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Tissue Ischemia Due to CO_2 Pressure during Laparoscopic Radical Prostatectomy

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A B S T R A C T

Laparoscopic radical prostatectomy is nowadays one of the most frequently performed urological surgical procedures. For insufflation in laparoscopic radical prostatectomy (LRP) CO_2 is used, with the pressure in the operative region between 12 and 15 mm Hg. At the microcirculation level, the pressure is lower, which raises the possibility of ischemic tissue damage during the procedure. The activity of glutathione peroxidase (GSH-px), superoxide dismutase (SOD) and catalase (CAT) was measured at the beginning and immediately after the end of the surgery in 44 patients who underwent LRP and in 11 who underwent retropubic radical prostatectomy (RRP). Capillary endothelial damage was assessed by applying immunohistochemical and morphometric methods to tissue samples from the urinary bladder neck, which contains all layers of the bladder wall. Measurement of the enzyme activity showed no significant increase of GSH-px (p-0.431), SOD (p-0.220) and CAT (p-0.434) levels. Neither immunohistochemical analysis of the bladder neck capillaries with i-nitric oxide synthase (i-NOS) nor morphometric analysis showed signs of endothelial ischemic damage.

Key words: *prostatic carcinoma, CO_2 pressure, tissue ischemia, free radicals, glutathione peroxidase, superoxide dismutase, catalase*

Introduction

CO_2, used by Zollikofer[1] for insufflation in celioscopy, is still in routine use in laparoscopic procedures. The gas pressure in the abdomen, retroperitoneally or preperitoneally, ranges between 12 and 15 mm Hg. In the last eight years, LRP has become a routine operative treatment in urological centres worldwide[2–4]. The method is minimally invasive and its operative, postoperative and oncological results are comparable to those achieved by the standard RRP[2–7]. Several operative methods of LRP have been described. Gulloneau and Vallancien[3] have described a descendent transperitoneal method named Montsouris. Rassweilwer and collaborators[8] discussed an ascendant operative method similar to the standard RRP, and called it Hellbron. Menon and collaborators from Vattikuti Institute from Detroit[9] described a technique of robotic radical prostatectomy, named Vattikuti method. Nowadays there are also numerous modifications of these methods. At our department, the first laparoscopic radical prostatectomy was performed in 1999[10] using transperitoneal approach according to Montsouris operative method.

The potential complications have become a matter of concern since the early beginnings of routine use of laparoscopy. One of them is the risk of ischemic tissue damage caused by CO_2 pressure exceeding the microcirculation pressure. Mesmer and others[11] wrote that microcirculation enables a direct connection between the blood and the tissue and, consequently, between the cell and the entire organism.

Regardless of the approach and the method, CO_2 pressure in the operative region is between 12 and 15 mm Hg. A laparoscopic radical prostatectomy lasts on average more than two and half hours[2–8]. Many authors have tried to answer the questions concerning the risk of ischemic tissue damage in the course of laparoscopic procedures caused by CO_2 pressure exceeding the microcirculation pressure. It is well known that a larger quantity

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of free oxygen radicals that follows the reestablishment of circulation after a certain period of ischemia may cause cellular damage. In 1954, Gerschman[12] wrote about the toxic effect of oxygen radicals. During ischemia, the quantity of calcium increases, which activates protease, which in turn catalyzes xanthine dehydrogenase into xanthine oxidase. This results in an increase in the quantity of free oxygen radicals. During hypoxia, energy expenditure increases and the catabolic product ATP hypoxanthine accumulates in the body[13,14]. The energy loss blocks protein synthesis and simultaneously activates numerous enzymes that decompose proteins and DNA[15]. The reestablishment of circulation is followed by the activation of protease, nuclease and nitric oxide synthase, which increases the quantity of nitric oxide (NO). That compound, in turn, reacts with superoxide radical and produces the toxic peroxinitrite radical that damages the cells[16]. Cu/Zn superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are enzymes that protect cells from free oxygen radicals in post-ischemic reoxygenation[17]. Measurements of their activity may be used for assessment of the tissue damage level. Their activity may also vary in different non-pathological situations[18]. For instance, it may increase during the aging process[19,20]. SOD catalyzes the reaction of dismutation of toxic superoxide radical into molecular oxygen and water, which is an important part of the antioxidative defensive mechanism[21]. It contains two equal parts of the same molecular weight, bonded by disulfide links, protecting hyaluronates from depolymerisation by simple oxygen radicals, and it may also have an anti-inflammatory effect. GPx is located in the mitochondrial cytoplasm and plays an important role in the elimination of toxic peroxides from the cell. It acts upon lipid hydroxides that are released from the phospholipid membrane. CAT is located in the peroxisomes of aerobic cells and it protects the cells from harmful effects of hydroxide peroxide, by catalyzing it into molecular oxygen and water[22,23]. Its concentration is higher in the liver than in the connective tissue. Cat in GPx in the cell decomposes hydrogen peroxide. CAT acts at high levels of hydrogen peroxide, GPx at low[24,25]. Hydrogen peroxide is a toxic product of normal metabolism as well as of pathological conditions. It is decomposed by catalase in dismutation reaction. Over 98% of CAT concentration is located in human erythrocytes.

