Cell Transplantation in Surgical Treatment of Familial Adenomatous Polyposis Coli

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We developed a method of surgical treatment of familial adenomatous polyposis coli giving an opportunity to prevent the growth of new polyps in the preserved part of the rectum and consisting in transplantation of fetal cells of the epithelial origin into the rectum wall after mucosectomy. Since the rectum is partially preserved, ileorectal anastomosis can be formed after colectomy, which preserves natural bowel passage. Complex examination 4 weeks after surgery revealed the formation of normal rectal mucosa. No new polyps were detected in the rectum 1-3 years after surgery.

Key Words: *familial adenomatous polyposis coli; cell transplantation*

Familial adenomatous polyposis coli (FAP) is an autosomal dominant genetic disorder characterized by numerous adenomatous polyps of the large intestine (LI), often with profound metabolic disturbances and inevitable colorectal carcinoma development without surgical treatment [1]. Adenomatous polyposis coli (APC) gene responsible for normal proliferation of the gastrointestinal mucosa was identified in the human genome. This gene is located in the long arm of chromosome 5 (locus 5q21) [5,8]. Numerous studies showed that different mutations in this gene often lead to FAP [2,7,8,12]. The mutations in this gene are inherited in a sex-independent manner.

Surgery is the only method of FAP treatment. In Research Center of Coloproctology, the results of treatment and follow-up of 1367 patients are available; of them 547 patients (40%) had one or more malignant colorectal tumors at the moment of primary surgical intervention. Surgery usually consists in total proctocolectomy with permanent incapacitating ileostomy, which leads to permanent disability of the patient. However, neither surgeons, nor patients are now satisfied with surgeries aimed only at saving patient's live without restitution of its social status. It is evident that any radical colorectal surgery can be more physiological if it is completed by restoration of the natural bowel passage. To this end, subtotal colorectal resection with bringing its right portion down to the anal canal and colectomy with the formation of ileorectal anastomosis are performed. The retained portions of LI require close endoscopic monitoring. However, inspection of residual LI after surgical treatment for FAP revealed intensive growth of polyps and carcinoma development in these patients at different terms after surgery. The only surgical method preserving natural bowel passage after total colorectal resection is the formation of a pelvic ileal pouch with ileoanal anastomosis. However, these interventions not always provide the desirable effect and sometimes lead to anal sphincter incontinence [10,12]. For solving the problem of surgical rehabilitation of these patients and achieve good functional results it is important to preserve a portion of the rectum providing evacuation function. The search for a new method of surgical rehabilitation of FAP patients drove us to an idea to use cell transplantation for creating transplanted rectal

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mucosa. This can help to retain natural bowel passage and prevent the growth of polyps. Recent reports suggest that full-value bowel segment can be created by methods of tissue engineering [3-4,6,9].

Here we assessed the potentialities of cell transplantation for creation of transplanted rectal mucosa in surgical treatment of FAP.

MATERIALS AND METHODS

The study aimed at the development of technologies for creation of transplanted rectal mucosa was carried out at Research Center of Coloproctology in cooperation with Laboratory of Clinical Immunology, V. I. Kulakov Research Center of Obstetrics, Gynecology, and Perinatology, according to the agreement about scientific cooperation. The aim of the study was to prevent the growth of polyps in patients after surgical treatment for FAP. We propose a surgical technique preserving natural bowel emptying in FAP patients due to preservation of tunica muscularis of the rectum providing its reservoir and evacuatory functions. For preventing polyp growth, rectal mucosa was completely removed with the lamina propria (mucosectomy) and allogenic fetal cells of the epithelial origin were transplanted to the mucosectomized rectum.

The culture of fetal allogenic somatic cells of the intestinal epithelium and liver and bone marrow mesenchymal cells enriched with stem and progenitor cells from human fetuses (gestation weeks 8-12) were obtained in Laboratory of Clinical Immunology, V. I. Kulakov Research Center of Obstetrics, Gynecology, and Perinatology. Abortion material was obtained from licensed institutions of Ministry of Health and Social Development of the Russian Federation. Karyotyping of the initial biological material was carried out in Institute of Medical Genetics. Only material tested for viruses and antibodies was used in the study.

For obtaining primary culture, the tissues were subjected to enzymatic disaggregation (0.025% collagenase-1 solution). The cells were cultured in complete nutrient medium DMEM/F12 (1:1, Gibco) supplemented with 10% FCS (HyClone), penicillin/streptomycin (20 ng/ml; Gibco), and L-glutamine. The cells in the initial suspension and after repeated dissociation of aggregates were counted in a Goryaev chamber; their viability was determined using standard trypan blue exclusion test. The cells were cultured for 7-14 days and then transplanted to mucosectomized rectum; 4×10^8 cells were transplanted; cell viability in cultures was 85-90%.

Surgical treatment of FAP followed by cell transplantation was performed in 9 patients (3 men and 6 women). All patients were informed about their disease and signed informed consent for participation in the study and cell transplantation. FAP with involvement of all portions of LI with polyps up to 1.5 cm in diameter without signs of their malignant transformation was diagnosed in all the patients and familiar history of the disease was verified. Patient's age varied from 17 to 36 years.

The surgery was performed as follows. The colon was mobilized and resectied together with in the upper half of the rectal ampulla. The rectum was partially mobilized, the lateral ligaments and the pelvic nervous plexus being preserved. The mucosa was removed by electrocoagulation after evagination of the rectum to perineum. Then cell transplantation was performed.

