Comparison of laparoscopic vs conventional technique in colonic and liver resection in a tumor-bearing small animal model

Impact on short-term and long-term results

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Abstract

Background: Laparoscopic procedures in oncological surgery are either done in curative or palliative intent. We present two experiments comparing laparoscopic to conventional surgery in the curative and palliative setting regarding short-term (stress and immune alteration) and long-term aspects (survival time and recurrence rate).

Methods: We established two syngenic tumor-bearing small animal models for curative liver resection (Morris hepatoma 3924A, ACI rats) and palliative colon resection (BSP73 ASML, BOX rats). Male rats were operated on, performing laparoscopic and conventional liver resection as well as laparoscopic and conventional colon resection; control groups (anesthesia) were included. The following parameters of the stress and immune system were measured: corticosterone, neopterin, IL-1β, IL-6, and body weight as a parameter of postoperative recovery. Analyzed long-term parameters were survival time, tumor weight, and recurrence rate (histology).

Results: After colon resection, analysis of variance showed significant differences in all short-term parameters, including body weight after laparoscopic versus conventional colon resection (p < 0.05). In the case of laparoscopic versus conventional liver resection, only IL-6 showed globally statistically significant differences for the short-term parameters (p = 0.05). Long-term parameters were not significantly different between the laparoscopic and conventional groups, regardless of the type of resection (colon or liver) or the operative setting (curative or palliative). However, there were differences after curative liver resection compared to the control group (anesthesia alone).

Conclusion: These results suggest that the type of intra-abdominal operation (colon or liver) may influence the degree of trauma of an operation more than the type of technique (laparoscopic or open). The perioperative alteration of stress and immune function has no implications on the long-term results, such as survival time or recurrence, neither in the curative nor in the palliative setting. The thesis that laparoscopic surgery results in less pain, which in turn means less stress and less alteration of the immune system and therefore results in a lower rate of postoperative metastasis is only valid for laparoscopic colonic resection in our model. The part of the thesis that states that fewer metastases should occur after laparoscopic oncological surgery cannot be confirmed in our study.

In the past 10 years, as laparoscopic surgery was introduced as a surgical technique, it was hypothesized that it results in less pain, which in turn means less stress and less alteration of the immune system, thus resulting in a lower rate of postoperative metastasis [7, 8, 10, 11, 16, 18, 29, 30, 32]. Whether this hypothesis is indeed true in the curative as well as the palliative setting is unproven.

For some types of operation parts of this hypothesis could be verified by clinical experience, by clinical retro- or prospective studies, or by randomized experimental studies partly done in small animal models [3, 4, 7–11, 14, 15, 28–30, 32]. For example, in the case of laparoscopic cholecystectomy the advantages were confirmed by non-randomized trials demonstrating significant differences in stress and immune parameters [7, 8, 11].
However, Majeed et al. [20] found no differences in postoperative recovery time and hospital stay when comparing laparoscopic to conventional cholecystectomy in a randomized study, suggesting that psychological effects could account for the favorable course associated with laparoscopic surgery. Therefore, it is unclear if the previous hypothesis is correct in all curative or palliative situations. For laparoscopic colon surgery, the following advantages have been demonstrated in prospective experimental and clinical studies: decreased postoperative pain, less postoperative disability, shorter hospital stay, and earlier return to work in comparison to conventional surgery [16–18, 24]. On the other hand, several other studies could not confirm these advantages [3, 20, 22]. Whether the differences in the stress and immune response lead to different outcomes in oncological surgery, especially regarding long-term survival, is still under discussion. Laparoscopic liver resections have been performed in only a few hospitals since 1995, and some articles have been published on this topic [2, 6, 23, 25]. Prospective data on the advantages of laparoscopic liver resection have not been published.

We performed a prospective randomized study focusing on the short- and long-term results of laparoscopic versus conventional liver resection with curative intent in a tumor-bearing small animal model, which is free from psychological influences. A liver tumor model (Morris hepatoma 3924A) and a laparoscopic liver resection model were established [13, 31]. Additionally, we performed a prospective randomized study on the short- and long-term results of laparoscopic versus conventional palliative colon resection in a tumor-bearing small animal model [14, 15]. This allowed us to prospectively compare the results of curative liver resection to the results of palliative colon resection with regard to stress and immune response and their influences on long-term results, such as survival time and recurrence rate, and with regard to the curative and palliative aspect of surgery.

