PATHOPHYSIOLOGICAL CONSEQUENCES OF PNEUMOPERITONEUM

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PATHOFYSIOLOGISCHE GEVOLGEN VAN HET PNEUMOPERITONEUM

PROEFSCHRIFT

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CONTENTS

'Life is what happens to you while you are busy making other plans'
John Lennon

To Saskia
and my parents

CHAPTER 1

INTRODUCTION

INTRODUCTION

Laparoscopic surgery has been performed for more than a century, although its use was mainly restricted to diagnostic purposes [1, 2]. Recent developments in instrumental design and methods of visualization have contributed to further implementation of laparoscopic techniques [3]. In 1985, Muhe performed the first laparoscopic cholecystectomy [4]. After further development of this technique by Mouret and Dubois [5, 6], laparoscopic techniques have gained wide acceptance in surgical practice. Except for laparoscopic gallbladder removal, minimally invasive techniques now have been established for other surgical procedures such as gastric fundoplication, appendectomy, splenectomy and (donor) nephrectomy [7-10]. The popularity of these techniques may be explained by the growing evidence that the minimally invasive approach is associated with a reduction in operative morbidity, such as less postoperative pain, decreased systemic stress response, shorter hospitalization and improved cosmesis [11-15].

The pneumoperitoneum is the crucial element in laparoscopic surgery. Each laparoscopic procedure requires a working space in the abdominal cavity to allow safe introduction of trocars and instruments and for exposure of the abdominal contents. Intraperitoneal insufflation of gas is the most common method to elevate the abdominal wall and suppress the viscera. Carbon dioxide (CO_2) is the preferred gas for establishing a pneumoperitoneum because it is non-flammable and inexpensive. However, CO_2 absorption through the peritoneal membrane leads to hypercapnia and acidosis and in order to reduce these effects, minute ventilation has to be adjusted. In addition, the increased intra-abdominal pressure due to intraperitoneal gas insufflation influences hemodynamic and respiratory function.

Other methods for creation of a working space include abdominal wall retraction, which may be performed either without gas insufflation or with low-pressure insufflation (5-7 mmHg) [16]. However, experience with gasless laparoscopy in humans is not very encouraging [17]. Low elasticity of the abdominal wall and lack of compression of abdominal contents limit the working space to such an extent that laparoscopic gasless surgery becomes feasible in only a few carefully selected cases.

In early years, the laparoscopic approach was mainly applied to young, healthy patients. However, with growing surgical expertise and continuing improvements in technology, minimally invasive surgery is applied to a much broader patient population, such as elderly patients with multiple comorbid conditions, the very young and the critically ill. Therefore, it is well recognized that the pathophysiological consequences of abdominal gas insufflation may become even more important [18].

Cardiovascular effects of pneumoperitoneum

Most studies investigating the cardiovascular changes associated with laparoscopic surgery report an increase in systemic vascular resistance, mean arterial blood pressure (MAP) and myocardial filling pressures, accompanied by a fall in cardiac index, with little change in heart rate [19-25] However, studies on the cardiovascular consequences of pneumoperitoneum are often contradictory. This discrepancy may be explained by the fact that alterations of cardiovascular function depend on the interaction of multiple factors such as intra-abdominal pressure (IAP), patient position, CO_2 absorption and duration of the procedure. Furthermore, the patient's intravascular volume status, pre-existing cardiopulmonary status, neurohumoral factors and administration of anesthetic agents all may influence the cardiovascular response to the induction of pneumoperitoneum.

In general, increased IAP (up to 12-15 mmHg) decreases venous return, which results in reduced preload and cardiac output, without adequate intravascular volume loading. Changes in body position, especially 'head-up' position (or: reversed-Trendelenburg position) augment these adverse effects of pneumoperitoneum [26], whereas 'head-down' position (or: Trendelenburg position) has a positive effect on venous return [27]. Furthermore, pneumoperitoneum increases sympathetic cardiac activity and may induce a hemodynamic stress response by activation of neurohumoral vasoactive factors such as catecholamines, anti-diuretic hormone and the renine-aldosterone system [16, 20]. This results in increased heart rate, systemic vascular resistance and MAP. Recently, it was concluded in a clinical practice guideline of the European Association for Endoscopic Surgery [28] that the hemodynamic and circulatory effects of pneumoperitoneum in patients without comorbidity (ASA I and II) are generally not clinically relevant. However, in patients with comorbidity (ASA III and IV), invasive monitoring of blood pressure and circulating volume must be considered [28]. Since the effects of increased IAP are volume dependent, adequate preoperative intravascular fluid administration is essential to prevent cardiovascular side-effects of a pneumoperitoneum [29].

Respiratory effects of pneumoperitoneum

Carbon dioxide, which is rapidly absorbed across the peritoneal membrane into the circulation, leads to a respiratory acidosis by the generation of carbonic acid. During laparoscopy, minute ventilation should be increased in order to maintain normocapnia. Anesthesia and surgery may cause a progressive cranial displacement of the diaphragm due to a sequence of events: assuming the supine position, induction of anesthesia and causation of paralysis of the diaphragm [30]. Pressure of the intra-abdominal contents further displaces the diaphragm cranially. Increased intra-abdominal pressure due to CO_2

insufflation impairs excursion of the diaphragm and leads to compression of the lower pulmonary lobes.

The cranial displacement of the diaphragm results in a decreased functional residual capacity (FRC). Decreased FRC may cause intraoperative atelectasis, intrapulmonary shunting and a ventilation-perfusion mismatch [31]. Assumption of different surgical positions will influence the degree of the diaphragmatic shift. In addition, increased intra-abdominal pressure decreases chest compliance and thus increases peak airway pressure [32, 33]. As a result the bronchial tree may expand which increases anatomical dead space. In patients with normal lung function, intra-operative respiratory changes are usually not clinically relevant [34]. However, in patients with limited pulmonary reserves, CO_2 pneumoperitoneum carries an increased risk of CO_2 retention, especially in the postoperative period ('hypercapnic hangover'). Therefore, intra- and postoperative arterial blood gas monitoring and continuous capnometry are generally recommended for patients with cardiopulmonary disease [31]. Alternative gases such as helium, argon, nitrous oxide and nitrogen have been suggested for creation of a pneumoperitoneum since these gases do not lead to hypercarbia and respiratory acidosis [35-38], and therefore may be beneficial in patients with limited pulmonary function.

Immunologic, metabolic and oncological consequences of pneumoperitoneum

Surgical trauma is associated with a modulation of the inflammatory and immune response. Clinical and experimental studies have reported that cell-mediated immunity is suppressed to a greater extent following open surgery compared with laparoscopic surgery [39-43]. The clinical correlation of these finding may be translated to a reduction in postoperative infections and diminished local tumor recurrence or metastases. Most studies comparing laparoscopic versus conventional surgery have focused on changes of serum cytokine levels such as IL-1 [44] and IL-6 [45, 46] to investigate systemic immunological function. Other studies have demonstrated that indices of the inflammatory and metabolic responses, such as C-reactive protein [13, 47], serum insuline-like growth factor 1 (IGF-1) [48] and glucose levels [49, 50] are affected to a lesser degree by the laparoscopic approach. In most studies comparing laparoscopic and open surgery, the immunological effects of the pneumoperitoneum and the surgical procedure overlap each other, which precludes the quantification of a specific effect. Furthermore, the influence of the specifics of the pneumoperitoneum in terms of intra-abdominal pressure and gas type on immunological function has only partly been studied. It has been demonstrated that helium insufflation preserves cell-mediated intraperitoneal immunity better than after insufflation with CO_2 [51, 52] and causes a less pronounced cytokine response [53]. Much

interest has focused on the occurrence of port site metastases and local tumor recurrence following laparoscopic surgery [54]. In experimental studies, beneficial effects of helium as an alternative insufflation gas have been reported. Jacobi et al. [55] observed that CO_2 pneumoperitoneum significantly increased tumor growth in comparison to helium, which appears to be attributed to differences in pH. Neuhaus et al. [56] investigated the impact of different insufflation gases on port-site development, and reported that tumor growth was significantly less after helium insufflation. However, evidence regarding the immunologic changes associated with laparoscopy remains inconclusive, mostly because of discrepancies in the qualitative and quantitative degree of changes of the immune response [28]. Although the use of alternative gases such as helium appears to be promising, it is generally considered that further studies are needed before their routine clinical use can be supported [57]. Additional clinical trials with adequate sample size are needed to confirm whether the better preservation of immune function is of clinical relevance.

Hypothermia and tissue desiccation
Operative hypothermia is caused by anesthetic agents which disturb thermoregulatory control [58], low temperature in the operating room [59], cold irrigating fluids and infusion of cold intravenous fluids [60, 61], as well as by losses incurred by the actual surgery itself. Hypothermia has been shown to be associated with hypokalemia [62], coagulation disorders [63] and increased oxygen consumption [64, 65]. In addition, perioperative normothermia appears to reduce the number of surgical-wound infections and shorten postoperative hospital stay [66, 67]. Corrective measures to prevent operative hypothermia include the use of heated body blankets, irrigation of the abdominal cavity with warm saline, and raising the operating room temperature. During prolonged, complex laparoscopic procedures, patients are exposed to large volumes of insufflation gas at room temperature, putting the patient at risk for hypothermia [68-70]. Therefore, identifying the effect of CO_2 on core body temperature is important. Heating of the insufflation gas has been proposed as a measure to prevent the decrease of body temperature during laparoscopic procedures [71], although others state that heating is only useful if the gas is also humidified [72].
An issue which has not fully been addressed is whether insufflation with cold, dry CO_2 contributes to an increase of pain in the immediate postoperative period. In most studies, postoperative pain is significantly reduced by the laparoscopic approach as compared with open techniques [73, 74]. However, many patients still experience considerable postoperative pain, which is mainly identified as right subcostal pain or shoulder tip pain [75, 76]. Suggested mechanisms for this pain include: ultracellular trauma to the peritoneum caused by the dry CO_2 gas, neuropraxia of the phrenic nerve, formation of carbonic acid

which lowers the pH of the peritoneum, and retained intraperitoneal gas [68]. In an experimental study by Volz et al [77], it was shown that the integrity of the parietal peritoneum is temporarily disturbed by insufflation with dry CO_2. Denudation of the mesothelial surface increases the risk for adherence of tumor cells and adhesions [78-80], which implies that peritoneal changes due to the pneumoperitoneum may render it susceptible to tumor growth or infection [81]. Currently, data on the influence of heating or humidifying the insufflation gas are contradictory [28], suggesting that this issue requires further study.

Perfusion of intra-abdominal organs

There are few data available on the relationship between the systemic and regional hemodynamic effects of pneumoperitoneum. In experimental studies, it was shown that blood flow in the superior mesenteric artery and hepatic portal vein is reduced during pneumoperitoneum [82]. In addition, increased intra-abdominal pressure compresses capillary beds, decreases splanchnic microcirculation and therefore may impair oxygen delivery to the intra-abdominal organs. In several clinical studies, pneumoperitoneum was associated with intramucosal acidosis which signifies inadequate perfusion [83-85]. However, other studies did not find any detrimental effect due to abdominal gas insufflation [86, 87]. Therefore, the clinical implications of these investigations remain largely unclear. In general, healthy patients seem to compensate changes in intra-abdominal perfusion without impairment of organ function [28]. However, in elderly patients, splanchnic circulation is very sensitive to elevated intra-abdominal pressure [88]. This suggests that especially in patients with already impaired perfusion, intra-abdominal pressure should be kept as low as possible.

Increased intra-abdominal pressure may lead to diminished renal function due to compression of renal parenchyma and renal vessels [89-92]. The decrease in renal blood flow and in cortical and medullary perfusion observed during pneumoperitoneum causes a reduction in glomerular filtration rate (GFR) [93, 94], urinary output [84] and creatinine clearance [89]. In addition, renal perfusion and function may be influenced by release of neurohumoral factors following increased intra-abdominal pressure [95]. Increased plasma renin activity, which can be triggered by vascular compression, may lead to renal vasoconstriction [16]. In an experimental study, elevated endothelin concentrations during pneumperitoneum were associated with a reduction in renal blood flow, GFR and sodium excretion [94]. Other studies report increased antidiuretic hormone (ADH) levels during increased intra-abdominal pressure [96-98], although the actual mechanism for elevated intra-abdominal pressure and increased ADH secretion remains poorly understood.

One of the most recent developments in laparoscopic surgery is the laparoscopic approach for live renal donation, which was first described by Ratner et al. [10] in 1995. In December 1997, laparoscopic donor nephrectomy was introduced at the Erasmus Medical Center, Rotterdam. Unlike most surgical operations, live donor nephrectomy involves a healthy individual which is subjected to major surgery for the benefit of another individual. Several studies have reported that LDN can be performed safely in selected candidates with a reduction in postoperative morbidity and shorter hospitalization compared to conventional donor nephrectomy [99-102]. It is mandatory that laparoscopic procurement of a kidney should provide excellent recipient graft outcomes, however, the effect of LDN on transplant graft function has not yet been fully elucidated. Concern has been raised about the reported incidence of primary dysfunction of transplanted kidneys after laparoscopic procurement [103]. Although retrospective studies comparing LDN with the conventional approach suggest similar graft function after one year [101], it has been shown that recipients of laparoscopically procured kidneys have higher serum creatinine levels and a greater need for dialysis in the first weeks after transplantation [103, 104]. It has been stressed that it will take years before data on long term graft function after LDN are available [105]. In view of these data, employment of the laparoscopic approach as an alternative for the 'gold' standard of open nephrectomy can only be justified if it is clear that allograft function after laparoscopic kidney procurement is not at stake. At present, no randomized controlled clinical trials are at hand and follow-up of most series has been relatively short. Furthermore, the current lack of adequate evidence-base for LDN necessitates institutions performing this technique to report on safety and effectiveness after engraftment [105].

OBJECTIVES OF THE THESIS

This thesis presents experimental and clinical studies in which pathophysiological consequences of the pneumoperitoneum are assessed. The objectives of this thesis can be summarized as follows:

1. To investigate the hemodynamic and respiratory responses related to the pneumoperitoneum in the laparoscopic rat model, using either CO_2 or helium insufflation or a gasless technique.

2. To determine the effect of abdominal gas insufflation on arterial oxygenation, used as a substrate for atelectasis formation, during mechanical ventilation with and without PEEP in rats.

3. To investigate the effect of heating and humidifying CO_2 used to create pneumoperitoneum on body temperature and peritoneal morphology.

4. To compare laparoscopic live renal donation with conventional, open donor nephrectomy, with specific emphasis on intraoperative variables, graft function and clinical outcome.

5. To measure plasma antidiuretic hormone (ADH) levels in patients before, during and after laparoscopic donor nephrectomy.

6. To investigate the impact of pneumoperitoneum used for laparoscopic donor nephrectomy in donors and recipients in a rat model, with specific emphasis on renal function, renal histomorphology and renal immunohistology.

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CHAPTER 2

IMPACT OF CARBON DIOXIDE AND HELIUM INSUFFLATION ON CARDIORESPIRATORY FUNCTION DURING PROLONGED PNEUMOPERITONEUM IN AN EXPERIMENTAL RAT MODEL

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ABSTRACT

Background: Experimental studies on laparoscopic surgery are often performed in rats. However, the hemodynamic and respiratory responses related to the pneumoperitoneum have not been studied extensively in rats. Therefore, the aim of this study was to investigate in spontaneously breathing rats the effects of CO_2 and helium, insufflation pressure and duration of pneumoperitoneum on blood pressure, arterial pH, pCO_2, pO_2, HCO_3^-, base excess and respiratory rate.

Methods: Five groups of 9 Brown Norway rats were anaesthetized and underwent either CO_2 insufflation (6 or 12 mmHg), helium insufflation (6 or 12 mmHg) or abdominal wall lifting (gasless control) for 120 min. Blood pressure was monitored by an indwelling carotid artery catheter. Baseline measurements of mean arterial pressure (MAP), respiratory rate, arterial blood pH, pCO_2, pO_2, HCO_3^- and base excess were recorded. Blood gases were analyzed at 5, 30, 60, 90 and 120 min during pneumoperitoneum, MAP and respiratory rate were recorded at 5 and 15 min and at 15 min intervals thereafter for 2 hours.

Results: CO_2 insufflation (at both 6 and 12 mmHg) caused a significant decrease in blood pH and increase in arterial pCO_2. Respiratory compensation was evident, since pCO_2 levels returned to pre-insufflation levels during CO_2 insufflation at 12 mmHg. There was no significant change in blood pH and pCO_2 in rats undergoing either helium insufflation or gasless procedures. Neither insufflation pressure nor type of insufflation gas had a significant effect on MAP over time.

Conclusion: The cardiorespiratory changes during prolonged pneumoperitoneum in spontaneously breathing rats are similar to those seen in clinical practice. Therefore, studies conducted in this animal model can provide valuable physiological data relevant to the study of laparoscopic surgery.

INTRODUCTION

Laparoscopic surgery requires creation of a working space in the abdominal cavity to allow safe introduction of trocars and instruments and for exposure of the abdominal contents. Intraperitoneal insufflation of gas is the most common method to elevate the abdominal wall and suppress the viscera. Carbon dioxide (CO_2) is the preferred gas for establishing a pneumoperitoneum because it is non-flammable and inexpensive. However, rapid transperitoneal absorption of CO_2 can cause hypercapnia and acidosis [1]. Therefore, use of inert gases, such as helium and argon, has been suggested for establishment of pneumoperitoneum, because these gases do not alter blood pH and pCO$_2$ [2, 3]. In addition, experimental studies indicated less tumor growth after helium pneumoperitoneum than after CO_2 pneumoperitoneum [4, 5].

Various studies have been performed in small animal models to evaluate the oncologic and immunologic consequences of laparoscopic surgery. All these studies involved spontaneously breathing rodents [6, 7]. Surprisingly, knowledge of physiologic changes occurring during pneumoperitoneum in rodents is limited. Some studies have investigated the influence of increasing insufflation pressures during CO_2 pneumoperitoneum on cardiorespiratory function in spontaneously breathing rats [8, 9]. However, to our knowledge, the effects of intraperitoneal gas insufflation with a constant pressure over a prolonged period of time have not been recorded in the rat model. The purpose of experimental studies is to extrapolate experimental findings to daily clinical practice. Since most clinical laparoscopic procedures are performed while the patient is mechanically ventilated, thus allowing correction of hypercarbia, knowledge on the physiologic changes occurring in spontaneously breathing rats exposed to a pneumoperitoneum is necessary. The aim of our study was to record the cardiorespiratory changes during prolonged intraperitoneal insufflation with either CO_2 or helium.

MATERIAL AND METHODS

Animals
Male rats of the inbred Brown Norway strain, weighing 250-300 g and aged 10-12 weeks were obtained from Charles River, Someren, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals (n = 45) were kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark), fed with laboratory diet (Hope Farms, Woerden, The Netherlands) and with

free access to water. The experimental protocols adhered to the rules laid down by the Dutch Animal Experimentation Act, and were approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Operative procedures

All procedures were performed in spontaneously breathing rats. After induction of anesthesia with 65% nitrous oxide/33% oxygen/2% isoflurane (Isoflurane; Pharmachemie BV, Haarlem, The Netherlands), a polyethylene catheter (0.8 mm outer diameter) was inserted into a carotid artery for blood pressure monitoring and for drawing arterial blood samples. Gaseous anesthesia was discontinued and replaced with pentobarbital sodium 20 mg/kg/h intraperitoneally (Nembutal®; Sanofi Sante Animale Benelux BV, Maassluis, The Netherlands), given every 30 min. The rat was placed in supine position, the abdomen was shaved and cleaned with 70% alcohol, and dried with gauze. After making a 5-mm skin incision in the midline of the abdomen, a shortened 5 mm trocar (Ethicon Endo-Surgery, Cincinatti, OH, USA) was introduced and secured with a purse-string suture. Baseline measurements of mean arterial pressure (MAP), respiratory frequency and arterial blood pH, pCO_2, pO_2, HCO_3^- and base excess (BE) were recorded using conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). Body temperature was kept within normal range by means of a heating lamp.

Pneumoperitoneum

Animals were randomly assigned to five groups of nine animals each: one group had a pneumoperitoneum by insufflation of CO_2 at 6 mmHg, another group had CO_2 insufflation at 12 mmHg, a third group had a pneumoperitoneum with helium insufflation at 6 mmHg and a fourth group was insufflated with helium at 12 mmHg. The fifth group served as gasless control by lifting the abdominal wall with a suture attached to the trocar. All animals were exposed to a procedure lasting 120 min.

Arterial pH, pCO_2, pO_2, HCO_3^- and BE were determined at 5, 30, 60, 90 and 120 min after start of either gas insufflation or gasless elevation of the abdominal wall. After start of the procedure, blood pressure and respiratory rate were recorded at 5, 15 and every 15 min thereafter for 2 h. After 120 min, the abdomen was desufflated in all animals followed 15 min later, by final measurement of arterial pH, pCO_2, pO_2, HCO_3^-, BE, blood pressure and respiratory rate. At the end of the experiment, all animals were killed with an overdose of pentobarbital sodium injected into the carotid artery.

Statistical analysis

Statistical analysis was performed utilizing the SPSS 10.0 (SPSS Inc., Chicago, IL) statistical software package. To analyze all data, mean values and standard deviations of arterial pH, pCO_2, pO_2, HCO_3^-, BE, blood pressure and respiratory rate were calculated. For all groups, values of arterial pH, pCO_2, pO_2, HCO_3^- and BE at 5, 30, 60, 90 and 120 min were compared to baseline values using paired t-tests. Subsequently, values of arterial pH, pCO_2, pO_2, HCO_3^- and BE were compared between groups using ANOVA. To study changes in blood pressure and respiratory rate in all groups, the mean of all values at 5, 15 and every 15 min for 2 hours was compared to the baseline value using ANOVA. In addition, the mean values of blood pressure and respiratory rates during either gas insufflation or gasless elevation of the abdominal wall were compared between groups using paired t-tests. Statistical significance was accepted at $p < 0.05$.

RESULTS

There were no significant differences in body weight of rats and time between induction of anesthesia and start of procedure. Before insufflation (t0), measurements of arterial pH, pCO_2, pO_2, HCO_3^-, BE, blood pressure and respiratory rate did not significantly differ among the experimental groups.

Arterial blood gases

Intraperitoneal insufflation with CO_2 caused a significant decrease in blood pH compared to baseline measurements (Fig. 1a). In the 6 mmHg CO_2 insufflation group, blood pH levels remained lower than pre-insufflation levels for 120 min, whereas in the 12 mmHg group the decrease in pH was no longer significant after 90 min. After desufflation, all pH values returned to pre-insufflation levels. There were no differences in pH levels between the 6 and 12 mmHg CO_2 groups. Blood pH levels in the gasless control group remained constant during the entire experiment. Although insufflation with helium at 12 mmHg caused a brief decrease in arterial pH, neither 6 nor 12 mmHg helium pneumoperitoneum had a significant effect on blood pH levels during 120 min (Fig. 1b).

Insufflation with CO_2 at 6 mmHg increased $PaCO_2$ at 5, 30, 60 and 90 min, whereas in the 12 mmHg group there was a significant increase in $PaCO_2$ at 5 min only (Fig. 2a). There were no significant differences in $PaCO_2$ values between the 6 and 12 mmHg CO_2 groups. There were no significant changes in $PaCO_2$ in either the gasless control group or either of the helium groups (Fig. 2b).
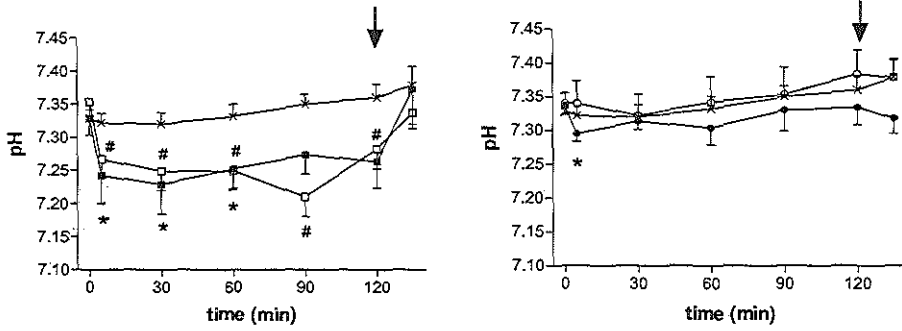
Figure 1a. Blood pH values of CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (X) over time.
Figure 1b. Blood pH values of helium insufflation at 6 mmHg (○) and 12 mmHg (●) and of the gasless control group (X) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
* $p < 0.05$ compared to pre-insufflation values (t0) for 12 mmHg
# $p < 0.05$ compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

Figure 2a. Arterial pCO_2 values of CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (X) over time.
Figure 2b. Arterial pCO_2 values of helium insufflation at 6 mmHg (○) and 12 mmHg (●) and of the gasless control group (X) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
* $p < 0.05$ compared to pre-insufflation values (t0) for 12 mmHg
# $p < 0.05$ compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

Arterial oxygenation showed no significant change with CO$_2$ insufflation at 6 mmHg, whereas CO$_2$ insufflation at 12 mmHg led to an immediate significant increase in PaO$_2$ which was maintained during 120 minutes (Fig. 3a). After desufflation, PaO$_2$ in both CO$_2$ insufflation groups was still significantly increased compared to baseline measurements. There were no differences in PaO$_2$ values between the 6 and 12 mmHg CO$_2$ groups. Neither helium insufflation nor abdominal wall lift had any effect on PaO$_2$ at any time point (Fig. 3b).

Insufflation with CO$_2$ at 12 mmHg caused a decrease in HCO$_3^-$ and negative BE (Figs. 4a and 5a). There were no changes in HCO$_3^-$ and BE in the CO$_2$ 6 mmHg group, both helium groups or the gasless control (Figs. 4b and 5b).
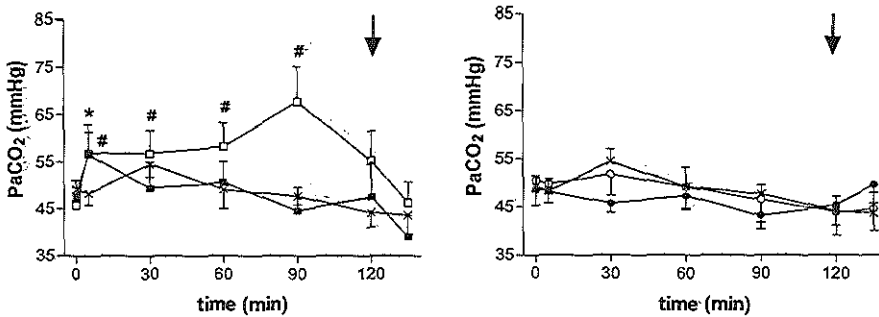
Figure 3a. Arterial pO$_2$ values of CO$_2$ insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (X) over time.
Figure 3b. Arterial pO$_2$ values of helium insufflation at 6 mmHg (O) and 12 mmHg (●) and of the gasless control group (X) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
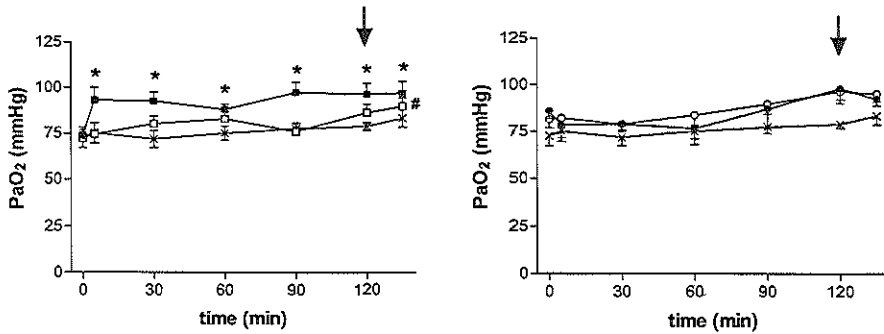* p < 0.05 compared to pre-insufflation values (t0) for 12 mmHg
# p < 0.05 compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

Figure 4a. Arterial HCO_3^- values of CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (X) over time.
Figure 4b. Arterial HCO_3^- values of helium insufflation at 6 mmHg (O) and 12 mmHg (●) and of the gasless control group (X) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
* $p < 0.05$ compared to pre-insufflation values (t0) for 12 mmHg
# $p < 0.05$ compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

Figure 5a. Base Excess (BE) values of CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (X) over time.
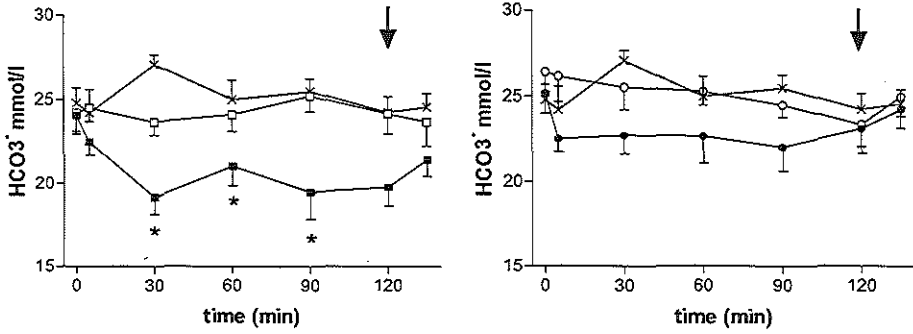**Figure 5b.** Base Excess (BE) values of helium insufflation at 6 mmHg (O) and 12 mmHg (●) and of the gasless control group (X) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
* $p < 0.05$ compared to pre-insufflation values (t0) for 12 mmHg
# $p < 0.05$ compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

Respiratory rate and blood pressure

Respiratory rate of the spontaneously breathing animals is shown in Fig. 6a, b. Gas insufflation was followed by an increase of respiratory rate in both CO_2 and helium groups. In the CO_2 12 mmHg group, respiratory rate was significantly increased during 90 min compared with pre-insufflation levels. CO_2 insufflation at 6 mmHg caused a significant increase at 45 and 60 min after start of insufflation. Insufflation with helium at 12 mmHg caused a significant increase in respiratory rate at all measured time points except for 60 and 90 min, whereas in the helium 6 mmHg group respiratory rate was significantly increased at 75 and 105 min. Gasless elevation of the abdominal wall did not change the respiratory rate. Comparison of respiratory rates during insufflation with either CO_2 or helium showed no differences; therefore, the increase of respiratory rate was independent of the type of insufflation gas and insufflation pressure. After desufflation of the peritoneal cavity, respiratory rates of all animals returned to pre-insufflation levels.

