

EVALUATION OF A NOVEL PRECLINICAL MODEL FOR BLADDER BARRIER EVALUATION USING WHOLE PORCINE BLADDERS

Hypothesis / aims of study

Preclinical experimental models for evaluation of bladder permeability are important for investigating novel therapies for Interstitial Cystitis / Bladder Pain Syndrome (IC/BPS). This is often done with *in vitro* or small rodent models, whilst larger animal bladders like the porcine bladder is more biocompatible with the human bladder. The aim of this study was to evaluate the applicability of a new bioreactor in which the barrier properties of an intact porcine bladder can be investigated. Key factor for applicability of the model was the usage of readily available porcine bladders from freshly dissected bladders from the local abattoir.

Study design, materials and methods

A bioreactor was developed by LifeSciences, that provided a sealed-off environment that contained different storage levels to position kidney's, ureters, bladders and urethra in the normal anatomical position. Porcine lower urinary tracts (bladder and urethra) from 3 month old pigs (mixed gender; n=12) were collected from the local abattoir and experiments were conducted on the same day. Before use in the experiments, the quality of the bladders was evaluated and classified as either compliant (flexible low-resistant bladder wall during filling) and non-compliant bladders (rigid high-resistant bladder wall during filling).

After placing of the organs in the model, closed access to the bladder was achieved by the insertion of 8 French catheter that was placed through the urethra with the tip of the catheter in the bladder base. Urethra was sealed from the outside with 2 tie ribs, to prevent urethral leakage. Bladders were checked for leakage and to see if they could be drained completely after which the exterior column of the bioreactor was filled with warm water (37 °C). Bladders were rinsed with warm saline (37 °C), before the instillation of 100ml of sodium-fluorescein – RPMI (culture media) solution (50mg/100ml, 37 °C) for one hour. 4 ml samples were collected from the bladder lumen at t = 1, 15, 30, 45, 60 min (replaced with stock solution) and samples from the exterior column fluid were taken at t = 60 min. After this, bladders were again rinsed with normal saline (37 °C) and bladders were carefully opened using surgical scissors. Standard 1 cm ϕ punch biopsies were taken and flash frozen in isopentane on dry ice (n=2 / bladder). Other biopsies were taken for immunohistochemistry. These were embedded in TissueTek® and flash frozen.

Bladder barrier properties were assessed with: **1)** Semi- quantitative immunohistochemical/immunofluorescence assessment e.g.: sodium fluorescein infiltration in bladder wall, standard H&E, cytokeratin 18 (umbrella cells), tight junction, uroplakin III and chondroitin sulfate (GAG) expression. **2)** Measuring sodium-fluorescein uptake in bladder biopsies with IVIS SpectrumBL High-Throughput In Vivo Optical Imaging System (Perkin Elmer Inc, Waltham, Massachusetts) and **3)** Decrease in time of sodium-fluorescein concentration using spectrofluorescence measurements in triplo (Victor3, Perkin Elmer Inc, Waltham, Massachusetts).

Results

No leakage occurred via ureters. Two bladders were not used because of bladder damage/leakage due to the slaughtering procedure in the abattoir. A total of 5 bladders were labelled non-compliant based on increased bladder wall stiffness mainly the bladder dome. Increased pressures during filling resulted in urethral leakage of intravesical fluid that resulted in exclusion of this specimen from the study. A total of nine bladders were included in the final evaluation. Immunohistochemical evaluation showed a clear pattern in the non-compliant bladders with an increased bladder wall permeability. Although these bladders seemed normal on standard H&E, these bladders did not express a cytokeratin 18 positive cell layer (umbrella cells) and almost no uroplakin layer was seen compared to the compliant bladders. This pattern was also seen with a dramatic increase in sodium-fluorescein uptake in the bladder wall seen under a fluorescent microscope and with the IVIS Spectrum imaging of the bladder biopsies. It was also observed with spectrofluorescence measurements on bladder installation solutions, where a decreased concentration of sodium-fluorescein in time was measured in the non-compliant bladders. A remarkable inverse correlation was seen with the presence of clear chondroitin sulfate layer expression in non-compliant specimens.

