

კვლევის მიზანია - ნერვული ბოჭკოებისა და სხვადასხვა თვისებების მქონე ზედაპირის კრემნიუმის ძაფისებრი კრისტალების ურთიერთქმედების მორფოლოგიური შეფასება პერიფერიული ნერვის მიმდებარე ტრამისა და ნეიროპლასტიკის მოდელზე.

ექსპერიმენტი ჩატარდა ვისტარის ჯიშის 50 მამრ-ვირთაგვებზე, წონით 180-250 გ. ექსპერიმენტული ჯგუფების ვირთაგვებზე მოდელირებული იყო საჯდომის ნერვის მიმდებარე დაზიანება (Sunderland 5), 10 მმ სიგრძის ნერვის ღეროს დეფექტის ფორმირებით, რომელიც ჩანაცვლებული იყო კონდუიტით, დეცელულარიზებული აორტით, კარბოქსიმეთილცელულოზური გელით და გრძივად ორიენტირებული p-ტიპის კრემნიუმის ძაფისებრი კრისტალებით, დიამეტრით 2-20 μm . Ia ჯგუფის ვირთაგვებში გამოყენებული იყო კრემნიუმის ძაფისებრი კრისტალების ბუნებრივი ოქსიდი, Ib ჯგუფის ვირ-

თაგვებში - კრისტალები, რომელთა ზედაპირი გაიწმინდა წყალბადის ნაკადში, Ic ჯგუფის ვირთაგვებში - კრისტალები ხელოვნურად თერმულად გამოყვანილი ოქსილით. დიასტაზი ნერვულ დაბოლოებათა შორის კონტროლის ჯგუფებში შეესრულებული იყო გელიანი აორტით (II ჯგუფი), ან აუტონეიროგრაფტით (III ჯგუფი). ოპერაციიდან 6 კვირის შემდეგ, კონდუიტის ადგილი, მიმდებარე ნერვული დაბოლოებით ამოღებული იყო სხივური მიკროსკოპისთვის. კრემნიუმის ძაფისებრი კრისტალების გამოყენება ბუნებრივი ოქსილით იწვევს კონდუიტის ადგილის უფრო სრულ და თანაბარზომიერ ნევროტიზაციას. P-ტიპის კრემნიუმის ძაფისებრი კრისტალები ბუნებრივი ოქსილით, როგორც ნაწილი რეგენერაციული იმპლანტისა წარმოადგენს უფრო შესაფერის მასალას ელექტროდების დასამზადებლად, ვიდრე სხვა ტიპის კრისტალები.

FEATURES OF HISTOCHEMICAL CHANGES IN THE ACTIVITY OF SUCCINATE DEHYDROGENASE OF ARTIFICIAL BLADDER IN DYNAMICS (EXPERIMENTAL STUDY)

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Bladder cancer remains an urgent problem, due to a steady increase in morbidity and mortality. About five thousand new cases and two, three thousand deaths from this pathology are registered in Ukraine every year [1]. The active development and improvement of the quality of anesthetic management, early diagnosis and the emergence of new methods of treating oncological diseases, led to an expansion of indications for reconstructive operations in oncurology.

The gold standard for the treatment of invasive bladder cancer recognized throughout the world is radical cystectomy with orthotopic ileocystoplasty using the ileal intestinal tract [2,3,4]. A segment of the ileum is isolated, to form a reservoir from the gastrointestinal tract. The latter is detubulated, and then sewn as S, W, U, M, N - anastomoses, turning into a capacity of the shape of the ball [11]. The radical cystectomy with orthotopic derivation of urine is performed throughout the world by an open, laparoscopic and robot-assisted method, sometimes with the formation of intracorporeal anastomoses, which reduces the wound surface, the number of complications and quickly restores patient activity [5-8].

The study of the effect of urine on the adaptation of the mucosa of the artificial bladder continues for the last twenty years. According to the researchers, the results are quite contradictory, as some scientists note the hypersecretion of sulphomucins, sialomucins, progressive atrophy of microvilli, adenomatous hyperplasia and dysplasia [9,10].

