Osmotic nephrosis due to the use of anti-adhesive membrane intraperitoneally

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Abstract

Background. A common strategy for the prevention of intra-abdominal adhesions post-operatively has been the application of adhesion barriers into the peritoneal cavity. Side effects of these barriers are infection, abscesses and inadequate wound healing. There is no information about such a side effect of these materials on renal function. The aim of this study was to evaluate the effect of two different, commercially available polysaccharide-based anti-adhesive materials on renal function.

Methods. In 24 adult Wistar rats, an abdominal midline incision was performed, and an anti-adhesion membrane was placed in the peritoneal cavity so as to cover its whole surface. Four rats were used as the control group. In 12 rats, a membrane of macromolecular polysaccharides, weighing 40 mg/cm², was placed intra-abdominally and in 8 rats, a hyaluronic acid-hydroxyacidmethylcellulose membrane weighing 0.4 mg/cm² was placed. At 24 or 70 h, the rats were sacrificed, and we evaluated changes in serum creatinine, urea, uric acid, K and Na, and histologic examination of the kidney was performed.

Results. The use of the thicker macromolecular membrane was associated with a rise in serum creatinine and urea levels, vacuolization of all the tubular epithelial cells and mild interstitial infiltration. Rats in which the hyaluronic acid-hydroxyacidmethylcellulose membrane was used did not show any creatinine elevation, and they presented milder histologic lesions.

Conclusion. Polysaccharide and cellulose anti-adhesive membrane cause renal damage with tubular cell vacuolization. The severity of kidney damage is relative to the quantity of the membrane material used.

Keywords: acute renal failure; anti-adhesive barriers; macromolecular polysaccharides; osmotic nephrosis

Introduction

Major intra-abdominal surgery and peritoneum inflammation are a common cause of adhesions in the peritoneal cavity. Post-operative adhesions often elicit symptoms such as abdominal pain and have been associated with bowel obstruction and female infertility [1]. A practical preventive technique used to minimize post-operative adhesions is the placement of adhesion-reducing agents in the abdomen intra-operatively.

A variety of different materials have been used as adhesion-reducing agents in experimental as well as in clinical trials, including substances with anti-inflammatory, fi-
brinolytic, vasoactive and antioxidant properties. One of the modalities that have been used showing promising results is the so-called barrier method. The concept of this technique is to keep surgically traumatized surfaces covered with biomaterial barriers during mesothelial regeneration, thus preventing formation of adhesions with adjacent organs. The compounds that have been used as barriers can be found in either liquid or solid form and consist of macromolecular polysaccharides like dextrane, mananitol, processed cellulose etc. The most common adverse effects that have been encountered when using such materials include infection, abscesses and inadequate wound healing [2–4]. There is no information in the literature about the effect of such material on renal function.

Having the experience of two patients, who presented with acute renal failure after abdominal surgical operation that included the use of a certain type of anti-adhesion membrane in the peritoneal cavity, we studied experimentally the effect of two types of anti-adhesive membranes on renal function.

Materials and methods

For the study, we used 24 adult Wistar rats. The study was performed in the Experimental Animal Research Laboratory of Physiology Department, Medical School of Aristotle University. The study protocol was in accordance with the rules for handling and protecting experimental animals. Mean body weight of the experimental animals was 288 ± 30 mg. The animals originally lived under stable temperature conditions (22 ± 2°C) day–night cycle and had free access to food and water. They were then isolated in individual cages for 24 h before the surgical procedure with water intake ad libitum.

The rats were anaesthetized with hydrated chloral in a dosage of 1 mg/100 g of body weight. An abdominal midline incision was performed, and an anti-adhesion membrane was placed in the peritoneal cavity so as to cover its whole surface. The midline incision was closed by continuous suture in two layers. Twenty-four to 70 h later, the animals were submitted to a second evaluation laparotomy before being sacrificed.

