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Research Article



Pathomorphological restructuring of the buccal mucosa grafts during ureteroplasty (experimental and clinical study)

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ABSTRACT

BACKGROUND: Currently for the plastic surgery of extended strictures of the pyeloureteral segment and proximal ureter grafts from the buccal mucosa are used. Histological changes in these grafts in the postoperative period have not been sufficiently studied.

AIM: is to study the histological changes in the graft from the buccal mucosa used for ureteroplasty in an experiment and in patients at different times after surgery.

MATERIALS AND METHODS: The experimental part of the study was carried out on 10 animals (rabbits). We studied histological changes in the wall of the buccal mucosa graft used for ureteroplasty. Under general anesthesia a median laparotomy was performed, the ureter was mobilized along the middle third, a defect of about 1 cm was created. Next a buccal graft of 1.5 × 1 cm was cut out, which was sutured to the ureteral defect using the onlay technique. After 6 months nephroureterectomy was performed. Three parts of the ureter were identified: the replacement zone, 3 cm sections above and below it, followed by histological examination. The clinical part of the study consisted of a histological examination of biopsy samples obtained from 5 patients during ureteroscopy by pinching graft biopsy 12 months and 24 months after buccal ureteroplasty.

RESULTS: The experimental part of the study demonstrated the possibility and effectiveness of ureteroplasty with a buccal graft, and also revealed the restructuring of squamous epithelium into transitional cell. In patients 12 and 24 months after buccal ureteroplasty similar changes in the mucous membrane of the graft were not observed, therefore, further study of the mucous membrane of the buccal graft in later postoperative periods is necessary.

CONCLUSIONS: The results of experimental and clinical studies indicate the possibility of using a graft from the buccal mucosa for plastic surgery of extensive and recurrent ureteral strictures. Unlike animals, where a restructuring of squamous to transitional cell epithelium was observed, similar morphological changes did not occur in patients after buccal ureteroplasty.

Keywords: ureteral stricture; ureteral plastic surgery; buccal mucosal graft; buccal ureteroplasty; stratified squamous epithelium; transitional cell epithelium.

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Научная статья

Патоморфологическая перестройка слизистой оболочки буккального лоскута при уретеропластике (экспериментально-клиническое исследование)

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АННОТАЦИЯ

Актуальность. В настоящее время при пластике протяженных стриктур пиелоуретерального сегмента и проксимального отдела мочеточника используют лоскуты из слизистой оболочки щеки. Гистологические изменения данных лоскутов в послеоперационном периоде исследованы недостаточно.

Цель — изучить гистологические изменения лоскута из слизистой оболочки щеки, используемого для уретеропластики, в эксперименте и у пациентов в разные сроки после операции.

Материалы и методы. Экспериментальную часть исследования проводили на 10 животных (кроликах). Изучали гистологические изменения в стенке лоскута из слизистой оболочки щеки, использованного для пластики мочеточника. Под общей анестезией выполняли срединную лапаротомию, мочеточник мобилизовали на протяжении средней трети, создавали дефект около 1 см. Далее выкраивали буккальный графт 1,5 × 1 см, который пришивали к дефекту мочеточника по методике onlay. Через 6 мес. выполняли нефроуретерэктомию. Выделяли три части мочеточника: зону замещения, участки выше и ниже нее по 3 см, с последующим гистологическим исследованием. Клиническая часть состояла в гистологическом исследовании биоптатов, полученных у 5 пациентов при уретероскопии путем щипковой биопсии лоскута через 12 и 24 мес. после буккальной пластики мочеточников.

Результаты. В экспериментальной части исследования показана возможность и эффективность пластики мочеточника буккальным лоскутом, а также выявлена перестройка плоского эпителия на переходо-клеточный. У пациентов через 12 и 24 мес. после буккальной уретеропластики подобные изменения слизистой оболочки лоскута не наблюдались, поэтому необходимо дальнейшее изучение слизистой оболочки лоскута из щеки в более поздние послеоперационные сроки.

Заключение. Результаты экспериментального и клинического исследований указывают на возможность использования лоскута из слизистой оболочки щеки для пластики протяженных и рецидивных стриктур мочеточника. В отличие от животных, где наблюдалась перестройка плоского на переходо-клеточный эпителий, у пациентов после буккальной уретеропластики подобные морфологические изменения не происходили.

Ключевые слова: стриктура мочеточника; пластика мочеточника; лоскут слизистой оболочки щеки; буккальная уретеропластика; многослойный плоский эпителий; переходо-клеточный эпителий.

