Protease Inhibition Protects Surfactant Protein B in an in vivo Model of Meconium Aspiration Syndrome

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BACKGROUND

- Meconium aspiration syndrome (MAS) is a complex disease.
- It has been shown that meconium inhibits natural surfactants.
- The current treatment strategy for the treatment of MAS includes surfactant administration.
- The meconium detrimental effects on the lipid fraction of surfactant are well described. However, the role of surfactant proteins (SP) in MAS pathogenesis remains largely unexplored.
- Subjects with defects in synthesis of surfactant protein B (SP-B) die with symptoms similar to neonatal respiratory distress syndrome (RDS).
- Meconium is known to contain proteolytic enzymes and there is evidence that proteinases might be involved in the pathogenesis of MAS.
- Previous studies have shown that digestive enzyme inhibitors offer protection in different models of MAS and prevent surfactant from inhibition by meconium.
- We hypothesize that meconium proteases degrade SP-B and other proteins.

OBJECTIVES

- To evaluate stability of human SP-B in the presence of meconium.
- To evaluate the proteolytic effect of protease inhibitors against this effect.
- To quantify meconium proteolytic activity in a standard assay and investigate if different pH could affect this activity.
- To establish an azocaseine assay as a method for quantification of proteolytic activity of meconium.

RESULTS

- The proteolytic effect of meconium continues up to 48 hours after exposure. The effect is seen up to 48 hours after exposure.
- Rate of protein degradation p < 0.0001 ANOVA.
- Proteolytic enzymes from meconium degrade SP-B. Meconium degrades SP-B. The degradation of protein is attenuated by protease inhibitors and ACC.

METHODS

- General proteolysis assays such as the azocaseine method could be useful tools for testing possible pharmacological grade ACC.
- SDS PAGE and Western Blot.
- Various pH.
- Different concentrations of meconium for 30 min at 37 ºC, with and without protease inhibitors and ACC.

CONCLUSIONS

- Proteolytic enzymes from meconium degrade SP-B.
- The proteolytic effect of meconium continues up to at least 48 hours after exposure.
- Effect is sensitive to alterations in pH, presence of inhibitors and inhibitor levels.
- General proteolysis assays such as the azocaseine method could be useful tools for testing possible therapeutic combinations of proteolytic enzyme inhibitors at different pH values.
- Proteolytic enzyme inhibitors should be considered and further studied as therapeutic option for MAS.
- Proteolytic enzyme inhibitors and alteration of pH will now be further tested in an animal model in vivo to screen for a possible therapy for MAS.

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