Two different types of anti-adhesion membranes were studied. There were differences in the type of the material used as well as in the quantity of the material per surface unit (the thickness of the membrane). The first membrane consisted of macromolecular polysaccharides and was quite condensed, weighing 40 mg/cm² of membrane surface, and the second one consisted of hyaluronic acid and hydroxyethylcellulose, weighing almost one-hundredth of the first membrane (0.4 mg/cm²). They had both been characterized as biocompatible, biodegradable material, expected to be completely absorbable and have been approved for intra-abdominal use as anti-adhesion membranes in humans. In order to investigate the hypothesis that the quantity of the membrane could affect the appearance of renal damage, two different quantities of each membrane were studied.

Four animals, in which 10 mL of saline water were placed intraperitoneally instead of anti-adhesion membrane, comprised the control group (Con).

The polysaccharide membrane was used at a dose of 12.25 cm² (sufficient to cover the inner peritoneal surface) in 8 out of the 24 animals (Su1). In four of them, the evaluation laparotomy was performed 24 h after membrane placement (Su1-24) and in the remaining four at 70 h (Su1-70). There were also four more animals in which we used the same membrane but in half of the original dose (6.12 cm²) (Su2), and these were also sacrificed at 70 h.

In the remaining eight animals, the cellulose and hyaluronic acid membrane was used. In four of them, we used 12.25 cm² of the membrane (Se1) and in another four the double dose (24.5 cm²) (Se2). In both Se1 and Se2 groups, evaluation laparotomy was performed 70 h later.

Evaluation laparotomy was followed by ligation of the renal vessels at the renal hilus. Blood samples were drawn from the aorta for the measurement of urea, creatinine, uric acid, potassium and sodium. Both kidneys were removed for histological examination.

### Histological analysis

Sagittal 0.5 cm kidney sections were taken and maintained in a 10% formalin solution. They were then embedded in paraffin blocks, cut in 2 μm sections and stained with haematoxylin–eosin. Renal histological examination included evaluation of the renal cortex and medulla. Apart from the renal glomeruli, we also examined the tubulointerstitial tissue, evaluating the following parameters: (i) vacuolization in tubular cells, (ii) degeneration of tubular cells and (iii) infiltration of the interstitial tissue by inflammatory cells. We evaluated 20 visual fields (10 from the renal cortex, 10 from the medulla) on each kidney (eight kidneys from each group of four animals), and the lesions were staged as follows: Stage 0, no lesions; and Stages 1, 2, 3 and 4: lesions expanding to zero–25%, 25–50%, 50–75% and >75% of the tissue examined, respectively.

Statistical analysis was performed using SPSS13.0 for Windows (SPSS Inc., Chicago, IL, USA) statistical software. All values are presented as mean and standard deviation. Differences between groups were evaluated by ANOVA with Bonferroni correction. P-values of <0.05 were considered statistically significant.

### Table 1. Serum biochemistry data from animal groups at evaluation laparotomy

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>K (mEq/L)</th>
<th>Na (mEq/L)</th>
<th>Ca (mEq/L)</th>
<th>Uric acid (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>48 ± 3</td>
<td>0.47 ± 0.02</td>
<td>4.0 ± 0.5</td>
<td>140.8 ± 0.2</td>
<td>4.0 ± 0.5</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Su1-24</td>
<td>65 ± 27</td>
<td>1.15 ± 0.43</td>
<td>5.8 ± 0.1</td>
<td>137.3 ± 0.6</td>
<td>5.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Su1-70</td>
<td>289 ± 73*</td>
<td>3.03 ± 1.02*</td>
<td>5.5 ± 0.2</td>
<td>138 ± 0.5</td>
<td>138.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Su2</td>
<td>38 ± 2</td>
<td>0.52 ± 0.00</td>
<td>5.0 ± 0.3</td>
<td>139.1 ± 0.2</td>
<td>140.1 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Se1</td>
<td>41 ± 3</td>
<td>0.52 ± 0.01</td>
<td>4.7 ± 0.2</td>
<td>140.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Se2</td>
<td>45 ± 4</td>
<td>0.51 ± 0.01</td>
<td>4.9 ± 0.1</td>
<td>141 ± 0.8</td>
<td>0.9 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

Con, control group.

*P<0.0001 (ANOVA).

