The aim of the study was to evaluate the effects of 0.9% NaCl and other solutions suitable for peritoneal lavage on in vitro viability and fibrinolytic activity of human peritoneal mesothelial cells. Material and methods. Mesothelial cells were isolated from the intra-operatively collected greater omentum specimens and then cultured. Subsequently, eight cultures (n=8) were incubated for six hours with M199 culture medium (control group) or culture medium with the addition of 0.9% NaCl, PD fluid Gambrosol-Trio 10 or Hanks’ solution. Immediately after exposure to the studied solutions, the amount of LDH released by the cultured cells was determined. Then, cultured cells were incubated for the next 24 hours; after this period their metabolism and fibrinolytic activity were determined by means of IL-6, t-PA and PAI-1 levels. Levels of these substances were compared to the amount of total cellular protein that was determined simultaneously.

Results. Hanks’ solution showed no significant influence on cellular culture architecture and mesothelium metabolism. PD fluid Gambrosol-Trio 10 damaged mesothelial cell structure and function as manifested by an increased release of LDH and enhanced t-PA synthesis, as well as decreased production of IL-6 and PAI-1. Exposure to 0.9% NaCl solution was found to cause a disturbance in cellular adhesion without significant structural damage (marked changes in cellular morphology without increased LDH release) and significant metabolism impairment as presented by reduced production of t-PA, IL-6 and PAI-1.

Conclusions. Saline solution and PD fluids should not be used for peritoneal lavage. Further investigations are required to identify the least harmful solution for mesothelial cells suitable for application in intra-operative peritoneal lavage.

Key words: peritoneum, saline solution, peritoneal lavage, peritoneal adhesions

Peritoneal mesothelial cells, which line the abdominal walls and intraperitoneal organs, demonstrate a high metabolic activity and take part in inflammatory reactions following peritoneal injury (through the production of pro-inflammatory cytokines such as interleukin 6 (IL-6)); they also induce the formation of fibrin deposits due to their procoagulant activity. Peritoneal mesothelial cells also participate in fibrinolytic processes, producing both fibrinolysis activating (tissue plasminogen activator (t-PA)) and urokinase plasminogen activator (u-PA)) and fibrinolysis inhibiting factors (plasminogen activator inhibitors (PAI-1 and PAI-2)). Peritoneal fibrinolytic activity is the most important mechanism preventing the formation of adhesions; in animal models, increased synthesis of t-PA was observed in response to fibrin deposition (1), and t-PA was shown to exert 95% of the peritoneal plasminogen activator activity (2). Peritoneal fibrinolytic activity is derived from two sources, mesothelial...
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541Effects of peritoneal lavage solutions on the morphology and function of in vitro mesothelial cells (3), of which mesothelium plays a particular role in the production of fibrinolysis activators (4).

There are factors involved in both fibrinogenic and fibrinolytic systems, such as kallikrein, tissue thromboplastin and high-molecular-weight kininogen; they are responsible for balance between these two antagonistic systems. Consequently, they take part in maintaining balance between the production and degradation of fibrin, as well as the formation and resorption of intraperitoneal adhesions (5). Imbalance between fibrinogenesis and fibrinolysis, in favor of the former, predisposes to the development of permanent adhesions. Inadequate peritoneal fibrinolysis may be caused by decreased t-PA or increased PAI-1 and PAI-2 (6).

Abdominal surgical interventions cause mechanical injury to the peritoneal mesothelium and also compromise its physiological function, which may lead to disproportion between production and degradation of fibrin, thus leading to the formation of peritoneal adhesions (6). Many things may influence postsurgical mesothelium activity, such as extension of the incision and other types of mechanical injuries (initial incision, coagulation, tissue drying, presence of sutures and other foreign bodies); also, the peritoneal lavage solution used during surgery plays a role in mesothelial activity.

Peritoneal lavage during abdominal surgery allows for the removal of excess residual secretions, tissue debris and deposits that develop due to inflammatory, mainly purulent, process. The following solutions are routinely used: 0.9% NaCl, Ringer’s solutions and electrolyte solution. So far there has been no agreement upon which solution is the most neutral for the peritoneum. Another relevant issue is whether 0.9% NaCl solution is neutral enough to be used as a control solution for other studied substances in experiments involving the culture of mesothelial and animal cells (which is a common practice) (7).

The aim of the study was to evaluate the effect of 0.9% NaCl and other solutions suitable for possible or common use for peritoneal lavage on in vitro viability and fibrinolytic capacity of human peritoneal mesothelial cells.