Figure 6a. Respiratory rate during CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (✕) over time.
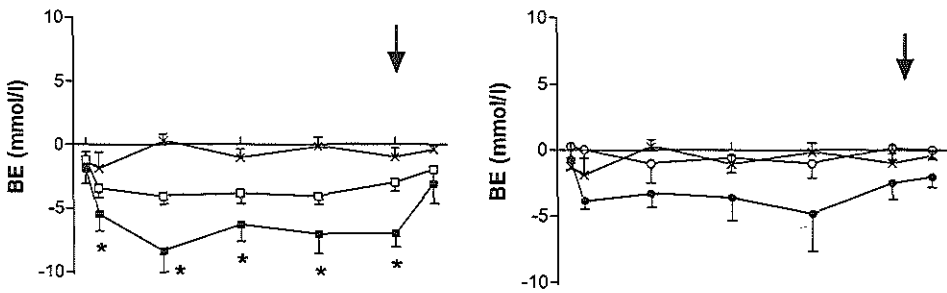**Figure 6b.** Respiratory rate during helium insufflation at 6 mmHg (○) and 12 mmHg (●) and of the gasless control group (✕) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
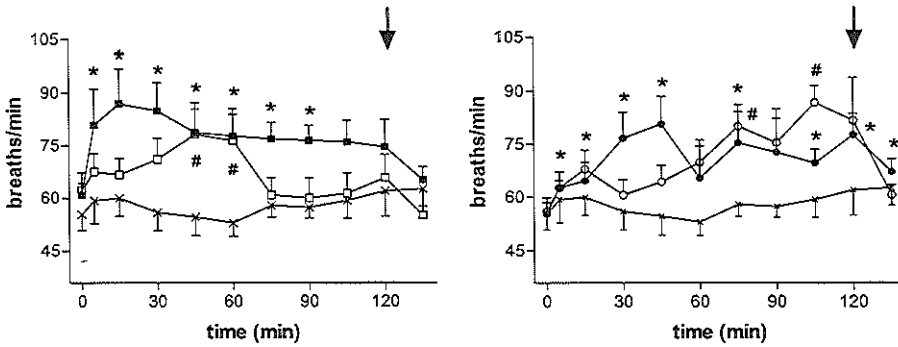* p < 0.05 compared to pre-insufflation values (t0) for 12 mmHg
# p < 0.05 compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

The effect of different insufflation gases and pressures on MAP is shown in Fig. 7a, b. During the experiment significant changes in MAP were recorded in all insufflation groups compared with pre-insufflation levels. Insufflation with CO_2 at 12 mmHg did not cause any changes in MAP, except for a significant decrease at 105 min. At 5 min after onset of the procedure and from 60 to 105 min onwards, blood pressure decreased in rats with CO_2 insufflation at 6 mmHg. Lowered blood pressure was recorded during 12 mmHg helium insufflation at all measurement points, except for those at 30, 45 and 120 min. Upon insufflation of helium at 6 mmHg, decreased blood pressure was recorded at 45 and 105 min. ANOVA analyses showed that there were no differences in blood pressure levels between the CO_2 and helium groups. Release of pneumoperitoneum led to an immediate increase in blood pressure in all insufflation groups.

Two rats died during the experiments; one rat was exposed to 12 mmHg CO_2 insufflation and the other to 12 mmHg helium insufflation. Both rats died after 90 min. Early hypoxia, despite a normal respiratory rate, followed by decreasing blood pressure was noted in both these animals.

Figure 7a. Mean arterial pressure (MAP) values of CO_2 insufflation at 6 mmHg (□) and 12 mmHg (■) and of the gasless control group (✕) over time.
Figure 7b. Mean arterial pressure (MAP) values of helium insufflation at 6 mmHg (○) and 12 mmHg (●) and of the gasless control group (✕) over time.
Data points represent mean ± SEM (error bars) for each group. Significant changes are described in the text.
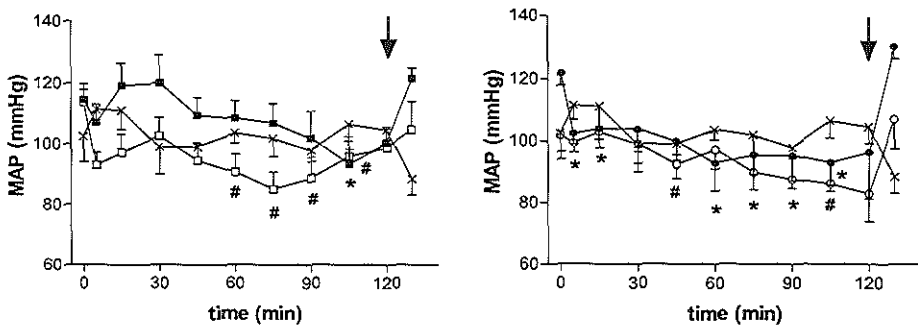* $p < 0.05$ compared to pre-insufflation values (t0) for 12 mmHg
# $p < 0.05$ compared to pre-insufflation values (t0) for 6 mmHg
↓ indicates desufflation

DISCUSSION

The main objective of this experimental study was to record the cardiorespiratory changes in spontaneously breathing rats exposed to a pneumoperitoneum. Earlier experimental studies in rats investigating changes due to laparoscopic surgery have all been performed in spontaneously breathing rats. Our concern was that these latter studies were negatively biased due to adverse cardiorespiratory consequences of intraperitoneal insufflation during spontaneous breathing. In the present study, acidosis was a consistent finding during CO_2 insufflation. The occurrence of respiratory acidosis following CO_2 pneumoperitoneum has been reported frequently, and it appears that the effect of CO_2 absorption across the peritoneal surface is primarily responsible for this phenomenon [1]. Using a porcine model, Ho et al. found that absorption of CO_2 from the peritoneal surface only, irrespective of insufflation pressure, is responsible for acidosis and hypercapnia [10]. In our study, we employed a constant pressure gradient similar to the study by Ho et al., eliminating the effect of incremental pressure increases and thus allowing the body to adapt to abdominal insufflation. Utilizing the study design of a fixed insufflation pressure is more representative of daily clinical practice and of models used in experimental laparoscopic studies. Only a few studies on changes in cardiorespiratory parameters during pneumoperitoneum have been performed in the rat model [8, 9]. In a study using stepwise increases in pneumoperitoneum pressure in spontaneously breathing rats, Berguer et al. demonstrated significant respiratory acidosis during CO_2 pneumoperitoneum at a pressure of 10 mmHg, whereas a pressure of 5 mmHg did not result in hypercarbia and acidosis [8]. However, it is possible that applying a constant pressure of 5 mmHg could also have led to respiratory acidosis over time or that the respiratory acidosis was due to the effects of accumulated CO_2 during the course of the experiment. In a recent study in rats, Kuntz et al. conclude that blood, subcutaneous and intra-abdominal pH are influenced differently by the type of gas used for laparoscopy and that this phenomenon is accentuated by increasing intra-abdominal pressure [9]. Again, the study design of using stepwise pressure increases did not allow proper evaluation of blood gas changes during pneumoperitoneum over time.

Hemodynamics during pneumoperitoneum have been studied extensively. Various hemodynamic patterns have been described. Decreases [11, 12], increases [10, 13] as well as no alterations [14-18] in MAP during pneumoperitoneum have been reported. These conflicting results may be due to differences in intravascular volume status, level of intra-abdominal pressure, level of CO_2 in the blood, anesthetic regimen and lack of adequate statistical power. In our study, changes in MAP were documented during insufflation of the

abdominal cavity with CO_2 or helium although, over time, there was no significant change in MAP due to type of gas or insufflation pressure. Our pilot studies have shown that particularly during laparoscopy in the rat, careful administration of anesthesia (pentobarbital) is mandatory, since injection of barbiturates can have an immediate hypotensive effect [19, 20]. Therefore, the effects of insufflation on blood pressure in the present study should be interpreted with some caution, since maintenance of anesthesia might have confounded some of these effects by decreasing blood pressure. The findings in this study imply that, over time, spontaneously breathing rats undergo a depression of hemodynamic function which can only be improved by release of pneumoperitoneum, suggesting that insufflation itself depressed hemodynamics, independent of pressure. However, it must be realized that applying high insufflation pressures, such as 12 mmHg, may increase the risk of respiratory depression in the spontaneously breathing rat, since two animals died after 90 min. To avoid the combined negative effects of anesthesia and pneumoperitoneum on cardiorespiratory function, mechanical ventilation may be preferable when studying the effects of high intra-abdominal pressures or if prolonged laparoscopic procedures are to be performed in the rat model [21].

In the present study, cardiorespiratory stress was minimal during gasless procedures, which underlines the aim to keep insufflation pressures as low as possible during laparoscopic surgery. However, experience with gasless laparoscopy in humans is not very encouraging. Low elasticity of the abdominal wall and lack of compression of abdominal contents limit the working space to such an extent that laparoscopic gasless surgery becomes feasible in only a few carefully selected cases. Of interest is the finding in this study that $PaCO_2$ was only elevated during 6 mmHg CO_2 insufflation whereas 12 mmHg CO_2 insufflation was associated with normal arterial pCO_2 levels. This finding could be attributed to hyperventilation during 12 mmHg insufflation, resulting in increased pulmonary CO_2 excretion and increased O_2 absorption. The clear advantages of low pressure CO_2 insufflation in this study were limited loss of bicarbonate reflected by smaller base deficits and the absence of operative deaths.

Helium insufflation was superior to carbon dioxide insufflation since acidosis, hypercarbia and changes in base excess were not observed. These findings are in concordance with results from studies in large animals and clinical studies comparing changes in arterial blood gases following intra-abdominal CO_2 or helium insufflation [3, 14-16, 18]. Further clinical studies with helium insufflation are mandatory to establish the role of helium in laparoscopic surgery.

The current study has shown that the cardiorespiratory changes due to prolonged pneumoperitoneum in spontaneously breathing rats are similar to those seen in clinical

practice. Therefore, studies conducted in this animal model can provide valuable physiological data relevant to the study of laparoscopic surgery.

ACKNOWLEDGMENTS

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CHAPTER 3

MECHANICAL VENTILATION WITH POSITIVE END-EXPIRATORY PRESSURE PRESERVES ARTERIAL OXYGENATION DURING PROLONGED PNEUMOPERITONEUM

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ABSTRACT

Background: Laparoscopic surgery usually requires a pneumoperitoneum by insufflating the abdominal cavity with CO_2. Increased intra-abdominal pressure causes diaphragmatic displacement resulting in compressed lung areas which leads to formation of atelectasis, especially during mechanical ventilation. Application of positive end-expiratory pressure (PEEP) can maintain pulmonary gas exchange. The objective of this study is to investigate the effect of abdominal gas insufflation on arterial oxygenation during mechanical ventilation with and without PEEP in rats.

Methods: In experiment 1, two groups of six rats were continuously insufflated with CO_2 at 12 mmHg for 180 min. Group I was ventilated with 8 cmH_2O PEEP and group II had 0 cmH_2O PEEP. A third group served as control. This group had abdominal wall lifting and was ventilated with 0 cmH_2O PEEP (group III). In experiment 2, two groups of six rats had abdominal CO_2 insufflation and were ventilated with or without PEEP during 180 min (groups IV and V). In this experiment, abdomens were desufflated in both groups for 5 min at 60 and 120 min. Blood pressure monitoring and measurement of arterial pO_2 was performed by placement of an indwelling carotid artery catheter in both experiments.

Results: In both experiments, PaO_2 values decreased significantly in insufflation groups which were ventilated with 0 cmH_2O PEEP (groups II and V). Insufflation groups ventilated with 8 cmH_2O PEEP had PaO_2 values comparable to the control group. There were no significant differences in mean arterial pressure between insufflation groups ventilated with or without PEEP.

Conclusion: PEEP preserves arterial oxygenation during prolonged pneumoperitoneum in rats with minimal adverse hemodynamic effects.

INTRODUCTION

Laparoscopic surgery requires the use of a pneumoperitoneum to create a working space in the abdominal cavity in order to allow safe introduction of trocars and instruments and exposure of the abdominal contents. Intraperitoneal insufflation with carbon dioxide (CO_2) is the most common method to elevate the abdominal wall and suppress the viscera. During laparoscopy CO_2 absorption through the peritoneal membrane leads to hypercapnia and acidosis and, in order to ameliorate these effects, minute ventilation has to be adjusted [1].

During anesthesia lungs are compressed by a cranial shift of the diaphragm and changes in the thoraco-abdominal configuration, resulting in the formation of compression atelectasis [2, 3]. During laparoscopic surgery the cranial shift of the diaphragm is enhanced by the increase in intra-abdominal pressure, which could lead to further atelectasis formation [4]. An increase of atelectasis will decrease the number of sufficiently ventilated alveoli, resulting in increased dead space, a ventilation-perfusion mismatch and a decrease in arterial oxygenation (PaO_2) [5, 6]. During short laparoscopic procedures, these changes in cardiorespiratory parameters seldom have a clinically significant adverse effect. However, it is well recognized that with expansion of laparoscopy, laparoscopic operations will become longer, more complex and applied to a broader patient population [7]. Minimally invasive techniques have already been applied in elderly patients or patients with poor cardiopulmonary reserves [8]. Application of positive end-expiratory pressure (PEEP) has been shown to reduce atelectasis formation during anesthesia [2, 3]. However, the use of PEEP in the presence of pneumoperitoneum remains controversial because their simultaneous application may reduce cardiac output [9].

The objective of this study was to determine the cardiopulmonary effects of pneumoperitoneum during mechanical ventilation with and without PEEP in a rat model. We investigated the effects of continuous CO_2 insufflation on arterial oxygenation and blood pressure. In addition, we studied the effects of abdominal desufflation during these two ventilation strategies.

MATERIAL AND METHODS

Animals

Male rats of the inbred Brown Norway (BN) strain, weighing 250-300 g and aged 10-12 weeks were obtained from Charles River (Someren, the Netherlands). Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark), fed with laboratory diet (Hope Farms, Woerden, the Netherlands). Before the experiment, all animals had free access to food and water. The experimental protocols adhered to the rules laid down by the Dutch Animal Experimentation Act and were approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Operative procedures

Rats were anaesthetized with 65% nitrous oxide/33% oxygen/2% isoflurane (Pharmachemie BV, Haarlem, the Netherlands) and a polyethylene catheter (0.8 mm outer diameter) was inserted into a carotid artery for blood pressure monitoring and for drawing arterial blood samples. After this, rats were tracheotomized and a metal cannula was inserted into the trachea (Fig. 1). Gaseous anesthesia was discontinued and replaced with pentobarbital sodium 20 mg/kg/h intraperitoneally (Nembutal; Sanofi Sante Animale Benelux BV, Maassluis, the Netherlands), given every 30 min.
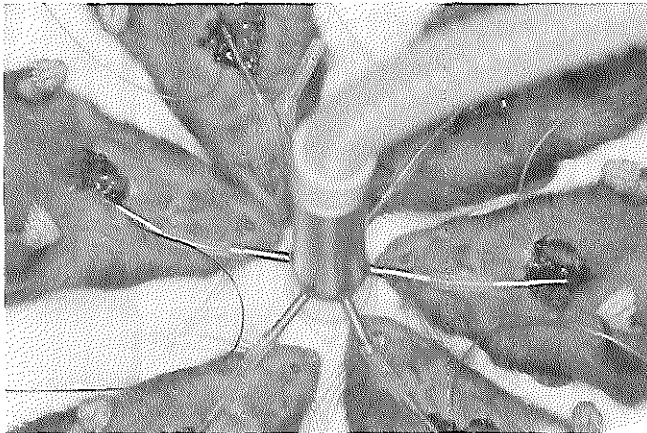
Figure 1.
Positioning of the rats during the experiment. Rats are mechanically ventilated through a tracheotomy.
An indwelling carotid artery catheter is placed for blood pressure monitoring and collection of blood samples.

Rats were placed at the operating table in supine position and, subsequently, muscle relaxation was attained and maintained by hourly intramuscularly injections of 2.0 mg/kg pancuronium (Pavulon; Organon Technika, Boxtel, the Netherlands). After muscle relaxation, the animals were connected to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna Sweden) in a pressure-controlled mode, at a peak inspiratory pressure (PIP) of 12 cmH$_2$O, a PEEP of 2 cmH$_2$O, an FiO$_2$ of 1.0, a frequency of 30 beats per minute (bpm) and an I/E ratio of 1:2. In order to re-open atelectatic lung areas induced by induction of anesthesia and the surgical procedure, PIP was increased to 26 cmH$_2$O for 30 seconds, after which ventilator settings were returned to starting values. Abdomens were shaved and cleaned with 70% alcohol and dried with gauze. After making a 5-mm skin incision in the midline of the abdomen at two-third between the xiphoid process and the pubis, a shortened 5-mm trocar (Ethicon Endo-Surgery, Cincinatti, OH, USA) was introduced and secured with a purse-string suture. Measurements for mean arterial pressure (MAP) and arterial blood gases were recorded using conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). For determination of PaO$_2$, a blood sample of 0.3 mL was drawn. After each sample, circulating volume was supplemented with 1 mL of saline. One milliliter was given instead of 0.3 mL to compensate for fluid loss from ventilation and pneumoperitoneum. Other means of volume expansion were not employed. There were no differences in fluid management between groups. Body temperature was kept within normal range by means of a heating lamp.

Experiment 1. Mechanical ventilation (PEEP versus no PEEP) during continuous pneumoperitoneum

The animals were allocated randomly to one of three groups (n = 6 per group). Group I had abdominal insufflation of CO$_2$ at 12 mmHg and underwent ventilation with PIP of 18 cmH$_2$O and a PEEP of 8 cmH$_2$O. Group II had CO$_2$ insufflation at 12 mmHg and was ventilated with a PIP of 12 cmH$_2$O and a PEEP of 0 cmH$_2$O. Group III served as gasless control by lifting the abdominal wall with a suture attached to the trocar, this group underwent ventilation with a PIP of 12 cm H$_2$O with 0 cmH$_2$O PEEP.

In preliminary experiments, tidal volumes of approximately 10 ml/kg proved sufficient in all groups. This volume is commonly used in humans undergoing mechanical ventilation. Although the ventilator settings (pressure levels) cannot be directly translated to patients they can serve as an indication for ventilator settings in humans. Respiratory rate was adjusted to maintain PaCO$_2$ within normal range. In the groups having pneumoperitoneum, respiratory rate was 100 bpm in the no-PEEP and 70 bpm in the PEEP group. In the gasless control group, respiratory rate was 25 bpm. All animals were

exposed to a procedure of 180 min. Because the ventilation rate was adjusted in all groups to maintain normocarbia, $PaCO_2$ and pH values are not described in this study.

PaO_2 was determined at baseline and at 5, 30, 60, 90, 120, 150 and 180 min after start of the procedure. Blood pressure was recorded at 5, 15 and every 15 min for 3 h, after start of the procedure. After 180 min, abdomens were desufflated in all animals, followed by a final measurement of PaO_2 and blood pressure 15 min later. At the end of the experiment, all animals were euthanized with an overdose of pentobarbital sodium injected into the carotid artery.

Experiment 2. Mechanical ventilation (PEEP versus no PEEP) during pneumoperitoneum, discontinued by abdominal desufflation

In two groups of 6 animals pneumoperitoneum was established by insufflation of CO_2 at 12 mmHg. One group underwent ventilation with a PIP of 18 cmH_2O and a PEEP of 8 cmH_2O (group IV) and another group was ventilated with a PIP of 12 cmH_2O and a PEEP of 0 cmH_2O (group V). In both groups, intraperitoneal insufflation was performed during 180 min, but at 60 and 120 min abdomens were desufflated for 5 min (D1 and D2, respectively). During these episodes of desufflation, respiratory rate was adjusted to maintain normocarbia. PaO_2 was determined at baseline and at 5, 60, 120 and 180 min after start of the procedure. In addition, a blood sample was collected directly after desufflation (D1 and D2) and again after resumption of gas insufflation. Blood pressure was recorded at baseline and at 5, 15 and every 15 min for 180 min, after start of the procedure. After 180 min, abdomens were desufflated in all animals (D3), followed by a final measurement of PaO_2 and blood pressure 15 min later. At the end of the experiment, all animals were killed as previously described.

Statistical analysis

Statistical analysis was performed utilizing the SPSS 10.0 statistical software package (SPSS Inc., Chicago, IL). Inter-group comparisons were analyzed with ANOVA. Intra-group comparisons were analyzed with repeated measures ANOVA. If ANOVA resulted in $p < 0.05$ a Tukey post-hoc test was performed. Statistical significance was accepted at $p < 0.05$.

RESULTS

There were no significant differences between the groups in body weight, time between induction of anesthesia and start of procedure. Baseline measurements did not differ among groups.

Experiment 1.

The effects of ventilation with and without PEEP during pneumoperitoneum on arterial oxygenation are shown in figure 2. In the group which had pneumoperitoneum with PEEP (group I), PaO_2 levels showed no significant differences during the 3 h study period. When no PEEP was applied during carbon dioxide insufflation (group II), PaO_2 values decreased. In the gasless control group (group III) there were no significant differences in PaO_2 levels during the experiment. Although there were no significant differences in arterial oxygenation between the three experimental groups at 5, 30 and 60 min, PaO_2 levels in group II were significantly lower compared to pre-insufflation levels after 90 min. From 120 min until the end of the experiment, PaO_2 levels in group II were significantly lower compared to PaO_2 levels in the group with PEEP and the gasless control group. After desufflation, PaO_2 levels decreased further in the group which had gas insufflation and 0 cmH_2O PEEP (group II) whereas PaO_2 remained stable in the group in which pneumoperitoneum was combined with PEEP (group I).

Figure 3 shows the data on MAP of groups I, II and III. For 180 min, there was no significant change in blood pressure in the gasless control group (group III). In groups I and II, MAP was significantly lower compared to pre-insufflation levels after 180 min. However, there were no significant differences in MAP between the group ventilated with PEEP (group I) and the group ventilated without PEEP (group II) during 180 min of pneumoperitoneum. Abdominal desufflation caused an increase in MAP in groups I and II, which was more pronounced in group II.

Experiment 2.

Figure 4 shows the effects of ventilation with and without PEEP on arterial oxygenation during CO_2 pneumoperitoneum with abdominal desufflation after 60, 120 and 180 min. At 60 min of intraperitoneal insufflation, there were no significant differences between the group in which PEEP was applied (group IV) and the group which had no PEEP (group V). PaO_2 levels remained stable in both groups during the first desufflation procedure
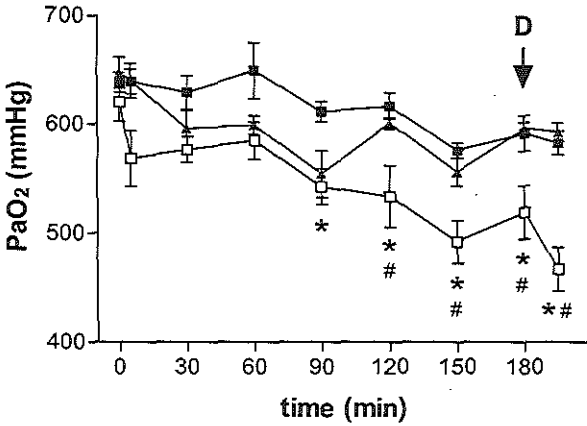
Figure 2. PaO_2 values in animals which had intraperitoneal CO_2 insufflation and 8 cmH$_2$O PEEP (group I,■), CO_2 insufflation and 0 cmH$_2$O PEEP (group II,□) and abdominal wall lifting and 0 cmH$_2$O PEEP (group III,▲). Data points represent mean ± SEM (standard error of the mean) for each group. D = abdominal desufflation. * $p < 0.05$ for group II compared to pre-insufflation levels. # $p < 0.05$ for group II compared to groups I and III.
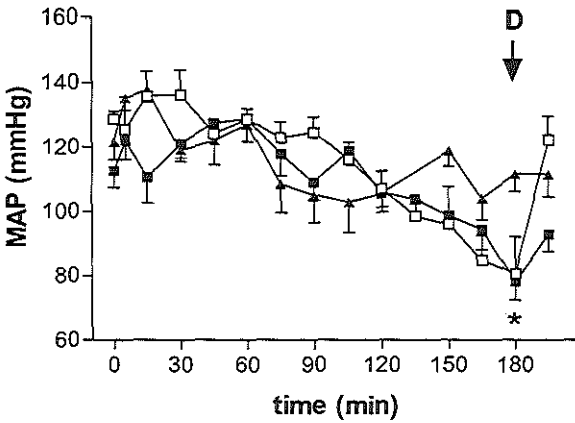
Figure 3. Mean arterial pressure (MAP) in animals which had intraperitoneal CO_2 insufflation and 8 cmH$_2$O PEEP (group I,■), CO_2 insufflation and 0 cmH$_2$O PEEP (group II,□) and abdominal wall lifting and 0 cmH$_2$O PEEP (group III,▲). Data points represent mean ± SEM (standard error of the mean) for each group. D = abdominal desufflation. * $p < 0.05$ for groups I and II compared to pre-insufflation levels.
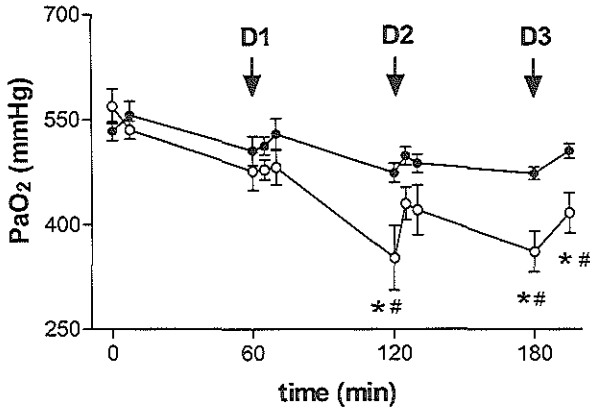
Figure 4. PaO_2 values in animals which had intraperitoneal CO_2 insufflation and 8 cmH_2O PEEP (group IV,●) and CO_2 insufflation and 0 cmH_2O PEEP (group V,○). Data points represent mean ± SEM (standard error of the mean) for each group. D1, 2 and 3 = abdominal desufflation after 60, 120 and 180 min, respectively. * $p < 0.05$ for group V compared to pre-insufflation levels. # $p < 0.05$ for group V compared to group IV.
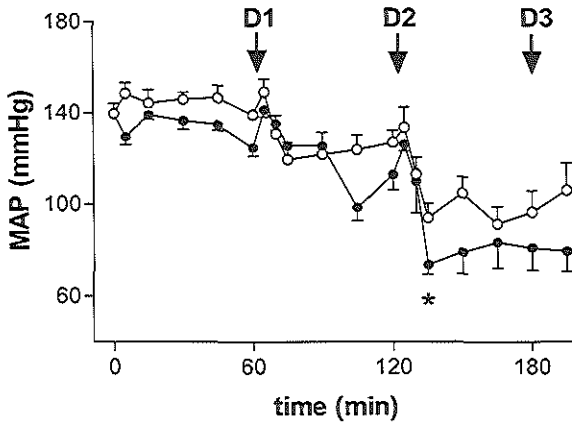
Figure 5. Mean arterial pressure (MAP) in animals which had intraperitoneal CO_2 insufflation and 8 cmH_2O PEEP (group IV,●) and CO_2 insufflation and 0 cmH_2O PEEP (group V,○). Data points represent mean ± SEM (standard error of the mean) for each group. D1, 2 and 3 = abdominal desufflation after 60, 120 and 180 min, respectively. * $p < 0.05$ for group IV compared to pre-insufflation levels.

(D1) and after start of insufflation at 65 min. However, 120 min after the start of pneumoperitoneum, PaO_2 levels in group V were significantly lower compared to pre-insufflation levels and compared to those in group IV. The second procedure of abdominal desufflation (D2) led to an increase in PaO_2 in both groups. However, after 180 min, PaO_2 levels were significantly lower in animals that had no PEEP (group V) compared to animals in group IV. Desufflation of the abdomen (D3) caused a small increase in PaO_2 in group V, but these levels remained significantly lower compared to pre-insufflation levels.

Figure 5 shows the data on MAP of groups IV and V. Over time, blood pressure decreased in both insufflation groups. In animals which had no PEEP (group V), MAP was not significantly changed compared to pre-insufflation levels during the 3 h study period. In rats that had PEEP (group IV), MAP was only decreased after 135 min compared to pre-insufflation levels. In both groups, the changes in MAP during the periods of desufflation and re-insufflation (D1, 2 and 3) did not reach statistical significance. During the experiment there were no significant differences between insufflation groups that were ventilated with a PEEP of 8 cmH_2O or with a PEEP of 0 cmH_2O.

DISCUSSION

This study was designed to investigate changes in arterial oxygenation as a substrate for atelectasis formation in animals undergoing pneumoperitoneum during mechanical ventilation with and without PEEP. The results of this study show that application of PEEP during mechanical ventilation maintains arterial oxygenation during CO_2 pneumoperitoneum. When no PEEP was applied during carbon dioxide insufflation, PaO_2 levels decreased significantly.

It has been shown that increased intra-abdominal pressure causes a cranial shift of the diaphragm resulting in compressed lung areas which leads to formation of atelectasis, particularly during mechanical ventilation [4]. Therefore, we first studied the effect of continuous gas insufflation on arterial oxygenation. In rats ventilated without PEEP, PaO_2 levels decreased after 90 min of abdominal insufflation. After 180 min of gas insufflation, arterial oxygenation was still significantly decreased. These findings demonstrate that increased intra-abdominal pressure results in formation of atelectatic lung areas. In the abdominal wall lift group, PaO_2 levels did not significantly change over time. In this group, the small changes in PaO_2 were most likely caused by end-expiratory alveolar

collapse due to the absence of PEEP, which prevents alveolar collapse. Therefore, rats ventilated without PEEP are prone to atelectasis formation, aggravated by supine position and muscular relaxants.

