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N-acetylcysteine (NAC) Protects Against Acute Kidney Injury (AKI) Following Prolonged Pneumoperitoneum in the Rat

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Background. Acute kidney injury (AKI) following prolonged laparoscopy is a documented phenomenon. Carbon dioxide pneumoperitoneum induces oxidative stress. Previous experimental studies have shown that the antioxidant, N-acetylcysteine, protects the rat from AKI following ischemia-reperfusion. The aim of this study was to evaluate the effects of N-acetylcysteine (NAC) on rat renal function after prolonged pneumoperitoneum.

Methods. Normal rats treated or not with NAC were submitted to abdominal CO₂ insufflation of 10 mmHg, at short and long periods of time of 1 and 3 h, respectively, and evaluated at 24, 72 h, and 1 wk after desufflation. Glomerular filtration rate (GFR) was measured by inulin clearance and oxidative stress was evaluated by serum thiobarbituric acid reactive substances (TBARS).

Results. No significant alterations in GFR were observed in normal animals submitted to the pneumoperitoneum of 1 h and evaluated after 24 h desufflation. With 3 h of pneumoperitoneum, a significant and progressive decrease in GFR occurred 24 and 72 h after desufflation with an increase in serum TBARS. GFR returned to normal levels a week later. In the NAC-treated rats, a complete protection against GFR drops was observed 24 and 72 h following 3 h of pneumoperitoneum associated with a decrease in TBARS.

Conclusion. These results suggest that NAC protects against acute kidney injury following prolonged pneumoperitoneum. These findings have significant clinical implications.

Key Words: pneumoperitoneum; AKI; glomerular filtration rate; NAC; oxidative stress.

INTRODUCTION

In general, laparoscopic surgery has begun to be more used with the incorporation of a video system since the first cholecystectomy [1]. In the beginning, laparoscopic surgeries were carried out in young patients without serious pathological antecedents. However, with the advance of technology and with surgeons using this technique, the profile of the population of patients submitted to the laparoscopic surgeries came to change.

Laparoscopic surgery represents a model of ischemia-reperfusion kidney injury in which the rise of the intra-abdominal pressure induced by pneumoperitoneum produces alterations in renal blood flow and reduction of the urinary output and creatinine clearance [2–6]. After desufflation of the abdomen, intra-abdominal pressure and splanchnic blood flow normalize, representing reperfusion.

Ischemia-reperfusion increases oxidative stress. Oxidative stress is an imbalance between oxidants and antioxidants, with increase of oxidants leading to tissue damage.

Reactive oxygen metabolites are important mediators of ischaemic renal failure. A recent review reported animal studies and human clinical trials indicating that pneumoperitoneum decreases splanchnic perfusion with the generation of oxidative stress [7].

N-acetylcysteine protects organs by scavenging the oxygen-free radicals (either directly or by increasing intracellular concentrations of glutathione). Previous studies have shown that N-acetylcysteine (NAC) improves...
renal function in rats and/or human with ischaemia/reperfusion-induced acute kidney injury, radiocontrast nephropathy, and ureteral obstruction [8–10].

The purpose of the present study was to evaluate a protective effect of NAC on the renal function of rats submitted to prolonged time of pneumoperitoneum.

METHODS

Experimental Animals

Adult male Wistar rats weighing 250 to 280 g were obtained from the animal facilities of the University of São Paulo School of Medicine. Selected rats were treated with NAC (440 mg/kg BW in drinking water and standard rat chow (Nuvilab, Colombo, Brazil). Animals were returned to their original cages and given free access to water and standard rat chow (Nuvilab, Colombo, Brazil).

The following groups were studied:

1) Control group (sham operated-rats, without pneumoperitoneum (PP), (n = 6);
2) Sham operated-rats + 1 h (PP) evaluated after 24 h (n = 6);
3) Sham operated-rats + 3 h (PP) evaluated after 24 h (n = 6);
4) Sham operated-rats + 3 h (PP) evaluated after 72 h (n = 6);
5) Sham operated-rats + 3 h (PP) evaluated after 1 wk (n = 6);
6) NAC treated without PP (n = 7);
7) NAC treated + 3 h (PP) evaluated after 24 h (n = 6);
8) NAC treated + 3 h (PP) evaluated after 72 h (n = 6).