Как цитировать

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BACKGROUND

Recurrent extended narrowing of the pyeloureteral segment (PUS) and the proximal ureter is an indication for buccal ureteroplasty if plasty with the use of urinary tract tissues is impossible. A buccal graft (BG) is a free flap without a vascular pedicle. Revascularization and morphological changes in the mucosa under the influence of urine remain insufficiently examined. The evaluation of the histological rearrangement of the epithelium of the cheek mucosa flap used for ureter replacement in the experiment and in different periods after ureteroplasty in clinical practice can predict the effectiveness of its use in reconstructive surgery of the upper urinary tract and identify potential complications of these operations and ways of their prevention. Earlier experimental studies have been conducted on the pathomorphologic changes in a BG during urethroplasty. These studies have shown that within two months, the squamous epithelium of a BG degenerated into transitional cellular epithelium [1–5]. In the Russian literature, only a few experimental studies have focused on the graftability of the buccal flap during urethroplasty [6]. However, unlike the urethra, the flap is constantly exposed to urine, which necessitates further studies.

The literature on buccal ureteroplasty includes only one experimental paper by Somerville and Naude [7]. In their experiment on three baboons, tubular ureteroplasty using a flap from the cheek mucosa with the omentum enveloping the operation zone was performed. Antegrade ureterograms showed good patency of the ureter, and histological studies showed that the epithelium of the graft remained intact and did not differ from its original structure. No further experimental studies on buccal ureteroplasty have been conducted. Pathomorphologic changes in the flap in patients who underwent buccal plasty were not examined. Thus, this paper presents the results of experimental work on 10 rabbits in which onlay replacement of the middle third of the ureter with a flap from the cheek mucosa was performed. In addition, the morphologic changes of the epithelium in different terms after ureter replacement in clinical practice are presented.

This study aimed to investigate the histological changes of the cheek mucosa flap used for ureteroplasty in both experimental and patient settings at various post-operative intervals.

MATERIALS AND METHODS

In this study, experiments used 10 male rabbits weighing >3 kg. The experimental model for ureteroplasty using a flap from the cheek mucosa was created as follows: a midline laparotomy was performed under general anesthesia, and the ureter was mobilized in the

middle third along 3–4 cm and held in place. At this point, the ureter was opened, and a 1-cm defect was created (Fig. 1).

A 3-ch ureteral stent was placed through the defect. A buccal flap measuring 1.5×0.5 cm was taken, and the fat layer was cleaned (Fig. 2). After completing the preparation, the flap was sutured to the ureteral defect using the onlay technique and Vicryl 7/0 (Fig. 3).

After closing the ureteral defect with a buccal flap, the surgical site was covered with retroperitoneal fat because the greater omentum is not present in rabbits. The wound was sutured layer by layer. Six months later, under general anesthesia, a repeated midline laparotomy was performed, consisting of the mobilization of the kidney and ureter throughout the length, followed by nephroureterectomy with bladder resection (Fig. 4). Three sections of the excised ureter were identified: the replacement zone and 3 cm above and below it. The specimens were fixed in 10% neutral formalin, labeled, and processed for paraffin embedding, sectioning, and staining.

Following nephroureterectomy, the anterior abdominal wall was sutured through all layers. The experimental animals were euthanized by administering sodium phenobarbital at a dose of 60–100 mg/kg body weight intraperitoneally and into the lung cavity. Animal corpses were disposed of in accordance with the SanPin regulations.

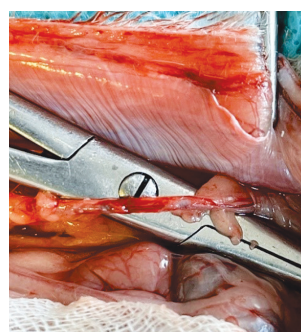


Fig. 1. Isolation of the middle third of the ureter, formation of a defect measuring 1.0 cm

Рис. 1. Выделение средней трети мочеточника, формирование дефекта размером 1,0 см



Fig. 2. A flap is cut out from the buccal mucosa and cleared of underlying tissue

Рис. 2. Выкроен лоскут из слизистой оболочки щеки, очищен от подлежащей клетчатки