We used arterial oxygenation during mechanical ventilation with 100% oxygen as an accurate indicator for atelectasis formation. In a 'normal' lung this will result in PaO_2 levels up to 600 mmHg [10], as observed in baseline values of PaO_2 in all animals. When the lung remains inflated, no atelectasis formation occurs and PaO_2 will not be affected [6, 11], as observed in the 8 cmH$_2$O PEEP group (Fig. 2). These findings are in concordance with a study in pigs by Loeckinger et al., in which they conclude that application of PEEP during intraperitoneal CO_2 insufflation can improve pulmonary gas exchange [12]. One of the main differences between the study by Loeckinger et al. and our study is that they evaluated different levels of PEEP during intraperitoneal CO_2 insufflation on the lung's ventilation-perfusion, whereas we studied the effect of mechanical ventilation with a constant level of PEEP during pneumoperitoneum. To mimic clinically relevant settings we kept PEEP levels constant and applied the pneumoperitoneum and PEEP levels during a 180 min study period, thus simulating prolonged laparoscopic procedures.

In our second experiment, we studied the effects of release of intra-abdominal pressure on pulmonary gas exchange. This study design is more representative for clinical laparoscopic procedures, since abdominal desufflation occurs during inadvertent removal of laparoscopic instruments and because of deflation of electrocautery smoke. Although moments of intra-abdominal pressure release are often incomplete and may occur randomly in clinical practice, procedures of abdominal desufflation were of the same length and were performed at defined time points to prevent any bias among the experimental groups. In rats ventilated with and without PEEP, PaO_2 levels did not significantly change after 60 min or during the first abdominal desufflation procedure (Fig. 4). However, after 120 min, PaO_2 was significantly decreased in animals ventilated without PEEP. At this time, abdominal desufflation led to an increase in PaO_2, suggesting that release of pneumoperitoneum improves ventilation-perfusion mismatch. After 180 min, PaO_2 levels were decreased again and remained significantly decreased after final abdominal desufflation. These findings suggest that there is a beneficial effect on pulmonary gas exchange caused by abdominal desufflation. However, this effect appears to be temporary since installation of pneumoperitoneum caused a further decrease in arterial oxygenation.

The use of PEEP in the presence of pneumoperitoneum has been controversial due to the decrease of venous return with subsequent reduced cardiac output [9]. However, others conclude that simultaneous application of PEEP and increased intra-abdominal pressure

results only in modest hemodynamic depression [12] or can be performed without adverse cardiovascular effects [13]. In our study, we did not measure cardiac output, because accurate measurement of cardiac output in rodents requires placement of a flow probe around the aorta by thoracotomy. Therefore, we measured MAP to investigate an effect of pneumoperitoneum on hemodynamic function. As was shown by Pizov et al. [14], measuring arterial blood pressure changes allows sufficient assessment of hemodynamic changes in the absence of cardiac output measurement. Our study revealed a significant drop in blood pressure during ventilation with PEEP in only two instances. This suggests that the adverse hemodynamic effects of PEEP during pneumoperitoneum are limited.

It is well recognized that, as laparoscopic procedures become longer and more complex, the altered physiology due to pneumoperitoneum becomes more important [7]. Therefore, insight in the potential side-effects inherent to laparoscopic surgery is mandatory.

Information concerning the use of PEEP in patients undergoing mechanical ventilation during pneumoperitoneum is scarce. However, Neumann et al. [6] showed that during general anesthesia, a PEEP level of 10 cmH_2O could prevent atelectasis formation in patients undergoing elective surgery, demonstrating that a PEEP level of at least 10 cmH_2O is required to prevent atelectasis formation in healthy lungs. In addition, prevention of atelectasis formation can reduce post-surgery respiratory morbidity [15, 16]. Our study demonstrates that pulmonary atelectasis formation, as measured by arterial oxygenation, induced by abdominal gas insufflation can be prevented by adding PEEP to mechanical ventilation. Issues concerning the pathophysiological consequences of PEEP ventilation during laparoscopic surgery should therefore be addressed in clinical trials.

We conclude that application of PEEP preserves arterial oxygenation during prolonged pneumoperitoneum in mechanically ventilated rats. In the current study, application of 8 cmH_2O of PEEP was feasible with minimal hemodynamic changes. Our data advocate the use of PEEP to prevent pulmonary atelectasis formation during laparoscopic surgery.

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CHAPTER 4

IMPACT OF TEMPERATURE AND HUMIDITY OF CO_2 PNEUMOPERITONEUM ON BODY TEMPERATURE AND PERITONEAL MORPHOLOGY

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ABSTRACT

Background: Insufflation of cold gas during laparoscopic surgery exposes the patients to the risk of hypothermia. The objectives of this study were to investigate if heating or humidification of insufflation gas could prevent peroperative hypothermia in a rat model, and, to assess whether the peritoneum was affected by heating or humidification of the insufflation gas.

Methods: Rats were exposed to insufflation with either cold, dry CO_2 (group I), cold, humidified CO_2 (group II), warm, dry CO_2 (group III), warm, humidified CO_2 (group IV) or gasless laparoscopy (group V). Core temperature and intraperitoneal temperature were registered in all animals during 120 min. Specimens of the parietal peritoneum were taken directly after desufflation and 2 and 24 hours after the procedure. All specimens were analyzed using scanning electron microscopy (SEM).

Results: During the 120 min study period, core temperature and intraperitoneal temperature were significantly reduced in group I, II and III. Animals which had warm, humidified insufflation (group IV) and gasless controls (group V) did not develop intraoperative hypothermia. At SEM, retraction and bulging of mesothelial cells and exposure of the basal lamina were seen in the four insufflation groups (group I-IV) but also in gasless controls (group V).

Conclusion: Insufflation with cold, dry CO_2 may lower body temperature during laparoscopic surgery. Hypothermia can be prevented by both heating and humidifying the insufflation gas. Changes of the peritoneal surface, occur after CO_2 insufflation, regardless of heating or humidifying, but also after gasless surgery.

INTRODUCTION

Laparoscopic surgery requires creation of a working space in the abdominal cavity to allow safe introduction of trocars and instruments and expose the abdominal contents. Intraperitoneal insufflation of carbon dioxide (CO$_2$) is the most common method to elevate the abdominal wall and suppress the viscera. During prolonged, complex laparoscopic procedures, patients are exposed to large volumes of insufflation gas at room temperature, putting the patient at risk for hypothermia [1-3]. Preventing hypothermia during surgery is important considering that hypokalemia [4], coagulation disorders [5] and increased oxygen consumption [6, 7] are associated with surgical hypothermia. Furthermore, countering hypothermia appears to reduce the number of surgical-wound infections and shorten postoperative hospital stay [8, 9]. In patients with cardiac risk factors, perioperative maintenance of normothermia was associated with a reduction of morbid cardiac events [10].

Although heating of the insufflation gas has been proposed as a measure to prevent the decrease of body temperature [11], others state that heating is only useful if the gas is also humidified [12]. We hypothesized that a more physiological intra-abdominal environment by insufflating heated, humidified CO$_2$ gas would reduce the effect of tissue desiccation with subsequent denudation of the mesothelial surface [13].

The objectives of this experimental study were to investigate whether heated and humidified CO$_2$ insufflation could prevent intraoperative hypothermia and to relate morphological changes of the peritoneal surface to various intraperitoneal conditions using scanning electron microscopy.

METHODS

Animals

Male rats of the inbred Brown Norway (BN) strain, weighing 250-300 g and aged 10-12 weeks were obtained from Charles River, Someren, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark), fed with laboratory diet (Hope Farms, Woerden, The Netherlands) and with free access to water. The experimental protocols adhered to the rules laid down by the Dutch Animal Experimentation Act, and were approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Operative procedures

Anesthesia was established with pentobarbital sodium 20 mg/kg/h intraperitoneally (Nembutal®; Sanofi Sante Animale Benelux BV, Maassluis, The Netherlands), given every 30 min. The rat was placed on the operating table in supine position, the abdomen was shaved and cleaned with 70% alcohol and dried with gauze. During the experiment, the rat was placed on a heating pad in order to maintain normothermia. Pilot studies showed that when external heating is not used in anesthetized rats, core body temperature decreases dramatically, even without performing either laparoscopy or laparotomy (data not shown). After making a small incision in the right lower quadrant of the abdomen, a shortened 10-mm trocar (Ethicon Endo-Surgery, Cincinatti, OH, USA) was introduced, secured with a purse-string suture and connected to a CO_2 insufflator. On the left side of the abdomen, a 5-mm trocar was placed. Abdomens were insufflated at a pressure of 6 mmHg. By partly opening the stopcock on the 5-mm trocar, a standardized gas leak of 0.3 L/min was created to simulate gas losses during clinical laparoscopic procedures.

Experimental set-up

The experimental set-up is presented in Fig.1. For humidification of the insufflation gas, an MR 600 anesthesia respiratory humidifier (Fisher & Paykel Healthcare, Auckland, New Zealand) was connected to the insufflation hose before entering the insufflation port. This device, which basically consists of a water chamber on a heater plate, could easily be adjusted to deliver either heated or unheated gas and humidified or non-humidified gas. In animals which had insufflation with dry gas, the insufflation hose was connected to the device without filling the water chamber. To maintain the CO_2 at 37 °C, the afferent gas line was equipped with a heating wire (Fisher & Paykel Healthcare, Auckland, New Zealand). An electronic servo control system provided constant temperature and humidity levels.

Through the insufflation port, an 8-mm humidity probe (Ahlborn GmbH, Holzkirchen, Germany) was introduced to the entry point of the trocar. A flexible temperature sensor was placed in the abdomen through the exsufflation port to measure intraperitoneal temperature. Body temperature was measured by a rectal PT100 temperature probe (Ahlborn GmbH, Holzkirchen, Germany). Ambient room temperature in the laboratory was recorded during the course of the experiment. All measurements were registered every 2 min by an ALMEMO 2290-8 data monitor (Ahlborn GmbH, Holzkirchen, Germany), connected to a laptop computer.

Figure 1. Experimental set-up.

A. Insufflator/ CO_2 tank
B. Heater/ humidifying device
C. Data monitor for registration of temperature (°C) and relative humidity (% RH)

Placement of sensors:
1. Insufflation port: measurement of gas temperature (°C) and relative humidity (% RH)
2. Abdomen: measurement of intraperitoneal temperature (°C)
3. Rectum: measurement of body temperature (°C)

IN = insufflation port
EX = exsufflation port
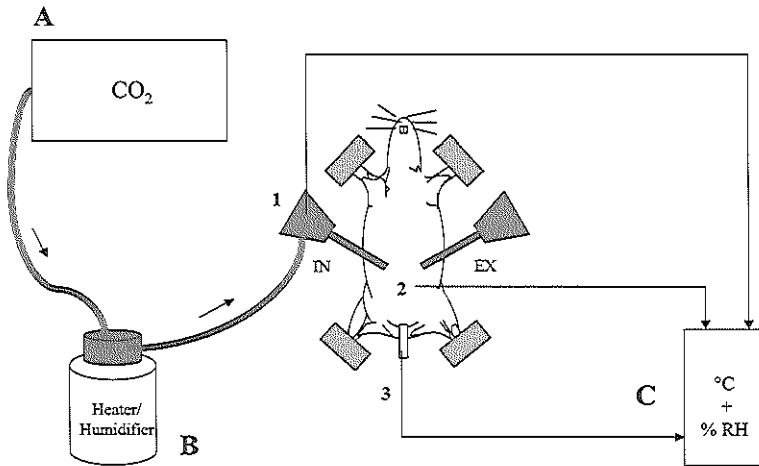
Study design

Sixty rats were randomly allocated to five groups (n = 12 per group). Group I had cold, dry CO_2 insufflation, group II had cold, humidified CO_2, group III had warm, dry CO_2 insufflation, group IV had warm, humidified CO_2 insufflation and group V had 120 min of abdominal wall lifting (gasless control). Duration of pneumoperitoneum was 120 min in all groups. Insufflation started 10 min after induction of anesthesia. In the gasless control group, abdominal wall lifting was established by attaching the trocars to a mechanical arm positioned over the rat.

Scanning electron microscopy (SEM)

Animals were sacrificed at three time points: directly after the insufflation procedure (t0), 2 h after insufflation (t2) and 24 h after insufflation (t24). Four additional animals which did not have either anesthesia or a surgical procedure, served as controls.

In 20 animals of the first group (t0), 10 ml of 2.5 % glutaraldehyde in 0.15 M cacodylate buffer was injected into the abdominal cavity for fixation of the peritoneum (n = 4 per group). Subsequently, the abdomen was desufflated and, after fixation for 5 min, a laparotomy was performed. In the remaining 40 animals (t2 and t24), abdomens were desufflated, trocar wounds were closed and animals were placed back in their cages. At time of sacrifice, 10 ml of 2.5 % glutaraldehyde in 0.15 M cacodylate buffer was injected into the abdominal cavity, and after fixation for 5 min, a laparotomy was performed.

Peritoneal tissue samples of the anterior abdominal wall were resected, distant from the trocar wounds, using a circular tissue clamp. After resection, specimens were further fixed in 2.5 % glutaraldehyde in 0.15 M cacodylate buffer and, subsequently, animals were sacrificed. After washing in cacodylate buffer the tissue was post-fixed in 1 % $OsO4 + 50$ mM k3Fe(CN)6 in cacodylate buffer. Following dehydration in graded alcohol, tissue samples were impregnated in hexamethyldisilazane (HMDS) and air dried. The dried specimens were mounted on stubs, and sputter coated with gold (Agar Scientific Ltd., Stansted, England). All peritoneal specimens were evaluated with a JSM-25 scanning electron microscope (JEOL, Tokyo, Japan). Descriptive analysis of the parietal surface of the peritoneum at five different fields per specimen was performed at various magnifications. The peritoneal surface was examined regarding destruction of cells, changes in mesothelial cell-size, denudation of the basal lamina and loss of tight-junctions.

Statistical analysis

Statistical analysis was performed utilizing the SPSS 9.0 statistical software package (SPSS Inc., Chicago, IL). Inter-group comparisons were analyzed with ANOVA. If ANOVA resulted in $p < 0.05$, a Bonferroni post-hoc test was performed. Intra-group comparisons were analyzed with repeated measures ANOVA using the S.A.S. 6.12 system (S.A.S. Institute Inc., Cary, NC). Statistical significance was accepted at $p < 0.05$. Data in the figures are presented as mean $\pm$ standard deviation (SD).

RESULTS

Values of core temperature and relative humidity of the insufflated gas in group I – IV are described in Table 1. There were no significant differences in body weight, time between induction of anesthesia and start of procedure. All animals survived the two-hour study period. Directly after onset of anesthesia, rats had a mean core body temperature of 35.9 °C. At start of insufflation (t0), core temperature was 35.1 ± 0.54 °C. Baseline measurements of core temperature and intraperitoneal temperature did not differ among groups. Mean room temperature during the experiment was 22.4 ± 0.31 °C.

Table 1. Temperature and relative humidity of the insufflated gas measured at the insufflation port. Values documented as mean (range).

	Cold dry CO_2 (group I)	Cold humidified CO_2 (group II)	Warm dry CO_2 (group III)	Warm humidified CO_2 (group IV)
Temperature (° C)	24.9 (24.4 – 25.9)	24.8 (24.2 - 25.9)	36.9 (35.7 – 37.3)	37.1 (36.6 – 37.4)
Relative humidity (%)	4 (2 – 6)	87 (83 – 90)	5 (3 – 7)	88 (84 – 91)

Data on core temperature of the five groups are depicted in Fig. 2. Repeated measures ANOVA showed that there were significant changes in core temperature in group I, II, III and IV ($p < 0.001$ in each group). There were no significant changes in core temperature in group V ($p = 0.42$). Repeated measures ANOVA further showed that the differences between groups significantly depended on time. Therefore, comparisons between the five groups were performed at each measured time point by one way ANOVA.

In animals which had cold, dry CO_2 insufflation (group I), body temperature was significantly lower after 10 min when compared to t0 ($p = 0.003$). After 2 hours of exposure to cold, dry CO_2, body temperature had fallen to 32.4 °C ($p < 0.001$). Insufflation with cold humidified gas (group II) caused a significant decrease in core temperature after 40 min ($p = 0.011$). In the group which had warm and dry insufflation (group III), body temperature was also significantly lower after 10 min ($p = 0.031$) and decreased further to 33.1 °C upon 120 min of insufflation. In the group which received heated and humidified insufflation gas (group IV), core body temperature significantly increased after 40 min ($p = 0.031$). After 2 hours of insufflation, body temperature was 36.4 °C. At that time, the difference in core body temperature between group I and IV was

4.0 °C (p < 0.001). Further analysis by inter-group comparisons showed that after 20 min, core temperature in group I was significantly lower compared to group V (p = 0.022). After 30 min, core temperature in group II and III was significantly lower compared to gasless controls (p = 0.046 and p = 0.009, respectively). After 40 min, core temperature in animals which had warm humidified gas insufflation (group IV) was significantly higher compared to control animals (p = 0.037). After 70 min, core temperature in animals which had cold insufflation was higher in group II when compared to group I (p = 0.014). There were no differences in core temperature between group I and III.
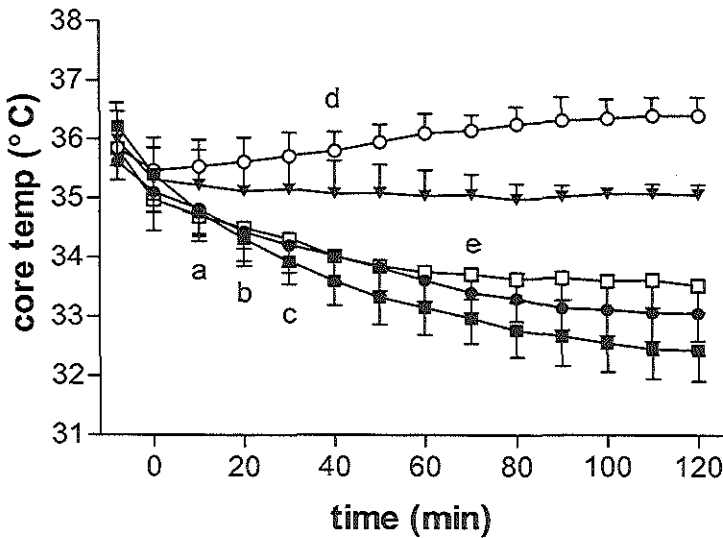
Figure 2. Core temperature in animals which had cold and dry CO_2 insufflation (group I,■), cold and humidified CO_2 insufflation (group II,□), warm and dry CO_2 insufflation (group III,●), warm and humidified CO_2 insufflation (group IV,○) and abdominal wall lifting (group V,▼). Data points represent mean ± SD for each group. Core temperature during the experiment was compared to pre-insufflation levels (t0) and among the five groups.
[a] $p < 0.05$ t0 vs. t10 in group I
[b] $p < 0.05$ t0 vs. t20 in group II and III
 $p < 0.05$ for group I vs. group IV and vs. gasless control (group V)
[c] $p < 0.05$ for group II vs. group IV and vs. gasless control (group V)
 $p < 0.05$ for group III vs. group IV and vs. gasless control (group V)
[d] $p < 0.05$ t0 vs. t40 group IV
 $p < 0.05$ for group IV vs. gasless control (groupV)
[e] $p < 0.05$ for group I vs. group II

Data on intraperitoneal temperature in the five groups are presented in Fig 3. Insufflation with cold and dry CO_2 (group I) caused a significant decrease in intraperitoneal temperature in comparison to pre-insufflation values after 10 min ($p < 0.001$). Intraperitoneal temperature in group I was significantly lower compared with group IV and V after 10 min ($p < 0.001$ for both). Intraperitoneal temperature in the group which had cold humidified insufflation (group II) also decreased significantly after 10 min ($p = 0.045$). In animals which had warm, dry insufflation (group III), intraperitoneal temperature was significantly decreased after 20 min ($p = 0.044$) and remained decreased until the end of the experiment. Intraperitoneal temperature was stable in animals which had warm humidified insufflation (group IV) and in the gasless controls (group V).
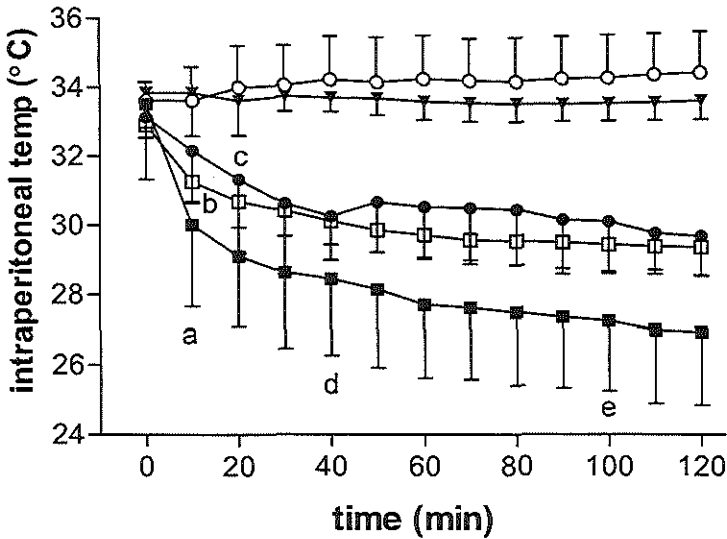
Figure 3. Intraperitoneal temperature in animals which had cold and dry CO_2 insufflation (group I,■), cold and humidified CO_2 insufflation (group II,□), warm and dry CO_2 insufflation (group III,●), warm and humidified CO_2 insufflation (group IV,○) and abdominal wall lifting (group V,▼). Data points represent mean ± SD for each group. Intraperitoneal temperature during the experiment was compared to pre-insufflation levels (t0) and among the five groups.

[a] $p < 0.05$ t0 vs. t10 in group I
  $p < 0.05$ for group I vs. group IV and vs. gasless control (group V)
[b] $p < 0.05$ t0 vs. t10 in group II
  $p< 0.05$ for group II vs group IV and vs. gasless control (group V)
[c] $p < 0.05$ t0 vs. t10 in group III
  $p < 0.05$ for group III vs. group IV and gasless control (group V)
[d] $p < 0.05$ for group I vs. group III
[e] $p < 0.05$ for group I vs. group II

Scanning electron microscopy

Analysis of all parietal peritoneum specimens exposed to abdominal gas insufflation showed that there were no significant differences in morphological changes between animals which had either cold insufflation (group I and II) or warm insufflation (group III and IV). Since values of core temperature and intraperitoneal temperature varied most among the cold, dry insufflation group (group I) and the warm, humidified insufflation group (group IV), the results of group II and III are not discussed.

Figs. 4 and 5 show the normal configuration of the parietal peritoneum in a control animal. The peritoneal surface is a monolayer of flat mesothelial cells with abundant microvilli. The hexagonally shaped mesothelial cells are confluenty distributed without intercellular clefts or visible basal lamina.

Figure 4. Parietal peritoneum of a control animal. Mesothelial cells form an intact, continuous cell layer. Magnification ×150.
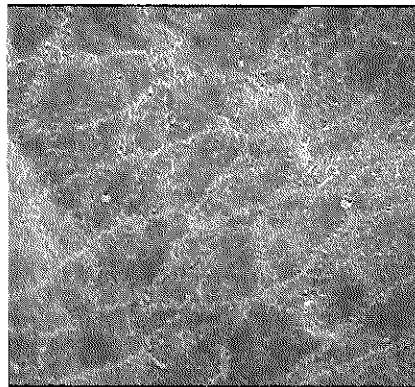
Figure 5. Parietal peritoneum of a control animal. The peritoneum consists of flat mesothelial cells covered with dense microvilli. There are no intercellular clefts or exposed basal lamina visible. Magnification ×700.
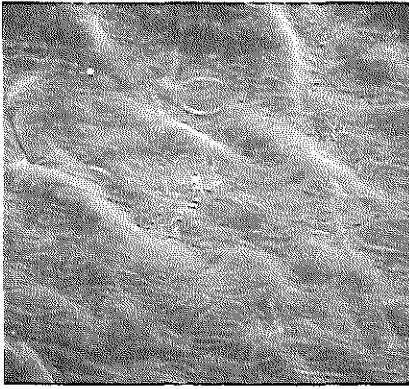
Figure 6. Parietal peritoneum directly after cold, dry CO_2 insufflation (t0). The hexagonal shape of the mesothelial cells disappeared and the number of microvilli is reduced. There are no intercellular clefts visible. Magnification 700X.
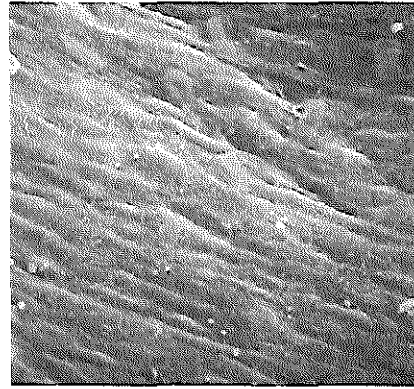
Figure 7. Parietal peritoneum directly after warm, humidified CO_2 insufflation (t0). Exposure to warm, humidified insufflation caused similar changes as detected after cold, dry insufflation (Fig. 6): disappearance of the hexagonal shape of mesothelial cells and a reduction in number of microvilli. Magnification 700X.
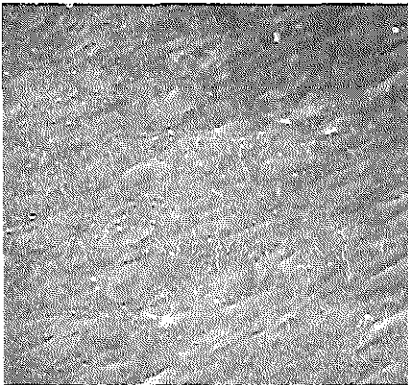
Figure 8. Two hours after abdominal wall lifting (t0), the pattern of mesothelial cell structure had changed to a similar degree as was detected after 2 hours of CO_2 insufflation (Fig. 6 and 7). Magnification 700X.
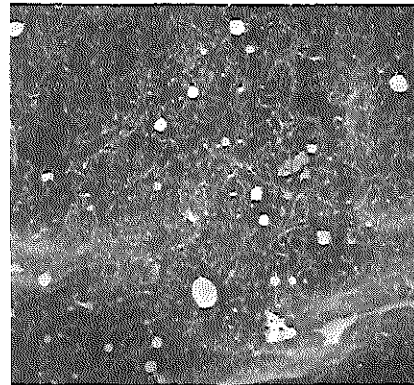
Figure 9. Parietal peritoneum after exposure to cold, dry CO_2 insufflation, 2 h after release of pneumoperitoneum (t2). Numerous peritoneal macrophages and lymphocytes are detected on the mesothelial surface. A similar aspect of sections of the peritoneum was found after either warm, humidified insufflation or abdominal wall lifting (not shown). Magnification 1500X.
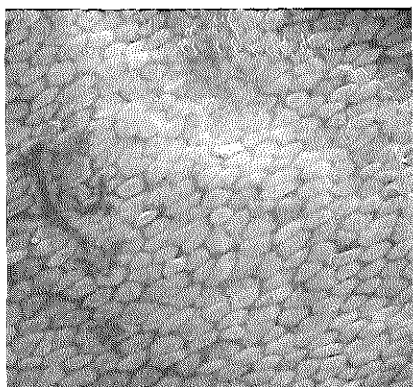
Figure 10. Parietal peritoneum after exposure to cold, dry CO_2 insufflation, 24 h after release of pneumoperitoneum (t24). Mesothelial cells have a retracted and bulged up appearance. Magnification 150X.
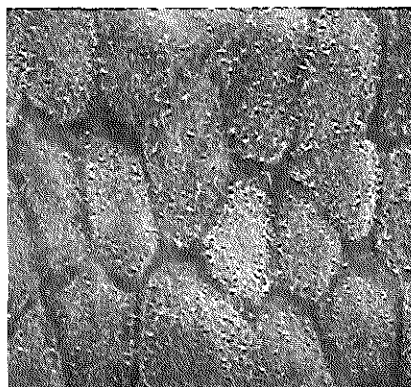
Figure 11. Same section of mesothelium as in Fig 10. (cold, dry CO_2, 24 h), only at a higher magnification. Intercellular clefts are clearly visible throughout the entire section of peritoneum. Magnification 700X.
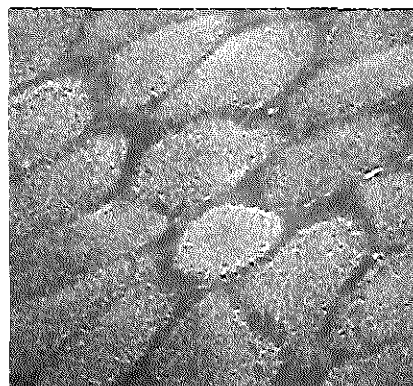
Figure 12. Parietal peritoneum after exposure to warm, humidified CO_2 insufflation, 24 h after release of pneumoperitoneum (t24). Insufflation with warm, humidified gas also caused retraction of mesothelial cells. In all specimens the underlying basal lamina was exposed. Magnification 700X.
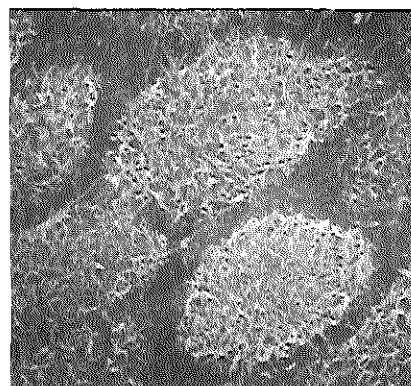
Figure 13. Parietal peritoneum in the gasless control group (t24). 24 h after abdominal wall lifting, mesothelial cells were retracted and the basal lamina was exposed. Magnification 1500X.

65

After two hours of either cold, dry insufflation or warm, humidified insufflation (t0), the hexagonal pattern of mesothelial cells disappeared and the number of microvilli was reduced (Figs. 6 and 7). There were no intercellular clefts visible. In animals which had 2 hours of abdominal wall lifting, a similar pattern of mesothelial cell structure was found (Fig. 8). Two hours after release of pneumoperitoneum (t2), peritoneal macrophages and lymphocytes could be detected on the mesothelial surface of animals insufflated with cold, dry gas and also in the group which had warm, humidified insufflation and in gasless control animals (Fig. 9).

