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The phagocytosis activity during conventional and laparoscopic operations in the rat

A preliminary study

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Abstract

Background: Numerous experimental and clinical investigations indicate that the mononuclear phagocyte system (MPS) has a relevant function in terms of physiological defense against tumor metastasis and bacterial infection. Consequently, a point of major interest is the influence of surgical techniques on the MPS function.

Method: The model investigation examines the phagocytosis activity of the rat's MPS during conventional fundoplication (group 1, $n = 10$), laparoscopic fundoplication using a pneumoperitoneum (group 2, $n = 10$), and gasless laparoscopic fundoplication (group 3, $n = 10$). The MPS function is evaluated by an intravascular carbon clearance test (G. Biozzi).

Results: The fastest carbon elimination half-life was found in group 3. By way of contrast, there was a significant increase of carbon half-life in group 2 ($p < 0.005$). Even group 1 caused less MPS depression ($p < 0.1$) than group 2.

Conclusion: Gasless laparoscopic procedures have a favorable effect on phagocytosis activity. The CO_2 pneumoperitoneum seems to be the main reason for a decreased antigen elimination in laparoscopic treatments.

Key words: Laparoscopy — Pneumoperitoneum — Gasless laparoscopy — Mononuclear phagocytes system — Rat model

The human and animal organism's answer to an injury is characterized by an early rise of the serum level for stress hormones and a drop in the immunoresponse conveyed by the cells. The stress reaction's force and duration are considered to be proportional to the severity of the injury. There is a correlation between a reduced perioperative cell-conveyed immunoresponse and an increased risk of postoperative infection and metastatic tumor spreading [6, 9, 10, 11, 13].

The investigation of the mononuclear phagocyte system (MPS)'s phagocytosis activity is determined by means of the Carbon-Clearance test which, itself, is carried out in a modified form, i.e., following the method as described by Biozzi et al. [3] and Lemperle et al. [7]. In the course of this test, the degree to which carbon particles are eliminated from the circulating blood by local macrophages of the MPS is determined. This phagocytosis activity depends on the kind and extent of an operative manipulation and can be judged to be a measure of interference with the organism caused by surgical interventions. Our investigation compares the phagocytosis activity during conventional operations (group 1) with that of laparoscopic surgery using a pneumoperitoneum (group 2) and gasless laparoscopic surgery without the use of a pneumoperitoneum (group 3) (Table 1).

Method

Laparoscopy in the rat

A surgical table for small animals (KAPS Company, Asslar, Germany) is used consisting of an operation platform and a base plate with a flexible supporting arm. In a supinated position, the narcotized animal lies on the raised operation platform which is set over the base plate. By slightly moving the operation platform via two micrometer screws with fixed laparoscope, the surgeon or his assistant can finely adjust the picture segment. The surgical instruments consist of a 4-mm arthroscope and micro-alligator forceps and microscissors (Aesculap, Tuttlingen, Germany). All instruments, as well as the suture material, can easily be inserted into the abdomen via 3-mm synthetic ports [2, 5].

Experimental procedure

Before the operation starts, the rats are injected via the v. dorsalis penis with 0.1 ml/100 kg of ''fount india'' shellac-free ink (Pelikan Company,

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Table 1. Nissen fundoplication was performed in three animal groups

Group 1	(n = 10)
Conventional laparotomy	
Group 2	(n = 10)
Laparoscopy with pneumoperitoneum	
Group 3	(n = 10)
Gasless laparoscopy	

Hannover, Germany) which, in order to obtain a better colloid stabilization, is mixed with gelatine. Periodically, 200 μl blood is taken from the animals. After the hemolysis in 2 ml of a 0.1% Na_2CO_3 solution, the mixture's extinction at a wavelength of 640 nm is determined as a blank value against Na_2CO_3/blood (before ink injection). The extinction depends on the ink particles' quantity (particle size approx. 250 Å)/volume and consequently represents a measure for the ink concentration in the peripheral blood. Throughout that intervention period, the ink's half-life in the animal's circulating blood can be determined by exploratory blood takings. The bigger the half-life, the longer the ink remains in the blood and the lower is the MPS's phagocytosis activity under the corresponding operation conditions. At first, the rats of the first group of animals (group 1, n = 10) are subject to a conventional laparotomy; then a fundoplication after Nissen is made. Throughout the operation, and in defined intervals (3, 15, 30, and 40 min after the ink injection), 200 μl of blood is taken from the animal's retrobulbar plexus venosi and analyzed. In the second group of animals (group 2, n = 10), the fundoplication is carried out under an endoscopic operation with a pneumoperitoneum of 7 mmHg. The rats belonging to the third group of animals (group 3, n = 10), as well, are subject to an endoscopic fundoplication; however, a pneumoperitoneum is renounced and, instead, the abdomen is stretched out by a specially constructed wire bow. Still narcotized, all animals are killed by carbon monoxide.