MATERIAL AND METHODS

Experiments were carried out in human mesothelial cell cultures. The cells came from the fragments of greater omentum sampled during abdominal surgeries performed at the Department of General and Vascular Surgery of Medical University in Poznan, and were isolated according to the technique described previously by other authors (2). Tissue samples from a total of 8 surgical patients were used in this experimental study (n=8).

Approval of the Bioethics Committee was gained for this entire protocol. Each time, patients were informed of the fact and purpose of the additional tissue fragment collection during the surgery and they signed an informed consent form prior to this procedure.

Cellular cultures were bred and further investigated at the Department of Pathophysiology of Medical University in Poznan. Peritoneal mesothelial cells were cultured in M199 medium (Sigma Aldrich, USA) with the addition of 10% bovine serum (Life Science Technologies, GIBCO, Germany) at 37°C and 5% CO₂. Initially, they were cultured in culture bottles; they were then transferred to 24-well plates. Experiments were performed following cellular monolayer formation on the bottom of the wells.

Human peritoneal mesothelial cells were incubated for six hours with the addition either 0.9% saline solution, Hanks’ electrolyte solution (used e.g. for tissue transfer), peritoneal dialysis (PD) fluid Gambrosol-Trio 10 or M199 culture medium (which was used as a control for the other solutions).

Prior to and after the experiment, light microscope pictures of the cultures were taken. Six hours after the addition of the solutions, samples were drawn up from each well and tested for lactate dehydrogenase activity (LDH) by means of enzymatic assays, as LDH is a marker of cell degradation. After the completion of the experiment, the cells were further incubated for 24 hours with M199 medium. Subsequent to this period, supernatant was collected and frozen at -80°C for further analyses. Afterward, cell lysis was performed in distilled water and the lysate was then frozen at -80°C. The collected supernatant was used for the measurement of tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1), and interleukin 6 (IL-6) activities. The activity of these substances was expressed as milligram per total cellular protein as determined in the mesothelium cell lysate.
Statistical analysis

The results were presented as means±standard deviation. Statistical analysis was performed using Instat GraphPad v 3.06 software and two nonparametric tests: Wilcoxon test for paired data and analysis of variance (ANOVA) with the Kruskal-Wallis test. The results were found statistically significant if p<0.05.

RESULTS

Microscopic pictures of cells incubated with the control solution, Hanks’ solution and PD fluid Gambrosol-Trio 10 did not change after the 6-hour exposure; however, cells incubated with 0.9% NaCl solution showed clear morphological changes, including exfoliation from the well surface, reduced volume and packet formation.

Despite major changes in the morphology of mesothelial cell cultures incubated with saline solution, no significant increase in LDH levels was observed in this group (fig. 1). Considerable LDH increase as compared to the control solution was only seen for PD fluid (23.27±10.69 vs 12.81±4.83 mU/ml).

There was also a significant reduction in the t-PA level observed in saline solution-added cultures (417.58±241.17 vs 971.17±321.01 pg/µg of total cell protein) (fig. 2). In addition, a statistically significant reduction in IL-6 production as compared to the control group was recorded (3558.29±944.56 pg/µg of total cell protein) in cells exposed to NaCl solution (1460.73±533.25; p<0.01) and PD fluid (2021.94±576.15; p<0.05) (fig. 3). A similar decrease in relation to the control group (1.74±0.41 ng/mg of total cell protein) was seen for PAI-1 production in cells incubated with NaCl solution (0.54±0.2; p<0.01) and PD fluid (0.96±0.44; p<0.05) (fig. 4).

It was proven that mesothelial cells incubated with Hanks’ electrolyte solution show metabolic activity patterns most approximate to control cells. Increases in PAI-1 levels observed in this group (2.52±0.55 ng/mg of total cell protein) compared to control culture failed to reach statistical significance.

DISCUSSION

Peritoneal cleaning and lavage during surgery provide for the removal of built up biological debris, bacteria and toxins. Lavage is thus a potentially beneficial procedure; however, despite its common use there is still no clear
and convincing evidence concerning the effectiveness and safety of electrolyte and antiseptic solutions used for peritoneal lavage (8). On the other hand, the procedure may compromise structure and function of the peritoneum. Intra-operative lavage may decrease the bacterial count in the abdominal cavity, and may remove blood, bile and exsudative residua. However, the applied solutions may induce inflammation and contribute to the formation and preservation of post-surgical adhesions (9). Early studies on this issue proved that use of peritoneal lavage was associated with a greater incidence of adhesions than in cases without lavage, and this was repetitively observed with various fluids (10, 11).

