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BUCCAL MUCOSA IN PRIMARY HYPOSPADIAS SURGERY IN PREPUBERTAL BOYS AND A POSSIBLE CAUSE OF HIGH COMPLICATION RATE IN HYPOSPADIAS SURGERY. MOLECULAR CHARACTERIZATION AND ANDROGEN RECEPTOR LOCALIZATION IN BUCCAL MUCOSA AND URETHRA.

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HYPOTHESIS / AIMS OF STUDY

After primary hypospadias surgery complication rate i.e. fistula, strictures, and dehiscence is high. Impaired wound healing might be explained by differences in tissue quality of hypospadic versus normal urethra. Besides during primary hypospadias surgery there may be lack of local tissue. Buccal mucosa (BM) is widely used for urethroplasties after failed hypospadias correction in adults. Little is known about the applicability of BM in prepubertal boys and the effect of puberty on BM. To investigate differences in tissue quality, we performed molecular characterisation of the urethral plate in hypospadias patients compared with non-hypospadic urethra in male-to-female transgender patients. Also we compared androgen receptor (AR) localisation in BM with AR localisation in human and rat urethra.

STUDY DESIGN, MATERIALS AND METHODS

After parents informed consent, surplus tissue of hypospadias surgery was collected under local biobank protocol and embedded in paraffin. BM from fertile male rats was harvested, and a part of the tissue was embedded in paraffin. Coupes of 3 µm were cut, hematoxylin-eosin (HE) and immunohistochemical staining for AR, Ki-67, p63, von Willebrand factor (vWf), elastin, and keratin 7 (CK 7) were performed. As a control tissue of male urethra from gender conformation surgery and male rat urethra was used. Part of the rat BM was used for isolation of primary keratinocytes. Cells were grown on a feeder layer of immortalised fibroblast according to protocol [1]. After approximately ten days colonies of keratinocytes were formed, cells were fixed and stained for AR.

RESULTS

HE staining showed a important difference between the epithelium of the urethra: in hypospadias patients the epithelium displayed a multi-layered morphology, compared with a single layer in the normal urethra. P63 was expressed in both epithelia of the urethra's. CK7, marker of single layered epithelium was absent in the hypospadic urethra, whereas it was expressed in the normal urethra. Vascular marker vWf was diminished in hypospadias, compared to the normal urethra, less capillaries and less vascular spaces were seen. Moreover, there was less elastin detected in the hypospadic urethra.

HE staining of BM showed a multi-layer epithelium. In BM the AR was nuclear localised in the basal layer, and cytoplasmatic localised in the apical layer. Moreover, there was less expression in the apical layer. The basal layer was Ki-67 positive, this is associated with cell proliferation. Preputial skin epithelium was also multi-layered as expected in skin: vascular markers showed capillaries in the sub-epithelial layer. We did not detect variations in elastin in preputial skin samples. In pre-pubertal boys AR was localised in the cytoplasm, whereas in adult male in the nucleus. Just like the human urethra, the rat urethra has a single layer epithelium. In both the AR was nuclear localised. In the isolated BM cells AR was homogeneously distributed, with a slight enrichment of the nucleus.

INTERPRETATION OF RESULTS

There are differences in the hypospadic urethra in comparison to the normal human urethra. In hypospadias patients there is a multi-layer epithelium, compared with a single layer in the normal urethra. Moreover, there are less capillaries and vascular spaces, and diminished elastin expression in the hypospadic urethra leading to less elasticity and vascularisation of the subepithelial layer as described before [2]. This could be an explanation for the high complication rate in hypospadias surgery. In BM the AR is localised in the nucleus in the basal layer, as in the case in human and rat urethra, indicating that the AR is active. This finding suggests that BM is sensitive to androgens and might grow during puberty like other hormone sensitive tissue.

CONCLUDING MESSAGE

Urethral tissue in hypospadias shows multilayer epithelium and less elasticity and vascularisation in the subepithelium compared with non-hypospadic urethra, as a possible cause of high complication rate in hypospadias surgery. Furthermore, the fact that nuclear localised, active AR is found in BM suggests that BM might be responsive to testosterone during puberty. Although further research is needed to confirm, these preliminary results, these data suggest that BM can be used in primary hypospadias corrections in prepubertal boys.

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