The changes in the activity of enzymes of oxidation-reduction reactions in the tissues of the artificial bladder remain outside the attention of researchers. Although their activity causes the course of the main processes of vital activity in the tissues of the body.

Aim - to study the features of the histochemically revealed activity of succinate dehydrogenase in the wall of the artificial bladder and ileum in experimental animals.

Material and methods. The material of the present study were the results obtained from the study of 18 female mini-pigs aged 4-5 months and weighing 8-10 kg. The modeling of the artificial bladder was performed in experimental animals, by cystectomy and subsequent ileo-cystoplasty. The choice of an experimental facility is due to anatomical considerations. The urethra in female is straight and 5-7 times shorter than males.

The procedure for surgical intervention was as follows. A cut of the abdominal wall in the middle line from the pubic symphysis to the navel is performed under intravenous anesthesia (thiopental) in the position on the back of the pig. The top of the bladder is captured by forceps and tucked up. The front wall of the bladder is separated. The urethra resects, the bladder is separated from the rectum. The bladder is removed. Hemostasis. Retreating 15 cm from the ileocecal valve, sew the end of the isolated intestinal segment with continuous serous-muscle sutures vikryl 4-0. During the antibriated region, the distal part of the idiopathic segment is dissected within about 10 cm. The cut part of the segment is U-shaped, the adjacent edges of both knees are sewn together by a series of continuous serous-muscle sutures, cured 4-0. The lower part of the resulting U-shaped segment is placed transversely upwards. The distal portion of the ileo-intestinal segment is dissected approximately 10 cm long in mesenteric margin. The dissected part of the segment is U-shaped, adjacent edges of both knees sewed together by one row of continuous serous-muscular sutures vikryl 4-0. The lower part of the resulting U-shaped segment is enclosed transversely upwards. The ureteral catheters №3 Fr are inserted, the ends of which are removed through the wall of the reservoir before stitching the free margins of the dissected segment into the ileal lobe. An orifice is made, in the most caudal part of the reservoir, to which the urethra is sutured with 6 seams of vicryl 4-0. The sutures are tied after conducting the urethra of the Foley catheter

Table. SDG activity in the ileum wall (con. un.)

| | | control | 3 months | 6 months | 12 months |
|-------|--------------------|------------|------------|------------|------------|
| ileum | Epithelium | 6,41±0,11 | 6,16±0,13 | 6,11±0,13 | 5,07±0,17* |
| | Sub/ mucosa | 3,02±0,09 | 3,12±0,17 | 4,12±0,13* | 2,11±0,11* |
| | The muscular layer | 5,03±0,184 | 6,02±0,09* | 5,09±0,20 | 4,02±0,11* |

note * - the differences between the indicators are significant $p < 0.05$

No. 8Fr. The reservoir is drained with a cystostomy tube 12Fr, which is removed together with the ureteral stents through the wall of the reservoir. The reservoir is laid in place, forming isoperistaltic bringing knee. The ileum is dissected at the level of the ureters resected previously, 10 cm above the ileal-intestinal reservoir. The ureters slant obliquely, dissect along and anastomose the end to the side with the proximal non-rosaceous part of the ileum-intestinal segment. The stents, which are located inside the segment, are passed to the ureters. Restore bowel continuity. The stents are withdrawn through the anterior abdominal wall; drainages are installed through the counterparts into the small pelvis. The wound is sewed up by the vikryl.