Patients and Methods

Patients

In a ten-month period from 1 June 2002 to 31 March 2003, 44 patients were operated at the Department of Urology of the General Hospital in Slovenj Gradec using laparoscopic transperitoneal method (Montsouris technique) and 11 patients at the Department of Urology of the Teaching Hospital in Maribor using RRP. The protocols were the same for all patients. The study was time--limited, which caused the difference in the number of patients. That, in our view, had no impact on the result of the study. The average patient age in both groups was similar (62.6: 61.8 years). The value of PSA in both groups was also similar (6.5:7.1), as well as of the Gleason score (5.7: 6.1). The laparoscopic procedure lasted significantly longer (218 minutes: 139 minutes, $p=0.000$), so the microcirculation in laparoscopic treatments was exposed to CO_2 pressure for longer time.

Our study aims to find out if CO_2 pressure in laparoscopic radical prostatectomy damages microcirculation and consequently causes ischemic tissue damage. We measured the activity of SOD, GPx and CAT enzymes. Namely, in oxidative stress the activity of the mentioned enzymes increases because they protect the cells from the increased concentration of free oxygen radicals, which are produced in oxidative stress. At the same time, we used immunohistochemical and morphometric methods to assess the level of capillary endothelial damage in the operative region. Two groups of patients were followed up and analyzed. The first group consisted of 44 patients that between 1 June 2002 and 31 March 2003 had undergone transperitoneal laparoscopic radical prostatectomy (Montsouris surgical technique) at the Department of Urology in Slovenj Gradec. The CO_2 pressure was between 12 and 15 mmHg. The second group included 11 patients that in the same time period had been operated using the standard retropubic method at the Department of Urology in Maribor. The protocols for both patient groups were the same. Samples of blood were taken from all patients at the beginning and after surgical procedure to assess the activity of SOD, GPx and CAT enzymes. Furthermore, samples of tissue from the bladder neck were taken for immunohistochemical and morphometric analysis of capillary endothelium before creation of the uretrovesical anastomosis. The blood was centrifuged, cooled down to –18 degrees and sent to the Central Chemical and Biochemical Laboratory of the University Clinical Centre in Ljubljana, while the tissue samples were sent to the Institute of Pathology of Ljubljana School of Medicine. There the capillary endothelium, from the surface to the mucous lining of the bladder, was examined using immunohistochemical and morphometric methods. Immunohistochemical analysis was conducted using endothelial cell markers –CD 31. Endothelial cells were then visualized following the antigen retrieval in the microwave oven Polnar Patent PP-780 using the MSAPE (Microwave Strept Avidine Peroxidase Enzyme) method. This was a triphasic reaction. The antigen became visible as cytoplasm grains under the electronic microscope. Morphometric methods were used to determine the number of capillaries and, using the image analysis system, their diameters on the surface of the tissue. For each sample 10 visual fields were examined under 20 X magnification.

The pathohistological tests were conducted to find malignant cells. In all patients we tried to preserve the neck of the urinary bladder because it has an important role in the continence preservation. At the same time, we did not want to jeopardize the radical nature of the procedure.

The statistical analysis consisted of the following descriptive methods:

Minimum, maximum

- mean
- standard deviation
- standard error of mean

And the following statistical methods were used

- t-test to test the difference between two population means,
- paired t-test to estimate the difference between two means in the same group, χ^2-test to test the difference between two distributions

The following labels were used:

N	number of patients
%	percentage of patients
max	maximal data
min	minimal data
mean	mean
Std	standard deviation
stderr	standard error of mean
P	probability
*	level of significance between 0.01 and 0.05
**	level of significance between 0.005 and 0.01
***	level of significance between 0.001 and 0.005
****	level of significance less than 0.001

The Microsoft Excel 2000 was used for the computational part of the analysis. This report was written using Microsoft Word 2000. Both files are enclosed.