Induction of Pneumoperitoneum

Animals were anesthetized with 50 mg/kg body weight (BW) of sodium pentobarbital, administered intraperitoneally. Pneumoperitoneum was induced by 10 mmHg CO2 insufflation by 1 and 3 h [11, 12]. After deinsufflation and recovery from the anesthesia, the animals were returned to their original cages and given free access to water and standard rat chow (Nuvilab, Colombo, Brazil).

Clearance Studies and Collection of Blood/Urine Samples

To determine glomerular filtration rates, inulin clearance studies were conducted. On the day of the experiment, the animals were anesthetized intraperitoneally with sodium thiopental (50 mg/kg BW). The trachea was cannulated with a PE-240 catheter and spontaneous breathing was maintained. To control mean arterial pressure and allow blood sampling, another PE-60 catheter was inserted into the right carotid artery. For the infusion of inulin and fluids, another PE-60 catheter was inserted into the left jugular vein. In order to collect urine samples, a suprapubic incision was made, and the urinary bladder was cannulated with a PE-240 catheter. After the surgical procedure had been completed, a loading dose of inulin (100 mg/kg BW diluted in 0.9% saline) was administered through the jugular vein. Subsequently, a constant infusion of inulin (10 mg/kg BW in 0.9% saline) was started and was continued at 0.04 mL/min/100 g BW throughout the experiment. Three urine samples were collected at 30-min intervals. Blood samples were obtained at the beginning and at the end of the experiment. Blood and urine inulin were determined using the anthrone method. Glomerular filtration rates are expressed as mL/min/100 g BW.

Reactive Oxygen Metabolites

Serum levels of thiobarbituric acid reactive substances (TBARS), which are markers of lipid peroxidation, were determined using thiobarbituric acid assay. In brief, a 0.2-mL serum sample was diluted in 0.8 mL of distilled water. Immediately thereafter, 1 mL of 17.5% trichloroacetic acid was added. Following the addition of 1 mL of 0.6% thiobarbituric acid, pH 2, the sample was placed in a boiling water bath for 15 min, after which it was allowed to cool. Subsequently, 1 mL of 70% trichloroacetic acid was added, and the mixture was incubated for 20 min. The sample was then centrifuged for 15 min at 2000 rpm. The optical density of the supernatant was read at 534 nm against a reagent blank using a spectrophotometer. The quantity of TBARS was calculated using a molar extinction coefficient of 1.56 × 10^5 M^−1 cm^−1. Serum levels of TBARS are expressed as nmol/mL [13].

Statistics Analysis

All data are given as means ± SEM. The comparison between groups was performed using ANOVA and Student Newman-Keuls post-test. Statistical significance was established at P < 0.05.

RESULTS

A short period of pneumoperitoneum (1 h) did not modify the glomerular filtration rate, measured 24 h after pneumoperitoneum. The inulin clearance measured 24 h following 1 h of pneumoperitoneum was 0.78 ± 0.10 mL/min/100 g BW, a value similar to sham rats (0.82 ± 0.05 mL/min/100 g BW).

As we can see in Table 1, with 3 h of pneumoperitoneum a significant and progressive decrease in inulin clearance occurred 24 and 72 h after deinsufflation. Inulin clearance measured 1 wk after 3 h of pneumoperitoneum returned to values not significantly different from sham (0.69 ± 0.04 mL/min/100 g BW). Decrease in inulin clearance observed 24 and 72 h following pneumoperitoneum was associated with an increase in serum TBARS, indicating that prolonged pneumoperitoneum induced acute kidney injury (AKI) associated with an increase in oxidative stress. No significant differences in blood pressure were observed in all groups. The administration of NAC provided complete protection against inulin clearance drops and increases in TBARS levels. These data demonstrated that this antioxidant protects against AKI following prolonged pneumoperitoneum.

DISCUSSION

Pneumoperitoneum decreases splanchnic perfusion with resulting oxidative stress. Several studies have demonstrated the beneficial effect of antioxidants such as NAC in ischemic and nephrotoxic AKI. Our results show that NAC administration completely protects against the decrease in glomerular filtration rate (GFR) induced by prolonged PP.

Acute kidney injury was detected in this study 24 h after 3 h of pneumoperitoneum. The inulin clearance decreased to 20% of normal values 72 h following pneumoperitoneum and recovered to near-normal values in 7 d.