A fragment of the wall of the ileum and the wall of the bladder was removed during the operative modeling of the bladder. The resulting material was placed on dry carbon dioxide (-70 s) for instant freezing. The cryostat sections obtained from the blocks with a thickness of 11 μm were made, on which, according to Loida's instructions, histoenzymatic reactions were carried out to reveal the activity of succinate dehydrogenase (SDG). The results obtained by studying the activity of enzymes in the wall of the ileum and bladder, isolated before the creation of the model of the bladder, were subsequently used as a control. Experimental animals with a bladder model in groups of 6 animals were withdrawn from the experiment 3, 6 and 12 months after operational modeling. The exclusion from the experiment was carried out by the method of thiopental sodium overdose, and the position of the «European Convention for the Protection of Vertebrate Animals used for experimental or other scientific purposes» (Strasbourg, 1986) was improved. A fragment of the wall of the ileum and an artificial bladder was removed from the animals. The resulting material was processed in accordance with the above-described algorithm. SDG activity was determined on the obtained cryostat preparations. SDG (1.3.99.1) is an enzyme belonging to the succinate oxidase system and is its first enzyme and participates in the Krebs cycle, so the histochemical detection of SDG gives valuable information about the activity of this cycle. Estimation of the results of histochemical studies was carried out in a semi-quantitative manner (Nasibullin, 1992), and based on the correlation of the scores of enzyme activity, cystospectrophotometry data, and measured in conventional optical density units (con. un.). The results of the studies were subjected to a standard statistical treatment.

Results and their discussion. As follows from the data of Table, in experimental animals, before the start of the experiment, the activity of SDG in the epithelium of the intestinal mucosa is high 6.08 ± 0.11 con. un. in myocytes is close to a high 5.03 ± 0.184 units, and in the submucosa is a moderate 3.02 ± 0.09 con. un.

It allows believing that the repair processes in the epithelium and contractile activity of the muscular shell are provided by energy due to the Krebs cycle.

Three months after the formation of the artificial bladder, histochemical determination of SDG activity in the ileal wall revealed no statistically significant differences from the activity of this enzyme in healthy animals. It allows us to believe that the Krebs cycle in the cellular elements of different layers of the intestinal wall retains its leading role. Histoenzymological studies of the ileum wall six months after the experimental modeling

of the neocyst did not reveal practically significant changes in SDG activity relative to healthy animals. It can be assumed that the completion of the postoperative period returns the functional activity of the intestinal wall structures to the initial state, which determines the normalization of the activity of the Krebs cycle enzymes, as well as the enzymes of the substrate maintenance cycles of the main cycle of energy supply of vital activity.

The activity of SDG in the submucosa after 12 months, is significantly lower by 30% (2.11 ± 0.11 con. un., $p < 0.05$) than in intact mini-pigs, possibly due to atrophic submucosal processes. We did not reveal significant changes in relation to the previous observation period as for the activity of the enzymes under investigation in the structures of the muscular membrane.

The activity of SDG in the epithelium of the intestinal wall tended to weaken according to intact animals and animals of the previous periods of the experiment twelve months after the formation of the formal bladder. The weakening of the activity of the studied dehydrogenase, determined in animals of this period of observation, may be related to the age-related weakening of bowel function.

The activity of SDH is similar, close to high and not statistically different in the structures of the bladder wall of intact animals. Obviously, the functional activity of these structures is very close, which requires a fairly high intensity of power supply. The changes in the activity of the enzymes under investigation occur in the wall of the artificial bladder.

The activity of SDH in three months is sharply reduced by 37.4% (4.01 ± 0.12 con. un., $p < 0.05$), in relation to native ileum according to Fig. 1 in the mucosal epitheliocytes. The activity of SDH corresponds to data (6.21 ± 0.06 con. un., 6.31 ± 0.21 con. un.) on the epithelium of the original ileum and it is slightly higher by 22.3% than in the epitheliocytes of the native bladder after 3 and 12 months.

The adaptation of the intestinal epithelium is completed by the 12th month, after the operation and the energy supply system passes to a genetically determined level of activity.

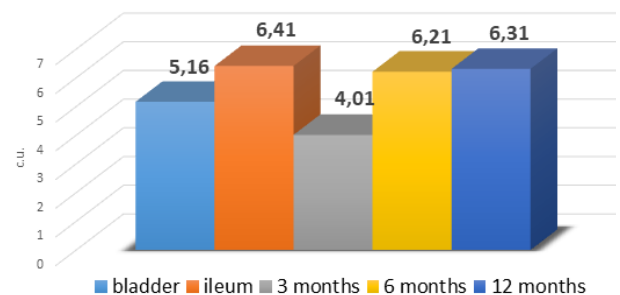


Fig. 1. Activity of SDG in the epithelial layer (con. un.)