Peritoneal reactions to injected substances, including different solutions, have not been thoroughly investigated. It seems that these reactions depend on a number of variables characterizing the substance: pH, temperature, osmolality, electrostatic charge, volume and exposure time (including peritoneal absorption ratio). One of the most important reports on peritoneal lavage showed a relationship between the number of adhesions and the temperature of the lavage fluid. Kappas showed that irrigation fluids at a temperature above the physiological level (37°C) encouraged adhesion formation following peritoneal lavage in rats (12). It was also demonstrated that adhesions were absorbed within the initial 12 weeks post surgery when a lavage solution at 30-40°C was used, whereas adhesions formed after irrigation with fluids at above 40°C persisted and did not disappear within this period.

Early studies on the influence of various solutions on mesothelial cell culture assessed only cell viability, without addressing the entire complexity of the harmful effects on mesothelial metabolism. In the presented study we attempted to evaluate not only morphological changes and cell viability, but also potential impairment of their metabolism.

Experimental use of 0.9% NaCl solution caused disturbances of surface and intercellular adhesion without a significant increase of LDH, a marker of cellular degradation. Analysis of other studied biochemical parameters showed extensive functional damage of cells with respect to both their ability to induce insignificant inflammatory reactions (decreased IL-6 production) as well as their fibrinolytic capacity (lower t-PA and PAI-1 levels). Reports supporting a disturbance of mesothelial cell viability and fibrinolytic capacity after intra-operative peritoneal lavage using a 0.9% saline solution have recently been published (13). Other investigators announced that 0.9% NaCl solution influences not only mesothelial morphology and metabolism but also cellular components of PD fluid. It has been shown in rats that continuous peritoneal lavage for as short as ten minutes with this solution resulted in lower total cell counts and a higher percentage of neutrophils in the peritoneal fluid, at the expense of macrophages, lymphocytes and mastocytes. This may in itself influence peritoneal reactivity and its protective functions (14).

In the light of these results, the weak spot of many studies on peritoneal lavage is use of 0.9% saline as a control fluid for other tested substances (7, 15, 16). Many investigators and clinicians simply denote this solution as a standard fluid for lavage of inflamed peritoneal cavities (17), and there is still no convincing evidence concerning the biocompatibility of this peritoneal lavage solution.

PD fluid Gambrosol-Trio 10 was another solution tested in our study, as dialysis fluids may seem optimal for peritoneal lavage. However, several essential reasons make this untrue. Dialysis fluids are not physiological fluids due to their low pH (5.2-5.5), high glucose levels (1.5-4.25%), and hyperosmolarity (18, 19). So far, available PD fluids contained lactates, which highly contributes to their biocompatibility and accounts for their low pH. A new generation fluids are buffered with sodium bicarbonate and lactate to physiological pH of 7.4, which favorably affects mesothelium viability and function (20). Gambrosol-Trio 10 used in this study is one of the old generation PD fluids that contains only lactate. Indeed, no significant damage to the morphology of cellular culture was observed microscopically, but markedly increased LDH release and decreased IL-6 synthesis were noted, which represent some damage to their structure and impairment of unspecific reactivity.

So far, a few conducted studies brought up the use of Hanks’ solution as a potential peritoneal lavage fluid. In one of those few experimental studies, the formation of adhesions was compared in rats following repetitive exposure of the peritoneum with a previously injured mesothelial layer to Hanks’ solution, as well
as 4.25% glucose and 7.5% icodextrin solutions. Occurrence of adhesions after exposure to Hanks’ solution was similar to the control group (without lavage) and significantly different than the number of adhesions formed after experimental use of highly osmotic glucose and icodextrin solutions (21). This study has also demonstrated that the mesothelial layer formed after peritoneal injury had only a slightly lower density of mesothelial cells, whereas lavage with the above-mentioned osmotic solution induced repair with connective tissue. Also, our study found Hanks’ solution a neutral fluid that affected the structure and metabolism of the mesothelium similar to the control agent. Reports regarding favorable effects of Hanks’ fluid on peritoneal cells initiated its use as a control solution for other fluids tested (22). However, it is rather limited to laboratory purposes, as relatively high costs limit its use in everyday clinical practice.