The cells were applied by the injection method starting from the upper edge of the anal canal. The needle was introduced into the muscular layer of the mucosectomized rectum. The cell material was injected in such a way that infiltration area was about 2 cm^2. Cell injections were performed in a staggered order from the upper edge of the anal canal towards the cupola of the rectal stump.

The rectal stump was stapled with a linear stapler and invaginated into the pelvic cavity. From the side of the abdominal cavity, an ileorectal anastomosis was formed. This technique was used in 5 patients.

In the other 4 patients, after colon resection, rectal mucosectomy, and injection of the cell transplant, an ileal pouch was formed and anastomosed with mucosectomized rectum using a stapler.

For stimulation of regeneration and engrafting processes in the mucosectomized rectum, fetal epithelial cells were additionally injected on day 14 after surgery. Single intravenous injection of 10^6 mesenchymal liver cells for their delivery into the focus of injury (mucosectomized rectum) was performed. A complex of bioactive growth factors of fetal origin was injected subcutaneously.

RESULTS

Two weeks after surgery, endoscopic examination of the mucosectomized rectum showed that its lumen was evenly moderately narrowed. The surface of the rectum was somewhere coated with fibrin and was moderately injured upon contact. At this stage, sites of formation of young immature epithelial lining looking like small pink-pale areas with smooth surface not injured upon contact appeared.

Examination of biopsy specimens obtained from mucosectomized rectum 2 weeks after surgery revealed a mucosa fragment with moderately deformed crypts containing fewer goblet cells. In the lamina propria, edema, small fresh hemorrhages, and moderate lymphocyte and plasma cell infiltration were seen between the crypts. A small connective tissue islet was

seen at the periphery of the preparation. Thus, foci of active epithelization in the mucosectomized rectum were seen as soon as 2 weeks after surgery.

Endoscopic examination 4 weeks after surgery showed pink glance rectal lining without signs of contact injury and fibrin depositions. Deformed vascular pattern was somewhere seen. The endoscopic picture corresponded to unchanged rectal mucosa.

Analysis of biopsy specimens showed a fragment of LI mucosa with deformed enlarged crypts enriched with goblet cells spreading to the mucosa surface. In the lamina propria, edema and few lymphocytes and plasmacytes were noted. Capillaries and venules were dilated (Fig. 1**)**.

X-ray examination 4 weeks after surgery showed that the rectum had usual shape with clearly seen rectal ampulla. It was able to perform the reservoir function; wall elasticity was completely preserved. Folds of the mucosa typical of the rectum were clearly seen. Evacuatory function was also preserved (Fig. 2).

The follow-up period was less than 1 year in 4 patients, and 5 patients were examined 1-3 years after surgery. Control examination was performed every 3 months in all the patients. Endoscopy showed that the rectal mucosa was glance and had normal color, the vascular pattern was clearly seen. No polyp growth was noted.

Thus, the use of cell transplantation for creation of the transplanted rectal mucosa in FAP patients after colectomy is a rational and perspective method of their rehabilitation. The rectal mucosa was practically completely restored as soon as 4 weeks after implantation of fetal stem cells. Preserved tunica muscularis provided the reservoir and evacuatory functions of the rectum, while newly created mucosa is expected to contain no genetically defect epithelial cells serving as the substrate for polyp growth. Many aspects of this problem require further investigation (evaluation of the functional state of the rectum, genetic and more detailed morphological examination of the mucosa). Nevertheless, these results give hope and open prospects for the use of cell technologies in coloproctology.

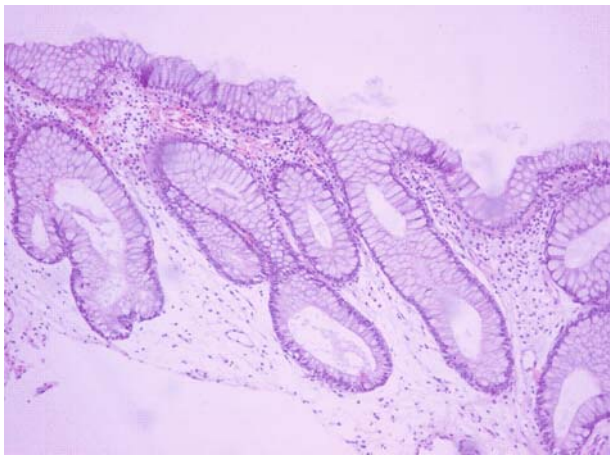

Fig. 1. Rectal mucosa biopsy specimen 4 weeks after surgery. Hematoxylin and eosin staining, ×200.

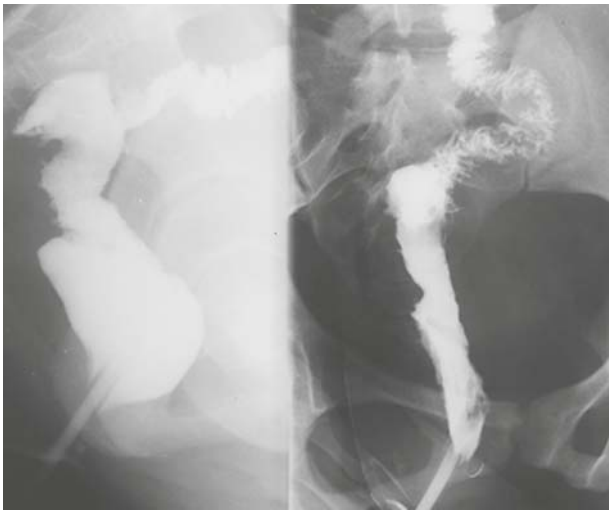

Fig. 2. X-ray examination of the rectum with transplanted mucosa 4 weeks after surgery.

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