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DEVELOPING A CHRONIC IN-VITRO MODEL FOR BLADDER PAIN SYNDROME.

Hypothesis / aims of study

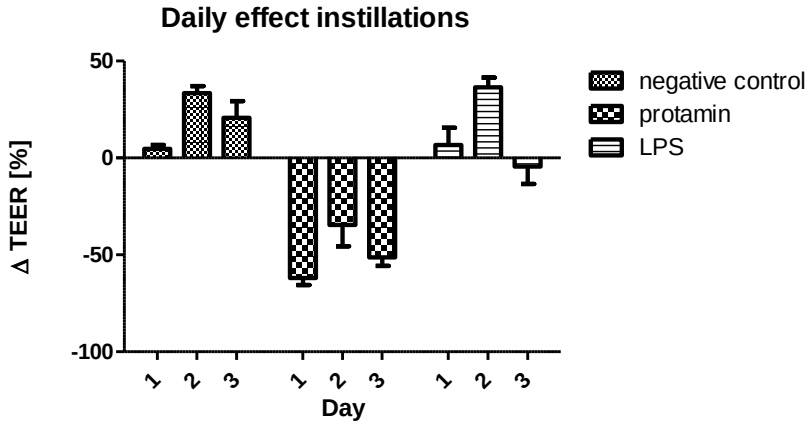
In bladder pain syndrome (BPS), the barrier of the urothelium is compromised. This enables solutes from the urine to leak into the bladder wall and cause irritative symptoms. To get a better understanding of this disease and to test therapies, different models are being developed. Many animal models use different irritants such as acids to directly damage the lining of the bladder wall. In our previous studies we used protamin to damage the urothelium and create a deficient barrier. In order to set up a chronic protocol we instil this enzyme repeatedly for longer periods of time. This could possibly interfere with glycosaminoglycan (GAG) replenishment therapies we want to evaluate with this model. We set out to prepare a chronic model in-vitro for urothelium with a deficient barrier. In this study we wanted to evaluate the effects of lipopolysaccharide (LPS) on barrier function in-vitro and compare this to protamine.

Study design, materials and methods

We used primary porcine cells and cultured these on Transwell inserts in medium supplied with fetal calf serum and calcium. With Trans Epithelial Electrical Resistance (TEER) we evaluated the barrier function. The 12 inserts were divided in groups of 4: negative control, protamine and LPS. Subsequent instillations with different solutions were performed in an attempt to break down the barrier. The protamine group was instilled daily for 6 hours with protamin 10%. The LPS group was instilled with 2% LPS continuously. The TEER was measured during 3 days.

Results

By instilling protamine, the TEER and thus the barrier, goes down. The TEER recovered every day in the protamine group (data not shown) after removal of the enzyme. There is no decrease in TEER in the negative control and the measurements in the LPS group shows similar results.



Interpretation of results

LPS shows no negative effect on the barrier function of cultured primary porcine cells. Instillation with protamin shows a daily drop in barrier function measured by TEER.

Concluding message

We evaluated the effect of protamine and LPS in search of the ideal chronic model in-vitro for inflamed urothelium with a deficient barrier. Protamine shows a nice decrease in TEER whilst the cells stay viable. The disadvantage of protamine is that it should be instilled daily because of the natural recovery of urothelium to a certain extent. Continuous instillation with this enzyme will possibly interfere with therapeutic instillations we want to test on this model.
Through measuring TEER, no negative effect of LPS could be detected for barrier function. For now, protamine is our best option to decrease the barrier in-vitro. These results prove the principle for animal experiments in which protamine is used to create a deficient barrier.

Disclosures

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