After 24 h, the mesothelial cells had a retracted and bulged up appearance (Fig. 10 and 11). Intercellular clefts were clearly visible throughout the entire section of the parietal peritoneum of animals exposed to either gas insufflation or abdominal wall lifting (Figs. 12 and 13). In all specimens, retraction of mesothelial cells was evident with exposure of the underlying basal lamina. There was no difference in size of the intercellular clefts between the insufflation groups and gasless control animals. In addition, animals which had either cold, dry insufflation or warm, humidified insufflation had a similar distribution of microvilli covering the mesothelial cells.

DISCUSSION

Operative hypothermia is caused by anesthetic agents which disturb thermoregulatory control [14], low temperature in the operating room [15], cold irrigating fluids and infusion of cold intravenous fluids [16, 17], in addition to losses incurred by the actual surgery itself. As a consequence, patient are put at risk for wound infections [9], longer postoperative hospital stay [8] and coagulation disorders [5]. Although the exact incidence of these physiological complications is not known, it is generally believed that if complications from hypothermia do occur, both morbidity and mortality, as well as the length and costs of hospitalization will increase [1, 18]. In anesthesiological literature, it has been shown that warming and humidifying of the inhalation gas are adequate measures to reduce the effects of hypothermia [19-22]. It has often been assumed that laparoscopy, in comparison to laparotomy, would decrease the risk of heat loss because the abdomen is sealed during laparoscopic surgery. However, during laparoscopic procedures, patients are exposed to large volumes of cool insufflation gas, which has been shown to decrease body temperature significantly [23, 24]. Cool, dry CO_2 at approximately 21 °C and 0 % relative humidity is the standard gas that exits the patient outlet from the majority of commercially available insufflators [25].

The findings of our study demonstrate that insufflation of the peritoneal cavity with CO_2 affects body temperature. Insufflation with CO_2 at room temperature caused a significant fall of core body temperature after 2 hours of insufflation. Simultaneous heating and humidifying of the gas stream before insufflating it into the abdomen prevented the decrease in body temperature. These results are in concordance with recent studies by Bessell et al., who concluded that warm, humidified insufflation gas prevented hypothermia during laparoscopic surgery [12]. In their study, the authors demonstrated that the majority of heat lost during laparoscopic procedures was due to intraperitoneal water evaporation induced by abdominal gas insufflation, which could be prevented by humidifying the insufflation gas. Therefore, current commercially available gas insufflators with built-in gas warming devices, but without humidification, would have no additional benefit on body heat homeostasis. Since humidifying devices as described in the study by Mouton et al. [26] were not commercially available in our country at the time of the research, we used an anesthesia respiratory humidifying device which we incorporated between the insufflator and the insufflation hose. Since some cooling of gas does occur in the insufflation hose, we measured gas temperature directly before it entered the abdominal cavity. The findings in our study suggest that humidifying the insufflation gas is a key factor in preventing hypothermia. Insufflation with warm, dry gas could not

prevent hypothermia in this study. In addition, when cold CO_2 was humidified, the decrease in core temperature was smaller when compared to the cold, dry gas.

Especially in advanced laparoscopic procedures, such as colorectal resection or esophageal surgery, repeated gas losses due to frequent removal and insertion of laparoscopic instruments or deflation of electrocautery smoke, may require insufflation of CO_2 at a high flow rate to maintain the pneumoperitoneum. Therefore, the risk for hypothermia increases during lengthy laparoscopic procedures.

In our study, we created a standardized leak to simulate the gas losses during laparoscopic operations. Although it must be considered that in clinical practice gas leakage will occur randomly and not continuously, a continuous leakage rate assured accurate heating of the insufflation gas and prevented any bias between the experimental groups. It can be estimated that the volume of the peritoneal cavity of a rat weighing 250 g is 200 mL. At a flow rate of 0.3 L/min, the intra-abdominal volume is being exchanged 1.5 times per min. Therefore, in a 65 kg human with an intraperitoneal cavity volume of 5 L, the leakage rate would approximately be 7.5 L/min. Although it is evident that such gas leakage is exaggerated to some extent, it was clearly shown that heating and humidifying the gas stream was responsible for the heat preserving effect in this animal model.

Several studies have described benefits associated with warming or heating of the insufflation gas during laparoscopic procedures. Bäcklund et al. described that warming of the insufflation gas resulted in a significantly higher urinary output in patients undergoing prolonged laparoscopic procedures [2]. They concluded that warm CO_2 insufflation may cause local vasodilatation in the kidneys which leads to improved renal function during laparoscopic procedures. In a clinical study by Puttick et al., insufflation of CO_2 at body temperature prevented intraoperative cooling and resulted in a reduced postoperative intraperitoneal cytokine response [24]. Mouton et al. described that humidification of the insufflation gas reduced postoperative pain in patients undergoing laparoscopic cholecystectomy, a finding which may be attributed to less irritation of the peritoneum by the CO_2 gas [26].

In a SEM study in mice by Volz et al., it was shown that the integrity of the parietal peritoneum is temporarily disturbed by insufflation with dry CO_2 [27]. Denudation of the mesothelial surface increases the risk for adherence of tumor cells and adhesions [28-30], which suggests that peritoneal changes due to pneumoperitoneum may render it susceptible to tumor growth or infection [31].

In our study, there was a close correlation between core temperature and intraperitoneal temperature. Although insufflation with warm, humidified CO_2 prevented the decrease in intraperitoneal temperature, there were no differences in morphological alterations of the

peritoneal surface when compared to insufflation with cold, dry CO$_2$. Furthermore, in animals having gasless laparoscopy, exposure of the basal lamina was also documented after 24 hours, which suggests that changes of the mesothelium are also influenced by abdominal distension due to mechanical lifting. As was previously suggested, further studies are needed to investigate if chemically inert gases such as helium also induce changes in mesothelial cell structure and if the visceral and parietal peritoneum react in a similar fashion to abdominal gas insufflation [32]. Studies investigating these insufflation-related effects are currently underway at our institution.

In summary, we conclude that intraperitoneal insufflation with 'standard' cold, dry CO$_2$ may cause a fall of body temperature during laparoscopic surgery. Hypothermia induced by abdominal gas insufflation can be prevented by heated, humidified gas. Changes of the peritoneal surface, occur after CO$_2$ insufflation, regardless of heating or humidifying, but also after gasless surgery. The results in this study warrant validation of heated, humidified insufflation gas in clinical practice.

ACKNOWLEDGMENTS

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CHAPTER 5

THE IMPACT OF INTRAOPERATIVE DONOR MANAGEMENT ON SHORT-TERM RENAL FUNCTION AFTER LAPAROSCOPIC DONOR NEPHRECTOMY

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ABSTRACT

Objective: To determine whether intraoperative diuresis, postoperative recovery and early graft function differ between laparoscopic donor nephrectomy (LDN) and open donor nephrectomy (ODN).

Summary background data: Laparoscopic donor nephrectomy can reduce donor morbidity in terms of decreased pain and shorter convalescence. Although its technical feasibility has been established, concerns have been raised about the impaired renal function due to pneumoperitoneum and short and long term function of kidneys removed by LDN.

Methods: Between December 1997 and December 2000, 89 LDNs were performed at our institution. These were compared to 83 ODNs performed between January 1994 until December 1997. Graft function, intraoperative variables and clinical outcome were compared.

Results: Laparoscopic donor nephrectomy was attempted in 89 patients and completed successfully in 91 % of cases (81/89). Length of hospital stay was significantly shorter in the laparoscopic group. During kidney dissection, the amount of fluids administered and intraoperative diuresis were significantly lower for LDN. In recipients, mean serum creatinine was higher after LDN compared with ODN 1 day after surgery. From postoperative days 2 until 28, there were no differences in serum creatinine. Graft survival rates were similar for LDN and ODN.

Conclusions: Donors can benefit from an improvement in postoperative recovery after LDN. Assessment of an adequate perioperative hydration protocol is mandatory to assure optimal kidney quality during laparoscopic procurement. The initial graft survival and function rates justify continued development and adoption of LDN.

INTRODUCTION

Laparoscopic donor nephrectomy (LDN) has been developed to reduce postoperative pain, shorten convalescence and improve cosmetic outcome of the kidney donor. LDN has the potential to increase the number of kidney donations by removing some of the disincentives inherent to donation [1]. Since the initial report by Ratner et al. [2] in 1995, LDN has been adopted by a number of institutions worldwide. However, concern has been raised about the reported incidence of primary dysfunction of transplanted kidneys after laparoscopic procurement [3]. Although retrospective studies comparing LDN with the conventional approach suggest similar graft function after 1 year, it has been shown that recipients of laparoscopically procured kidneys have higher serum creatinine levels and a greater need for dialysis in the first weeks after transplantation [3-5]. Mechanical injury to the graft, longer warm ischemia time and pneumoperitoneum have all been suggested as causative factors. Clinical and experimental studies have shown that increased intra-abdominal pressure can cause transient renal dysfunction (oliguria) due to impaired renal blood flow, caused by compression of the renal parenchyma and renal vein [6-8]. In view of these data, employment of the laparoscopic approach as an alternative to the gold standard of open nephrectomy can be justified only if it is clear that allograft function after laparoscopic kidney procurement is not at stake. At present, no randomized controlled clinical trials are at hand and follow-up of most series has been relatively short. Further, the current lack of adequate evidence-base for LDN obligates institutions performing this technique to report on safety and effectiveness after engraftment [9].

This article compares our 3-year experience with LDN and that of a control cohort of patients undergoing open donor nephrectomy (ODN) to determine whether early graft function, intraoperative diuresis and postoperative recovery differ between LDN and ODN.

PATIENTS AND METHODS

Patient selection

Laparoscopic donor nephrectomy was performed in 89 patients from December 1997 through December 2000. These laparoscopic donors were compared to a cohort of 83 patients undergoing ODN at our institution from January 1994 through December 1997. After the introduction of the laparoscopic technique in December 1997, ODN was performed in 6 patients, but these were excluded from analysis. The reasons for

performing the open technique were either obesity (n = 4) or multiple renal arteries (n = 2). Patient data were collected from medical records and were compared for age, gender, body mass index, and comorbidity. Operative and postoperative data collected included blood loss, warm ischemia time, length of operation, length of postoperative hospital stay, and complications. Complications were defined as events within the perioperative period that altered patient recovery, prolonged hospital stay, or technically changed the surgical procedure. Pre- and postoperative serum creatinine clearance were calculated using the Cockcroft-Gault formula [10] and were compared between ODN and LDN. During surgery, the amount of administered intravenous hydration fluids, osmotic diuretics administered and urine output in the donor until the moment of nephrectomy were compared. Graft function and survival were compared for the open and the laparoscopic group. Delayed graft function was defined as the need for dialysis in the postoperative period. Quantification of urine production after engraftment was documented in both groups. Mean serum creatinine levels at 1, 2, 3, 4, 5, 7, 14 and 28 days after transplantation was compared for all recipients. Fluid intake and diuresis from the moment of transplantation until 10 hours postoperatively were compared for all recipients. Four living donor transplants with pediatric recipients (younger than 16 years) were excluded from the study.

Candidates for donor nephrectomy were screened thoroughly by medical history, physical examination, blood and urine chemistry, immunologic and infectious diseases studies.

Standard preoperative screening included: renography, Seldinger angiography and selective renal artery angiography in case of more than one renal artery. Ultrasonography was performed to exclude the presence of kidney deformities. Preoperatively, it was decided from which side the kidney would be procured. If both kidneys had normal function and normal anatomy, the right kidney was preferred for LDN, because on this side the gonadal and adrenal veins do not originate from the right renal vein.

Operative technique

All ODNs were performed by the same transplant surgeon. In the first 30 cases, LDN was performed by the transplant surgeon and a general surgeon with advanced laparoscopic training. After 30 cases, LDN was performed by the transplant surgeon, assisted by one of the surgical residents.

All ODNs were performed through a retroperitoneal flank incision, without partial rib resection. LDN was performed under general endotracheal anesthesia with the patient in semilateral decubitus position. The operating table was flexed maximally to expose the space between the iliac crest and the costal margin. Positioning of the patient allowed, if

necessary, conversion to ODN by standard lumbotomy. Orogastric suction and bladder catheterization were used routinely, antibiotics were not routinely administered.

A 30° laparoscope was introduced through a Hasson trocar, placed through a small midline incision, just caudally to the umbilicus. At the end of the procedure this incision was extended to 5 to 6 cm to enable extraction of the kidney. A pneumoperitoneum of no more than 12 mmHg was created and four additional trocars were inserted. One 10-mm trocar port was placed at the lateral margin of the rectus muscle, equidistant of the umbilicus and the superior iliac spine. The second 10-mm trocar was placed laterally between the costal margin and the iliac crest. Two 5-mm ports were placed in the midline between the xiphoid process and the umbilicus. The more cephalad of these two ports was used to insert a small endo-Babcock clamp for retraction of the liver (right nephrectomy) or the spleen (left nephrectomy) by fixing it to the lateral abdominal wall.

The operation for right nephrectomy was conducted as follows: mobilization of the right colon using an ultrasonic device (Ultracision, Ethicon, Sommersville, NJ), opening of the renal fascia and division of the renal fat. The renal vein was dissected up to its entrance in the caval vein and encircled with a rubber vessel-loop to enable gentle traction and correct positioning of the stapling device. Attention was given to occasional lumbar veins at the confluence of the renal and caval veins. In case of multiple renal arteries, medial rotation of the kidney enabled dorsal view and access to hilar structures. Left donor nephrectomy was conducted in a similar fashion: mobilization of the left colon and spleen, dissection of the renal vein up to its crossing with the aorta, dissection of the renal artery, ligation of adrenal and ovarian or spermatic vein with titanium clips, dissection of the ureter, creation of an extraction incision, anticoagulation, division of the ureter, renal artery, renal vein and extraction of the kidney.

After administering 5000 U heparin systemically, the ureter, renal artery, and renal vein were divided using a linear vascular laparoscopic stapler (EndoGIA 30, US Surgical, Norwalk, CT). A plastic extraction bag (Endocatch, US Surgical, Norwalk, CT) was used to extract the kidney through the subumbilical incision. Directly after kidney extraction, hemostasis was restored by protamine sulfate. The kidney was perfused with Euro-Collins solution at 4 °C and stored on ice awaiting transplantation. After closure of the extraction incision, pneumoperitoneum was reestablished and inspection of the operative field was performed. After adequate hemostasis was ensured, ports were removed under direct visualization, the abdomen was desufflated and incisions were closed.

Statistical analysis

Statistical analysis was performed utilizing the SPSS 9.0 (SPSS Inc., Chicago, IL) statistical software package. Comparisons between ODN and LDN were performed using the Mann-Whitney test. Categorical data were reported as absolute number of patients and/or percentage of the group studied and were compared using 2x2 contingency tables and χ^2 tests. Adjustments for multiple covariates were made using linear regression for continuous outcomes. Survival analyses were performed using Kaplan-Meier techniques, compared with log-rank tests. Statistical significance was accepted at $p < 0.05$.

RESULTS

Patient demographics and operative data are shown in Table 1. LDN was attempted in 89 patients and completed in 81 patients (91 %). In six cases, a pneumatic sleeve (Omniport, ASC, Bray, Ireland) was used to allow performance of hand-assisted nephrectomy to control vascular bleeding or to facilitate dissection. Eight patients required conversion to flank laparotomy. In one patient undergoing left LDN, laceration of the splenic capsule required lumbotomy and subsequent splenectomy. Six conversions occurred after vascular injuries to either the lumbar vein or the renal vein. Although blood loss was limited in these cases, the technical difficulty to repair these lesions laparoscopically and the desire to prevent damage to the kidney from prolonged ischemia warranted conversion to lumbotomy. In one patient, bleeding from a trocar site resulting from a lesion from the epigastric vessels necessitated conversion after kidney excorporation.

Postoperative complications after LDN and ODN are listed in Table 2. Both techniques had low complication rates and the number of patients with complication rates was not different. One patient in the ODN group died 6 days after an uneventful donation procedure as a result of cardiac ischemia.

Table 1. Patient demographics and operative data. LDN, laparoscopic donor nephrectomy. ODN, open donor nephrectomy.

		LDN (n = 89)	ODN (n = 83)	*p-value*
Age (yr)(mean + range)		46.9 (20 - 76)	47.1 (20 - 77)	NS
Sex	Male	48 (54 %)	34 (41 %)	NS
	Female	41 (46 %)	49 (59%)	NS
BMI * (mean + range)		25.4 (17 - 35)	25.5 (16 - 36)	NS
ASA †	I	73 (82 %)	68 (82 %)	NS
	II	16 (18 %)	15 (18 %)	NS
Origin	Living-related	69 (78 %)	77 (93 %)	< 0.001
	Living-unrelated	20 (22 %)	6 (7 %)	< 0.001
Side	Left	26 (29 %)	47 (57 %)	0.002
	Right	63 (71 %)	36 (43 %)	0.002
Conversion	LDN	8 (8.9 %)		

* BMI = Body Mass Index
† ASA = American Society of Anesthesiologists classification

Table 2. Postoperative complications in kidney donors. LDN, laparoscopic donor nephrectomy. ODN, open donor nephrectomy.

LDN		ODN	
Hemorrhage, conservative treatment	1	Hematoma	1
Hemorrhage small laparotomy wound, reoperation	1	Urinary tract infection	2
Subcutaneous emphysema	1	Infection epidural catheter	1
Fever	2	Fever	4
Urinary tract infection	2	Exanthema after epidural	1
Bronchitis	1	Incisional hernia	2
Pneumonia	2	Bowel perforation	1
Number of patients with > 1 complication	1		2
Death	0		1

Perioperative data for ODN and LDN are shown in Table 3. Overall, mean operative time from skin incision to closure was longer for LDN (235 vs. 155 min). Mean intraoperative blood loss was comparable between both groups. Length of hospital stay was significantly shorter in the LDN group (3.9 vs. 6.2 days). The reduction in creatinine clearance 2 days after nephrectomy was more pronounced in the laparoscopic group.

Intraoperative hydration and diuresis during kidney dissection were documented until extraction of the graft (Table 4). Patients undergoing ODN had a significantly higher amount of fluids administered (colloids and crystalloids) during kidney dissection (8.1 and 22.4 vs. 3.4 and 16.2 ml/kg/h, respectively). Comparison of intraoperative urine output until the moment of nephrectomy showed that this was significantly lower for the laparoscopic group (1.6 vs. 2.8 ml/kg/h). Information on medication administered to promote diuresis could be obtained in 86 LDNs and 69 ODNs. In 47 % of LDNs, osmotic diuretics or dopamine were administered. Warm ischemia times were significantly longer in the LDN group (7.8 vs. 4.8 min).

Table 3. Perioperative data in kidney donors. Data are given as mean (range). LDN, laparoscopic donor nephrectomy. ODN, open donor nephrectomy.

	LDN	ODN	*p-value*
Length of operation (min)[*]	235 (105 – 420)	155 (75 - 310)	< 0.001
Estimated blood loss (ml)	375 (50 – 2300[†])	352 (100 - 1000)	NS
Hospital stay (days)	3.9 (2 - 9)	6.2 (3 - 31[‡])	< 0.001
Creatinine clearance, preoperatively (ml/min)[§]	104 (55 – 179)	109 (50 - 197)	NS
Creatinine clearance, 2 days postoperatively (ml/min)[§]	72 (41 - 117)	82 (40 - 132)	0.011

[*] Defined as time from skin incision to closure
[†] One patient required blood transfusion due to a lesion from the epigastric vessels.
[‡] Patient requiring reoperation due to bowel perforation; stay was 31 days.
[§] Calculated using the Cockcroft-Gault formula.

Table 4. Intraoperative data in kidney donors. Data are given as mean (range). LDN, laparoscopic donor nephrectomy. ODN, open donor nephrectomy.

	LDN	ODN	*p-value*
IV fluid hydration			
Colloid (ml/kg/h)	3.4 (0 - 11.7)	8.1 (0 - 92.7)	< 0.001
Crystalloid (ml/kg/h)	16.2 (4.3 - 51.4)	22.4 (2.0 - 45.5)	< 0.001
Administration of diuretics[*] or dopamine			
(number of cases)	40/86 (47 %)	27/69 (39 %)	NS
Urine output until nephrectomy (ml/kg/h)	1.6 (0.3 - 6.1)	2.8 (0.4 - 11.8)	< 0.001
Warm ischemia time (min)	7.8 (2 -17)	4.8 (2-12)	< 0.001

[*] Mannitol and/or furosemide.

Graft function and survival are compared in Table 5. Information on intraoperative urine production of the kidney during engraftment was documented in the operative charts of 62 LDNs and 78 ODNs. There was a significant difference between LDN and ODN grafts that were producing urine, subsequent to reperfusion (73 % vs. 88 %). Fluid intake of recipients from the moment of transplantation until 10 hours postoperatively was similar in both groups. During this period urine production was lower in recipients of a kidney removed by LDN (2.9 vs. 3.9 liters, p = 0.004).

Mean serum creatinine levels in the first month after transplantation are also shown in Table 5. Preoperatively, there were no differences in serum creatinine levels between LDN and ODN. On postoperative day 1, mean serum creatinine was significantly higher in the laparoscopic group (covariate analysis). From day 2 on, the differences in mean serum creatinine levels between LDN and ODN were no longer significant.

Duration of follow-up was longer for ODN patients because they were performed in the years preceding the introduction of LDN. Graft survival has been maintained in 86 (97 %) of 89 transplanted kidneys after LDN with a mean follow-up of 18.3 months (range 1 - 37 months). Graft failure resulting from arterial trombosis occurred in one patient, 1 day after transplantation, after uneventful laparoscopic harvest, necessitating transplant nephrectomy. We observed delayed graft function in three patients in the LDN group, requiring posttransplant dialysis in the first week.

Table 5. Graft function and survival. * Data expressed as mean ± standard error of mean. LDN, laparoscopic donor nephrectomy. ODN, open donor nephrectomy. POD = postoperative day.

	LDN	ODN	*p-value*
Immediate urine production	45/62 (73 %)	69/78 (88 %)	0.016
Fluid intake until 10 h postoperatively (liters)*	4.9 ± 0.17	4.7 ± 0.15	NS
Urine production until 10 h postoperatively (liters)*	2.9 ± 0.17	3.9 ± 0.23	0.004
Mean serum creatinine (µmol/l)*			
Preoperatively	819 ± 36.6	782 ± 33.9	NS
POD1	416 ± 26.5	344 ± 21.5	0.043
POD2	227 ± 23.5	182 ± 14.9	NS
POD3	195 ± 23.6	147 ± 12.0	NS
POD4	199 ± 23.9	142 ± 11.3	NS
POD5	200 ± 22.9	146 ± 13.3	NS
1 week	197 ± 23.5	157 ± 16.4	NS
2 weeks	155 ± 14.8	138 ± 12.9	NS
4 weeks	136 ± 9.1	127 ± 9.2	NS
Delayed graft function	3/89 (3.4 %)	3/83 (3.6%)	NS
One-year graft survival	86/89 (97 %)	76/83 (92 %)	NS
One-year patient survival	86/89 (97 %)	77/83 (93 %)	NS

DISCUSSION

There are several concerns with the application of the laparoscopic approach for live renal donation. Live donor nephrectomy involves a healthy individual who is subjected to major surgery for the benefit of another individual. At all times, the operation must be safe. Further, laparoscopic procurement of a kidney should provide excellent recipient graft outcomes. Several studies have reported that LDN can be performed safely in selected candidates with a reduction in postoperative morbidity and shorter hospital stays compared to ODN [4, 11-14].

Laparoscopic kidney removal does take more time when compared to the conventional approach. However, it has been shown that operating times of LDN reduce with increased experience [14]. Although most studies comparing LDN with the conventional approach suggest similar graft function after 1 year, concerns have been raised about the reported incidence of primary dysfunction after laparoscopic kidney procurement [3].

The impaired postoperative short-term function of LDN kidney transplants may be due to diminished intraoperative blood flow associated with the pneumoperitoneum, traumatic removal of the kidney graft through a small incision, and longer warm ischemia time [3]. Clinical and experimental studies have shown that increased intra-abdominal pressure can lead to transient renal dysfunction (oliguria) by reducing renal blood flow, caused by compression of the renal parenchyma and renal vein [7, 8, 15, 16]. However, the effects of procurement of a renal allograft in the altered physiologic environment of pneumoperitoneum are not fully understood. One of the aims of the present study was to retrospectively evaluate perioperative intraoperative fluid requirements for LDN and ODN. Analysis of the operative charts of all kidney donors between 1994 and 2000 revealed that during LDN, a significantly lower amount of fluid was administered during kidney dissection, and that urine production until the moment of kidney excorporation was lower when compared to the conventional approach. The importance of maintaining urine output at approximately 300 mL/h during LDN has been stressed [13], however, details on intraoperative diuresis during LDN are seldom reported. In our series, only 47 % of cases undergoing LDN received administration of mannitol or dopamine to encourage renal perfusion. At the initiation of this study, it was our impression that some recipients of a laparoscopically procured kidney do not have immediate function after reperfusion. Analysis of recipients' operative charts showed that after engraftment, confirmation of a brisk diuresis after declamping the renal artery was reported in 88 % after ODN and in 73 % after LDN. Although there was no difference in the amount of fluid administered from start of the transplantation procedure until 10 hours

postoperatively, during this period urine production was significantly lower in LDN recipients. This difference in postoperative graft function is reflected in a higher mean serum creatinine on postoperative day 1 after LDN. However, from day 2 onwards the differences in mean serum creatinine levels between LDN and ODN were no longer significant. These findings suggest that LDN grafts have a slower initial function compared with ODN, but there is no difference in longer-term renal function. In addition, there have been no differences in the incidence of delayed graft function or graft survival between the two groups.

Mean warm ischemia time after LDN in this study was 7.8 min, which is longer than reported in other studies [4]. Despite a longer warm ischemia time, there were no differences in delayed graft function between LDN and ODN. It is not clear what constitutes an acceptable limit, but with increased experience warm ischemia time can be reduced [4].

Unlike other groups, we performed a substantial number of right-sided LDNs, which is often considered to be one of the drawbacks of the laparoscopic approach [13, 17]. In all right-sided LDNs performed, an adequate length of the renal vein could be obtained. In addition, no problems occurred when performing the venous anastomosis.

In eight patients, the laparoscopic procedure was converted to ODN, and subsequently completed. In our opinion, adopting a low threshold to convert to an open procedure is necessary to assure procurement of the kidney graft in absolutely pristine condition with minimal risk for the donor.

Previously, it has been stressed that it will take years before data on long term graft function after LDN are available [9]. Therefore, the current lack of adequate evidence base for LDN obligates institutions performing this technique to report on safety and effectiveness after engraftment [9]. The present study shows our 3-year experience with LDN. In general, it must be stressed that development of a successful living-donor program requires a dedicated, coordinated multidisciplinary approach. Operating times of LDN are longer and, therefore, hydration protocols should be adjusted accordingly. The disparity in intraoperative fluid requirements and diuresis as reported in this study effected improvements in our anesthetic protocol for patients undergoing LDN. In an attempt to ameliorate the decrease in venous return resulting to increased intra-abdominal pressure, vigorous hydration is now employed intraoperatively to promote adequate diuresis [18, 19]. Preoperative fluid administration to the donor may be another measure to improve hemodynamics in the kidney [4]. We have started a prospective randomized clinical trial comparing LDN with ODN that will allow a more valid comparison of recuperation, complications, and graft function.

In conclusion, LDN is a technically feasible but demanding procedure that can be performed with morbidity and mortality rates comparable to ODN. Donors can benefit from an improvement in postoperative recovery. Although initial graft function and survival rates after LDN are good, long-term follow-up is needed. Special care should be given to intraoperative fluid administration and anesthesiologists should be trained in the hemodynamic consequences of a pneumoperitoneum during LDN.

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CHAPTER 6

ANTIDIURETIC HORMONE RELEASE DURING LAPAROSCOPIC DONOR NEPHRECTOMY

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ABSTRACT

Background: During laparoscopic procedures, increased intra-abdominal pressure may cause transient renal dysfunction due to impaired renal blood flow and induction of neurohumoral factors. However, the relationship between antidiuretic hormone (ADH) secretion and increased intra-abdominal pressure is poorly understood.

Hypothesis: Laparoscopic donor nephrectomy (LDN) is associated with an increase of plasma ADH concentration which influences renal function in both the donor and transplanted graft.

Objective: To evaluate plasma ADH levels during LDN and to correlate ADH levels with graft function.

Design and Interventions: In 30 patients who underwent LDN, plasma ADH levels were collected before insufflation, during surgery, after desufflation and 24 h after the procedure. In 6 patients who had open donor nephrectomy, blood samples were obtained as controls. Furthermore, graft function, operative characteristics and clinical outcome were compared.

Setting: University hospital.

Results: In the LDN group, mean ADH levels during pneumoperitoneum and 30 min post-insufflation were significantly higher compared to pre-insufflation values ($p < 0.001$). Twenty-hours after LDN, mean ADH levels had returned to normal values. There were no significant differences in ADH levels in the open donor nephrectomy group. No significant differences in either intra-operative diuresis, blood pressure readings or postoperative graft function were documented among the two groups.

Conclusion: In this study, LDN was associated with an increase in plasma ADH that appeared to be related to increased intra-abdominal pressure. We conclude that the increased ADH concentrations during LDN are not associated with clinically significant changes in either the kidney donor or the transplanted graft.

INTRODUCTION

Laparoscopic surgery requires the use of a pneumoperitoneum to create a working space in the abdominal cavity for safe introduction of trocars and other instruments as well as exposure of the abdominal contents. Intraperitoneal insufflation with carbon dioxide (CO_2) is the most commonly used method to elevate the abdominal wall and suppress the viscera. Clinical and experimental studies have shown that increased intra-abdominal pressure is associated with transient renal dysfunction (oliguria) due to impaired renal blood flow, as a consequence of compression of the renal parenchyma and renal vessels, and increased systemic resistance [1-3]. In addition, renal function may be influenced by the release of neurohumoral factors such as catecholamines, endothelin or antidiuretic hormone (ADH) following increased intra-abdominal pressure [4]. Several studies have reported elevated ADH levels during increased intra-abdominal pressure [5-8], although the actual mechanism for this occurrence is poorly understood. The major stimuli of ADH secretion are an increase in plasma osmolality and a decrease in the effective circulatory volume. Increased ADH secretion promotes renal water reabsorption, reducing plasma osmolality and increasing plasma volume [9]. However, the clinical significance of ADH release during pneumoperitoneum is still unclear.