Peritoneal lavage is a routine procedure and the irrigation fluid used should be an isosmotic solution of physiological pH and have a minimal impact on structure and metabolism of mesothelial cells. The absorption ratio of the solution is less important due to the short time of peritoneal contact limited by the lavage duration; therefore, crystalloids may be used. According to our analyses, the characteristics of Hanks’ solution are the most optimal of the solutions tested. Routine daily use in general surgical procedures calls for a solution which does not impact the peritoneum and could replace the commonly used 0.9% saline solution or, also permissible, peritoneal dialysis fluids. Further investigations are required to establish the suitability of lactated Ringer’s solution or multi-electrolyte solution.

CONCLUSIONS

1. Saline solution (0.9% NaCl) should not be used as a routine surgical peritoneal lavage fluid due to its unfavorable influence on the structure and metabolism of peritoneal mesothelial cells and impairment of their fibrinolytic capacity.

2. Further studies are essential to indicate fluid which demonstrates less harmful peritoneal effects (possibly lactated Ringer’s solution or a multi-electrolyte solution).

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COMMENTARY

There is absolutely no doubt that any interference in the peritoneal cavity leads towards a live reaction of active mesothelial cells. Doubts arise whether peritoneal lavage is beneficial during the postoperative period. Most surgeons terminate peritonitis surgery by means of lavage. However, as M. Schein mentioned: “there is no evidence that patients treated by means of antibiotics and subjected to lavage were diagnosed with a lower number of sepsis complications, which reduced mortality” (1).

There is absence of clinical evidence demonstrating the advantage of one peritoneal lavage solution over any other. Physiological saline lavage is a common procedure because there is no unfavorable effect of the activity, in spite of the fact that experimental investigations demonstrated possible damage (2). The wide range of solutions and accessories, mostly antiseptics, renders it difficult to choose the best solution. Even distilled water was mentioned to have an influence on neoplastic cells localized in the peritoneal cavity, prolonging survival (3). Solution temperature and pH are an additional difficulty, significantly influencing the degree of peritoneal cavity endothelial cell damage. As you can see, the problem is complex and every attempt at solving it is worth mentioning. The presented study results demonstrated the non-beneficial effect of different solutions used during lavage (mainly NaCl) on the metabolism of endothelial cells. According to the Authors, this could be of significance, considering inflammation control and the development of peritoneal adhesions. However, do the above-mentioned experimental studies authorize the Authors to come to the following conclusion: “the solution of physiological saline, due to its unfavorable effect on the structure and metabolism of peritoneal mesothelium cells and fibrinolytic system disturbances should not be used routinely during intraoperative peritoneal lavage”? In my opinion, this conclusion is far-reaching.

REFERENCES


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The presented study is difficult to comment on since it is composed of two diverse parts. The first part of the study concerns the influence of physiological saline and other solutions on the function and morphology of mesothelial cells (in vitro investigations), and without reservation possesses several novelties. Only the methodology section lacks information concerning which fragments of the omentum were investigated (normal or pathologically changed), as well as which particular elements were measured. It is difficult to guess whether the activity or concentration of the plasminogen activator was investigated, especially when t-PA concentration was mentioned in the summary, while t-PA activity in the methodology section is noted, without its measurement methods. These detailed data may be omitted in a surgical journal.

The second part of the study compared the in vitro and in vivo phenomena. Many expressions are imprecise, vague, erroneous and intermixed, which might be responsible for any false conclusions the surgeon may draw. Due to many incomprehensible and controversial views, the study requires an extensive, explanatory commentary, considering differences in the function and role of endothelial cells of the peritoneal cavity, both in the physiology and pathology. It is impossible to transfer in vitro results to clinical conditions due to differences in the vitality and activity of culture and tissue cells, as well as differences in the exposure to solutions.

The peritoneum is the thinnest serous membrane covering the abdominal cavity wall (parietal peritoneum, thickness of 100 µm) and abdominal cavity organs (visceral peritoneum, thickness of 50 µm). The surface of the peritoneum is enormous, ranging between 1.5 and 2.25 m². The above-mentioned is covered by polygonal mesothelial cells (thickness of 0.6-3 µm). The large surface supplied by blood, lymph and nerves gives the peritoneum its physiological and pathophysiological properties, which enable it to safely perform numerous operations. On the other hand, peritoneal pathologies are usually clinically relevant in determining the future prognosis of diseases and the patients’ mortality. The following properties should be considered: absorption, exudate and transudate production, and bactericidal activity. An important and favorable feature of the peritoneum is its ability to form conglomerations which seal the intestinal sutures and small internal organ damages, limiting inflammatory processes and delimiting suppuration from the rest of the peritoneal cavity. Adhesions which develop as a consequence of the above-mentioned conglomerations might lead to complications including intestinal obstruction.