The results of SDG activity in the submucosal layer of the artificial bladder are shown in Fig. 2. Thus, the activity of SDG in the intact bladder is 5.06 ± 0.11 con. un., in the ileum is 3.02 ± 0.09 con. un. A sharp decrease in SDG activity was observed in 60% (2.01 ± 0.07 con. un., $p < 0.05$) in relation to the bladder and 33.4% with respect to the ileum in the neobladder at 3, 6 and 12 months

after formation. Since a significant structural rearrangement of the submucosal layer was observed during the previous observation periods, it can be assumed that the vascular bed changes, which disrupts the transport of substrates and oxygen, and as a result, chronic hypoxia of this layer is formed.

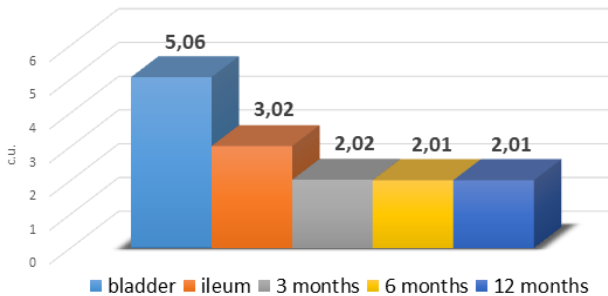


Fig. 2. SDG activity in the submucosal layer (con. un.)

This evidence of the activity of SDG in myocytes of the muscle layer is shown in Fig. 3. There is a decrease in SDG activity by 19%, in relation to the muscular layer of the initial bladder 5.02 ± 0.23 con. un. and the original ileum (5.03 ± 0.18 con. un.) at the stages of experimental studies, 3, 6 and 12 months (4.05 ± 0.21 con. un., 4.01 ± 0.1 con. un., 4.03 ± 0.1 con. un.) It can be assumed that hypertrophy of myocytes changes the transport capabilities of the vascular system of the formal bladder, which on the one hand causes the shift of the substratum of the substrate to the side of the intermediate metabolites, and insufficient general supply, contributes to the preservation of life-support processes at a relatively low level.

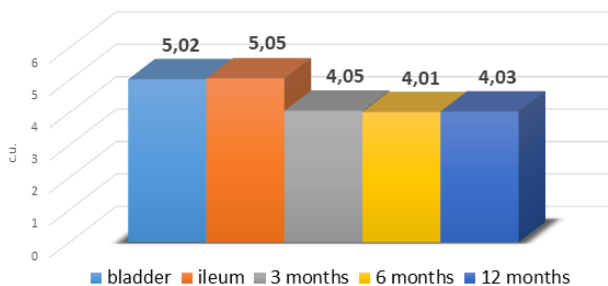


Fig. 3. Activity of SDG in the muscle layer (con. un.)

Conclusion. The results of studies showed that in the wall of the ileum, part of which forms artificial bladder, changes in SDG activity for 3, 6 and 12 months after the operation to form an uncontaminated disease does not occur. The marked unreliable fluctuations in activity recorded by us are most likely associated with seasonal changes in vital activity.

As for the wall of the official bladder, the changes in the activity of the studied enzymes were significant and showed not only possible changes in the activity of the Krebs cycle, but also about periodic displacements of the accents of substrate maintenance.

For example, a sharp decrease in SDG activity by 60% (2.01 ± 0.07 con. un., $p < 0.05$) in epithelial cells at 3 months relative to the bladder and by 33.4% in relation to the ileum, with the subsequent restoration of activity by the 6th and 12th months of the experiment. Depression of SDG activity by 19% was observed in the submucosal and muscle layers without a tendency to recovery in the end of the experiment.

These changes, in our view, are related to the transformation processes in the structural elements of the ileum wall, from which an unproblem has been formed to fulfill new functional

duties. Signs of a violation of energy metabolism indicate the processes of hypoxia in the tissue of the artificial bladder and require further study and observation.