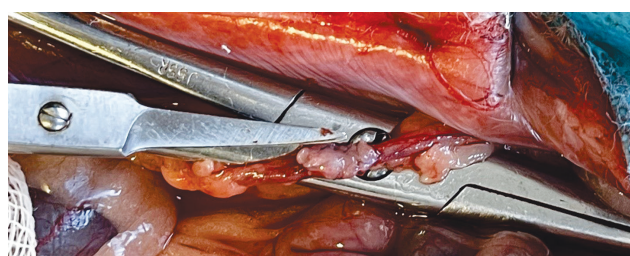


Fig. 3. The flap is sewn to the ureteral defect

Рис. 3. Лоскут пришит к дефекту мочеточника



Fig. 4. Removed kidney with ureter

Рис. 4. Удаленная почка с мочеточником

The clinical group consisted of 30 patients with extended and recurrent strictures of the proximal ureter, including PUS, who underwent surgery at the Urology Center of the City Mariinsky Hospital between 2018 and 2023. Of these patients, 25 (83.3%) were hospitalized routinely, whereas the remaining 5 (16.7%) were admitted as emergencies. The group comprised 18 (60.0%) male and 12 (40.0%) female patients, with a mean age of 49.5 ± 16.6 years (range, 19–77 years). All patients underwent laparoscopic onlay buccal plasty using four trocars. The first trocar was placed in the iliac region on

the side of the camera. After insufflation of the abdominal cavity to 12 mmHg, three more trocars were inserted. Two 6-mm trocars were placed along the subclavian line in the iliac region and below the rib arch, and one 6-mm trocar was placed along the posterior axillary line. The colon was mobilized and withdrawn medially. During the procedure, the scar tissue in the retroperitoneum was removed to identify the ureter in the upper third. The ureter was then identified above and below the narrowing to preserve as much unchanged tissue as possible. In cases of PUS stricture, the pelvis was adequately mobilized before dissecting the ureter along the narrowed area, 1 cm above and below the affected region. In cases of PUS obstruction, the incision was continued proximal to the pelvis. The degree of stricture was determined using a ureteral catheter. Then, a piece of cheek mucosa of appropriate length was taken and used to suture the defect with continuous sutures. Subsequently, the flap was prepared for plasty and passed through the trocar into the abdominal cavity. Several knotted sutures were used to secure the flap to the distal and proximal edges of the narrowed ureter, which facilitated further suturing. Initially, a continuous suture (Vicryl 4/0) was placed between the bladder and the lateral edge of the dissected ureter. After antegrade stent placement, the suture was placed on the medial edge of the ureter. The surgery was completed by enveloping the replacement area with a large omentum and placing a drain in the surgical site. In 5 patients, a pinch biopsy of the flap was performed at ureteroscopy 12 and 24 months after surgery. A detailed study of cells and tissues was conducted using histological sections prepared using a Sakura Accu-Cut SRM 200 rotary microtome. Slice thickness was 3 μ m, and staining was performed with hematoxylin and eosin. The microstructures were examined using an Olympus microscope at 40 and 400 magnification.

RESULTS

A histological examination of a frontal slice of the rabbit ureter in the surgical area revealed numerous long mucosal folds with well-developed connective tissue rods that remained connected to the ureteral mucosa over a wide area (Fig. 5, *a*). A considerable portion of the mucosa and its folds exhibited signs of productive inflammation. The epithelium of the folds had transitional cellular areas throughout their lengths, including the base (Fig. 5, *b*), middle part (Fig. 5, *c*), and apex (Fig. 5, *d*). The multilayer epithelium on the surface of the graft mucosa has distinct features that differentiate it from the epithelium of the cheek or the urothelium of a healthy organism. However, it still bears the predominant features of the transitional epithelium. The basal layer is represented by areas where the basal cells, which are in contact with the basal membrane, are either

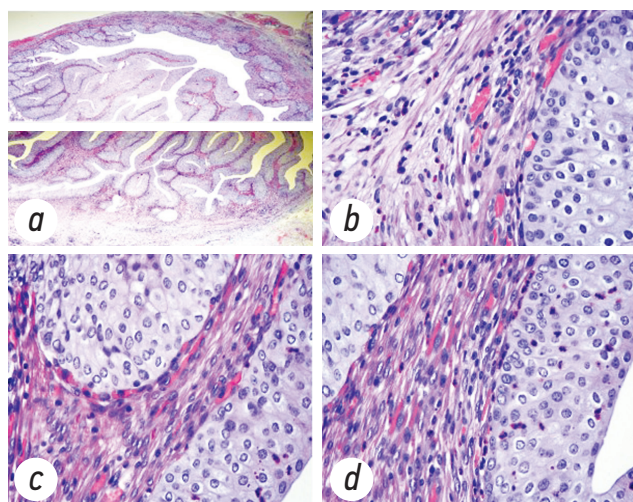


Fig. 5. Results of histological examination of the area of buccal plastic surgery of the rabbit ureter. The replacement of the squamous epithelium of the buccal mucosa with transitional cell epithelium is determined. Explanations in the text. Hematoxylin and eosin staining. Magnification $\times 40$ (*a*), $\times 400$ (*b*, *c*, *d*)