Recently, the laparoscopic approach has been adopted for the procurement of a kidney from a living donor for transplantation purposes [10]. Concern has been raised about the reported incidence of primary dysfunction of transplanted kidneys after laparoscopic procurement [11]. To our knowledge, no other study to date has investigated the relationship between renal function of the donor and renal graft and fluctuations in ADH levels.

The objective of this study was to evaluate plasma ADH levels during laparoscopic donor nephrectomy (LDN) and to correlate ADH levels with graft function.

PATIENTS AND METHODS

Patient selection

From November 1999 through December 2000, 38 live donor nephrectomies were performed. Laparoscopic donor nephrectomy (LDN), currently the standard approach for this procedure in living donors at our institution, was performed in 32 patients; a conventional open donor nephrectomy (ODN) was performed in 6 patients. The open procedures were performed in patients with severe obesity (n = 4) or multiple renal arteries on both sides (n = 2).

Candidates for donor nephrectomy were thoroughly screened using medical history, physical examination, blood and urine chemistry, immunological studies and screening for infectious diseases. Informed consent was obtained in all cases. The standard preoperative work-up included: renography, Seldinger angiography and selective renal artery angiography in cases of more than one renal artery. Ultrasonography was performed to exclude the presence of kidney deformities. In patients with normal function and anatomy of both kidneys, the right kidney was preferred for LDN; on the right side the gonadal and adrenal veins do not insert into the renal vein leaving vascular dissection less time-consuming. All LDNs and ODNs were performed by a single transplant surgeon (JNM IJ.).

Patient data were compared for age, sex, body mass index, and American Society of Anesthesiologists score. In all donors, we documented blood loss, length of operation, mean arterial pressure and urinary output from time of anesthesia induction until nephrectomy. In recipients, mean serum creatinine levels at 1, 2, 3, 4, 5, 7, 14 and 28 days after transplantation were documented to assess graft function.

Operative technique

Laparoscopic donor nephrectomy (LDN) was performed using general endotracheal anesthesia with the patient in the semi-lateral decubitus position. Details of this procedure have been reported previously [12, 13]. A 30° laparoscope was introduced through a Hasson trocar and placed through a small midline incision, just caudally to the umbilicus. A pneumoperitoneum of less than 12 mmHg was created and 4 additional trocars were inserted. Right nephrectomy was conducted as follows: mobilization of the right side of the colon, opening of the renal fascia and division of the renal fat. The renal vein was dissected up to its entrance into the caval vein and encircled with a rubber vessel-loop to enable gentle traction and correct positioning of the stapling device. After systemically administering 5000 U of heparin, the ureter, renal artery and renal vein were divided using a linear vascular laparoscopic stapler (EndoGIA 30, US Surgical, Norwalk, Conn.). Left nephrectomy was conducted in a similar fashion: mobilization of the left side of the colon and spleen, dissection of the renal vein up to its point of crossing with the aorta, dissection of the renal artery, ligation of the adrenal and ovarian /or spermatic veins with titanium clips, dissection of the ureter, creation of an extraction incision, anticoagulation, division of the ureter, renal artery, and renal vein, and extraction of the kidney. A plastic extraction bag (Endocatch, US Surgical, Norwalk, Conn.) was used to remove the kidney through the enlarged subumbilical incision. Directly after kidney extraction, hemostasis was restored using protamine sulfate. The kidney was perfused with Eurocollins solution at 4

°C and stored on ice awaiting transplantation. After closure of the extraction incision, pneumoperitoneum was re-established and inspection of the operative field was performed. After assurance of adequate hemostasis, the ports were removed using direct visualization, the abdomen was desufflated and the incisions were closed. Renal transplantation was commenced following LDN.

Hormonal measurements

Blood samples were obtained after the induction of anesthesia (T0), 30 min after the installation of pneumoperitoneum (T1), and 90 and 150 min after the start of insufflation (T2 and T3, respectively). At 30 min after abdominal desufflation and extraction of the kidney, another sample was obtained (T4). A final blood sample was obtained, 1 day postoperatively (T5). In the 6 ODNs, blood samples (approximately 5 mL of blood at each time point) were obtained at similar times. Samples were obtained in tubes primed with EDTA (K3 15%, 0.054 mL, 0.34 M/10 mL) for the measurement of ADH. Samples were immediately placed on ice and centrifuged for 10 min at 4 °C (3000 rpm), and aliquots were stored at −20 °C until analysis. Antidiuretic hormone was analyzed by radioimmunoassay using a commercial kit (Bühlmann Laboratories, Basel, Switzerland).

Statistical analysis

Statistical analysis was performed with the supervision of a statistician (E.W.S.) using SPSS 9.0 (SPSS Inc., Chicago, Ill.). Patients undergoing ODN and LDN patients were compared using non-parametric analysis of variance (Mann-Whitney U test). The Wilcoxon rank sum test was used for within group comparisons. Percentile values (P_5, P_{25}, P_{50}, P_{75} and P_{95}) were calculated for selected patients at T0 to illustrate the variability and distribution of ADH values during donor nephrectomy. Correlations were determined between urinary output, plasma ADH concentrations and blood pressure readings. Data are summarized as mean ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics and intra-operative data for kidney donors are presented in Table 1. There was a significant difference in body mass index (BMI) between the two groups: 25.6 (range: 19-32) for LDN and 32.6 (range: 29–37) for ODN (p < 0.001). The two groups were similar regarding all other characteristics. Two patients in the LDN group required conversion to flank laparotomy after vascular injuries to either the lumbar or renal vein. Data from these patients were excluded from analysis. The mean operative time from skin incision to closure was similar for both groups (168 vs. 145 min). Intraoperatively, there were no significant differences regarding estimated blood loss, mean arterial pressure, intravenous volume administration or urinary output.

Table 1. Patient characteristics and operative data in kidney donors. Values of continuous variables are means with ranges.

	LDN (n = 30)	ODN (n = 6)	*p-value*
Age (years)	50.6 (25 – 75)	39.5 (24 – 55)	0.06
Sex (male : female)	18 : 12	4 : 2	0.19
BMI *	25.6 (19 –32)	32.6 (29 – 37)	0.001
ASA† class I : II	25 : 5	4 : 2	0.09
Duration of operation (min)	168 (90 – 270)	145 (120 – 175)	0.19
Estimated blood loss (mL)	313 (50 – 1000)	480 (130 – 1200)	0.60
Mean arterial pressure (mmHg)	83 (63 – 110)	79 (73 – 85)	0.81
i.v. fluid hydration (mL/kg/h)	21.4 (12.5 - 42.4)	19.5 (13.8 – 26.9)	0.58
Urinary output until nephrectomy (mL/kg/h)	1.8 (0.5 – 4.6)	2.0 (0.3 – 3.5)	0.79

* BMI = Body Mass Index
† ASA = American Society of Anesthesiologists classification

Fig. 1a. shows plasma ADH levels during LDN. After insufflation (T1), ADH levels were significantly increased compared with pre-insufflation levels (T0) ($p < 0.001$). During laparoscopic dissection, ADH levels remained significantly increased (T2 and T3) ($p < 0.001$ and $p = 0.003$, respectively). Thirty min after kidney extraction and subsequent desufflation (T4), ADH levels were still significantly higher ($p < 0.001$). Twenty-four hours after the procedure (T5), plasma ADH levels decreased to control values, but were still significantly higher compared with T0 ($p = 0.003$).

Fig. 1b. shows the plasma ADH concentrations during ODN. There were no significant increases in ADH levels during or after donor nephrectomy.

In Fig. 2, percentile values (P_5, P_{25}, P_{50}, P_{75} and P_{95}) of ADH levels during LDN (T0, T1, T2, T3 and T4) are presented. This figure illustrates the large variability in plasma ADH concentration during the laparoscopic procedures and shows that the relative increase in ADH concentration is similar in all percentiles. During almost the entire duration of the operation, the median value (P_{50}) of the plasma ADH concentration was still within the normal range (0.20 - 4.7 ng/L). In 53 % of patients (16/30) who had LDN, the increase in plasma ADH levels during pneumoperitoneum was still within this range (data not shown).

Figs. 3 a-c show the results of the regression analysis that was performed to investigate if urinary output was influenced by either ADH or blood pressure. During pneumoperitoneum, no significant correlation was found between an increase in mean ADH concentration and intra-operative urinary production ($R = 0.24$, $p = 0.18$) (Fig. 3a), neither was there a correlation between mean arterial pressure and intra-operative urinary production ($R = 0.11$, $p = 0.52$) (Fig. 3b.). Fig. 3c shows that for individual patients who had LDN, a relative increase in ADH concentration was not correlated with diminished intraoperative urinary output ($R = 0.25$, $p = 0.23$).

In all cases, transplantation was successful. Graft function, reflected by mean serum creatinine levels in recipients, was not significantly different at any time point following the laparoscopic or open procedure (Table 2).

mean ADH levels during LDN
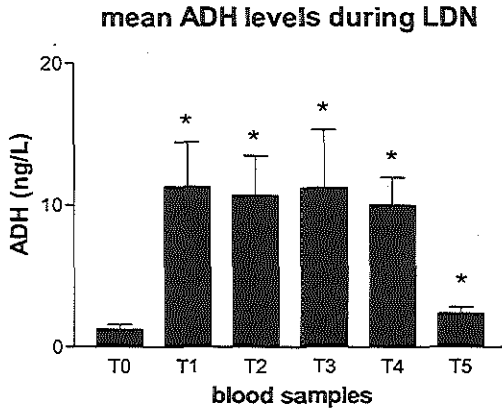
Figure 1a. Plasma antidiuretic hormone (ADH) levels in patients having laparoscopic donor nephrectomy (LDN). T0 indicates pre-insufflation; T1, T2 and T3 indicate 30, 90 and 150 min after the start of insufflation, respectively; T4 indicates 30 min after desufflation and T5 indicates 24 h after the procedure.
Data are presented as mean ± SEM. Normal values for plasma ADH concentrations range between 0.20 and 4.7 ng/L.
* indicates $p < 0.05$ compared to T0.

mean ADH levels during ODN
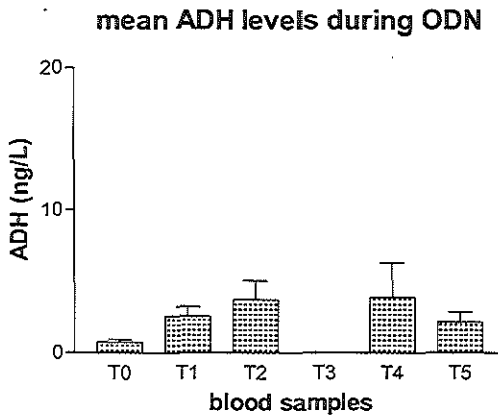
Figure 1b. Plasma ADH levels in patients having open donor nephrectomy (ODN). T0 indicates before skin incision; T1 indicates 30 min after skin incision; T2 indicates 90 min after skin incision; T4 indicates 30 min after kidney extraction; T5 indicates 24 h after the procedure. Data are presented as mean ± SEM. Normal values for plasma ADH concentrations range between 0.20 and 4.7 ng /L. No value was measured at T3, because of the shorter operating time for ODN.

distribution of ADH levels during LDN
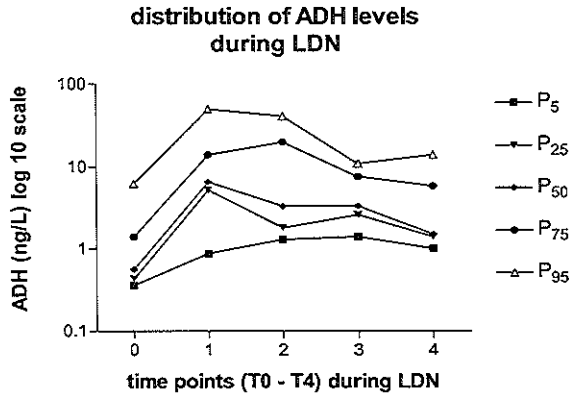
Figure 2. Percentile values (P_5, P_{25}, P_{50}, P_{75} and P_{95}) of antidiuretic hormone (ADH) levels during laparoscopic donor nephrectomy (LDN) were calculated at T0 (pre-insufflation) to illustrate the large variability in plasma ADH concentration during the laparoscopic procedures. The logarithmic scale demonstrates that during pneumoperitoneum, there is a relative increase in ADH concentration compared with T0, which is similar in all percentiles. In addition, it shows that during almost the entire length of the operation, the median value (P_{50}) of plasma ADH is still within the normal range (0.20 - 4.7 ng/L).
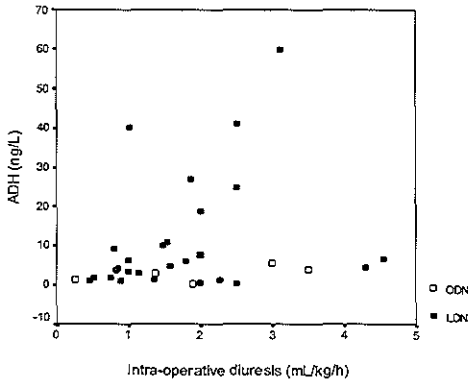
Figure 3a. Correlation between mean antidiuretic hormone (ADH) concentration and intra-operative urine production during laparoscopic donor nephrectomy (LDN) and open donor nephrectomy (ODN). In both groups, there was no significant correlation between mean ADH concentration and intra-operative urine production (R = 0.24, p = 0.18).
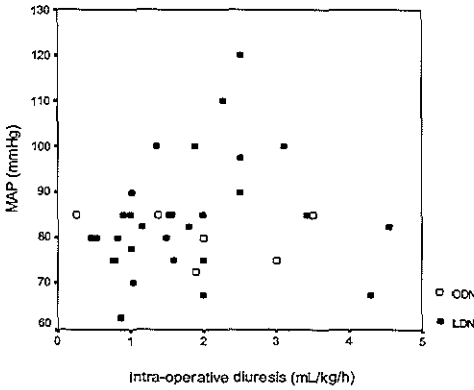
Figure 3b. Correlation between mean arterial pressure (MAP) and intra-operative urine production. Blood pressure did not affect intra-operative urine production in either LDN or ODN. (R = 0.11, p = 0.52).
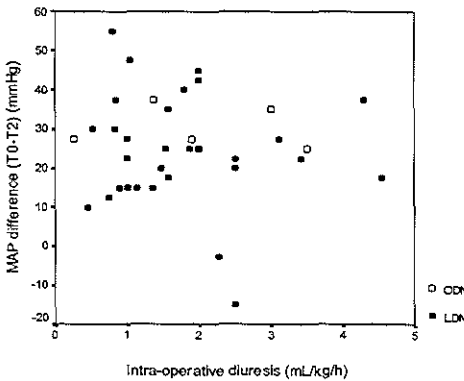
Figure 3c. Correlation between the relative increase in ADH concentration (calculated as T2 - T0/T0 x 100%) and intra-operative urine production during LDN and ODN. In all patients, the increase in ADH concentration did not correlate with a reduction in intra-operative urine production (R = 0.25, p = 0.23).

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Table 2. Recipient graft function. POD = postoperative day. Data are expressed as mean ± SEM (standard error of mean).

Mean serum creatinine (μmol/l)	LDN	ODN	*p-value*
Preoperatively	843 ± 152	594 ± 108	0.16
POD1	399 ± 37.5	258 ± 66.9	0.19
POD2	176 ± 15.5	162 ± 49.9	0.63
POD3	139 ± 11.6	168 ± 39.3	0.60
POD4	147 ± 12.2	169 ± 32.7	0.51
POD5	146 ± 10.7	130 ± 19.9	0.77
1 week	142 ± 9.4	112 ± 13.8	0.23
2 weeks	125 ± 7.7	122 ± 11.6	0.81
4 weeks	127 ± 7.7	130 ± 12.8	0.71

DISCUSSION

Laparoscopic donor nephrectomy can reduce donor morbidity in terms of decreased post-operative pain and shorter convalescence. As in most laparoscopic procedures, LDN is usually performed using intraperitoneal insufflation with CO_2 to facilitate the required working space. However, the effects of procuring of a renal allograft in the altered physiologic environment of pneumoperitoneum are not fully understood. Although transient renal dysfunction during a prolonged period of increased intra-abdominal pressure has been well documented [14], various mechanisms such as renal vessel compression, renal parenchymal compression and systemic hormonal effects have been described to explain these changes [1-3]. In a previous study comparing 89 LDNs with 83 ODNs, we found that intra-operative urinary output was significantly lower during the laparoscopic procedure, a finding that may be caused by insufficient intra-operative hydration of the donor [13]. However, it has been suggested that increased plasma ADH levels contribute to oliguria due to pneumoperitoneum [15]. Therefore, the objective of our study was to prospectively evaluate plasma ADH levels during LDN and to correlate ADH levels with graft function.

This study shows that ADH levels may increase during LDN. After start of insufflation with CO_2, mean ADH levels were significantly higher compared with pre-insufflation values. During laparoscopic kidney procurement, ADH levels remained significantly elevated even until 30 min after abdominal desufflation. Twenty-four hours after LDN, mean ADH levels had returned to normal, but were still significantly higher compared with pre-insufflation values. In patients who had ODN, ADH levels did not significantly change. Therefore, the cause of the increase in plasma ADH levels appears to be the increased intra-abdominal pressure, not the induction of general anesthesia or the mere act of surgery.

An increase in ADH in response to elevated intra-abdominal pressure has been demonstrated both in animal studies [7] and clinical studies [5, 6]. Melville et al. reported a 45-fold increase in ADH levels at an intra-abdominal pressure of 45 mmHg compared with values before the induction of pneumoperitoneum [6]. The mechanism of this massive release of ADH may be explained by reduced cardiac filling pressure due to the impairment of venous return, as was recently suggested by Odeberg et al. [16]. In other studies, no stimulatory effect of increased intra-abdominal pressure on ADH secretion was found [17], indicating that the ADH response due to pneumoperitoneum is still a matter of controversy [18]. In our study, the increase in mean plasma ADH levels during pneumoperitoneum was only 2- or 3-fold compared with normal values. At our institution, maximal insufflation pressure during LDN does not exceed 12 mmHg, which suggests that vasopressor substances may be activated at pressures higher than those currently used for clinical pneumoperitoneum [16]. Because there was a large variability in plasma ADH concentrations during LDN, we calculated percentiles to illustrate the distribution in ADH levels. In more than 50 % of patients who had LDN, plasma ADH concentrations increased during pneumoperitoneum, but were still within the normal range. In theory, it is possible that a 2-fold increase in plasma ADH level in one patient is consistent with that of a patient in whom the plasma ADH level did not increase following insufflation. Therefore, we calculated the relative increase in plasma ADH concentration during LDN to correlate this data with intraoperative urine production. Comparison of patients with an increased ADH response and their ADH-unchanged counterparts did not reveal a single clinically significant difference. Furthermore, intraoperative blood pressure readings and urinary production were comparable in both groups, and the kidney graft functioned equally well afterwards. Therefore, the clinical significance of the increase in ADH observed during LDN appears limited.

In summary, LDN was associated with an increase in plasma ADH which appears to be related to increased intra-abdominal pressure. However, the increased ADH levels during

LDN were not associated with clinically significant changes in either the kidney donor or the transplanted graft.

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CHAPTER 7

SHORT-TERM IMPACT OF CARBON DIOXIDE, HELIUM AND GASLESS LAPAROSCOPIC DONOR NEPHRECTOMY ON RENAL FUNCTION AND HISTOMORPHOLOGY IN DONOR AND RECIPIENT

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ABSTRACT

Background: Laparoscopic donor nephrectomy has the potential to increase the number of living kidney donations by reducing donor morbidity. However, studies have shown that raised intra-abdominal pressure can result in transient renal dysfunction. Therefore, laparoscopically procured kidneys might be at higher risk for suffering a period of ischemia during pneumoperitoneum. The objective of this study was to investigate the short-term impact of pneumoperitoneum used for laparoscopic donor nephrectomy on renal function and histomorphology in donor and recipient.

Methods: Experiment 1. Kidney donor: Initially, 36 Brown Norway (BN) rats were randomized for three procedures: 2 h of CO_2 insufflation, 2 h of helium insufflation 2 h of gasless technique. After this, a unilateral nephrectomy was performed in all animals. *Experiment 2. Recipient:* Subsequently, 36 donor BN rats were subjected to a similar insufflation protocol, but after nephrectomy, a syngeneic kidney transplantation (BN-BN) was performed. Urine and blood samples were collected on postoperative days 1, 3, 7 and 14 for determination of renal function. Subsequently, donor and recipient kidneys were removed for histomorphological and immunohistochemical analysis.

Results: In both donors and recipients, no significant changes in serum creatinine, proteinuria or glomerular filtration were detected between the CO_2, the helium and the gasless control groups. In both experiments, histological analysis of kidney specimens did not show any deleterious effects caused by abdominal gas insufflation. Although kidney grafts exposed to CO_2 showed significantly higher numbers of CD45+ leukocytes three days after transplantation, immunohistochemical analysis did not show significant differences in number of infiltrating cells (CD4, CD8, ED1, OX6, OX62) between the two insufflation groups and the gasless control subjects.

Conclusions: Abdominal gas insufflation does not have an adverse effect on renal function of the kidney donor, 1 week after laparoscopic donor nephrectomy. No differences in renal function and histomorphology were detected between syngeneic kidney grafts exposed to pneumoperitoneum and gasless controls.

INTRODUCTION

Renal transplantation is considered the treatment of choice for patients with end-stage renal failure. A method of addressing the growing shortage of organs for kidney transplantation involves the use of living kidney donors. Although unilateral nephrectomy can be performed safely in selected candidates, length of hospital stay, reconvalescence, postoperative pain and poor cosmesis face the donor. Laparoscopic resection of a kidney has the potential to reduce donor morbidity and, therefore, lower the threshold for kidney donation [1].

Although retrospective studies comparing laparoscopic donor nephrectomy (LDN) with conventional nephrectomy suggest similar graft function one year after transplantation, it has been shown that recipients of laparoscopically procured kidneys have higher serum creatinine levels and a greater need for dialysis in the first weeks after transplantation [2-4]. Mechanical injury of the graft, longer warm ischemia time and pneumoperitoneum have been suggested as causative factors inherent to the laparoscopic approach. Clinical and experimental studies have shown that increased intra-abdominal pressure is associated with transient renal dysfunction (oliguria) due to impaired renal blood flow caused by compression of the renal parenchyma and renal vein [5-7]. Carbon dioxide (CO_2) is the preferred gas for establishing pneumoperitoneum because it is non-flammable and low in cost. However, rapid absorption of CO_2 through the peritoneal surface can cause respiratory acidosis, which may affect renal vascular resistance because of increased sympathetic activity [8]. Although these pathophysiological effects appear rarely to have a clinically significant impact in short laparoscopic procedures, the effect of prolonged pneumoperitoneum during LDN on donor outcome has been poorly studied. In addition, LDN can only be the new golden standard, if it is clear that allograft function after laparoscopic kidney procurement is not at stake. The objective of this study was to determine the short-term impact of pneumoperitoneum on renal function, histomorphology and immunohistology in both donor and recipient.

MATERIAL AND METHODS

Animals

Male rats of the inbred Brown Norway (BN) strain, weighing 250-300 g and aged 10 to 12 weeks were obtained from Charles River, Someren, The Netherlands. These were bred under specific pathogen-free conditions. The animals were kept under standard laboratory

conditions (temperature 20-24°C, relative humidity 50-60%, 12 h light/12 h dark), fed with a laboratory diet (Hope Farms, Woerden, The Netherlands) and had free access to water. The experimental protocols adhered to the rules laid down by the Dutch Animal Experimentation Act and were approved by the Committee on Animal Research of the Erasmus University, Rotterdam.

Operative procedures

Experiment 1: Kidney donor

Anaesthesia was established with pentobarbital sodium (20 mg/kg/h) administered intraperitoneally (Nembutal®; Sanofi Sante Animale Benelux BV, Maassluis, The Netherlands) every 30 min. The rat was placed on the operating table in the supine position, after which the abdomen was shaved and cleaned with 70% alcohol and dried with gauze. A 5-mm skin incision was made in the midline of the abdomen at two-third between the xiphoid process and the pubis, through which a shortened 5-mm trocar (Ethicon Endo-Surgery, Cincinatti, OH, USA) was introduced and secured with a purse-string suture. Rats randomized to a gaseous pneumoperitoneum were insufflated with CO_2 or helium to a maximum pressure of 8 mmHg. In the gasless control group, abdominal wall lifting was established by a suture attaching the trocar to a mechanical arm positioned over the rat. In all animals, the duration of pneumoperitoneum was 120 min. Body temperature was kept within the normal range by means of a heating pad.

After 120 min of pneumoperitoneum, the abdomen was desufflated and the trocar was removed. Next, a unilateral nephrectomy was performed through a small laparotomy wound. The incisions were closed in one layer with continuous 2-0 silk sutures (B. Braun, Melsungen, Germany) and animals were placed in their cages.

Experiment 2: LDN and kidney transplantation

In this second experiment, rats were randomized for CO_2, helium or gasless pneumoperitoneum as described in experiment 1. Rats were insufflated to a maximum pressure of 12 mmHg. After desufflation, heparin (100 IU) was injected intravenously. Subsequently, the left donor kidney was excised and stored at 4 °C. Kidney transplantation was performed using a modification of the technique described by Fisher and Lee [9]. To ensure fast postoperative recuperation, recipient rats were anesthetized with ether. In the recipient, the kidney graft was transplanted heterotopically. Donor renal artery and vein were anastomosed end-to-side to recipient aorta and vena cava, respectively, using continuous 9-0 prolene (B. Braun, Melsungen, Germany). During surgery, the graft was wrapped in a gauze moisturized with phosphate-buffered saline

(PBS) of 4 °C. The perioperative ischemia time was 30 min. After revascularization, the ureter was anastomosed end-to-end to the distal third part of the recipient's ureter using interrupted 10-0 prolene sutures. Both native kidneys were removed at time of transplantation.

Experimental design

In the first experiment, 36 rats were randomized for either CO_2, helium or gasless pneumoperitoneum. After desufflation, animals were unilaterally nephrectomized. Functional, morphological and immunohistological evaluations were performed on days 1, 3 and 7.

In the second experiment, the donor procedure was repeated, after which a kidney transplantation was performed in 36 animals. In these recipients, functional, morphological and immunohistological evaluations were performed on days 3, 7 and 14 to investigate short-term graft function. The choice of these time points was based on results from previous experiments in our laboratory [10, 11].

Functional measurements

Urine was collected by placing the rats individually in metabolic cages (Tecniplast, Buguggiate (Va), Italy) for 24 h. Protein excretion was measured colorimetrically by addition of pyrogallol red [12]. The glomerular filtration rate (GFR) per 100 g of body weight was based on the clearance of creatinine (GFR [ml/min] = [creatinine]$_{urine}$ multiplied by 24h volume/ [creatinine]$_{serum}$). Serum and urinary creatinine was determined using the Jaffé method without deproteinization.

Macroscopy and histology

At the time of the rats were sacrificed, the ureter of the recipient kidney was inspected for its diameter. The kidney transplants were examined for signs of ischemic damage, such as swelling and infarction. Kidney grafts with early hydronephrosis were excluded from the study.

Donor and recipient kidneys were harvested and fixed by immersion for 48 h in a 3.6% buffered formaldehyde solution after longitudinal bisection and embedded in paraffin. Sections 1 µm thick were stained with hematoxylin and eosin, silver (modified Jones staining) and periodic acid Schiff (PAS) and evaluated by two investigators (I.M.B. and D.P.H.). Briefly, glomerulopathy, interstitial fibrosis, tubular atrophy and capillary or vascular changes were assessed in a blind fashion.

Immunohistology

Representative portions of all kidneys were stained on 5-μm cryostat sections by a three-layer immunoperoxidase technique. After fixation with acetone for 10 min, tissues were dehydrated through graded alcohols to block endogenous peroxidase activity by incubation for 10 min in methanol/0.03% H_2O_2. After rehydration the nonspecific binding was blocked by preincubation with 10% normal rabbit serum (Dako, Copenhagen, Denmark), in PBS/bovine serum albumin 5%. This was followed by 1 h of incubation with primary monoclonal antibodies (Serotec, Oxford, United Kingdom) for identification of CD45+ leukocytes (OX-1), CD4+ cells (W3/25), CD8+ cells (OX-8), monocytes/macrophages (ED-1), dendritic cells (OX-62) and major histocompatibility complex (MHC) class II antigens (OX-6). After each incubation, slides were washed in PBS-Tween 20, 0.1%. A second layer, rabbit anti-mouse IgG (Dako) was then applied for 30 min and after washing, slides were incubated with the third layer, mouse peroxidase-anti peroxidase (Dako) for 30 min. After washing in PBS, the reaction was developed by the addition of diaminobenzidine substrate (Dako) and slides were counterstained in Mayer's hematoxilin for 40 sec, washed, dehydrated and mounted.

The analysis was performed blindly as to the experimental group. Positive cells were counted at 400x magnification using a calibrated micro-ocular grid in more than 16 fields of view and expressed as the number of positive cells per 0.1 mm^2.

Statistical analysis

Statistical analysis of all data on urine production, creatinine, proteinuria, GFR, histomorphology and immunohistology was performed using the Kruskal-Wallis one way analysis of variance (ANOVA) followed by the Mann-Whitney test. Statistical significance was accepted at a p-value less than 0.05.

RESULTS

Renal function
Experiment 1: Kidney donor

Fig. 1 shows urinary production, serum creatinine, proteinuria and GFR of rats that underwent donor nephrectomy after CO_2 and helium insufflation and gasless technique. On postoperative days (PODs) 1 and 3, there were no significant differences in urinary production between the CO_2, helium and gasless control group. On POD 7, urinary production in the kidney donors that had undergone CO_2 insufflation was higher than in the gasless control group ($p = 0.018$). Serum creatinine levels did not significantly differ between insufflation groups on PODs 1, 3 and 7. All rats had a stable urinary protein excretion during the first week after nephrectomy. As a result of the unilateral nephrectomy, GFR was decreased on POD 1, but there were no differences between the three groups.