REFERENCES

1. National Cancer Registry of Ukraine: short description of the database as of January, 2017.
2. Leonardo L. Monteiro and Wassim Kassouf Radical Cystectomy is the best choice for most patients with muscle-invasive bladder cancer? Opinion: Yes Int Braz J Urol. 2017 Mar-Apr; 43(2): 184–187.
3. Hautmann R. E. 25 years of experience with 1,000 neobladders: long-term complications / R. E. Hautmann, R. C. de Petriconi, B. G. Volkmer // J. Urol. – 2011. – Vol. 185. – P. 2207–2212.
4. Yong Seong Lee, Ha Bum Jung, Don Kyoung Choi, Sung Tae Cho, Ki Kyung Kim, and Young Goo Lee Functional Assessment of the Hautmann Ileal Neobladder with Chimney Modification Using Uroflowmetry and a Questionnaire BioMed Research International Volume 2016 (2016).
5. Raza S.J., Tawfeeq M., Al-Daghmin A., Guru K.A. Robot-assisted intracorporeal urinary diversion: where do we stand in 2014? Urol Clin North Am. 2014;41:503–509.
6. Zehnder P., Gill I.S. Cost-effectiveness of open versus laparoscopic versus robotic-assisted laparoscopic cystectomy and urinary diversion. Curr Opin Urol. 2011;21:415–419.
7. Sandberg J.M., Manny T.B., Hemal A.K. Robot-assisted and laparoscopic continent urinary diversion. In: Bishoff J.T., Kavoussi L.R., editors. Atlas of laparoscopic urologic surgery. 3rd ed. Elsevier; New York: 2016.
8. Jason M. Sandberg and Ashok K. Hemal Robot-assisted laparoscopic radical cystectomy with complete intracorporeal urinary diversion Asian J Urol. 2016 Jul; 3(3): 156–166.
9. Parenti A. Aragona F. et al. Abnormal Patterns of Mucin Secretion in Ileal Neobladder Mucosa: Evidence of Preneoplastic Lesion? Eur Urol 1999;35:98–101.
10. Gatti R., Ferretti S., Bucci G., et al. Histological Adaptation of Orthotopic Ileal Neobladder Mucosa: 4-Year Follow-Up of 30 Patient Eur Urol 1999;36:588–594s.

SUMMARY

FEATURES OF HISTOCHEMICAL CHANGES IN THE ACTIVITY OF SUCCINATE DEHYDROGENASE OF ARTIFICIAL BLADDER IN DYNAMICS (EXPERIMENTAL STUDY)

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The material of the present study were the results obtained from the study of 18 female mini-pigs aged 4-5 months and weighing 8-10 kg. The modeling of the artificial bladder was performed in experimental animals, by cystectomy and subsequent ileo-cystoplasty. Experimental animals with a bladder model in groups of 6 animals were withdrawn from the experiment 3, 6 and 12 months after operational modeling.

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Keywords: cystectomy, ileocystoplasty, energy homeostasis, succinate dehydrogenase.

РЕЗЮМЕ

ОСОБЕННОСТИ ГИСТОХИМИЧЕСКИХ ИЗМЕНЕНИЙ АКТИВНОСТИ СУКЦИНАТДЕГИДРОГЕНАЗЫ АРТИФИЦИАЛЬНОГО МОЧЕВОГО ПУЗЫРЯ В ДИНАМИКЕ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

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Золотым стандартом лечения инвазивного рака мочевого пузыря признанным во всём мире, является радикальная цистэктомия с ортотопической илеоцистопластикой, с использованием участка подвздошной кишки. Изучение влияния мочи на адаптацию слизистой искусственного мочевого пузыря продолжается на протяжении последних двадцати лет. По данным исследователей, результаты достаточно противоречивы: некоторые учёные отмечают гиперсекрецию сульфомуцинов, сиаломуцинов, прогрессирующую атрофию микроворсинок, аденоматозную гиперплазию и дисплазию.

Целью исследования явилось изучение особенностей гистохимически выявляемой активности сукцинатдегидрогеназы в стенке искусственного мочевого пузыря и в илеуме у экспериментальных животных. Материалом настоящего исследования послужили результаты, полученные при наблюдении 18 самок mini-pigs в возрасте 4-5 месяцев и весом 8-10 кг. У экспериментальных животных моделирование искусственного мочевого пузыря выполняли путём цистэктомии, с последующей илеоцистопластикой, контрольные исследования проведены 3, 6 и 12 месяцев спустя.

