THE IMPORTANCE OF ANATOMICAL DISSECTION IN COLORECTAL CANCER SURGERY AND IN CLINICAL RESEARCH

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THE IMPORTANCE OF ANATOMICAL DISSECTION IN COLORECTAL CANCER SURGERY AND IN CLINICAL RESEARCH (Abstract): Study objectives. This study involves the development of an experimental ex-vivo model with utility in colorectal cancer therapy studies. For that we studied the colonic cells resistance to ischemia under normothermia and continuous perfusion. Materials and methods. We collected two surgical specimens and evaluated the possibility of maintaining the cell viability for a sufficient time to conduct fundamental research experiments. A third surgical specimen was used as a control model. Cell viability was tested by microscopic examination of the biopsies taken at 10 and 15 minutes from the surgical specimens. Results. Histopathological examination showed the preservation of cell viability of samples taken from the proposed experimental model specimens, with no microscopic changes suggestive for necrosis. Discussion. Colorectal cancer is one of the most accessible experimental models in the cancer study. In the long term, we believe that these results are the starting point for creating an ex-vivo colorectal cancer experimental model, in normothermic conditions for testing the effectiveness of selective photothermal therapy using various forms of energy and targeted chemotherapy. Conclusions. Our experiment proved the feasibility of achieving an ex-vivo experimental model of ascending colon with in situ adenocarcinoma. Achieving these models requires a “clean” surgical technique, respecting the anatomical planes, the preservation and maintenance of viable surgical specimens not being possible without this. Key words: COLON CANCER, ANATOMICAL DISSECTION, EX VIVO MODEL, CELL VIABILITY

INTRODUCTION

Surgical dissection done within the proper anatomical planes, is essential to minimize the risk of intraoperative and postoperative complications such as hemorrhage and lymphorrhagia. Furthermore, the standardization of this technical approach has improved the outcome: survival and quality of life. A good example is colorectal cancer surgery. In this one, the anatomical dissections, with complete mesocolon and mesorectum excision decreases the rate of local and lymphatic recurrence (1). Respecting the anatomical plans and preserving the nervous plexus (hypogastric plexus) the surgeon decreases the risks of sexual and urinary dysfunctions, with a major impact on the quality of life (2).

Since the 80s, the dissection for rectal cancer has been standardized. The moment was when Heald demonstrated the superiority of total mesorectum excision (3, 4). These technique, well known by now between the digestive surgeons, involve “in block” resection of the anatomical piece, composed of rectum and perirectal areolar tissue with the terminal branches of inferior mesenteric artery (mesorectum) (1).
In order to achieve this, using “sharp” dissection (dissection done with scissors or monopolar electrocautery) (2), “the holy plane” must be found, this one being situated between two different embryological origin structures, as stated by Heald (5). Maintaining this plane of dissection we ensure the preservation of the presacral venous plexus and the pelvic nervous structures located between the peritoneum and endopelvic fascia (1).

Following the same principles, in colon cancer surgery, the sharp anatomical dissection of the plane situated between the parietal and visceral fascia, while maintaining the integrity of the last one, allows full exposure of the mesocolon, with high central ligation of the arterial pedicle. Thus, by maintaining the integrity of the embryological envelop around the mesocolon, we ensure the harvesting of maximum number of lymph nodes (6).

Taking in consideration the anatomical distribution of lymph nodes and the fact that lymphatic metastasis appear for certainty at the level of the arterial pedicle of the affected organ, attempts in the standardization of the resection were tried, same way as in rectal cancer (7, 8). It this way, it was shown that maximum benefits regarding survival rate are brought by D3 lymphadenectomy, with surgical resection of the paracolic, intermediate and central lymph nodes (9, 10). Taking in account the tumor location in the colic framework, this technique can demand removal of lymph nodes located till the origin of ileo-colic, right colic and middle colic artery at the level of the superior mesenteric artery (for ascending colon cancer) or lymph nodes located in between the origin of inferior mesenteric artery and left colic artery (for descending colon cancer). In both cases, the optimal lymphadenectomy (D3) can be achieved only by respecting the anatomical planes mentioned above, with “in block” extraction of the surgical specimen without damaging the mesocolon (6, 8).