Results

It has already been said that the main goal of our study was to establish if CO_2 pressure during laparoscopic radical prostatectomy affects microcirculation and causes ischemic tissue damage in the operative region. Measurements of the systemic marker (SOD, GPx, CAT) activity showed no increase, which speaks against damage of the microcirculation and the consequent ischemic tissue damage in the operative region. The SOD activity did not significantly increase in patients operated using the laparoscopic method (p 0.220: 0.971). (Table 1, Figure 1). The GPx activity also did not significantly increase (Table 2, Figure 2). The activity of CAT was similar in both groups (p0.434: 0.279) (Table 3, Figure 3) and it failed to show a significant increase in laparoscopically treated patients.

Immunohistochemical analysis of a tissue sample from the neck of the urinary bladder was negative and non-specific in all operated patients. The samples was taken before suturing urethra-vesical anastomosis. Morphometric analysis of capillaries in the same tissue sample showed no signs of ischemic endothelial damage. A minor capillary dilatation in laparoscopically treated patient

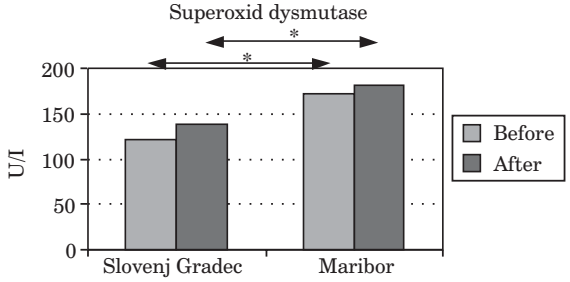

Fig. 1. Superoxide dismutase activity.

TABLE 1
SUPEROXIDE DISMUTASE ACTIVITY IN U/L (COMPARISION BY T-TEST)

		Before	After	P	Significance level
Slovenj Gradec	N	40	40		
	Max	239	323		
	Min	13	45		
	Mean	122.2	137.5	0.220	
	SD	48.558	66.092		
	Stderr	7.678	10.450		
Maribor	N	9	9		
	Max	244	244		
	Min	82	129		
	Mean	172.0	182.0	0.971	
	SD	46.505	46.184		
	Stderr	15.502	15.395		
P		0.014	0.029		
Significance level		*	*		

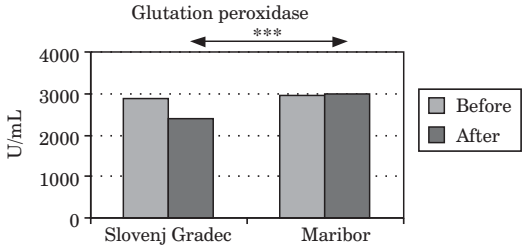

Fig. 2. Glutathione peroxidase activity.

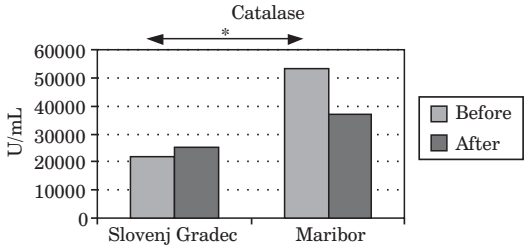

Fig. 3. Catalase activity.

TABLE 2
GLUTATIONE PEROXIDASE ACTIVITY IN U/ML (COMPARISION BY T-TEST)

		Before	After	P	Significance level
Slovenj Gradec	N	40	40		
	Max	26661	3711		
	Min	1189	1935		
	Mean	2871.0	2376.4	0.431	
	SD	3872.506	388.240		
	Stderr	612.297	61.386		
Maribor	N	9	9		
	Max	3711	3325		
	Min	2403	2694		
	Mean	2961.0	2978.0	0.850	
	SD	396.892	249.725		
	Stderr	132.297	83.242		
P		0.886	0.000		
Significance level			****		

was observed and explained by a direct impact of CO_2 on the capillary wall (Table 4), rather than ischemic damage.