Material and methods

Tumor cells and animals

As the tumor model with curative operative intent, we used the liver carcinoma Morris hepatoma 3924A, which is well described by Yang et al. [31]. The Morris hepatoma is a moderately differentiated hepatocellular carcinoma and a syngenic carcinoma in ACI rats. We used Yang et al.’s technique of tumor implantation into the liver, with an implantation rate of nearly 100%. From the literature it is well-known that 20% of the rats develop pulmonal metastasis 28 days after tumor implantation [31]. We operated on the rats 10 days after tumor implantation because at this time the primary tumor is at its largest and metastasis are not expected at this time [13]. We resected the tumor-bearing part of the liver and performed the operation in curative intent.

A tumor model with a solitary adenocarcinoma of the ascending colon has not been described in the literature. All established colon carcinoma models use chemically induced carcinomas that usually occur at multiple unknown localizations in the colon and therefore uncontrolled tumor growth. As an adenocarcinoma of the colon we used the colon carcinoma B5p73 ASML tumor cell line, which is a syngenic, spontaneously metastasizing, adenocarcinoma of BDX rats. It spreads primarily lymphogenically. We injected $5 \times 10^5$ tumor cells into the right hindleg in accordance with experiments published by Zöller et al. [33]. We then operated on the rats 7 days after tumor implantation because metastases are not expected at this time [21, 33]. As already described, the tumor is not resected (as described in the literature); the operation was performed merely in palliative intent.

We used BDX and ACI rats with a body weight of 230–280 g because they are known to be susceptible to the implanted tumor cell line. The rats were kept under pathogen-free and standardized conditions: the room temperature ranged between 22 and 24°C and the relative humidity of the air was 50–60%. There were 12 h of light and 12 h of darkness. They were fed a standard laboratory diet and allowed free access to food and water. Food was withdrawn 12 h before the surgical procedure. Food and water were offered to the rats immediately after the operation. The protocols were approved by the Animal Research Committee.

Anesthesia

Each operation was performed under anesthesia with ketamine (100 mg/kg body weight) intramuscularly (im) and xylazine (8 mg/kg body weight) im. Postoperative analgesia was provided by adding tramadol to each bottle of water over 24 h (40 mg tramadol/100 ml water).

Operative procedures

For the laparoscopic procedure, a 2.7-mm trocar for the laparoscope was placed in the midline just above the penis vein. Laparoscopic operations were performed with a pneumoperitoneum of CO$_2$ and an intraabdominal pressure of 3 mmHg. After insufflation of CO$_2$ a second trocar (2 mm) was inserted in the left upper quadrant of the abdomen. If necessary, a third trocar (2 mm) was inserted into the right abdomen.

In the case of laparoscopic colon resection ($n = 10$), the cecum and the ascending colon were localized using a 1.7-mm tissue grasper. After mobilization of this area a resection of 1 cm of the ascending colon was performed, followed by an end-to-end anastomosis with single sutures (prolene 6-0) outside of the abdomen. This technique is comparable to laparoscopically assisted colon resection in humans [13, 14].

In the case of open or conventional colon resection ($n = 10$), an incision of the skin was performed in the midline from the penis vein to the xiphoid. After localization of the cecum the ascending colon was removed from the abdominal cavity. A resection of 1 cm was performed followed by an end-to-end anastomoses with single sutures (prolene 6-0). After removing the colon, the muscle and skin were closed in separate layers.

In the case of laparoscopic liver resection ($n = 10$), the liver lobe of interest was mobilized from the omentum minus and its adhesions. Through the second working trocar a polypectomy wire for colonoctomy was inserted and the wire placed around the central part of the liver lobe (the rat generally has five nearly completely isolated liver lobes). Then the liver lobe was resected by cutting and coagulating. After removing one trocar and dilating the incision, the liver lobe was removed from the abdomen. The muscle and skin were closed in separate layers [13]. This method is similar to the method described by Krahenbuhl et al. [12]; however, they did not use a polypectomy wire.

In the case of open or conventional liver resection ($n = 10$), an incision of the skin was performed in the midline from the penis vein to the xiphoid. The liver lobe of interest was mobilized from the omentum minus and its adhesions. A polypectomy wire for colonoctomy was inserted and the wire placed around the central part of the liver lobe of interest. Then, the liver lobe was resected by cutting and coagulating. The muscle and skin were closed in separate layers.

Blood sampling

Before the operation and after anesthesia (time point A), 1 ml of venous blood was obtained from the retrobulbar venous plexus. Directly after the operation (B) and 24 h (C), 7 days (D), and 21 days (E) later, 1 ml of venous blood was taken from the retrobulbar venous plexus. The operation and blood sampling were always scheduled between 6 and 8 pm to avoid alterations of parameters because of their circadian