В стенке искусственного мочевого пузыря изменения активности сукцинатдегидрогеназы были значительны и свидетельствовали не только о возможных изменениях активности цикла Кребса, но и о периодических смещениях акцентов субстратного обеспечения. Эти изменения, на наш взгляд, связаны с процессами трансформации в структурных элементах стенки подвздошной кишки, из которых формировался неobladder для выполнения новых функций. Признаки нарушения энергетического метаболизма свидетельствуют о процессах гипоксии в ткани искусственного мочевого пузыря и требуют дальнейшего изучения и наблюдения.

რეზიუმე

ხელოვნური შარდის ბუშტის სუქცინატდეჰიდროგენაზას აქტივობის ჰისტოქიმიური ცვლილებების თავისებურებანი დინამიკაში (ექსპერიმენტული კვლევა)

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³ბ. ნასიბულინი, ⁴ა. იაცინა

¹ოდესის ეროვნული სამედიცინო უნივერსიტეტი; ²ეროვნული სამხედრო სამედიცინო კლინიკური ცენტრი, უროლოგიის კლინიკა, კიევი; ³უკრაინის სამედიცინო რეაბილიტაციის და კურორტოლოგიის სამეცნიერო-კვლევითი ინსტიტუტი, ოდესა, უკრაინა; ⁴კიბოს ეროვნული ინსტიტუტი, კიევი, უკრაინა

შარდის ბუშტის ინვაზიური კიბოს მკურნალობის ოქროს სტანდარტად მსოფლიოში ითვლება რადიკალური ცისტექტომია ორთოპედიული ილეოციტოპლასტიკით, თეძოს ნაწლავის ნაწილის გამოყენებით. ბოლო ოცი წლის განმავლობაში მიმდინარეობს შარდის გაგულენის კვლევა ხელოვნური შარდის ბუშტის ლორწოვანზე. მკვლევართა მონაცემებით, შედეგები მეტად წინააღმდეგობრივია; ასე მაგალითად, ზოგი მეცნიერი აღნიშნავს სულფომუცილების, სიალომუცილების ჰიპერსეკრეციას, მიკროხაოების პროგრესირებად ატროფიას, ადენომატოზურ ჰიპერპლაზიას და დისპლაზიას.

კვლევის მიზანს წარმოადგენდა სუქცინატდეჰიდროგენაზას აქტივობის ჰისტოქიმიურად გამოვლინებადი თავისებურებების შეფასება ექსპერიმენტული ცხოველების ხელოვნური შარდის ბუშტის და ილეუმის კედელში. კვლევის მასალად გამოყენებულია 4-5 თვის ასაკის, 8-10 კგ წონის მდედრი Mini-Pigs-ის კვლევის შედეგები. ექსპერიმენტულ ცხოველებში ხელოვნური შარდის ბუშტი მოდელირდებოდა ცისტექტომიით და შემდგომი ილეოციტოპლასტიკით; საკონტროლო კვლევები ჩატარდა 3, 6 და 12 თვის შემდეგ.

ხელოვნური შარდის ბუშტის კედელში აღინიშნა სუქცინატდეჰიდროგენაზას აქტივობის მნიშვნელოვანი ცვლილებები, რაც მოწმობს არა მარტო კრების ციკლის აქტივობის სავარაუდო ცვლილებებს, არამედ მიუთითებს სუბსტრატული უზრუნველყოფის აქცენტების პერიოდულ ძვრებზეც. ეს ცვლილებები, ავტორთა მოსაზრებით, დაკავშირებულია ტრასფორმაციულ პროცესებთან თეძოს ნაწლავის სტრუქტურულ ელემენტებში. ენერგეტიკული მეტაბოლიზმის დარღვევების ნიშნები ადასტურებს ჰიპოქსიური პროცესების არსებობას ხელოვნური შარდის ბუშტის კედელში და შემდგომ კვლევას და დაკვირვებას საჭიროებს.