Experiment 2: Recipient

As shown in Fig. 2, urinary production in recipients of a kidney graft exposed to CO_2, helium and gasless pneumoperitoneum was significantly higher on POD 7 compared to PODs 3 and 14, but this increase occurred independent of the pneumoperitoneum type. Serum creatinine, protein excretion and GFR remained stable in all recipient groups during the 14-day follow-up period.

Macroscopic appearance and histology

All remaining kidneys in animals that underwent LDN had a normal macroscopic appearance. No histologic changes were identified 120 min after establishment of pneumoperitoneum, or 1, 3 or 7 days postoperatively. In the three recipient groups, all kidney grafts had a normal macroscopic appearance. Throughout the 14 days of follow-up, all ureters had a normal caliber, without signs of obstruction or leakage .

In the donor groups, no significant histologic changes were identified. Transplanted kidneys showed mild tubular vacuolisation in all three groups at each time point. Although some grafts showed tubular dilatation, no significant differences between insufflation groups and the gasless control group could be detected. On POD 3, no vascular damage was observed, but on days 3 and 14, capillary dilatation was found in the CO_2, helium and gasless group. No significant differences in glomerular aspect were found between the insufflation groups. On POD 14, interstitial cell infiltration was

urine production

A

creatinine

B

proteinuria

C

GFR

D
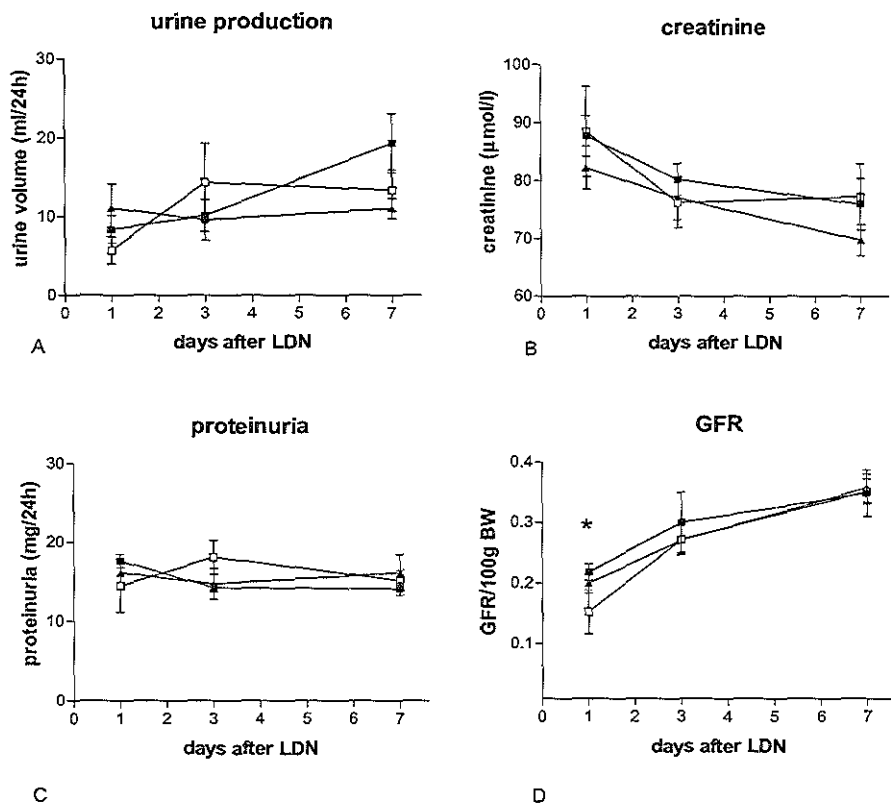
Figure 1 A-D. Renal function in kidney donors after insufflation with carbon dioxide (CO_2) (■), helium (□) and gasless technique (▲). Data are presented as mean ± SEM (error bars). On postoperative days (POD) 1, 3 and 7 there were no significant differences among the three groups in urine production, serum creatinine and proteinuria (Figure 1 A-C). On POD 1, the glomerular filtration rate (GFR) was significantly decreased, but there were no differences between the CO_2, helium and gasless groups (Figure 1 D).
* = $p < 0.05$ vs. pre-insufflation values (data not shown) and compared with POD 7.

urine production

serum creatinine

proteinuria

GFR
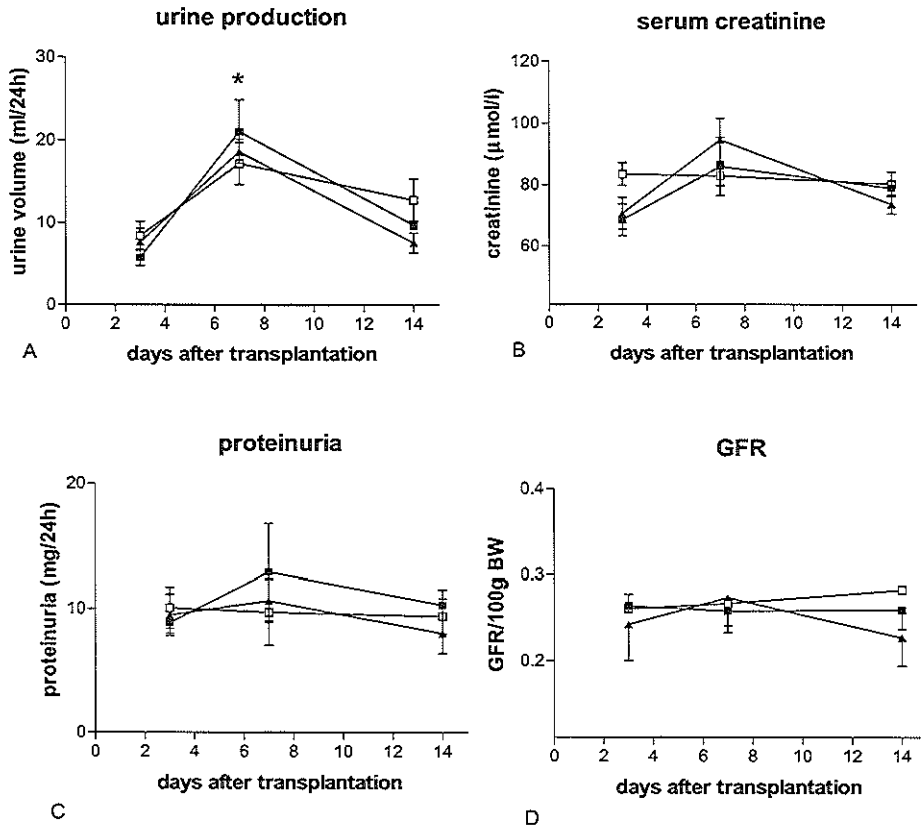
Figure 2 A-D. Renal function in recipients of a syngeneic kidney after exposure of the donor to insufflation with carbon dioxide (CO_2) (■), helium (□) or abdominal wall lifting (▲). Data are presented as mean ± SEM (error bars). On postoperative day (POD) 7, urine production was increased in all animals compared with PODs 3 and POD 14. There were no differences in urine production between the CO_2, helium and the gasless groups (Figure 2 A). There were no differences in serum creatinine, proteinuria or glomerular filtration rate (GFR) on PODs 3, 7 and 14 (Figure 2 B-D).

* = $p < 0.05$ vs. POD 3 and POD 14.

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apparent in one specimen in the helium group and in a gasless control graft, but again these differences were not significant.

Immunohistology

Experiment 1: Kidney donor

The type and number of infiltrating cells in kidneys exposed to CO_2 or helium insufflation and gasless technique are depicted in Fig. 3. After 120 min, there were no differences detected in number of CD45+ leukocytes, CD4+ cells, CD8+ lymphocytes, ED1+ macrophages, dendritic cells (OX-62) or MHC class II+ cells (OX-6). On PODs 1 and 3, kidney specimens did not show a deviant pattern in cellular composition. However, on POD 7, kidneys exposed to CO_2 insufflation showed significantly higher number of CD45+ leukocytes in comparison to gasless controls (p = 0.028). Other cellular infiltration markers did not show any differences between kidneys exposed to CO_2, helium or abdominal wall lifting.

Experiment 2: Recipient

The type and number of infiltrating cells in transplanted kidneys exposed to CO_2, helium insufflation and abdominal wall lifting are depicted in Fig. 4. At 3 days after transplantation, significantly higher numbers of CD45+ leukocytes had infiltrated the interstitium of kidney grafts exposed to CO_2 insufflation compared to the helium insufflation group (p = 0.034) and the gasless control group (p = 0.034). The number of CD4+ cells, CD8+ lymphocytes and ED1+ macrophages was not significantly different between the three groups on PODs 3, 7 or 14. Furthermore, kidney grafts exposed to CO_2 showed a higher number of dendritic cells (OX 62) on POD 7 compared to the gasless technique (p = 0.019). On POD 14, the number of MHC class II+ cells was higher in the CO_2 group than in the helium group (p = 0.046).
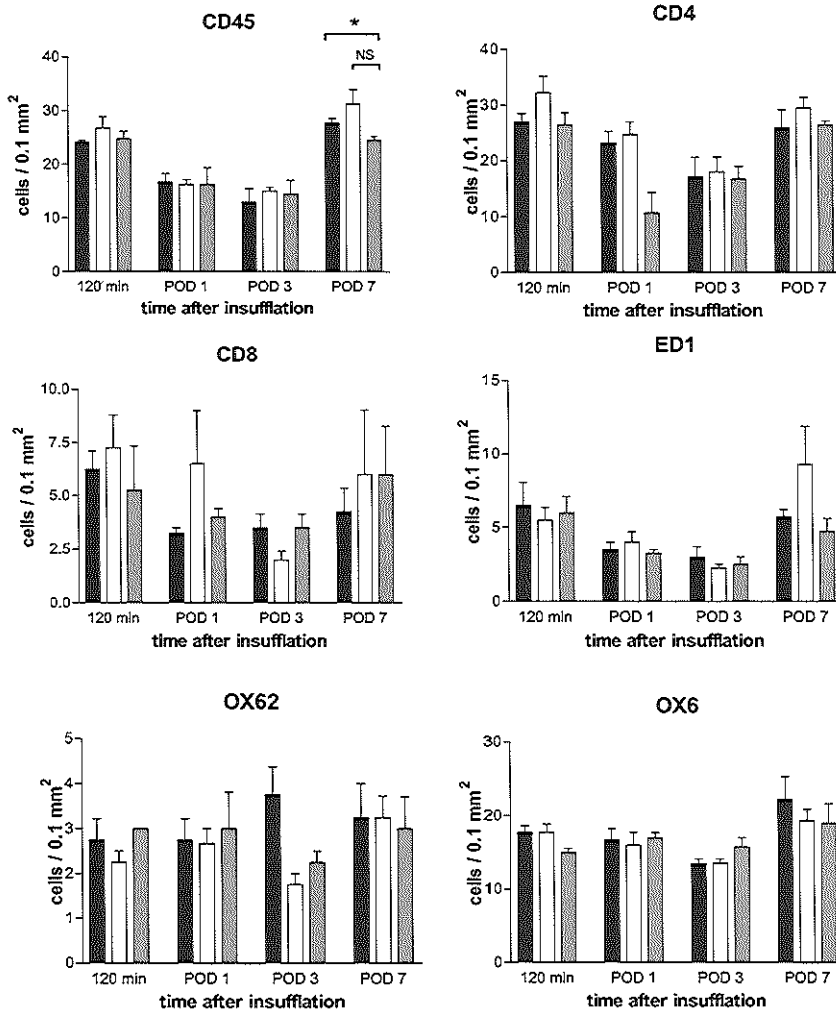
Figure 3. Immunohistologic cell infiltration in kidney donors after insufflation with carbon dioxide (CO_2)(▩), helium (□) or gasless technique (▨). On postoperative day (POD 7), there was a higher number of CD45+ leukocytes in the CO_2 group compared to the gasless control group. Other cellular infiltration markers did not show any differences between the groups after 120 min nor on PODs 1, 3 and 7. CD45 = pan leukocytes, OX62 = dendritic cells, OX6 = MHC class II.
* = $p < 0.05$ for CO_2 vs. gasless control.
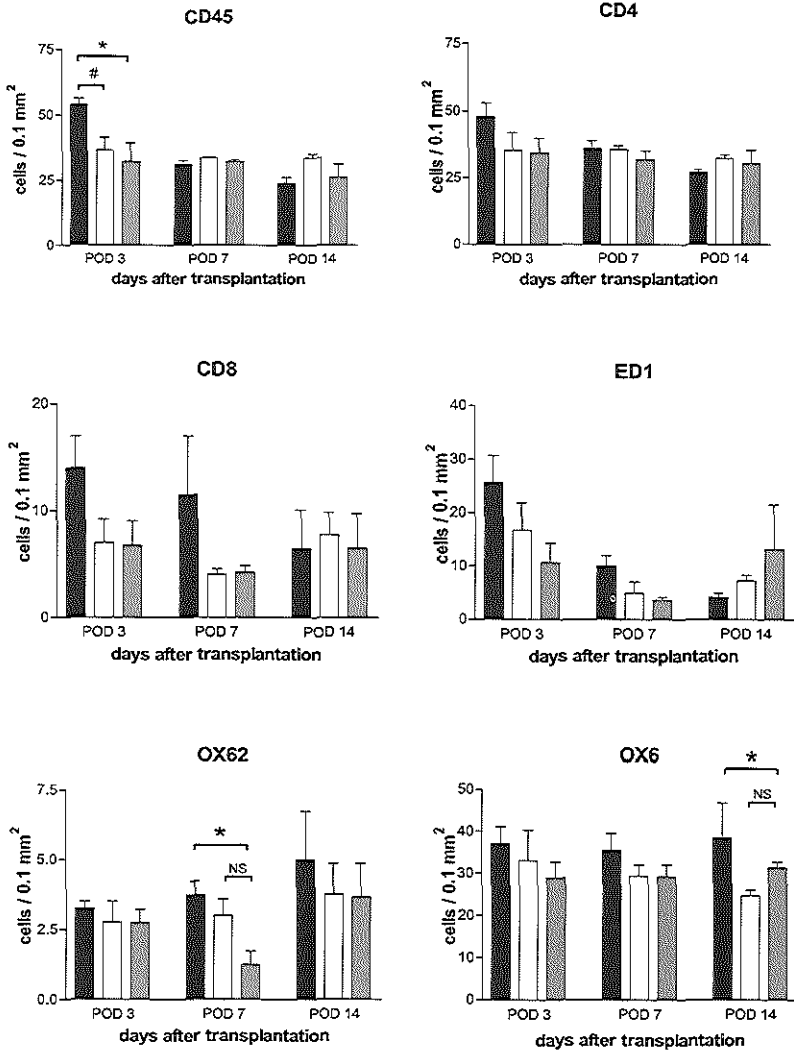
Figure 4. Immunohistologic cell infiltration in transplanted kidneys after insufflation with carbon dioxide (CO_2)(■), helium (□) or gasless technique (▨). On postoperative day (POD) 3, there was a higher number of CD45+ leukocytes in the CO_2 group than in the helium and the gasless control groups. On PODs 3, 7 and 14 there were no differences in CD4+ cells, CD8+ lymphocytes or ED1+ macrophages. CD45 = pan leukocytes, OX62 = dendritic cells, OX6 = MHC class II.
* = p < 0.05 for CO_2 vs. gasless control, # = p < 0.05 for CO_2 vs. helium.

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DISCUSSION

Laparoscopic donor nephrectomy was first described by Ratner et al.[13] in 1995. Concern has been raised about the potential deleterious impact of this new technique on recipient outcome. The greatest difference between the open and the laparoscopic technique is the use of a pneumoperitoneum. Elevated intra-abdominal pressure has been shown to decrease renal blood flow, rendering the graft at risk for the deleterious effect of warm ischemia [4]. The objective of this study was to determine the impact of pneumoperitoneum on donor and recipient outcome in terms of early postoperative renal function and histology. The length of the insufflation period was limited to 2 hours because in our experience longer insufflation periods may cause respiratory depression in spontaneously breathing rats. To differentiate between the potential negative effects of CO_2 absorption, we used helium as an alternative insufflation gas because this inert gas does not influence arterial pH and partial pressure of CO_2 (pCO_2) [14].

This study demonstrates that renal function in a unilaterally nephrectomized rat is not significantly influenced by a prolonged period of CO_2 or helium insufflation, as compared with a gasless control group, in the first week after donor nephrectomy. A 2-h period of abdominal gas insufflation did not show any deleterious effects on renal function in the first 2 weeks after transplantation. In a study using rats, Kirsch et al. [5] demonstrated that 1 h of CO_2 insufflation at a pressure of 10 mmHg reduced renal blood flow, with subsequent oliguria. They showed that 22 h after release of pneumoperitoneum, urinary production had returned to control levels. In our study, we did not measure intra-operative renal function, but the observation that all donor and recipient animals had normal urinary production and normal creatinine levels supports their theory that renal function fully recuperates within 1 day after abdominal desufflation.

Only few data exist concerning the effect of pneumoperitoneum on renal histology. Lee et al.[15] showed that a prolonged period of pneumoperitoneum does not lead to histological changes in rat kidneys within 3 months after the procedure. These findings are consistent with our results, although we used a different study design by simulating a laparoscopic donor nephrectomy and, subsequently, a kidney transplantation. Histopathological evaluation of all donor and recipient kidneys did not show significant differences between the CO_2 and helium insufflation groups and the gasless control group within 2 weeks, suggesting that abdominal insufflation does not have a deleterious effect on histomorphology.

It has been shown that ischemia can increase immunogeneity of the kidney graft by inducing MHC class-II expression [16, 17]. To investigate whether abdominal insufflation

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leads to additional cellular infiltration of the kidney graft, we performed immunohistochemical analysis of all donor and recipient kidneys at three defined time points in the early postoperative phase. By using a syngeneic rat model, it was possible to study the influence of pneumoperitoneum as an isolated factor on cell infiltration and function of the kidney graft, in the absence of an allogeneic response. Kidney grafts exposed to a 2-h period of CO_2 insufflation showed a significantly higher number of CD45+ leukocytes 3 days after transplantation than the grafts exposed to helium insufflation or the gasless technique. Although it appeared that the increase in CD45+ leukocytes consisted of CD8+ lymphocytes and ED-1+ macrophages, these numbers did not reach significance. In addition, analysis of other markers did not show a systematic pattern in cellular infiltration caused by abdominal gas insufflation on PODs 3, 7 or 14. In kidney donors, there was a significantly higher number of CD45+ leukocytes on POD 7 in the CO_2 group compared to the gasless control group. However, there were no significant differences in number of CD45+ leukocytes between the three groups after 2 h of insufflation nor on POD 1 and 3. In addition, there were no differences in CD4+ cells, CD8+ lymphocytes or ED-1+ macrophages at these time points. Therefore, it appears that the observed increased leukocyte count on POD 7 in the CO_2 group is part of normal biologic variation.

Several studies have reported beneficial effects of helium as an alternative insufflation gas. Jacobi et al. [18] observed that CO_2 pneumoperitoneum significantly increased tumor growth in comparison to helium, which appears to be attributed to differences in pH. In theory, CO_2 pneumoperitoneum could impair immunologic processes in the kidney graft by causing cellular acidification. However, our study demonstrates that 2 weeks after kidney transplantation, there is no significant influence on immunohistologic parameters in rat kidney grafts caused by either CO_2 or helium insufflation.

Laparoscopic donor nephrectomy has evolved as an attractive alternative to the conventional approach by reducing donor morbidity. Initial reports of delayed graft function after laparoscopic kidney procurement has not stopped surgeons from adopting this technique, but skepticism concerning recipient outcome persists [19]. Despite many reports on laparoscopic versus open donor nephrectomy, no randomized clinical trials exist [3, 20, 21]. Follow-up evaluation of graft function has been relatively short in most studies. Therefore, it is possible that any alterations in long-term function have not been encountered yet. The results from our study using an established kidney transplantation model are in concordance with data from clinical studies, which imply that the reported initial delayed graft function could be due to factors other than the pneumoperitoneum. Some authors have described a substantial learning curve for LDN, which in some cases

resulted in mechanical damage to the graft and inadequate dissection of renal vasculature or the allograft ureter [2, 22]. Others have noted that warm ischemia times shortened after the surgical team gained more experience [4]. Until results from large studies with adequate length of follow-up evaluation become available, experimental studies may help to gain further insight into kidney graft function after laparoscopic donor nephrectomy.

In summary, we conclude that a prolonged period of insufflation with CO_2 or helium does not impair renal function and renal histomorphology in donors and recipients during the first weeks after donor nephrectomy. Further studies to assess the long-term sequelae of pneumoperitoneum used for LDN are mandatory.

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CHAPTER 8

LONG TERM IMPACT OF PNEUMOPERITONEUM USED FOR LAPAROSCOPIC DONOR NEPHRECTOMY ON RENAL FUNCTION AND HISTOMORPHOLOGY IN DONOR AND RECIPIENT RATS

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ABSTRACT

Objective: To investigate the long term impact of pneumoperitoneum used for laparoscopic donor nephrectomy on renal function and histomorphology in donor and recipient.

Summary background data: Laparoscopic donor nephrectomy (LDN) has the potential to increase the number of living kidney donations by reducing donor morbidity. However, function of laparoscopically procured kidneys might be at risk due to ischemia as a consequence of elevated intra-abdominal pressure during laparoscopy.

Methods: Exp 1. Kidney donor: 30 Brown Norway (BN) rats were randomized for three procedures: 2 hours CO_2 insufflation, 2 hours helium insufflation and 2 hours gasless laparoscopy. After this, a unilateral nephrectomy was performed in all animals. Another 6 rats were used as controls. *Exp 2. Recipient:* 36 donor BN rats were subjected to a similar insufflation protocol, but after nephrectomy, a syngeneic renal transplantation was performed. All rats had a follow-up period of 12 months. Urine and blood samples were collected each month for determination of renal function. After one year, donor and recipient kidneys were removed for histomorphological and immunohistochemical analysis.

Results: In donors as well as in recipients, no significant changes in serum creatinine, proteinuria or GFR were detected between the CO_2, the helium and the gasless control group after one year. No histological abnormalities due to abdominal gas insufflation were found. Immunohistochemical analysis did not show significant differences in number of infiltrating cells (CD4, CD8, ED1, OX62 and OX6) and adhesion molecule expression (ICAM-1) between the three groups.

Conclusions: Abdominal gas insufflation does not impair renal function in the donor, one year after LDN. One year after transplantation, no differences in renal function or histomorphology were detected between kidney grafts exposed to either pneumoperitoneum or gasless procedure.

INTRODUCTION

Renal transplantation is life-saving for patients with end-stage renal failure. A method to address the growing shortage of organs for kidney transplantation is the use of living kidney donors. Although open donor nephrectomy can be performed safely in selected candidates, lengthy hospital stay, reconvalescence, postoperative pain and poor cosmesis face the donor. Laparoscopic resection of a kidney has the potential to reduce donor morbidity and, therefore, lower the threshold to donate a kidney [1].

Although retrospective studies comparing laparoscopic donor nephrectomy (LDN) to conventional nephrectomy suggest similar graft function one year after transplantation, it has been shown that recipients of laparoscopically procured kidneys have higher creatinine levels and a greater need for dialysis in the first weeks post-transplantation [2-4]. Mechanical injury of the graft due to atraumatic handling, longer warm ischemia time caused by more time-consuming extraction and renal ischemia due to pneumoperitoneum have been suggested to cause early graft dysfunction. Clinical and experimental studies have shown that during laparoscopic procedures, increased intra-abdominal pressure can cause transient renal dysfunction (oliguria) due to impaired renal blood flow, caused by compression of both renal parenchyma and renal vessels [5-7]. However, the effect of prolonged pneumoperitoneum on renal function of the remaining kidney of the donor, has been poorly studied. Moreover, LDN can only become the new golden standard, when it is clear that graft function after laparoscopic kidney procurement is not at stake.

The objective of this experimental study is to determine the long term impact of pneumoperitoneum on renal function, histomorphology and immunohistology in both donor and recipient.

METHODS

Animals

Male rats of the inbred Brown Norway (BN) strain, weighing 250-300g and aged 10-12 weeks were obtained from Charles River, Someren, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark), fed with laboratory diet (Hope Farms, Woerden, The Netherlands) and free access to water. The experimental protocols adhered to the rules laid down by the Dutch Animal

Experimentation Act and were approved by the Committee on Animal Research of the Erasmus University Rotterdam.

Operative procedures

Experiment 1. Kidney donor

Anesthesia was established with pentobarbital sodium 20 mg/kg/h intraperitoneally (Nembutal®; Sanofi Sante Animale Benelux BV, Maassluis, The Netherlands), given every thirty min. The rat was placed on the operating table in supine position, the abdomen was shaved and cleaned with 70% alcohol and dried with gauze. After making a 5-mm skin incision in the midline of the abdomen, a shortened 5-mm trocar (Ethicon Endo-Surgery, Cincinatti, OH, USA) was introduced and secured with a purse-string suture. Rats which had a gaseous pneumoperitoneum were insufflated with CO_2 or helium to a maximum pressure of 12 mmHg. In the gasless control group, abdominal wall lifting was established by a suture attaching the trocar to a mechanical arm positioned over the rat. Duration of pneumoperitoneum was 120 min. Body temperature was kept within normal range by a heating pad.

After 120 min of pneumoperitoneum, the abdomen was desufflated and the trocar was removed. Following this, a unilateral nephrectomy was performed through a small midline laparotomy. The incisions were closed in one layer with continuous 2-0 silk sutures (B. Braun, Melsungen, Germany) and animals were placed in their cages.

Experiment 2. LDN and kidney transplantation

In this experiment, rats were randomized for CO_2, helium or gasless pneumoperitoneum as described in experiment 1. Rats were insufflated to a maximum pressure of 12 mmHg for 120 min. After desufflation, heparin (100 IU) was injected intravenously and subsequently the left donor kidney was excised through a midline laparotomy and stored at 4 °C. Recipient rats were anesthetized with ether, after which kidney grafts were transplanted heterotopically, using a modification of the technique described by Fisher and Lee [8]. Donor renal artery and vein were anastomosed end-to-side to recipient aorta and vena cava, respectively, using continuous 9-0 prolene (B. Braun, Melsungen, Germany). During surgery, the graft was wrapped in a gauze moisturized with PBS of 4 °C. The perioperative ischemic time was 30 min. After revascularization, the ureter was anastomosed end-to-end to the distal third part of the recipient's ureter using interrupted 10-0 prolene sutures. The left kidney was removed at time of transplantation, while the contralateral kidney was resected one week later.

Experimental design

In Exp. 1, 30 rats were randomized for either CO_2, helium or gasless pneumoperitoneum. After desufflation, animals were unilaterally nephrectomized. Six rats served as native controls. In these animals, no anesthesiological or surgical procedure was performed. In Exp. 2, the donor procedure was repeated in 36 rats, and subsequently, a syngeneic kidney transplantation was performed.

In all animals, renal function was determined monthly. After 52 weeks, all animals were sacrificed and kidneys were removed for histomorphological and immunohistological evaluation.

Functional measurements

Urine was collected monthly by placing the rats individually in metabolic cages (Tecniplast, Buguggiate (Va), Italy) for 24 hours. Protein excretion was measured colorimetrically by addition of pyrogallol red [9]. The glomerular filtration rate (GFR) per 100 g body weight (BW) was based on the clearance of creatinine {GFR (ml/min) = $[creatinine]_{urine}$ multiplied by 24h volume/ $[creatinine]_{serum}$}. Serum and urinary creatinine was determined using the Jaffé method without deproteinization.

Macroscopy and histology

Kidneys of donor and recipient animals and native controls were harvested at the end of the follow-up period. At the time of sacrifice, the diameter of the ureter of the recipient kidney was measured. The kidney transplants were examined for signs of ischemic damage, such as swelling and infarction. All kidneys were weighed and fixed by immersion for 48 hours in a 3.6% buffered formaldehyde solution after longitudinal bisection and embedded in paraffin. Sections (1 μm) were stained with hematoxylin/eosin, silver (modified Jones staining) and periodic acid Schiff (PAS) and evaluated by 2 investigators (I.M.B. and D.P.H.). histological signs of chronic transplant dysfunction were assessed according to the Banff-criteria [10]. Briefly, glomerulopathy, interstitial fibrosis, tubular atrophy and intimal hyperplasia were determined separately with a score ranging from 0 = normal, 1 = mild, up to 25 % affected, 2 = moderate, 25-50 % affected, and 3 = severe, more than 50 % changes.

Immunohistology

Representative specimens of all kidneys were stained on 5 µm cryostat sections by a three-layer immunoperoxidase technique. After fixation with acetone for 10 min, tissues were dehydrated through graded alcohols. To block endogenous peroxidase activity, tissues were incubated for 10 min in methanol/0.03% H_2O_2. After rehydration the non-specific binding was blocked by preincubation with 10% Normal Rabbit serum (Dako, Copenhagen, Denmark), in PBS/Bovine Serum Albumin 5%. This was followed by one hour incubation with primary monoclonal antibodies (Serotec, Oxford, United Kingdom) for identification of CD4+ cells (W3/25), CD8+ cells (OX-8), monocytes/macrophages (ED-1), dendritic cells (OX-62), major histocompatibility complex (MHC) class II antigens (OX-6) and intercellular adhesion molecule 1 (ICAM-1). After each incubation, slides were washed in PBS-Tween 20, 0.1%. A second layer, rabbit anti-mouse IgG (Dako) was then applied for 30 min and after washing, slides were incubated with the third layer, mouse peroxidase-anti peroxidase (Dako) for 30 min. After washing in PBS, the reaction was developed by the addition of diaminobenzidine substrate (Dako) and slides were counterstained in Mayer's hematoxilin for 40 sec, washed, dehydrated and mounted.

The analysis was done blindly as to the experimental group. Positive cells were counted at 400x magnification using a calibrated micro-ocular grid in >16 fields of view and expressed as the number of positive cells / 0.1 mm^2. For ICAM-1 expression, the intensity on endothelium and tubules was measured semi-quantitatively on a 0-3 scale (0 = none, 1 = mild, 2 = moderate, 3 = dense).