Reactive oxygen species (ROS) are produced by mitochondria during conversion of molecular oxygen to...
Glomerular Filtration Rate (GFR); Serum Thiobarbituric Acid Reactive Substances (TBARS) and Blood Pressure (BP) in Sham Operated and Rats Submitted to 3 h of Pneumoperitoneum (PP) Not Treated and Treated with N-Acetylcysteine (NAC)

<table>
<thead>
<tr>
<th>Groups</th>
<th>GFR (mL/min/100 g BW)</th>
<th>TBARS (nM/mL)</th>
<th>BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 6) Sham without PP</td>
<td>0.82 ± 0.05</td>
<td>2.80 ± 0.14</td>
<td>120.0 ± 0.5</td>
</tr>
<tr>
<td>2 (n = 6) 1 hPP (24 h after)</td>
<td>0.78 ± 0.10</td>
<td>2.47 ± 0.10</td>
<td>116.4 ± 2.0</td>
</tr>
<tr>
<td>3 (n = 6) 3 hPP (24 h after)</td>
<td>0.42 ± 0.06*</td>
<td>3.71 ± 0.20</td>
<td>125.0 ± 0.5</td>
</tr>
<tr>
<td>4 (n = 6) 3 hPP (72 h after)</td>
<td>0.20 ± 0.06*</td>
<td>4.25 ± 0.18*</td>
<td>120.0 ± 0.2</td>
</tr>
<tr>
<td>6 (n = 7) Sham+NAC</td>
<td>1.02 ± 0.03</td>
<td>1.97 ± 0.01</td>
<td>120.0 ± 0.4</td>
</tr>
<tr>
<td>7 (n = 6) NAC+3 hPP (24 h after)</td>
<td>0.84 ± 0.07</td>
<td>2.46 ± 0.34</td>
<td>118.0 ± 0.3</td>
</tr>
<tr>
<td>8 (n = 6) NAC+3 hPP (72 h after)</td>
<td>0.96 ± 0.07</td>
<td>2.19 ± 0.17</td>
<td>123.0 ± 0.5</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*P < 0.05 versus sham without PP.

**P < 0.01 versus sham without PP.

TABLE 1

Water. The cells have antioxidant mechanisms such as superoxide dismutase and glutathione. Oxidative stress occurs when there is an imbalance between antioxidants and antioxidants. During oxidative stress, ROS may damage lipids, proteins, and cause oxidation of DNA. It is well demonstrated that kidney markers of oxidative stress (MDA) rise in a time-dependent manner [14]. A progressive increase of TBARS was observed in our study from 24 to 72 h following pneumoperitoneum in the untreated rats.

A recent study with children submitted to laparoscopic surgery under general anesthesia and mechanically ventilated demonstrated an increase in total plasma oxidant status and a decrease in total antioxidant status, suggesting that reactive oxygen species are produced during laparoscopy and cause oxidative stress in these children [15].

In another study, with adult patients who underwent a laparoscopic cholecystectomy and who were compared with patients submitted to an abdominal wall hernia surgery under general anesthesia, paraoxonase and arylesterase levels, markers of oxidative stress, were higher in the first group [16].

Pharmacologic agents have been tested to reverse oxidative stress consequent to pneumoperitoneum. The administration of erythropoietin before the pneumoperitoneum significantly decreased the plasma levels of LDH, TNF-α, and MDA, compared with those found in the laparoscopy group [17].

Melatonin administered before insufflation and immediately before deinsufflation significantly reduced mean malondialdehyde levels in the kidney [18]. Similarly, zinc, pentoxifyline and NAC decreased MDA kidney levels, however, no functional data of renal function were evaluated in this studies [19].

Our study was the first to demonstrate a protection of NAC on the glomerular filtration rate by the gold standard method- the inulin clearance. NAC benefits in humans following ischemia-reperfusion kidney injury have yet to be proven. However, a recent study in neonates undergoing arterial switch operation for dextro-transposition of the great arteries demonstrated that perioperative treatment with NAC resulted in improved urine output, shorter time to negative fluid balance, and attenuation of the rise in creatinine [20].

NAC is an inexpensive drug that produces no major collateral side effects. Studies in patients are needed in order to determine the protective effect of NAC on ischemia-reperfusion injury induced by PP in laparoscopic surgeries.

REFERENCES

1. Reynolds W Jr. The first laparoscopic cholecystectomy. JSLS 2001;5:89.