.jpg

Cells were cultivated until ~80% confluency in RPMI 1640 (H441) or DMEM (A549) media with 10% of FBS. Then media were replaced for a serum-free media and after 24 hours insulin was added (concentration 250 or 2500 ng/µL).

mRNA isolation

mRNA Isolation Kit (Roche Diagnostics) was used according to the manufacturer's instructions.

RealTime PCR

Quantitative PCR was performed using TaqMan probes (Applied Biosystems) on 7500 Real Time PCR System (Applied Biosystems). Expression of individual genes was determined by comparative Ct method. GAPDH was used as a reference gene.

VI. Acknowledgements

This work was supported by the Ministry of Education of the Czech Republic grant NPVII B06052) and Ministry of Health of the Czech Republic grant NS9681-4/2008.

VII. References

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III. Results

Figure 1: Concentration-dependent effect of insulin on SFTP mRNA.. Cells were cultured for 48 hours in a serum-free media containing insulin (0, 250, and 2500 ng/µL). Graphs show relative changes in the amount of mRNA against the calibrator (no insulin added) normalized to GAPDH.

SFTPD – insulin inhibits its transcription in both cell lines, more apparently in H441 cells; A549 cells contain twice as much SFTPD mRNA than H441 cells.

SFTPC – insulin inhibits its transcription in both cell lines; A549 cells contain five times as much SFTPD mRNA than H441 cells

SFTPB – insulin inhibits its transcription in H441 cells; no SFTPB mRNA was detected in A549.

SFTPA1 – insulin inhibits its transcription in H441 cells; no SFTPA1 mRNA was detected in A549 cells.

SFTPA2 – insulin inhibits its transcription in H441 cell; no SFTPA2 mRNA was detected in A549 cells.

IV. Conclusion

We demonstrated that insulin serves as a negative regulator of transcription of proteins associated with lungs surfactant in H441 and A549 cell lines. Therefore it appears that the potential beneficial effects of insulin supplied during lung cancer resections are not linked with lung surfactant regeneration.

V. Next steps

To analyze changes in transcription when insulin is used together with known activators of SFTP genes.

To analyze insulin induced changes in surfactant associated proteins levels by western blot.

To analyze changes in both mRNA and protein level in samples of lung parenchyme from lung cancer patients.

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