Ex-vivo experimental models are of particular importance in research, providing the closest properties to in-vivo models. Basically, ex-vivo experiments should allow the study of tissues under conditions easier to control than in in-vivo environment, with minimal alteration of natural, normal conditions. Ex-vivo experimental models are few and were done in hypothermic conditions, situation that leads to metabolic cellular changes (11). Experimental ex-vivo model kept under normothermic conditions is superposable with the normal biological human body conditions.

Achieving these models requires a “clean” surgical technique, respecting the anatomical planes, the preservation and maintenance of viable surgical specimens not being possible without this.

**MATERIALS AND METHODS**

This study involves the development of an experimental ex-vivo model with utility in colorectal cancer therapy studies.

To do this, we decided to collect two surgical specimens and to evaluate the possibility of maintaining the cell viability for a sufficient time to conduct fundamental research experiments. Basically, our objective was to study colonic cells resistance to ischemia under normothermic conditions and continuous infusion. A third surgical specimen was used as a control model.

Considering the type of experiment, which requires specimen collection of surgical resection piece resulting from surgeries performed for colorectal cancer, the patients signed an informed consent that was attached to the specimen collection protocol, protocol approved by the Ethics Committee of the Regional Institute of Gastroenterology and Hepatology “Prof. Dr. Octavian Fodor”, Cluj-Napoca, the institution in which the experiment was conducted.

For our experiment, we decided to use surgical pieces resulted from resections for ascending colon cancers, this pathology providing easy surgical resections, respecting the principles described above.

Applying the principles of oncologic resection in colorectal cancer surgery, the resection pieces resulting from the right hemicolectomy technique were prepared for the experiment. After establishing the resection margins, the intraoperative dissection of the resection piece was performed. In both cases these were at 10 cm from the ileo-cecal valve, on the ileum, and on the colon, on the right third of the transverse colon, proximal to the right colic branch of the middle colic artery. Next, we did the dissection at the level of main vascular pedicles with individualization of ileo-colic vein and artery. In the first specimen, the cannulation of the artery was performed after its proximal ligation and
intestinal resection (the cannulation was performed immediately after surgical piece removal). For the second specimen, the cannulation was made intraoperatively (in situ) by the classical vascular catheterization, arterial ligation being performed proximal to the catheter after its stabilization by ligatures. (Fig. 1) In both cases, ileo-colic artery was cannulated using a transfusion kit.

The specimen was perfused with Hemosol B0® (Gambro) (12) hemodialysis solution at 36-37°C, immediately after transfusion kit fitting for both cases. The solution was maintained at this temperature and perfused at a constant 120 mmHg pressure using the “enFlow IV Fluid / Blood Warming”® (Vital Signs) (13). The perfused resection pieces, were washed with Custodiol HTK® (Sandor) (14) and transported to the laboratory. The experimental specimens were kept at 36-37 °C by immersion in marine bain with Dianeal® solution (Baxter) (15). Temperature was maintained by means of an equipped tank with heater and thermostat. The infusion time was 15 minutes. Biopsy specimens were taken at 10 and 15 minutes for both surgical pieces. In the case of the control surgical specimen, no infusion and immersion was done.

Noted that the protocol described above does not affect in any way the cancer staging protocol and no alteration of the anatmopathological findings were done.

The histopathological elements assessed to determine the viability of the tissues are those previously described by Green et al (2009) for determining the ex vivo viability of tumors. Namely, the percentage of necrosis, presence of nucleoli and pyknosis and mitotic index were assessed by scanning the entire slide or, in the case of mitotic index by counting the mitotic figures on 10 randomly chosen high power (ob x40) microscopic fields.