Рис. 5. Результаты гистологического исследования зоны буккальной пластики мочеточника кролика. Определяется замещение плоского эпителия слизистой оболочки щеки переходно-клеточным эпителием. Объяснения в тексте. Окраска гематоксилином и эозином. Увел. $\times 40$ (*a*), $\times 400$ (*b*, *c*, *d*)

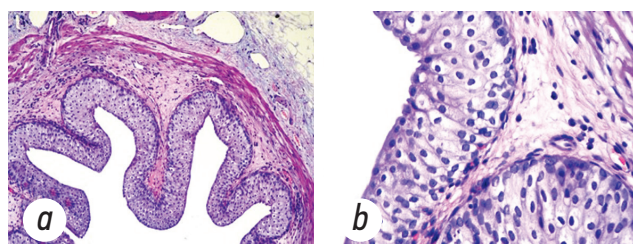


Fig. 6. Frontal section of the rabbit ureter above the buccal plasty area. Hematoxylin and eosin staining. Magnification $\times 40$ (a), $\times 400$ (b)

Рис. 6. Фронтальный срез мочеточника кролика выше зоны буккальной пластики. Окраска гематоксилином и эозином. Увел. $\times 40$ (a), $\times 400$ (b)

at the same or different levels. The intermediate layer contains more cells than both types of epithelium in a healthy organism. As it approaches the free surface of the tissue, the cell size increases while maintaining a polygonal shape and a nucleus structure that corresponds to cells with preserved mitotic ability. The outer layer is primarily composed of large elongated cells. However, unlike the outer squamous layer of the cheek epithelium in a healthy organism, it comprises tall, flattened cells that contain an oval, light-colored nucleus and chromatin. Most cells in the intermediate and basal layers exhibit signs of edema, including single cells of inflammatory infiltrate (granulocytes) and dying or dead epitheliocytes located between the cells.

Histological examination was performed on the ureter above and below the surgical area to evaluate the condition of the buccal flap replacement zone. The mucosal folds were small and lined with transitional epithelium that had undergone reactive changes. The basal layer consisted of typical low-differentiated epithelial cells arranged in 1–2 rows. The intermediate layer had several rows of cells with signs of pronounced edema. The cells in the outer layer are mainly cubic, unlike the intact urothelium. They are small, have only one nucleus, and exhibit low differentiation, such as a clumped form of chromatin and an indistinguishable nucleus (Figs. 6 and 7).

After replacement, the epithelium of the cheek mucous membrane flap may die because of impaired blood circulation in its vessels. The preserved transitional epithelium along the graft edges is activated by the surgical defect in the area of adjacent cells of the basal layer, and it regenerates according to known regularities. Thus, the basal and neighboring cells proliferate along the basal membrane beneath the dead multilayered epithelial layer and displace it toward the surface. This process leads to the recanalization of the mucosal vessels and the restoration of the epithelial layer, which retains the characteristics of the surrounding tissue. However, after a certain period, it undergoes inflammatory postregenerative changes.

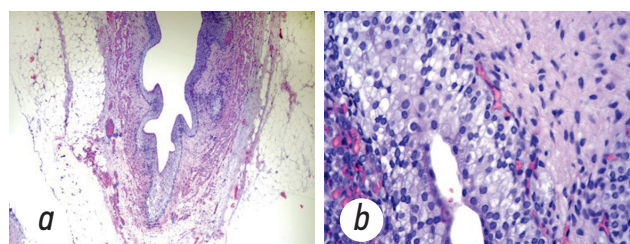


Fig. 7. Oblique longitudinal section of the rabbit ureter below the buccal plasty. Hematoxylin and eosin staining. Magnification $\times 40$ (a), $\times 400$ (b)

Рис. 7. Косо-продольный срез мочеточника кролика ниже буккальной пластики. Окраска гематоксилином и эозином. Увел. $\times 40$ (a), $\times 400$ (b)