Fibrin plays a major role in the formation of conglomerations. The non-thrombogenic, smooth and moist surface of the peritoneal cavity is covered by fibrin during irritation by means of mechanical, chemical, bacterial, thermal and xerosis factors. Mesothelial cells covering the peritoneum are hemostatically active, continuously synthesizing and releasing elements which form and dissolve fibrin. Under normal conditions, one can observe a balance between the coagulation system and fibrinolysis. The ratio between tissue plasminogen activator (t-PA) and its inhibitor (PAI) is considered as the most important factor influencing the antithrombotic and fibrinolytic potential of the serous surface of the peritoneum. Under pathological conditions, peritoneal tissue is infiltrated by macrophages, which are a rich source of procoagulants (mainly tissue factor), and prothrombotic cytokines activate the process of fibrinogenesis (transition of fibrinogen into stabilized fibrin). The above-mentioned forms conglomerates from pathologically changed peritoneal surfaces lacking endothelium.

The integrative view (formulated by T. Astrup in 1966) concerning the dynamic balance between fibrin formation and dissolution, which plays a major role in the regulatory process of tissue repair, remains up-to-date. The production of fibrin is part of the hemostatic process preventing extravasation after rupture of vessel continuity. However, Astrup demonstrated that the significance of fibrin plays an even more important physiopathological role than hemostasis. The production and dissolution of fibrin by means of the fibrinolytic system is observed during numerous processes including the following: tissue repair, malignant transformation, ovulation and embryo implantation. Fibrin produced on the pathological surface of the peritoneum forms conglomerations between intestinal loops, being a medium for migrating fibroblasts and the matrix of connective tissue.
(adhesions). However in practice, the above-mentioned situation is more complex in comparison to theoretical dissertations concerning fibrinolytic potential. Experimental investigations demonstrated the formation of peritoneal adhesions in 100% of dogs, which after abdominal operations, prophylactically received (intraperitoneally) an active fibrinolytic enzyme (plasmin). This is in contradiction to the good results obtained in cases of intravenous plasmin administration prior to surgery. A normal peritoneum inactivates the above-mentioned enzyme. The presence of circulating plasmin in the bloodstream leads towards a hyperfibrinolytic condition during which fibrin is produced (as a consequence of operative trauma), dissolves, and is absorbed prior to its organization and formation of adhesions. None of the mentioned dogs subjected to intravenous plasmin administration developed adhesions.

According to many teachers of surgeons and pioneers of peritoneal pathology treatment in Poland (Michejda, Rutkowski, Bogusz), peritoneal lavage is only indicated in cases of the most severe complications of diffuse peritonitis in order to remove purulent and fecal contents after damage or perforation of internal organs or when the exudate turned purulent, forming large collections during inflammation. Large amounts of fluids should be used during lavage in order it be efficient. Under pathological conditions, the entire peritoneal membrane is significantly thickened with inflammatory cell infiltration and is reddened, matte and often covered with fibrin. It is essential to compare the functional and structural condition of endothelial cells subjected to changes during peritonitis lasting for at least 12-24 hours and lavage of 10 minutes with the functional and structural condition of mesothelial cells collected from the greater omentum and subjected to at least 6 hours of lavage. Thus, to draw the first conclusion based on these “scientific investigations” is inappropriate.

Considering peritoneal treatment in case of damaged endothelial cells, the exchange of 0.9% NaCl solution for other, more expensive and unavailable solutions seems absurd. Therefore, I cannot agree with the content of the first conclusion. In cases of the above-mentioned pathological conditions, expensive fluids should not be sought after, and peritoneal lavage should be performed using the 0.9% NaCl solution, being easily available in every Polish surgical department. I can assure you that greater influence on the extent of postoperative adhesions is connected with the delicate management of intestinal loops rather than the type of solution coming into contact with the surface of the bowels during peritoneal lavage.

I am deeply convinced that the reader of the „Polish Journal of Surgery” will be able to come to proper practical conclusions after becoming acquainted with the study, as well as the critical and explanatory commentary.

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