Statistical analysis

Statistical analysis of all data on urinary production, creatinine, proteinuria, GFR, histomorphology and immunohistology was performed using the Kruskal-Wallis one way ANOVA followed by the Mann-Whitney test. Statistical significance was accepted at $p < 0.05$.

RESULTS

Renal function

Experiment 1. Kidney donor

One rat in the CO_2 group died after 44 weeks without any signs of renal dysfunction. All other animals survived the 1-year study period. Figs. 1 a-d show the data on urinary production, creatinine, proteinuria and GFR of rats which underwent donor nephrectomy after insufflation with CO_2 and helium or abdominal wall lifting. There were no significant differences in urinary production among kidney donors in the CO_2, helium or gasless control group, although at one time point (44 weeks) urinary production was higher in the CO_2 group when compared to the gasless control group and the native control group ($p = 0.023$ and $p = 0.022$, respectively) (Fig. 1a).

Mean serum creatinine levels did not significantly differ among the CO_2, helium or gasless control groups. However, at 8 weeks and at 52 weeks, serum creatinine was significantly lower in the native controls when compared to the CO_2, helium and gasless control group ($p < 0.001$ and $p = 0.005$, respectively) (Fig. 1b).

Kidney donors had a stable urinary protein excretion during the 1-year follow-up, although rats in the CO_2 and the native control group had a higher urinary protein excretion when compared to gasless controls at 4 weeks after nephrectomy ($p = 0.043$ and $p = 0.002$, respectively) (Fig. 1c). Significant differences in GFR were documented at two time points during the one year study period (Fig. 1d). At 4 weeks, GFR was significantly higher in the CO_2 group when compared to the gasless and the native control group ($p = 0.006$ and $p = 0.036$, respectively). At 48 weeks, GFR was significantly higher in gasless controls compared to the CO_2 group ($p = 0.010$).

Experiment 2. Recipient

Four rats with a renal transplant did not survive the study period. Two rats in the CO_2 group died after 3 months, one rat in the helium group died after 5 months and one rat in the gasless control group died one week before sacrifice. In all cases, mortality was not associated with signs of renal dysfunction, such as an increase in mean serum creatinine or proteinuria. In addition, kidneys had a normal appearance at autopsy. Figs. 2 a-d show the data on urinary production, creatinine, proteinuria and GFR in recipients of a graft exposed to CO_2 or helium insufflation or abdominal wall lifting. There were no significant differences in urinary production between the CO_2, helium or gasless control group during the study period (Fig. 2a). Mean serum creatinine in the three experimental groups did not significantly differ over time (Fig. 2b).
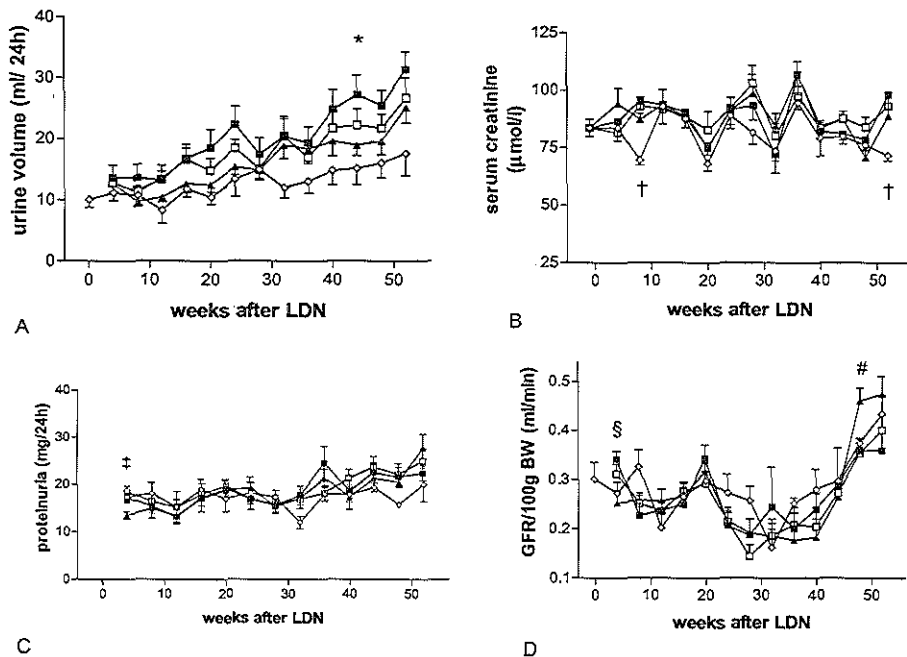
Fig. 1 a - d. Urinary production, serum creatinine, proteinuria and GFR/100g BW (body weight) in *kidney donors* after insufflation with carbon dioxide (CO_2) (■) or helium (□), gasless control (▲) and in the native control group (◇). Data are presented as mean ± SEM (standard error of mean).

*p < 0.05 for CO_2 vs. gasless and native control

† p < 0.05 for native vs. CO_2, helium and gasless

‡ p < 0.05 for gasless vs. CO_2 and native control

§ p < 0.05 for CO_2 vs. gasless

# p < 0.05 for CO_2 vs. gasless and native control

Urinary protein excretion was significantly higher in the helium group when compared to the CO_2 group at 20 weeks (p = 0.013) and in the CO_2 group when compared to the gasless control group at 36 weeks (p = 0.020) (Fig. 2c). After 44 weeks, urinary protein excretion showed a tendency to increase, however, there were no significant differences between the CO_2, helium or gasless control group. At 20 weeks, GFR was lower in the CO_2 group when compared to the helium group (p = 0.001) and after 36 weeks, GFR was higher in the CO_2 group when compared to the helium group and the gasless control group (p = 0.002 and p = 0.038, respectively) (Fig. 2d). Except for these time points, GFR was stable throughout the study period in all recipients.

Macroscopic appearance and histology
Experiment 1. Kidney donor
After one year, all remaining kidneys in the three donor groups and in native controls had a normal macroscopic appearance. Mean kidney weight in native control rats was 1.53 g. One year after nephrectomy, there was a significant increase in kidney weight in the CO_2, the helium and the gasless control group (mean weight (g): 2.22, 2.19 and 2.17, respectively)(p < 0.001 for each group). The differences in kidney weight were not significant among the experimental groups. Histomorphologically, all kidneys showed some propulsion of intratubullary capillary vessels. Signs of chronic ischemia, such as 'wrinkling' of the basal membrane, were found in all specimens. Signs of membranoproliferative glomerular nephritis, characterized by a double basal membrane and dilated capillaries, were detected in one specimen in the helium group, in two specimens in the gasless control group and in two native control kidneys.
Furthermore, mild focal interstitial infiltrates were found in kidney specimens in the CO_2 (n = 2), helium (n = 2) and the gasless control group (n = 1).

Experiment 2. Recipient
There were no significant differences in weight of kidney grafts exposed to CO_2, helium or abdominal wall lifting, one year after transplantation (mean weight (g): 2.84, 2.69 and 2.65, respectively). Mild glomerular ischemic damage and endocapillary proliferation was detected in kidneys of all three experimental groups. Focal infiltration of lymphocytes and proliferation of fibroblasts was mostly found in the proximity of the ureter. Some of the tubules contained protein cylinders. In all animals, Banff scores varied from 0 to 1, indicating that less than 25 % of the tissue showed either glomerular, vascular or interstitial changes. One year after transplantation, total Banff scores were: 0.6 for the CO_2 group, 0.6 for the helium group and 0.5 for gasless controls (p = 0.57).
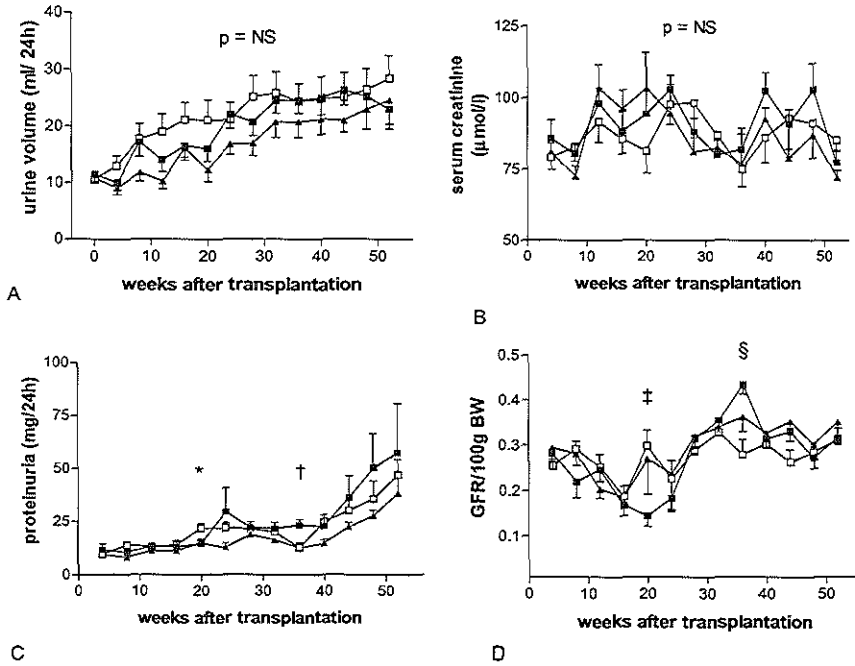
Fig. 2 a - d. Urinary production, serum creatinine, proteinuria and GFR/100g BW (body weight) in *recipients* of a syngeneic kidney graft after abdominal insufflation with carbon dioxide (CO_2) (■) or helium (□), and gasless controls (▲). Data are presented as mean ± SEM (standard error of mean). p = NS (not significant).
* $p < 0.05$ for helium vs. CO_2
† $p < 0.05$ for CO_2 vs. gasless
‡ $p < 0.05$ for helium vs. CO_2
§ $p < 0.05$ for CO_2 vs. helium and gasless

Immunohistology

Experiment 1. Kidney donor

The type and number of infiltrating cells and ICAM-1 expression in kidneys exposed to either CO_2 or helium insufflation, abdominal wall lifting and in native control rats are shown in Table 1.

One year after LDN, there were no significant differences in cellular infiltration (CD4+ cells, CD8+ cells, ED1+ monocytes/macrophages, dendritic cells and MHC class II+ cells) among the CO_2, helium or gasless control group. In addition, no significant differences were detected when compared to native control rats. Furthermore, expression of ICAM-1 was similar among all four groups.

	CO$_2$	helium	gasless	native control	*p-value*
CD4$^+$ lymphocytes	12 ± 4.5	9 ± 4.6	10 ± 3.9	12 ± 0.9	NS
CD8$^+$ lymphocytes	5 ± 2.5	5 ± 2.9	5 ± 1.3	5 ± 1.9	NS
ED1$^+$ macrophages	3 ± 2.0	3 ± 1.5	3 ± 1.7	2 ± 0.9	NS
OX-62$^+$ dendritic cells	2 ± 1.0	2 ± 1.2	1 ± 0.8	1 ± 0.4	NS
MHC class II$^+$cells	8 ± 3.7	7 ± 3.6	9 ± 3.4	11 ± 2.3	NS
ICAM-1	1 ± 0.3	1 ± 0.7	1 ± 0.3	1 ± 0	NS

Table 1. *Kidney donors (Exp. 1).* Cellular infiltration and ICAM-1 expression in donor kidneys, one year after insufflation with CO_2 or helium or abdominal wall lifting. Native control animals did not undergo any procedure. Cell counts are expressed as mean ± cells/ 0.1 mm^2. For ICAM-1 expression, the intensity on endothelium and tubules was measured semi-quantitatively on a 0-3 scale (0 = none, 1 = mild, 2 = moderate, 3 = dense).
One year after LDN, there were no significant differences in either cellular infiltration or ICAM-1 expression among the CO_2, helium or gasless control group. In addition, no significant differences were detected when compared to native control rats.

Experiment 2. Recipient

The type and number of infiltrating cells and adhesion molecule expression in transplanted kidneys exposed to CO_2 insufflation, helium insufflation and abdominal wall lifting are shown in Table 2. One year after transplantation, there were no significant differences in cellular infiltration in kidney grafts in either the CO_2, helium or gasless control group. In addition, there were no differences in ICAM-1 expression between the three experimental groups.

	CO_2	helium	gasless	*p-value*
CD4^{+} lymphocytes	32 ± 9.6	33 ± 23.8	33 ± 16.2	NS
CD8^{+} lymphocytes	4 ± 2.7	4 ± 2.8	4 ± 2.4	NS
ED1^{+} macrophages	4 ± 2.9	5 ± 3.0	3 ± 2.6	NS
OX-62^{+}dendritic cells	2 ± 1.4	2 ± 1.6	2 ± 1.4	NS
MHC class II[+]cells	14 ± 2.1	13 ± 2.4	14 ± 2.6	NS
ICAM-1	2 ± 0.8	2 ± 0.6	2 ± 0.7	NS

Table 2. *Recipients (Exp. 2).* Cellular infiltration and ICAM-1 expression in renal isografts, one year after transplantation. Before transplantation, kidney donors were exposed to a 120 min period of CO_2 or helium insufflation or abdominal wall lifting. Cell counts are expressed as mean ± cells/ 0.1 mm². One year after transplantation, there were no significant differences in cellular infiltration among the CO_2, helium or gasless control group.
For ICAM-1 expression, the intensity on endothelium and tubules was measured semi-quantitatively on a 0-3 scale (0 = none, 1 = mild, 2 = moderate, 3 = dense). There were no significant differences between the CO_2, helium or gasless control group.

DISCUSSION

Laparoscopic donor nephrectomy was first described by Ratner et al. [11]. Concern has been raised about the potential negative impact of this new technique on renal graft function. One of the main differences between the open and the laparoscopic technique is the use of a pneumoperitoneum. Elevated intra-abdominal pressure, due to abdominal gas insufflation, has been shown to decrease renal blood flow [7], rendering the graft susceptible for ischemia [4]. In addition, rapid absorption of CO_2 through the peritoneal surface causes respiratory acidosis, which may affect renal vascular resistance due to increased sympathetic activity [12]. The objective of this study was to determine the impact of pneumoperitoneum on donor and recipient outcome in terms of long term renal function and histomorphology. A follow-up period of one year was based on results from previous experimental studies, in which exposure of kidney grafts to a period of ischemia led to renal dysfunction from 20 weeks post-transplantation [13]. To differentiate between the potential negative effects of CO_2 absorption, we used helium as an alternative insufflation gas, because this inert gas does not influence arterial pH and pCO_2 [14]. The use of a syngeneic rat model enabled us to study the influence of pneumoperitoneum as an isolated factor on cell infiltration and function of the kidney graft, in the absence of an allogeneic response with subsequent rejection.

The present study demonstrates that 52 weeks after donor nephrectomy, renal function in a unilaterally nephrectomized rat is not significantly influenced by a prolonged period of CO_2 or helium insufflation when compared to normal age-matched rats or to rats which had gasless laparoscopy. Furthermore, a two hour period of abdominal gas insufflation did not show any adverse effects on renal function one year after transplantation. Although some significant differences were found in some renal parameters at various time points, a consistent pattern of renal dysfunction due to abdominal gas insufflation could not be detected in this study.

Only few data exist on the effect of pneumoperitoneum on renal histology. In an experimental study in rats, Lee et al. showed that a prolonged period of pneumoperitoneum does not cause any histological changes in kidneys within three months after the procedure [15]. These findings are in concordance with our results, although we used a different study design by simulating a laparoscopic donor nephrectomy, and subsequently, performing a kidney transplantation.

Histopathological evaluation of all donor and recipient kidneys did not show significant differences between CO_2 and helium insufflation groups and the gasless control group after 52 weeks, suggesting that abdominal insufflation does not have a deleterious effect

on histomorphology. It has been shown that ischemia can increase immunogenicity of the donor kidney by inducing MHC class-II expression [16, 17]. To investigate whether abdominal insufflation causes additional cellular infiltration of the kidney graft, we performed immunohistochemical analysis of kidneys 52 weeks after either nephrectomy or transplantation. Several studies have reported beneficial effects of helium as an alternative insufflation gas. Jacobi et al. found that CO_2 pneumoperitoneum significantly increased tumor growth in comparison to helium, a finding which is attributed to changes in pH [18]. In theory, cellular acidification caused by CO_2 pneumoperitoneum could impair immunological processes in the kidney graft resulting in renal dysfunction. Our study demonstrates that 52 weeks after kidney transplantation, there is no significant influence on immunohistological parameters in rat kidney grafts due to either CO_2 or helium insufflation.

Laparoscopic donor nephrectomy has evolved as an attractive alternative to the conventional approach by reducing donor morbidity. Initial reports of delayed graft function after laparoscopic kidney procurement has not stopped surgeons from adopting this technique, but skepticism concerning recipient outcome still persists [19]. In recent years, several studies have reported similar recipient outcomes after laparoscopic donor nephrectomy when compared with the open approach [3, 20-22]. However, at present there are no randomized clinical trials on this topic and follow-up of graft function in most series has been relatively short. Therefore, it is possible that any alterations in long-term function have not been encountered yet. The results from our study, utilizing an established kidney transplantation model, are in concordance with data from clinical studies, suggesting that the reported initial delayed graft function may be due to factors other than the pneumoperitoneum. Some authors have described a substantial learning curve for LDN, which in some cases resulted in mechanical damage to the graft and inadequate dissection of renal vasculature or the graft ureter [2, 23]. Others describe that warm ischemia times shortened after the surgical team gained more experience [4]. In their extensive review, Merlin et al. stressed that it will take years before data on long term graft function after LDN are available [19]. Until results from large series with long term follow-up become available, experimental studies may help to gain further insight in kidney graft function after laparoscopic donor nephrectomy.

In summary, we conclude from this study that a prolonged period of abdominal insufflation with CO_2 or helium does not impair renal function or affect renal histomorphology in donors and recipients in the long term. Therefore, continued development and adoption of the laparoscopic approach for live donor nephrectomy appears justified.

ACKNOWLEDGMENTS

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CHAPTER 9

GENERAL DISCUSSION

Laparoscopic surgery requires a working space in the abdominal cavity to allow safe introduction of trocars and instruments and for exposure of the abdominal contents. Intraperitoneal insufflation of gas is the most common method to elevate the abdominal wall and suppress the viscera. Carbon dioxide (CO_2) is the preferred gas for establishing a pneumoperitoneum because it is non-flammable and inexpensive. However, CO_2 absorption through the peritoneal membrane leads to hypercapnia and acidosis. In addition, the increased intra-abdominal pressure due to intraperitoneal gas insufflation may influence hemodynamic and respiratory function, and perfusion of intra-abdominal organs.

The objectives of our studies were:

1. To investigate pathophysiological consequences of abdominal gas insufflation regarding cardiorespiratory function, lung physiology, body temperature and peritoneal morphology in an animal model.
2. To investigate whether intraoperative variables, postoperative recovery and early graft function differ between laparoscopic and conventional, open donor nephrectomy.
3. To investigate the impact of pneumoperitoneum in a rat model of laparoscopic donor nephrectomy on renal function, histomorphology and immunohistology in donors and recipients.

Since the first description of a laparoscopic rat model by Berguer et al. [1], many studies have been performed in this model to investigate changes associated with laparoscopic surgery. However, knowledge of the cardiorespiratory effects during pneumoperitoneum in rats is limited. Because in most studies rats are allowed to breathe spontaneously, we hypothesized that these studies could be negatively biased due to adverse cardiorespiratory consequences of intraperitoneal insufflation during spontaneous breathing. Therefore, the main objective of the experimental study described in *chapter 2* was to record the cardiorespiratory changes in spontaneously breathing rats exposed to a pneumoperitoneum.

Respiratory acidosis following CO_2 pneumoperitoneum has been reported frequently, and it appears that absorption of CO_2 across the peritoneal surface is primarily responsible for this phenomenon [2]. Using a porcine model, Ho et al. found that absorption of CO_2 from the peritoneal surface only, irrespective of insufflation pressure, is responsible for acidosis and hypercapnia [3]. In our study, we employed a constant pressure similar to the study by Ho et al., eliminating the effect of incremental pressure increases and thus allowing the body to adapt to abdominal insufflation. Utilizing the study design of a fixed insufflation

pressure is more representative of daily clinical practice and of models used in experimental laparoscopic studies. In our study, helium insufflation was superior to carbon dioxide insufflation since acidosis, hypercarbia and changes in base excess were not observed. These findings are in concordance with results from studies in large animals and clinical studies comparing changes in arterial blood gases following intra-abdominal CO_2 or helium insufflation [4-8]. From the study described in *chapter 2*, we conclude that the cardiorespiratory changes due to prolonged pneumoperitoneum in spontaneously breathing rats are similar to those seen in clinical practice. Therefore, studies conducted in this animal model can provide valuable physiological data relevant to the study of laparoscopic surgery.

During anesthesia, lungs are compressed by a cranial shift of the diaphragm and changes in the thoraco-abdominal configuration, resulting in the formation of compression atelectasis [9]. Application of positive end-expiratory pressure (PEEP) has been shown to reduce atelectasis formation during anesthesia [10]. During laparoscopic surgery, the cranial shift of the diaphragm is enhanced by the increase in intra-abdominal pressure, which could lead to further atelectasis formation. The study described in *chapter 3* was designed to investigate changes in arterial oxygenation used as a substrate for atelectasis formation in animals undergoing pneumoperitoneum during mechanical ventilation with and without PEEP. We used arterial oxygenation during mechanical ventilation with 100% oxygen as an accurate indicator for atelectasis formation. In a normal (or: healthy) lung this will result in PaO_2 levels up to 600 mmHg [11], as observed in baseline values of PaO_2 in all animals. When the lung remains inflated, no atelectasis formation occurs and PaO_2 will not be affected [11, 12]. First, we studied the effect of a period of continuous gas insufflation on arterial oxygenation. In rats ventilated without PEEP, PaO_2 levels decreased after 90 min of abdominal insufflation and were still significantly decreased after 180 min of gas insufflation. These findings imply that increased intra-abdominal pressure results in formation of atelectatic lung areas. In a second experiment, we studied the effects of release of intra-abdominal pressure on pulmonary gas exchange. This study design is more representative for clinical laparoscopic procedures, since abdominal desufflation occurs during inadvertent removal of laparoscopic instruments and because of deflation of electrocautery smoke. Although moments of intra-abdominal pressure release are often incomplete and may occur randomly in clinical practice, procedures of abdominal desufflation were of the same length and were performed at defined time points to prevent any bias among the experimental groups. Similar to the first study, PaO_2 values significantly decreased in animals ventilated without PEEP. A short period of abdominal

desufflation led to an increase in PaO_2, which suggests that release of pneumoperitoneum improves the ventilation-perfusion mismatch. However, this effect appears to be temporary since installation of pneumoperitoneum caused a further decrease in arterial oxygenation. These findings suggest that pulmonary atelectasis formation induced by abdominal gas insufflation can be prevented by adding PEEP to mechanical ventilation. Issues concerning the pathophysiological consequences of PEEP ventilation during laparoscopic surgery should therefore be addressed in clinical trials.

The studies described in *chapter 4* were designed to investigate the impact of temperature and humidity of CO_2 pneumoperitoneum on body temperature and peritoneal morphology. During lengthy laparoscopic procedures, patients are exposed to large volumes of cool insufflation gas, which has been shown to decrease body temperature significantly [13, 14]. Furthermore, in an experimental study using scanning electron microscopy (SEM), Volz et al. demonstrated that the integrity of the parietal peritoneum is temporarily disturbed by insufflation with dry CO_2 [15]. It has been shown that denudation of the mesothelial surface increases the risk for adherence of tumor cells, adhesions and infections [16-18]. We hypothesized that creation of a more physiological intra-abdominal environment by insufflating heated, humidified CO_2 gas would reduce the effect of tissue desiccation with subsequent denudation of the mesothelial surface. In our study, insufflation with CO_2 at room temperature caused a significant fall of core body temperature during the two-hour study period. The decrease in body temperature was prevented when the gas stream was simultaneously heated and humidified before it was insufflated into the abdomen. However, SEM analysis of the peritoneal surface revealed that changes in peritoneal morphology occurred after CO_2 insufflation, regardless of heating or humidifying, but also after gasless surgery. Therefore, it is highly likely that the mechanical effect of abdominal distension caused the retraction of mesothelial cells. Further studies are needed to investigate if chemically inert gases such as helium also induce changes in mesothelial cell structure and if the visceral and parietal peritoneum react in a similar fashion to abdominal gas insufflation.

Several studies have described benefits associated with warming or heating of the insufflation gas during laparoscopic procedures. Bäcklund et al. described that warming of the insufflation gas resulted in a significantly higher urinary output in patients undergoing prolonged laparoscopic procedures [19]. They concluded that warm CO_2 insufflation may cause local vasodilatation in the kidneys which leads to improved renal function during laparoscopic procedures. In a clinical study by Puttick et al. [14], insufflation of CO_2 at body temperature prevented intraoperative cooling and resulted in a

reduced postoperative intraperitoneal cytokine response. Mouton et al.[20] described that humidification of the insufflation gas reduced postoperative pain in patients undergoing laparoscopic cholecystectomy, a finding which may be attributed to less irritation of the peritoneum by the CO_2 gas. At present, there is no consensus concerning the additional benefits of warming and humidifying of insufflation gas, because most studies have small sample sizes with possible type II error[21]. In our opinion, the findings of our study warrant further validation of heated, humidified insufflation gas in clinical practice.

The laparoscopic approach for live renal donation has recently been established to reduce some of the disincentives inherent to organ donation. Several studies now have reported that laparoscopic donor nephrectomy (LDN) can be performed safely in selected candidates with a reduction in postoperative morbidity and shorter hospitalization compared to conventional donor nephrectomy[22-25]. Although its technical feasibility has been established, concerns have been raised about the impaired renal function due to pneumoperitoneum and short and long term function of kidneys removed by LDN[26]. Previously, it has been stressed that it will take years before data on long term graft function after LDN are available[27]. Therefore, employment of the laparoscopic approach as an alternative for the 'gold' standard of open nephrectomy can only be justified if it is clear that allograft function after laparoscopic kidney procurement is not at stake. The studies described in *chapters 5-8* were undertaken to evaluate our experience with LDN and, to investigate the impact of pneumoperitoneum on renal function and histomorphology in an animal model.

The objective of the study described in *chapter 5* was to determine whether intraoperative diuresis, postoperative recovery and early graft function differ between LDN and conventional, open donor nephrectomy (ODN). From this retrospective review it was concluded that length of hospital stay was significantly shorter in the laparoscopic group (mean: 3.9 vs. 6.2 days), which suggests that kidney donors can benefit from an improvement in postoperative recovery after LDN. Similar to other studies comparing LDN with ODN, we found that duration of operation was longer for laparoscopic kidney removal. However, it has been shown that operating times of LDN shorten with increased experience[28]. One of the most striking findings in our study was that during kidney dissection, the amount of administered fluids and intraoperative diuresis were significantly lower for LDN. This may have resulted in a higher serum creatinine level in the LDN group on the first day after transplantation. From the second postoperative day onwards the differences in mean serum creatinine were no longer significant, therefore, the effect is probably minor. In general, it must be stressed that development of a

successful living-donor program requires a dedicated, co-ordinated multidisciplinary approach. Operating times of LDN are longer and, therefore, hydration protocols should be adjusted accordingly. The disparity in intraoperative fluid requirements and diuresis as reported in this study ensued improvement of our anesthetic protocol for patients undergoing LDN. In an attempt to ameliorate a decrease in venous return due to increased intra-abdominal pressure, vigorous hydration is now employed intraoperatively to promote adequate diuresis [29, 30].

It has been suggested that renal function is influenced by release of neurohumoral factors such as antidiuretic hormone (ADH) caused by increased intra-abdominal pressure [31]. Therefore, we prospectively evaluated plasma ADH levels in 30 patients who underwent a laparoscopic donor nephrectomy and in 6 patients who were not eligible for the laparoscopic approach and had a conventional donor nephrectomy (*chapter 6*). In this study, it was shown that mean ADH levels increased during the laparoscopic procedure. Twenty-four hours after LDN, mean ADH levels had returned to normal values, but were still significantly higher compared to pre-insufflation values. In patients who had a conventional ODN, ADH levels did not significantly change. A relationship between increased ADH response, changes in intra-operative blood pressure and diuresis could not be detected. Therefore, the clinical significance of the increase of ADH observed during LDN appears limited.

The studies described in *chapter 7 and 8* aimed to determine the impact of pneumoperitoneum on short- and long-term renal function and histomorphology of the kidney donor and recipient in an experimental model of LDN. The use of a syngeneic rat model enabled us to study the influence of pneumoperitoneum as an isolated factor on cell infiltration and function of the kidney graft, in the absence of an allogeneic response with subsequent rejection. Both studies demonstrate that after donor nephrectomy, renal function in a unilaterally nephrectomized rat is not significantly influenced by a prolonged period of abdominal gas insufflation when compared to normal age-matched rats or to rats which had gasless laparoscopy. Furthermore, a two hour period of abdominal gas insufflation did not result in any adverse effects on renal function even until one year after transplantation. Although significant differences were found in the parameters of renal function and in the immunohistochemical analyses, differences among the experimental groups occurred at various time points, and, a consistent pattern of renal dysfunction due to abdominal gas insufflation could not be detected. From the results of these experimental studies, utilizing an established kidney transplantation model, we conclude

that a prolonged period of abdominal gas insufflation does not impair renal function or affect renal histomorphology in donors and recipients in either the short or the long term.

Previously, it has been stressed that it will take years before data on long-term graft function after LDN are available [27]. Therefore, the current lack of adequate evidence-base for LDN necessitates institutions performing this technique to report on safety and effectiveness after engraftment. Experimental studies may help to gain further insight in kidney graft function after laparoscopic donor nephrectomy. The studies described in this thesis imply that the reported initial delayed graft function following LDN is not related to the pneumoperitoneum Whether surgery-related factors such as a longer warm ischemia time, or mechanical damage to either the graft, renal vasculature or ureter play a role may be the subject of future studies. Currently, we have started a prospective randomized clinical study (*LiDo-trial; Living Donors: laparoscopic or open kidney donation*) which compares LDN with ODN. This study will allow a more valid comparison of recuperation, morbidity and graft function. In addition, issues concerning cost-effectiveness and quality of life will be addressed.