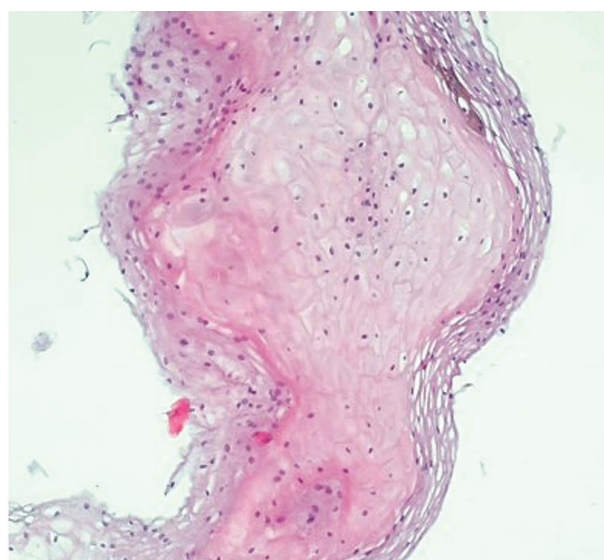


Fig. 8. Mucosa of a patient's buccal graft after buccal ureteroplasty. Hematoxylin and eosin staining. Magnification $\times 40$

Рис. 8. Слизистая оболочка буккального графта пациента после буккальной пластики мочеточника. Окраска гематоксилином и эозином. Увел. $\times 40$

In the clinical section, the histological study of the biopsy specimen of the BG showed that the mucous membrane of the graft corresponded to the multilayer squamous epithelium in all cases. No atrophy or inflammatory infiltration, which is characteristic of the unchanged epithelium of the cheek mucosa, was observed (Fig. 8). Unlike the experimental data, no transformation of the squamous epithelium into transitional cellular epithelium occurred under clinical conditions. This is likely due to the short observation period of patients after buccal ureteroplasty.

DISCUSSION

The cheek mucosa flap is currently used to replace long urethral strictures. Some clinics are gaining experience in the plasty of extended urethral defects using this graft [8–14]. Thus, studying the graftability of the BG,

determining the terms of its final vascularization, and examining the pathomorphological changes occurring in the epithelium of the flap used for ureteroplasty are important. The literature includes only one experimental study on ureter replacement using a flap from the cheek mucosa [7]. The study was conducted by Somerville and Naude in 1984 on three baboons that share similar anatomophysiological features with humans. However, their results did not demonstrate any degeneration of the squamous epithelium into the transitional cellular epithelium in any of the cases. This may be attributed to the short observation period of the animals. Furthermore, the use of tubular ureteroplasty may have resulted in the contact between the ureter epithelium and the flap only in the areas of the upper and lower anastomoses. Our experiment revealed the reorganization of the cheek mucosa into transitional cellular epithelium by the 6th month of observation, which can be attributed to the greater expression of regenerative processes in rabbits. In the clinical part of the study, flap biopsy was performed on 5 patients at 12 and 24 months after buccal ureteroplasty during ureteroscopy. Histological studies revealed that the transformation of the squamous epithelium into the transitional cellular epithelium did not occur in humans during these periods. These findings are consistent with the results of an experimental study conducted by Somerville and Naude [7]. Regenerative processes in humans are slower than those in rabbits. Therefore, no significant reorganization of the BG mucosa was observed during the short follow-up period. Further observation of patients after buccal ureteroplasty will allow for the determination of the possible transformation of the squamous epithelium into the transitional cellular epithelium.

CONCLUSIONS

The experimental study on rabbits demonstrated the possibility and effectiveness of ureteroplasty using a buccal flap and the reorganization of the squamous transitional cell epithelium. In patients who underwent buccal ureteroplasty 12 and 24 months earlier, no changes in the flap mucosa were observed. Therefore, further study of histological changes in the cheek flap mucosa during later postoperative periods is necessary.

ADDITIONAL INFORMATION

Authors' contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. Personal contribution of each author: B.G. Guliev — development of the design of the study, analysis of the data obtained, editing the text of the manuscript; Zh.P. Avazkhanov — collection of material, writing the text of the manuscript, manuscript design, analysis of the data obtained; A.V. Droblenkov — analysis of the data obtained, editing the text of the manuscript; S.A. Vinnichuk — performing morphological studies, analysis of the data obtained, editing the text of the manuscript; O.Sh. Abdurakhmanov — collection of material, manuscript design.

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Competing interests. The authors declare that they have no competing interests.

Ethics approval. The present study protocol was approved by the local Ethics Committee of the North-Western State Medical University named after I.I. Mechnikov (No. 10, 30.10.2019)

Consent for publication. Written consent was obtained from the patient for publication of relevant medical information within the manuscript.

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