Discussion

Laparoscopic radical prostatectomy is safe, comfortable for patients and less invasive than the standard retropubic prostatectomy. The procedure takes longer but has fewer peri- and post-operative complications than the open prostatectomy, while oncological results are equally good. In our study, the percentage of incontinent patients was manifestly lower and sexual potency was better. They used less painkillers, wore catheters and stayed in hospitals for shorter periods of time, and returned to normal physical activity faster. But the pressure during procedures is higher than the pressure in microcirculation.

The goal of the study was to establish if in laparoscopic radical prostatectomy CO_2 pressure damages microcirculation and causes ischemic tissue damage in the operative region. Namely, during a laparoscopic procedure, CO_2 pressure is higher than the blood pressure in microcirculation. This may cause an increased release of harmful free oxygen radicals post-desufflation, after a longer operative procedure such as laparoscopic radical prostatectomy. Numerous studies have tried to ascertain if CO_2 pressure in laparoscopic surgical treatments may cause microcirculation and ischemic tissue damage. Schelling et al.[26] measured microcirculation changes in the splanchnic region during laparoscopic cholecystectomy. They have established that at gas pressure of 15 mm Hg blood flow decreases for 40–54% in the stomach, 32% in the jejunum, 44% in the colon, 39% in the liver and 60% in the peritoneum. Elefteriadis et al.[27] measured liver microcirculation using a laser probe during laparoscopic cholecystectomy. They have found out that both liver microcirculation and stomach mucosa Ph were reduced. Quickly after desufflation the condition normalized. By contrast, Odeberg et al.[28] have found no such changes in their study of laparoscopic cholecystectomy. Bukan et al.[29] measured the level of malondialdehyde (MDA) in two groups of operated patients. One group was operated using the standard method and the other laparoscopically. The value of the variable increased during the procedure, but the increase was higher in open cholecystectomy. The result has shown that both cholecystectomy methods cause oxidative stress, but the condition normalizes quickly after the procedure. Ozmen et al.[30] measured MDA and pH in the stomach. They have con-

TABLE 3
CATALASE ACTIVITY IN U/ML (COMPARISION BY T-TEST

		Before	After	P	Significance level
Slovenj Gradec	N	39	38		
	Max	64529	89645		
	Min	579	1868		
	Mean	21812.8	25467.8	0.434	
	SD	16899.42	25251.20		
	Stderr	2706.073	4096.285		
Maribor	N	10	9		
	Max	122618	84879		
	Min	14683	3864		
	Mean	53407.8	37022.8	0.279	
	SD	32945.51	25700.93		
	Stderr	10418.28	8566.98		
P		0.015	0.247		
Significance level		*			

cluded that laparoscopic cholecystectomy has no harmful effect on the intestinal perfusion. Polat et al.[31] assessed oxidative stress in laparoscopic and open hernioplasty. They measured the markers of oxidative stress: MDA, carbonyl protein and sulphide protein. They have concluded that in both methods these markers of oxidative stress increase significantly. Urena and al.[32] compared oxidative stress in longer-lasting pneumoperitoneum with open operative procedures. They took urine samples before, immediately after and 6 and 18 hours after the procedure to measure the concentration of F2a prostaglandin. Immediately before the procedure the concentration increased in both groups. It normalized 18 hours after the surgery. In patients who were operated laparoscopically the increase was somewhat higher and the return to normal values somewhat slower.

In laparoscopic surgery, CO_2 pressure increases the plasma concentration of catecholamines regardless of the bodily posture during the operation. This causes an increase in vascular resistance in particular in the lungs.[33]. A longer-lasting ischemia may cause damage and dysfunction of capillaries, which complicates the normalization of the nutritive circulation in capillaries. This event is variously explained. The cause may be haemoconcentration with subsequent thrombosis, leukocyte plug, endothelial cell oedema, vasomotor dysfunction and interstitial oedema[34]. Microscopic studies in vivo have shown that the leukocyte plug is the original cause and the obstacle to the post-ischemic re-establishment of circulation in striated muscles. Histological examinations have confirmed that the endothelium is damaged and that the fluid is lost into the intercellular space with consequent haemoconcentration, endothelial and intercellular oedema. These events further reduce the vascular diameter, increase the vascular resistance and decrease perfusion. It has been shown that 30 minutes after a myocardial infarction the capillary walls become oedematous and capillary lumens, as well as the flow, are reduced[35]. In a study on dogs, Arminger et al.[36] have found similar changes after a coronary arterial occlusion. Ward and McCarthy[37] have discovered that a 60-minute ischemia is followed by endothelial cell oedema, which reduces the capillary diameter and consequently decreases the blood flow.