Statistics

The mean carbon half-life and the standard deviation (SD) of the collected data were recorded. Carbon half-life was calculated by using simple linear regression (Pearson-regression). To calculate p values a Kruskal-Wallis test was used.

Results

The lowest elimination half-life of ink particles in the laboratory animal's bloodstream ($t_{1/2}$ = 12.86 min) could be ascertained in group 3 (gasless laparoscopic). Compared to this group, the elimination half-life is significantly increased ($t_{1/2}$ = 21.91 min; p value < 0.005) in group 2 (laparoscopic with pneumoperitoneum). In group 1 (conventional laparotomy) the ink particles' elimination half-life ranged below the one of group 2 ($t_{1/2}$ = 16.1 min; p value < 0.1) (Fig. 1).

Discussion

Repeatedly, literature has described the phenomenon of the implantation of tumor cells during laparoscopies [4, 10, 13]. Why this problem occurs and how often are still unclear. Yet there are hypotheses according to which suboptimal technique and instrumentation are said to play a decisive part in that matter [1, 12]. Under certain conditions, even the pneumoperitoneum seems to support the intraperitoneal dissemination of tumor cells [8].

The mononuclear phagocyte system (MPS) represents an important component of the endogenous defense against

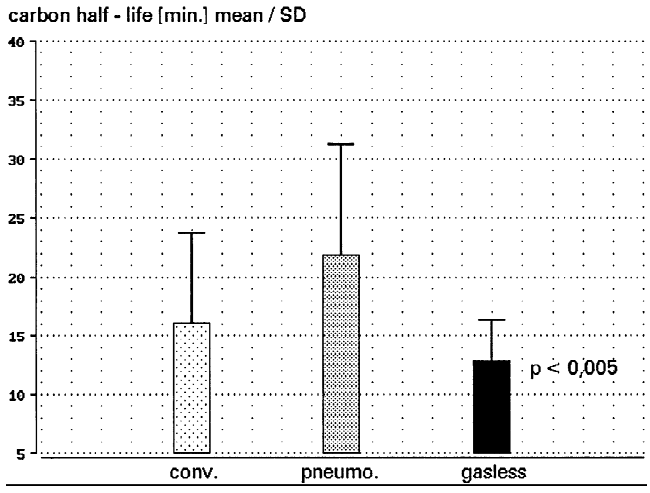

Fig. 1. Average carbon elimination half-life of the experimental groups, graphically described.

circulating tumor cells. A decreased perioperative, cell-conveyed immunoresponse correlates not only with an increased metastatic tumor spreading risk but also with postoperative infections; the phagocytotic activity of the MPS consequently seems to be of significance when judging the etiology of implantation metastases during laparoscopic interventions.

The Carbon-Clearance test is a well-established method of measuring the phagocytosic activity of the mononuclear phagocytes system (MPS). In conformity with the method described by Biozzi et al. [3] and Lemperle et al. [7], it is applied in a modified form which offers the advantage of measuring in vivo whole the extent of a hematogen antigen elimination. Since the phagocytosis activity depends on the type and extent of an operation it, therefore, can be considered a measure of the disturbance of the organism by any such interventions. Therefore, a highly standardized proceeding, as well as the choice of an anesthesia method not influencing the test procedure, is a basic condition. From a technical point of view, the rat fundoplication is a simple operation which, without any problems, can be carried out both conventionally and laparoscopically [5].

As shown by the results of this investigation, the fastest way to eliminate carbon particles from the bloodstream by local macrophages of the MPS is via gasless laparoscopic (group 3). Compared with the conventional method (group 1), the minor disturbance of the organism seems to be the minimally invasive access. The use of a pneumoperitoneum (group 2) has an even stronger influence on the antigen elimination from the circulating blood than the choice of surgical access. Although, in case of the rat, with a pneumoperitoneum of 7 mmHg there is no long-term decrease in the cardiac output, the intraperitoneal pressure seems to cause a reduced circulation speed of carbon particles in the blood. Further impacts that may prejudice the organism, apart from the circulatory influences of the CO_2 pneumoperitoneum, are not excluded. The results of the present investigation show that a pneumoperitoneum in the rat is an invasive arrangement leading to a significant reduction of the phagocytosis activity of the MPS.

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