After accomplishing the perfusion protocol, tissues from the tumor and surrounding healthy areas were immediately harvested and fixed in 10% neutral-buffered formalin (pH 7). After complete fixation, the tissues were trimmed and subsequently dehydrated in ethanol and afterwards embedded in paraffin wax following the routine processing protocol (Prophet 1992). Multiple, seriate tissue sections were cut from the chilled paraffin block at 5 µm thickness with a rotary microtome, mounted on salinized histological slides (VWR-Q Path® plus) and stored in a thermostatically controlled oven at 37°C until stained. Histological sections from the tumor and surrounding tissue were stained using a routine hematoxylin and eosin (H&E) protocol and finally examined under an Olympus BX41 light microscope. Images were acquire,
using an 3.2 megapixel resolution Olympus UC30 Microscope Digital Camera and processed using Olympus Stream micro-imaging analysis software (OLYMPUS Stream Basic package).

All surgical specimens were confirmed pre-operatory with the diagnostic of colon adenocarcinoma (by colonoscopy with biopsy). After harvesting the experimental tissue pieces, the surgical specimens were sent to pathology for examination as standard behavior of our Institute.

RESULTS
Histopathological examination of the perfused surgical specimens showed the preservation of cell viability of samples taken from the proposed experimental model specimens, with no microscopic changes suggestive for necrosis. (Fig. 2)

In the case of control specimen (which was not infused), microscopic analysis revealed changes of necrobiosis (dystrophic irreversible cell damage). The timing of the cannulation (in situ vs immediately after extraction) did not influence the occurrence of microscopic cellular changes.

Also, infusion time (10 minutes vs 15 minutes) did not significantly affect the microscopic changes between the two specimens.

DISCUSSION
Despite the material and human efforts, cancer remains a major public health problem and one of the main concerns of research teams worldwide. Colorectal cancer is the third most common cancer in men and the second in women, with an overall incidence of about 6%. It is one of the leading causes of death, accounting for 9% of all cancer deaths, percentage that ranks it on the second place (16, 17).

Colorectal cancer is one of the most accessible experimental models in the cancer study (18-22). Once with the development of nanotechnology, a growing number of researchers have concerns about the use of nanoparticles in the treatment of cancers (23-25). Their desire is to develop a targeted therapy, trying to concentrate desired drug or nanoparticles with photothermal properties into the tumor (26). To achieve this goal, we must find a carrier that has affinity for specific protein in the cell membrane and then to achieve the internalization of the
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nanobioconjugate, process that normally does not occur (27).

Most experiments concerning the internalization of the nanobioconjugate and photothermal therapy were made on in-vitro models. In-vitro experimental models are different from those of ex-vivo as some properties of the nanoparticles (absorption capacity, loading surface, catalytic activity, optical and magnetic properties) are changed.

Surgical technique, based on anatomical dissection, respecting the principles of oncological surgery, allowed us to harvest and maintain viable two ascending colon adenocarcinoma specimens. Separate dissection of the vascular elements, in particular the origin of the main arterial trunk, is mandatory for achieving optimal lymphadenectomy, and to facilitate the early vascular pedicle cannulation, with a possible positive impact on the maintainence of the viability of surgical piece.

In the long term, we believe that these results are the starting point for creating an ex-vivo colorectal cancer experimental model, in normothermic conditions for testing the effectiveness of selective photothermal therapy using various forms of energy and targeted chemotherapy.

The stability of this experimental model will have to be tested using immunohistochemical tests that will reveal the histopathological changes inside the colon tissue.

CONCLUSIONS

Our experiment proved the feasibility of achieving an ex-vivo experimental model of ascending colon with in situ adenocarcinoma.

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In the first 2 groups (column 1 and 2), the overall histomorphology of the intestine is maintained. In the group 2, a discrete desquamation of the superficial epithelium is observed (indicated by arrow). In both groups, an mild interstitial infiltration with lymphocytes, plasma cells, macrophages and eosinophils (indicated by the arrow from the second line of the column 2) is observed.

In the group 3, the tumoral cells (adenocarcinoma) are organized in ducts and acini, in most of the cases with an patent lumen, arranged on abundant fibro-vascular stroma (desmoplastic reaction, indicated by asterisk); small areas of intratumoral necrosis and cell desquamation (indicated by the arrow) (H&E, Obx20 for the first line of images and x100 for the second).

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