### Fig. 1. Renal biopsy. Tubular vacuolization in group Su1 HE 20×.
Results

In the four animals that were sacrificed at 24 h, evaluation laparotomy revealed that the anti-adhesive membrane had been dissociated, was in a gelous form and had been partially absorbed. On evaluation laparotomies at 70 h after membrane placement, we found that the membrane had been completely absorbed and that there appeared to be peritoneal oedema. Macroscopically, the kidneys revealed no significant findings.

Concerning biochemistry, we found an increase in the levels of urea and creatinine compared with controls, in both Su1-24 and Su1-70 groups, that was significant only for the latter (P<0.0001), while in the remaining groups all of the biochemical parameters examined remained at normal levels (see Table 1).

The histological examination did not reveal any glomerular lesions, but there were important findings from the renal tubules and the interstitial tissue. The most typical lesions that we found included micro- and macrovacuolization of the tubular epithelial cells, with apical degeneration which was focally quite severe, leading to apoptosis. Tubular vacuolization was almost uniformly present in all treated groups. The interstitium appeared focally mildly oedematous, and there were areas of mild lymphocytic infiltration. Epithelial degeneration and interstitial infiltration seemed to be rather dependent upon the dose rather than the molecular composition of the membrane used, as Su2 values did not differ from the two Se groups, but they were strikingly different from those observed with higher doses of polysaccharide membrane (Su1-24, Su1-70) (Figures 1, 2 and 3). Among these last two groups that used the original dose of the thick polysaccharide membrane, the severity of epithelial degeneration and interstitial infiltration lesions seemed to be also time dependent, probably reflecting an active ongoing inflammatory procedure. The evaluation of tubulointerstitial lesions in each group of animals, according to the grading system that we described above, is presented in Table 2. It is worth mentioning that the presence of histologic lesions in Su2, Se1 and Se2 was not associated with serologic evidence of renal function deterioration (i.e. a rise in urea or creatinine).

Discussion

The present study is the first to show that adhesion-reducing agents placed in the peritoneal cavity during laparotomy can cause renal damage. The damage was detected at the renal tubules and the interstitial space and histologically presented as osmotic nephrosis. The extent of the damage depended on the composition of the membrane, i.e. the kind and the quantity of the polysaccharides that they contain.

![Fig. 2. Renal biopsy. Tubular vacuolization in group Su2 HE 20×.](image1)

![Fig. 3. Renal biopsy. Tubular vacuolization in group Se1 HE 20×.](image2)

Table 2. Mean severity scores per group of animals for each pathological parameter of tubulointerstitial damage examined

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Su1-24</th>
<th>Su1-70</th>
<th>Su2</th>
<th>Se1</th>
<th>Se2</th>
</tr>
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<tbody>
<tr>
<td>Tubular vacuolization</td>
<td>0</td>
<td>3.1 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4 ± 0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tubular epithelial degeneration</td>
<td>0</td>
<td>2.4 ± 0.78&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.4 ± 0.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.9 ± 0.2</td>
<td>0.83 ± 0.25</td>
<td>0.86 ± 0.32</td>
</tr>
<tr>
<td>Interstitial infiltration</td>
<td>0</td>
<td>1.1 ± 0.2&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.9 ± 0.15&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.3 ± 0.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.

<sup>a</sup>Su1-24 vs Con P<0.0001, vs Se1 P<0.01 and vs Se2 P<0.003.
<sup>b</sup>Su1-70 vs Se1 P<0.0001, vs Su2 P<0.01 and vs Se2 P<0.003.
<sup>c</sup>Su2, Se1 and Se2 vs CON P<0.0001.
<sup>d</sup>Su1-24 vs Con P<0.0001, vs Su2, Se1 and Se2 P<0.01.
<sup>e</sup>Su1-70 vs Con, Su2, Se1, Se2 P<0.0001.
<sup>f</sup>Su1-24 and Su1-70 vs OE, Su2,Se1, Se2 P<0.0001.
Heavier lesions were found in the animals where we used the barriers with polysaccharides of especially dense composition, since the membrane weight was 100 times greater than that of the hydroacidmethylcellulose membrane with hyaluronic acid.

Surgical re-exploration of the abdominal cavity either in 24 or in 70 h revealed that the two membranes were broken down and partially or completely absorbed from the peritoneum. Intraperitoneally, enzymes such as α-amylase caused the hydrolysis of both hydrocellulose and the polysaccharides to oligosaccharides. The degradation products reached the kidneys fast through the circulation, as there were already lesions in the tubules in the 24 h group.