In summary, the initial graft survival and function rates following LDN at our institution are similar to conventional, open donor nephrectomy and are in concordance to reports by other centers performing this technique. In our opinion, continued development and adoption of laparoscopic donor nephrectomy appears justified.

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CHAPTER 10

SUMMARY AND CONCLUSIONS

SUMMARY

Chapter 1 is the general introduction to this thesis. An overview is presented regarding the general pathophysiological effects of pneumoperitoneum during laparoscopic procedures. This chapter concludes with a summary of the objectives of this thesis.

Experimental studies on laparoscopic surgery are often performed in spontaneously breathing rats. However, the hemodynamic and respiratory responses related to the pneumoperitoneum have not been studied extensively in rats. *Chapter 2* describes an experimental study, in which the effects of CO_2 and helium, insufflation pressure and duration of pneumoperitoneum on blood pressure, arterial pH, pCO_2, pO_2, HCO_3^-, base excess and respiratory rate are investigated. In this study, rats were anaesthetized and underwent either CO_2 insufflation (6 or 12 mmHg), helium insufflation (6 or 12 mmHg) or abdominal wall lifting (gasless control) for 120 min. Blood pressure was monitored by an indwelling carotid artery catheter. Mean arterial pressure (MAP), respiratory rate, arterial blood pH, pCO_2, pO_2, HCO_3^- and base excess were recorded at defined time points during 120 min. In this study, acidosis was a consistent finding during CO_2 insufflation (at both 6 and 12 mmHg). There was no significant change in blood pH and pCO_2 in rats undergoing either helium insufflation or gasless procedures. Neither insufflation pressure nor type of insufflation gas had a significant effect on MAP over time. This study shows that the cardiorespiratory changes during prolonged pneumoperitoneum in spontaneously breathing rats are similar to those seen in clinical practice. Therefore, studies conducted in this animal model can provide valuable physiological data relevant to the study of laparoscopic surgery.

Increased intra-abdominal pressure causes diaphragmatic displacement resulting in compressed lung areas which leads to formation of atelectasis, especially during mechanical ventilation. Application of positive end-expiratory pressure (PEEP) can maintain pulmonary gas exchange. In *chapter 3*, a study is presented in which the effect of abdominal gas insufflation on arterial oxygenation, used as a substrate for atelectasis formation, during mechanical ventilation with and without PEEP is evaluated in rats. In this study, PaO_2 values decreased significantly in insufflation groups which were ventilated with 0 cmH_2O PEEP. Insufflation groups ventilated with 8 cmH_2O PEEP had PaO_2 values comparable to the control group. There were no significant differences in mean arterial pressure between insufflation groups ventilated with or without PEEP during the 180 min study period. These results suggest that PEEP preserves arterial

oxygenation during prolonged pneumoperitoneum in rats with minimal adverse hemodynamic effects.

Chapter 4 evaluates the impact of temperature and humidity of CO_2 pneumoperitoneum on body temperature and peritoneal morphology. Insufflation of cold gas (room temperature) during laparoscopic surgery exposes the patients to the risk of hypothermia. In an experimental model, it was investigated if heating or humidification of insufflation gas could prevent peroperative hypothermia. Furthermore, it was assessed whether the peritoneum was affected by heating or humidification of the insufflation gas. In this study, rats were exposed to insufflation with either cold dry CO_2, cold humidified CO_2, warm dry CO_2, warm humidified CO_2 or gasless laparoscopy. Core temperature and intraperitoneal temperature were registered in all animals during 120 min. Specimens of the parietal peritoneum were taken directly after desufflation and 2 and 24 hours after the procedure. All specimens were analyzed using scanning electron microscopy (SEM). During the 120 min study period, core temperature and intraperitoneal temperature were significantly reduced in animals which either had cold dry CO_2 or cold humidified CO_2, but also in animals which had warm dry CO_2 gas. Animals which had warm, humidified insufflation or gasless laparoscopy did not develop intraoperative hypothermia. At SEM, retraction and bulging of mesothelial cells and exposure of the basal lamina were seen after 24 h in the four insufflation groups but also in gasless controls. The results in this study suggest that insufflation with cold dry CO_2 may lower body temperature during laparoscopic surgery. Furthermore, hypothermia can be prevented by simultaneously heating and humidifying the insufflation gas. Finally, changes of the peritoneal surface occur after CO_2 insufflation, regardless of heating or humidifying, but also after gasless surgery.

Chapter 5 describes our experience with laparoscopic donor nephrectomy, a minimally invasive technique which has been introduced at our institution in 1997. Laparoscopic donor nephrectomy (LDN) can reduce donor morbidity in terms of decreased pain and shorter convalescence. Although its technical feasibility has been established, concerns have been raised about the impaired renal function due to pneumoperitoneum and short and long term function of kidneys removed by LDN. The objective of this study was to determine whether intraoperative diuresis, postoperative recovery and early graft function differ between LDN and conventional open donor nephrectomy (ODN). Between December 1997 and December 2000, 89 LDNs were performed at our institution. These were compared to 83 conventional open donor nephrectomies (ODN) performed between

January 1994 until December 1997. Graft function, intraoperative variables and clinical outcome were compared. LDN was completed successfully in 91 % of cases. Length of hospital stay was significantly shorter in the laparoscopic group (mean: 3.9 vs. 6.2 days). Duration of operation was longer for the laparoscopic group (mean: 235 vs. 155 min). During kidney dissection, the amount of administered fluids and intraoperative diuresis were significantly lower for LDN. When recipient graft function was compared between the two groups, it was shown that mean serum creatinine was higher after LDN compared with ODN, one day after transplantation. From the second postoperative day onwards, the differences in mean serum creatinine were no longer significant. Graft survival rates were similar for LDN and ODN. The results in this study suggest that kidney donors can benefit from an improvement in postoperative recovery after LDN. In our opinion, assessment of a sufficient perioperative hydration protocol is mandatory to assure optimal kidney quality during laparoscopic procurement. Finally, the initial graft survival and function rates following LDN suggest that continued development and adoption of this technique are justified.

Chapter 6 presents the results of a study in which plasma antidiuretic hormone (ADH) levels were prospectively evaluated in patients which had a laparoscopic donor nephrectomy. During laparoscopic procedures, increased intra-abdominal pressure may cause transient renal dysfunction (oliguria) due to impaired renal blood flow. In addition, renal function may be influenced by release of neurohumoral factors such as ADH, although the relationship between ADH secretion and increased intra-abdominal pressure is poorly understood. The objective of this study was to evaluate plasma ADH levels during laparoscopic donor nephrectomy and to correlate ADH levels to graft function. In 30 patients who underwent LDN, plasma ADH levels were collected before insufflation, during surgery, after abdominal desufflation and 24 h after the procedure. In 6 patients who had open donor nephrectomy (ODN), blood samples were collected as controls. Furthermore, graft function, operative characteristics and clinical outcome were compared. In this study, it was shown that mean ADH levels increased during the laparoscopic procedure. Twenty-four hours after LDN, mean ADH levels had returned to normal values, but were still significantly higher compared to pre-insufflation values. In patients who had ODN, ADH levels did not significantly change. There were no significant differences in ADH levels in the ODN group. Furthermore, no significant differences in either intra-operative diuresis, blood pressure or postoperative graft function were documented among the two groups. From this study, it is concluded that LDN was associated with an increase in plasma ADH which appeared to be related to

increased intra-abdominal pressure. However, the increased ADH concentrations during LDN were not associated with clinically significant changes in renal function in either the patient donating a kidney or the transplanted graft.

Chapter 7 describes an experimental study in which the effects of abdominal gas insufflation on short-term renal function of kidney donors and recipients were investigated. Clinical and experimental studies have shown that raised intra-abdominal pressure due to abdominal gas insufflation can result in transient renal dysfunction. Therefore, laparoscopically procured kidneys might be at higher risk for ischemia during pneumoperitoneum. The objective of this study was to investigate the short-term impact of pneumoperitoneum used for LDN on renal function and histomorphology in donor and recipient.

In the first experiment, rats were randomized for three procedures: 2 h CO_2 insufflation, 2 h helium insufflation and 2 h gasless technique. After this, a unilateral nephrectomy was performed in all animals. In the second experiment, rats were subjected to a similar insufflation protocol, but after nephrectomy, a syngeneic kidney transplantation was performed. Urine and blood samples were collected on postoperative day (POD) 1, 3, 7 and 14 for determination of renal function. Subsequently, donor and recipient kidneys were removed for histomorphological and immunohistochemical analysis. In donors as well as in recipients, no significant changes in serum creatinine, proteinuria and GFR were detected between the CO_2, helium and the gasless control group. In both experiments, histological analysis of kidney specimens did not show any deleterious effects caused by abdominal gas insufflation. Furthermore, immunohistochemical analysis of all renal specimens did not show any consistent differences in number of infiltrating cells (CD4, CD8, ED1, OX6, OX62) between the two insufflation groups and the gasless controls. The results in this study suggest that abdominal gas insufflation does not have an adverse effect on renal function of the kidney donor in the first week after LDN. Moreover, no differences in renal function and histomorphology were detected between kidney grafts exposed to pneumoperitoneum and gasless controls.

Chapter 8 describes an experimental study which evaluates the long term impact of pneumoperitoneum used for LDN on renal function and histomorphology in donor and recipient. In the first experiment, rats underwent either insufflation with CO_2 or helium, or had a gasless procedure. After this, a unilateral nephrectomy was performed in all animals. In the second experiment, rats were subjected to a similar insufflation protocol, but after nephrectomy, a syngeneic renal transplantation was performed. In both

experiments, rats had a follow-up period of 12 months. Urine and blood samples were collected monthly for determination of renal function. After one year, donor and recipient kidneys were removed for histomorphological and immunohistochemical analysis. In donors as well as in recipients, no significant changes in serum creatinine, proteinuria or GFR were detected between the CO_2, the helium and the gasless control group after one year. No histological abnormalities due to abdominal gas insufflation were found. Furthermore, immunohistochemical analysis did not show significant differences in number of infiltrating cells (CD4, CD8, ED1, OX62 and OX6) and adhesion molecule expression (ICAM-1) between the three groups. The results in this study suggest that abdominal gas insufflation does not impair long-term renal function in the kidney donor. Moreover, no differences in renal function or histomorphology were detected between kidney grafts exposed to either pneumoperitoneum or a gasless procedure, one year after transplantation.

CONCLUSIONS

- Cardiorespiratory changes during prolonged pneumoperitoneum in spontaneously breathing rats are similar to those seen in clinical practice. Studies conducted in this animal model can provide valuable data relevant to the study of laparoscopic surgery.
- Abdominal insufflation with helium and gasless laparoscopy are not associated with acidosis in spontaneously breathing rats.
- Application of PEEP during mechanical ventilation preserves arterial oxygenation during prolonged pneumoperitoneum in rats with minimal adverse hemodynamic effects.
- Abdominal insufflation with cold, dry CO_2 contributes to a reduction of body temperature during laparoscopic surgery. Hypothermia may be prevented by simultaneously heating and humidifying the insufflation gas.
- Morphological changes of the peritoneal surface occur after CO_2 insufflation, regardless of heating or humidifying, but also after gasless surgery.
- Kidney donors can benefit from an improvement in postoperative recovery after laparoscopic donor nephrectomy.
- Graft survival and renal function following laparoscopic donor nephrectomy are similar to those after conventional, open donor nephrectomy.
- Laparoscopic donor nephrectomy is associated with an increase in plasma antidiuretic hormone (ADH) concentration which appears to be related to increased intra-

abdominal pressure. These increased ADH levels were not associated with clinically significant changes in either the kidney donor or the transplanted graft.

- Abdominal gas insufflation does not impair either short or long term renal function, histomorphology or immunohistology in the kidney donor.

- Short- and long-term function of transplanted kidney grafts are not impaired by exposure to either CO_2 or helium pneumoperitoneum or a gasless procedure.

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SAMENVATTING

Hoofdstuk 1 is de algemene introductie van dit proefschrift. Er wordt een overzicht gepresenteerd betreffende de algemene pathofysiologische gevolgen van het pneumoperitoneum tijdens laparoscopische procedures. Dit hoofdstuk besluit met een overzicht van de doelstellingen van dit proefschrift.

Experimentele studies op het gebied van laparoscopische chirurgie worden vaak verricht in spontaan ademende ratten. De hemodynamische en respiratoire effecten ten gevolge van het pneumoperitoneum zijn echter nog niet in detail bestudeerd in deze situatie. *Hoofdstuk 2* beschrijft een experimentele studie waarin de effecten van CO_2 en helium, insufflatiedruk en duur van het pneumoperitoneum op de bloeddruk, arteriële pH, pCO_2, pCO_2, HCO_3^-, base excess en ademfrequentie worden bestudeerd. In deze studie werden ratten in narcose gebracht en ondergingen CO_2 insufflatie (6 of 12 mmHg), helium insufflatie (6 of 12 mmHg) of gasloze laparoscopie gedurende 120 min. Bloeddrukregistratie vond plaats via een canule in de a. carotis. Gemiddelde arteriële bloeddruk, arteriële pH, pCO_2, pCO_2, HCO_3^-, base excess en ademfrequentie werden geregistreerd op vastgestelde tijden gedurende 120 min. In de twee groepen die met CO_2 werden geïnsuffleerd (6 en 12 mHg) trad acidose op. Er werden geen veranderingen in pH en pCO_2 geregistreerd tijdens insufflatie met helium en tijdens de gasloze procedure. Gedurende het experiment was noch de insufflatiedruk, noch het gebruikte type gas van invloed op de bloeddruk. Deze studie toont aan dat de cardiorespiratoire veranderingen tijden een langdurig pneumoperitoneum in spontaan ademende ratten vergelijkbaar zijn met gegevens die in klinische studies worden beschreven. Er kan geconcludeerd worden dat studies die verricht worden in dit proefdiermodel waardevolle fysiologische gegevens kunnen opleveren voor het bestuderen van laparoscopische chirurgie.

Verhoogde intra-abdominale druk leidt tot verplaatsing van het middenrif, hetgeen resulteert in samengedrukte longgebieden (atelectasen). Dit fenomeen treedt met name op tijdens kunstmatige beademing. De toepassing van positieve eind-expiratoire druk (PEEP) kan de gasuitwisseling in de longen in stand houden. *Hoofdstuk 3* beschrijft een studie in een rattenmodel waarin het effect van abdominale gasinsufflatie op de arteriële zuurstofspanning (PaO_2) tijdens beademing met PEEP en zonder PEEP wordt bestudeerd. In dit model maakt men gebruik van de relatie tussen een daling in PaO_2 en het optreden van atelectasen. In de insufflatiegroepen die beademd werden zonder PEEP daalde de PaO_2 waarden. In de dieren die beademd werden met 8 cmH_2O PEEP bleef de PaO_2

waarde gelijk aan die van de controlegroep. Gedurende het 180 min durende experiment waren er geen significante verschillen in bloeddruk tussen de insufflatiegroepen die beademd werden met of zonder PEEP. De resultaten in deze studie suggereren dat PEEP de arteriële oxygenatie in stand kan houden gedurende langdurig pneumoperitoneum met minimale nadelige hemodynamische gevolgen.

In *hoofdstuk 4* wordt de invloed van de temperatuur en vochtigheidsgraad van CO_2 pneumoperitoneum op lichaamstemperatuur en morfologie van het pneumoperitoneum. Tijdens laparoscopische ingrepen worden patiënten blootgesteld aan insufflatie met koud gas (kamertemperatuur) hetgeen kan leiden tot het ontstaan van hypothermie. In een experimenteel model werd onderzocht of peroperatief ontstane hypothermie voorkomen kan worden door het verwarmen dan wel bevochtigen van insufflatiegas. Tevens werd bestudeerd of het peritoneum aangetast werd door het verwarmde of bevochtigde gas. In deze studie werden ratten blootgesteld aan insufflatie met koud droog gas, koud bevochtigd gas, warm droog gas, warm bevochtigd gas of aan gasloze laparoscopie. In alle dieren werden lichaamstemperatuur en intraperitoneale temperatuur geregistreerd gedurende 120 min. Biopten van het peritoneum van de buikwand werden direct na desufflatie, alsmede 2 en 24 uur na de procedure genomen. Alle biopten werden geanalyseerd met behulp van een scanning electronen microscoop (SEM). Gedurende de studieperiode van 120 min waren lichaams –en intraperitoneale temperatuur significant lager in dieren die koud droog of koud bevochtigd gas geïnsuffleerd kregen en in dieren die warm droog gas geïnsuffleerd kregen. In dieren waarin het gas verwarmd en bevochtigd gas werd en in de gasloze controlegroep trad peroperatief geen hypothermie op. Bij het SEM onderzoek van de biopten welke genomen zijn na 24 uur, werden teruggetrokken en opgebolde mesotheelcellen gezien met blootliggen van de basaalmembraan in zowel de vier insufflatiegroepen als in gasloze controle dieren. De resultaten van deze studie suggereren dat insufflatie met koud droog CO_2, zoals gebruikt tijdens laparoscopische ingrepen, bijdraagt aan een afname van de lichaamstemperatuur. Hypothermie kan voorkomen worden indien het insufflatiegas niet alleen verwarmd, maar ook bevochtigd wordt. Veranderingen van het peritoneum treden op na insufflatie met CO_2, onafhankelijk van verwarming of bevochtiging, maar ook bij gasloze chirurgie.

Hoofdstuk 5 beschrijft de ervaring met laparoscopische donornefrectomie, een minimaal invasieve techniek die geïntroduceerd werd in het Academisch Ziekenhuis Rotterdam-Dijkzigt in 1997. Laparoscopische donornefrectomie (LDN) kan leiden tot een reductie in morbiditeit van de donor, zoals minder postoperatieve pijn en een korter

ziekenhuisverblijf. Ondanks dat de technische toepasbaarheid van LDN al een plaats heeft gevonden, bestaat er bezorgdheid omtrent de verminderde nierfunctie ten gevolge van het pneumoperitoneum, alsmede de korte en lange termijn functie van nieren die verwijderd zijn middels een LDN. Het doel van deze studie was te bepalen of peroperatieve urineproduktie, postoperatief herstel en vroege termijn transplantaatfunctie verschilden tussen LDN en de conventionele, open donornefrectomie (ODN). In de periode van december 1997 tot december 2000 werden 89 laparoscopische nierdonaties uitgevoerd. Deze werden vergeleken met 84 open nierdonaties die verricht werden tussen januari 1994 en december 1997. Transplantaatfunctie, peroperatieve data en het klinische beloop werden vergeleken. In 8 van de 89 LDNs (91 %) bleek het niet mogelijk om de operatie geheel laparoscopisch uit te voeren en werd geconverteerd naar een open procedure. De lengte van het ziekenhuisverblijf was significant korter in de laparoscopische groep (gemiddelde: 3.9 vs. 6.2 dagen). De duur van de operatie was langer in de laparoscopische groep (gemiddelde: 235 vs. 155 min). Tijdens de dissectie van de nier, waren de hoeveelheid toegediend vocht en de peroperatieve urineproduktie lager in de laparoscopische groep. Bij het vergelijken van transplantaatfunctie in de twee groepen, bleek dat het gemiddelde serum creatinine hoger was na LDN op de eerste dag na transplantatie. Vanaf de tweede postoperatieve dag waren deze verschillen in serum creatinine bij de ontvangers niet langer significant. De transplantaatoverleving was vergelijkbaar voor beide groepen. De resultaten in deze studie suggereren dat nierdonoren een voordeel kunnen hebben wat betreft postoperatief herstel na LDN. Het opstellen van een adequaat hydratieprotocol rondom de laparoscopische procedure lijkt noodzakelijk om een optimale kwaliteit van het niertransplantaat te kunnen garanderen. De voorlopige data betreffende transplantaatfunctioneren en –overleving na LDN suggereren dat verdere ontwikkeling en toepassing van deze techniek gerechtvaardigd is.

Hoofdstuk 6 presenteert de resultaten van een prospectieve studie waarin de concentratie van plasma antidiuretisch hormoon (ADH) werd bepaald in patiënten die een laparoscopische donornefrecomtie. Tijdens laparoscopische procudures kan de verhoogde intra-abdominale druk leiden tot een tijdelijke afname van de nierfunctie (oligurie) hetgeen deels verklaard kan worden door een verminderde bloeddoorstroming van de nier. Tevens zou de nierfunctie beïnvloed kunnen worden door de afgifte van neurohumorale factoren zoals ADH, echter de relatie tussen ADH afgifte en verhoogde intra-abdominale druk is niet geheel duidelijk. Het doel van deze studie was de plasma ADH concentratie tijdens laparoscopische donornefrectomie te evalueren en deze te correleren met transplantaatfunctie. In 30 patiënten die een LDN ondergingen werden ADH concentraties

bepaald vóór insufflatie, tijdens chirurgie, na abdominale desufflatie en 24 uur na de ingreep. In 6 patiënten die een open donomefrectomie ondergingen (ODN) werden bloedmonsters bepaald ter controle. Tevens werd transplantaatfunctie, operatieve data en klinisch beloop vergeleken. In deze studie werd aangetoond dat de gemiddelde ADH concentraties stegen tijdens de laparoscopische procedures. 24 uur na LDN waren de ADH concentraties teruggekeerd naar normaalwaarden, maar waren nog steeds significant hoger in vergelijking met de waarden voor gasinsufflatie. In patiënten die een ODN ondergingen traden er geen significante veranderingen in ADH concentratie op. Er waren geen significante verschillen in peroperatieve urineproduktie, bloeddruk en postoperatieve transplantaatfunctie tussen de twee groepen. Uit deze studie kan geconcludeerd worden dat LDN gepaard kan gaan met een stijging in plasma ADH concentratie, hetgeen gerelateerd is aan de verhoogde intra-abdominale druk. De stijging in ADH concentratie tijdens LDN ging echter niet gepaard met klinisch significante veranderingen in nierfunctie bij de donor en het niertransplantaat.

Hoofdstuk 7 beschrijft een experimentele studie waarin de invloed van abdominale gasinsufflatie op de nierfunctie van donor en ontvanger wordt bestudeerd. In klinische en experimentele studies is aangetoond dat verhoogde intra-abdominale druk ten gevolge van abdominale gasinsufflatie kan resulteren in een tijdelijk verminderde nierfunctie. Laparoscopisch verwijderde nieren zouden zodoende een hoger risico op ischemie kunnen hebben door het pneumoperitoneum. Het doel van deze studie was te bepalen wat de korte termijn invloed is van het pneumoperitoneum op de nierfunctie en histolomorfologie van de nierdonor en ontvanger. In het eerste experiment werden ratten gerandomiseerd over 3 groepen welke de volgende procedures ondergingen: 2 uur CO_2 insufflatie, 2 uur helium insufflatie, 2 uur gasloze techniek. Vervolgens werd bij alle dieren een unilaterale nefrectomie verricht. In het tweede experiment werd hetzelfde insufflatieprotocol uitgevoerd, maar na nefrectomie werd een syngene niertansplantatie verricht. Urine en bloedmonsters werden verzameld ter bepaling van de nierfunctie op 1, 3, 7 en 14 dagen na de operatie. Tenslotte werd bij alle donoren en ontvangers de resterende nier verwijderd voor histomorfologisch en immunohistologisch onderzoek. Bij zowel donoren als ontvangers werden geen significante verschillen in serum creatinine, proteïnurie of glomerulaire filtratiesnelheid waargenomen tussen de CO_2, helium en gasloze controlegroep. In beide experimenten liet histologische analyse van de nieren geen nadelige gevolgen zien ten gevolge van abdominale gasinsufflatie. Tevens waren er bij immunohistochemisch onderzoek geen consistente verschillen in aantal infiltrerende cellen (CD4, CD8, ED1, OX6, OX62) tussen de twee insufflatiegroepen en de gasloze

controlegroep. De resultaten in deze studie suggereren dat abdominale gasinsufflatie geen nadelig effect heeft op de nierfunctie van de donor in de eerste week na LDN. Ook werden er geen verschillen aangetoond in nierfunctie, histomorfologie en immunohistologie van niertransplantaten die bloot werden gesteld aan pneumoperitoneum of aan een gasloze procedure.

Hoofdstuk 8 beschrijft een experimentele studie waarin de lange termijn invloed van pneumoperitoneum op nierfunctie en histomorfologie in donor en ontvanger wordt bestudeerd. In het eerste experiment ondergingen kregen ratten een periode van CO_2 insufflatie, helium insufflatie of een gasloze procedure. Vervolgens werd bij alle dieren een nier verwijderd. In het tweede experiment werd hetzelfde insufflatieprotocol uitgevoerd, maar na nefrectomie werd een syngene niertransplantatie verricht. In beide experimenten was de duur van follow-up 1 jaar. Urine en bloedmonsters werden maandelijks verzameld ter bepaling van de nierfunctie. Na een jaar werd bij alle donoren en ontvangers de nier verwijderd voor histomorfologisch en immunohistologisch onderzoek. Bij zowel donoren als bij ontvangers werden gedurende het jaar geen significante verschillen in serum creatinine, proteïnurie of glomerulaire filtratiesnelheid waargenomen tussen de CO_2, helium en gasloze controlegroep. Er traden geen histomorfologische veranderingen op ten gevolge van de abdominale gasinsufflatie. Tevens was er geen significant verschillend patroon waarneembaar in aantal infiltrerende cellen (CD4, CD8, ED1, OX6, OX62) en expressie van het adhesiemolecuul ICAM-1 tussen de insufflatiegroepen en de gasloze controlegroep. De resultaten van deze studie suggereren dat abdominale gasinsufflatie geen nadelige invloed heeft op de nierfunctie van de donor op lange termijn. Een jaar na transplantatie zijn er geen verschillen in nierfunctie en histomorfologie tussen niertransplantaten die bloot werden gesteld aan pneumoperitoneum of een gasloze procedure.

CONCLUSIES

- In spontaan ademende ratten zijn de cardiorespiratoire veranderingen die optreden tijdens een langdurig pneumoperitoneum vergelijkbaar met gegevens die gerapporteerd worden in klinische studies. Studies die uitgevoerd worden in dit proefdiermodel kunnen daarom waardevolle gegevens opleveren voor laparoscopisch chirurgisch onderzoek.
- Abdominale gasinsufflatie met helium en gasloze laparoscopie gaan niet gepaard met acidose in spontaan ademende ratten.
- Toepassing van PEEP tijdens kunstmatige beademing zorgt voor behoud van arteriële oxygenatie tijdens langdurig pneumoperitoneum in ratten en gaat gepaard met minimale nadelige hemodynamische gevolgen.
- Abdominale gasinsufflatie met koud en droog CO_2 gas draagt bij aan een afname in lichaamstemperatuur. Hypothermie kan worden voorkomen indien het insufflatiegas gelijktijdig verwarmd en bevochtigd wordt.
- Morfologische veranderingen van het peritoneale oppervlak treden zowel na CO_2 insufflatie als na gasloze chirurgie op. Verwarming of bevochtiging van insufflatiegas is niet van invloed op deze veranderingen.
- Nierdonoren kunnen profiteren van een sneller postoperatief herstel na laparoscopische nierdonatie.
- Transplantaatfunctie en –overleving na laparoscopische donornefrectomie zijn vergelijkbaar met conventionele, open donornefrectomie.
- Laparoscopische donornefrectomie gaat gepaard met een stijging van de plasma antidiuretisch hormoon (ADH) concentratie hetgeen gerelateerd lijkt te zijn aan de verhoogde intra-abdominale druk. De verhoogde ADH respons ging niet gepaard met klinisch significante veranderingen in nierfunctie bij de donor en het niertransplantaat.
- Abdominale gasinsufflatie heeft geen nadelige invloed op korte en lange termijn nierfunctie, histomorfologie of immunohistologie van de nierdonor.
- Korte en lange termijn nierfunctie van niertransplantaten worden niet nadelig beïnvloed door blootstelling aan een CO_2 of helium pneumoperitoneum danwel gasloze laparoscopie.

DANKWOORD

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Laurens, na enkele bijzondere jaren als popmuzikant deden wij samen de eerste wetenschappelijke ervaring op tijdens ons verblijf in Australië. Op 18 september aanstaande, precies vier jaar na ons artsexamen, staan we weer zij aan zij. Jullie beiden dank ik voor de jaren van vriendschap en gezelligheid en voor de ondersteuning op deze dag.

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En tenslotte lieve Sas, jij verdient het meeste lof want samen met jou is het leven mooi.
♫*... it ain't so hard to do if you know how...*
...Listen to the music!

CURRICULUM VITAE

Eric Jasper Hazebroek werd geboren op 29 maart 1971 te Leiden. Het voorbereidend wetenschappelijk onderwijs (gymnasium β) volgde hij aan het Emmaus College te Rotterdam. In 1990 werd aangevangen met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Het afstudeeronderzoek verrichtte hij in het Royal Children's Hospital, Department of General Surgery in Melbourne, Australië (hoofd: Prof. J.M. Hutson). De eerste praktijkervaring deed hij op als lid van het chirurgisch studententeam 'les Forgerons' in het Ikazia Ziekenhuis te Rotterdam. In 1998 behaalde hij het artsexamen (cum laude).

Na een korte periode als AGNIO op de afdeling Spoedeisende Hulp van het Academisch Ziekenhuis Rotterdam-Dijkzigt, kreeg hij in 1999 een aanstelling als arts-onderzoeker op de afdeling Heelkunde (hoofd: Prof.dr. J. Jeekel) waarbij hij verantwoordelijk was voor de coördinatie van de COLOR trial (COlon cancer: Laparoscopic or Open Resection). In deze periode verrichtte hij tevens de studies beschreven in dit proefschrift, waarvan een groot deel werd uitgevoerd in het laboratorium voor Experimentele Chirurgie, Erasmus Universiteit Rotterdam (begeleiders: dr. R.L. Marquet en dr. R.W.F. de Bruin). In 2001 begon hij met de opleiding tot algemeen chirurg in het Academisch Ziekenhuis Rotterdam-Dijkzigt (opleider: Prof.dr. H.J. Bonjer), welke vanaf 2003 zal worden voortgezet in het Medisch Centrum Rijnmond-Zuid, lokatie Clara (opleider: dr. J.F. Lange).