Interpretation of results

Porcine bladders from the abattoir are readily available. In the Netherlands, no ethical approval is needed for the use of these bladders for experiments. To advance translational research with a focus on bladder barrier affecting therapies, there is a need for economically feasible models that are biocompatible with the human bladder and are easy to standardize. This model can be used to evaluate bladder permeability on a human scale and this model or a comparable one that uses abattoir derived porcine bladders, could decrease the amount of laboratory animals used, and reduce cost and administrative burden of these experiments. The challenge of using abattoir derived porcine bladders is to get the appropriate control over the quality and viability of the specimens used, and initiate the appropriate timeframes for the experiments.

Concluding message

This study shows that it is feasible to create a standardized preclinical model for the investigation of bladder barrier properties that is and very biocompatible with the human bladder, but does not have high cost or makes use of laboratory animals.

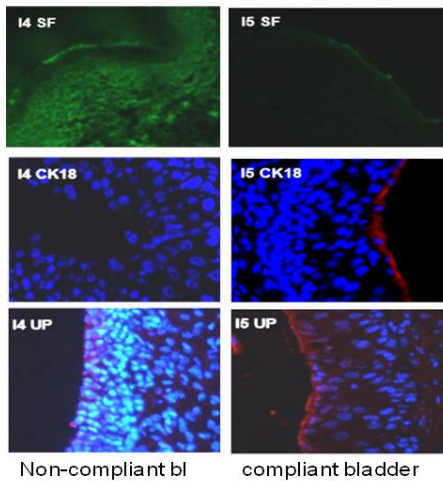
A: the model



B: Overall evaluation p. ex.

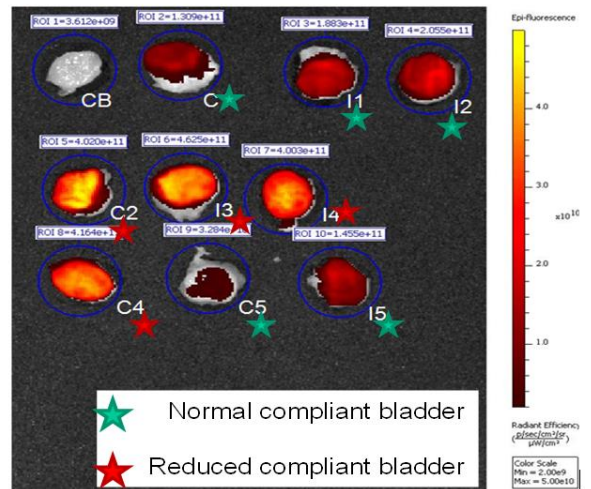
	Non-compliant 1	Compliant 1	Non-compliant 2	Compliant 2
% decrease in fluorescence count of intravesical solution (Victor)	14%	4%	21%	5%
Total radiant efficiency of biopsies (IVIS)	++	--	++	-
IH Na-FI penetration bladder wall	+	-	++	-
Cytokeratin 18	-	++	-	++
Tight junctions	+	+	+/-	+
Uroplakin III	+/-	+	+/-	+
Chondroitin sulfate (GAG)	++	-	++	-

C: IF images examples



Non-compliant bl compliant bladder

D: IVIS measurements biopsies



Legend to figure.

A: the experimental bioreactor where a porcine bladder is positioned where a 8Fr catheter is inserted into the bladder base via the urethra. B: Examples of quantitative and semi quantitative measurements of barrier properties between compliant and non-compliant bladders. C: Examples of IF images of non-compliant and compliant bladders. Evident abnormalities seen in non-compliant bladders: increased Sodium fluorescein (SF) uptake, absence of CK18 (umbrella cell marker), hardly any uroplakin layer expression. D: IVIS Fluorescence measurements on bladder biopsies from compliant and non-compliant bladders shows an increased SF uptake in the non-compliant bladders.

References

- Eichel L, Scheidweiler K, Kost J, Shojaie J, Schwarz E, Messing E, Wood R: Assessment of murine bladder permeability with fluorescein, validation with cyclophosphamide and protamine. *Urology* 2001; 58:113-118

Disclosures

Funding: AGIKO Grant; Dutch Government scientific grant. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** porcine **Ethics Committee:** We used abattoir derived porcine bladders (not laboratory animals). Under Dutch law; the use of these bladders do not require ethical committee approval. However, our local ethical committee is aware of investigation and consents and encourages the use of these specimens for research,