These lesions were first described in 1930 after i.v. infusion of hypertonic saccharose [5,6]. In 1951, Allen [7] named these lesions ‘osmotic nephrosis’ because he attributed the creation of vacuoles to osmotic forces between tubular cells and tubular lumen. The same renal lesions were later described in cases of i.v. infusion of oligo- or polysaccharide solutions to treat oedema (i.e. mannitol in cerebral lesions) or as a plasma substitute for intravascular volume expansion [8–10]. There are also reports of similar lesions after administration of immunoglobulins or iodine-containing contrast agents [11].

The mechanism is not clear. The obstruction of tubules by macromolecular substances and the increase in plasma osmotic pressure were the first theories that tried to explain the formation of vacuoles [7].

In the last years, a new theory has emerged: tubular cells ingest the oligosaccharides through pinocytosis and entrap them into cysts which later pile up in lysosomes and gradually create large vacuoles that tend to occupy the cytoplasm [12–14]. Whether this vacuole formation is simply a coincidence or the reason for the renal failure has been a matter of intense debate. The reason for this debate was the fact that similar lesions have appeared in patients with intact renal function. However, we think that the presence of increased serum creatinine, which is a late and not sensitive marker of renal damage, is only restricted to cases where tubular damage is extended, severe and is followed by renal failure. In accordance with that, early markers of renal damage have been shown to increase in kidneys infiltrated with hydroxyethylstarch (HES), long before the appearance of renal failure [15]. The magnitude of the lesions is probably associated with the molecular weight of the saccharides used.

In the present study, we found out that serologic markers of renal dysfunction such as urea and creatinine increased approximately 6-fold of the baseline levels. This increase appeared in the animals in which the thick composition membrane was used. Those animals also demonstrated the heavier histological damages. Unfortunately, a limitation of our study was that we did not measure the by-products of membrane degradation in order to discover whether the oligosaccharides that were produced by hydrolysis of the two different membranes. It was reasonably assumed though that the thicker membrane should have resulted in greater oligosaccharide burden.

Hüter et al. [15] administrated HES of different density and molecular weight and found out that the animal kidneys that were injected with high-molecular-weight HES had more serious histological damages, extended presence of vacuoles in the tubular cells and more intense infiltration of interstitial space with inflammatory cells. Additionally, Citanova et al. [16] found out that renal transplants from donors that had previously received high-molecular-weight HES presented delayed onset of diuresis and worse survival rate compared with transplants from donors who had received gelatin instead for volume expansion. This was attributed to the fact that gelatin has a lower molecular weight and can be detected in the circulation for a much smaller period as it has a shorter half elimination time.

Further evidence to support this theory came from the study of Schortgen et al. [17] where they compared the effects of a macromolecular HES and gelatin on renal function, and discovered that HES was an independent risk factor for renal failure in septic patients.

All these studies suggest that high-molecular-weight polysaccharides are correlated with a greater risk of renal failure. Besides the molecular weight, it seems that polysaccharide quantity, duration and speed of administration also play an important role in creating tubular damage [18]. These lesions are usually reversible. There have been reports though of cases of patients with pre-existing renal failure or advanced age where renal failure persisted and they consequently remained in dialysis [19].

What we found out from this study was that macromolecular polysaccharide membranes placed post-operatively in the endoperitoneum in order to prevent adhesion can cause severe kidney damage that may lead to renal function deterioration.

What was also impressive was that there was also significant histologic damage in the groups of animals that were exposed to a lesser burden of anti-adhesion membranes, even though this was not reflected in the serum levels of urea and creatinine. This implies that we should be cautious in evaluating the impact of such therapeutic interventions, which may have been systematically underestimated due to the poor sensitivity of our current markers of renal injury.

Finally, the hydroacidmethylcellulose/hyaluronic acid membranes that were used in our study appeared to cause milder kidney damage and did not affect renal function, as compared with the polysaccharide membrane. This may have been associated with the metabolic by-products or rather a matter of greater polysaccharide burden.

Conflict of interest statement. None declared.

References
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