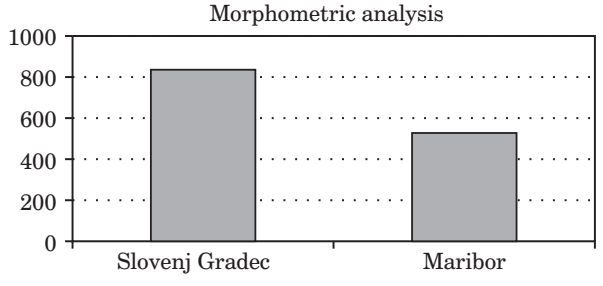

Fig. 4. Morphometric analysis of the area of capillaries at the surface of the urinary bladder.

The goal of our study was to find out if CO_2 pressure in laparoscopic radical prostatectomy causes oxidative stress and ischemic tissue damage in the operative region. By measuring the activity of SOD, GPx and CAT enzymes, we tried to determine the extent to which free oxygen radicals are released. Immunohistochemical and morphometric methods were applied to assess the potential capillary damage caused by oxidative stress in the region of the urinary bladder neck.

CO_2 pressure in the operative region exceeds the microcirculation pressure. Immunohistochemical and morphometric methods were used to evaluate the condition of capillary endothelium in the region. Immunohistochemistry applied a specific endothelial marker, CD31, to visualize capillaries in tissue samples from the bladder neck and evaluate endothelial damage[38]. Morphometric analyses used i-nitric oxide synthase (iNOS), the enzyme

that catalyze nitric oxide synthesis. It belongs to the group of isosomes that convert L-arginine into L-citrulline and produce NO, which is a short-lived radical that transmits cellular signals of the vascular wall tension and thus influences the blood pressure[39], neurotransmission and cytotoxicity. It has been shown that ischemia-reperfusion causes the endothelial cells to swell and burst and the blood to leak into the perivascular space[40,41]. The morphometric method was used to visualize and count the number of capillaries in the tissue sample, as well as to measure their diameters. The image analysis system »Cell and Tissue Analysis« was used. Ten visual fields were examined for each sample.

Conclusion

In our patients, we have found no increased activity of SOD, GPx and CAT enzymes and neither have immunohistochemical and morphometric analyses shown any signs of capillary endothelium ischemic damage. The samples were taken before suturing urethrovesical anastomosis.

The answer to our original question, »does CO_2 pressure causes ischemic tissue damage?« is negative. This additionally increases the safety of laparoscopic radical prostatectomy. Still, the best method is, in our opinion, the one that is impeccably mastered by the surgeon.

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ISHEMIJA TKIVA ZBOG TLAKA CO_2 ZA VRIJEME LAPAROSKOPSKE RADIKALNE PROSTATEKTOMIJE

SAŽETAK

Laparoskopska radikalna prostatektomija je danas jedna od najčešćih operativnih zahvata u urologiji. Za insuflaciju kod laparoskopske radikalne prostatektomije (LRP), upotrebljavamo CO_2 sa tlakom od 12–15 mm stupca žive u operativnom području. Na nivou mikrocirkulacije je tlak niži zbog čega postoji mogućnust izhemijskog oštećenja tkiva za vrijeme operativnog zahvata. Aktivnost glutation peroxidase (GSH-px), superoksid dismutaze (SOD) i katalaze (CAT) smo mjerili na početku i poslije završenog operativnog zahvata kod 44 bolesnika, kod kojih je bila napravljena LRP i kod 11 bolesnika, kod kojih je bila napravljena retropubična radikalna prostatektomija (RRP). Oštećenje endotela kapilara smo kontrolirali imunohistokemijskom i morfometrijskom metodom na uzorku tkiva iz vrata mokraćnog mjehura, koji je sadržavao sve slojeve stijenke mokraćnog mjehura. Mjerenje aktivnosti encima nije pokazalo signifikantni porast GSH-px (p=0,431), SOD (p=0,220), CAT (p=0,434). Niti imunohistokemijska analiza kapilara iz mokračnog mjehura sa i-dušičnom oksid sintazom (i-NOS) kao ni morfometrijska analiza nisu pokazali ishemijsko oštećenja endotela.