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Time to reconsider saline as the ideal rinsing solution during abdominal surgery

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Abstract

Background: Peritoneal mesothelial cells lining the peritoneal cavity play a primary role in prevention of formation of peritoneal adhesions, which depends mainly on their fibrinolytic activity. During surgical procedures, the abdominal cavity is in most cases rinsed with normal saline solution, which may modify the fibrinolytic activity of the peritoneal mesothelium and predispose to formation of adhesions. The goal of our experiments was to evaluate how normal saline and other solutions affect the fibrinolytic properties of the peritoneal mesothelial cells.

Material and methods: Experiments were performed on in vitro cultures of human peritoneal mesothelial cells. Mesothelial monolayers were exposed during 6 hours to the following solutions: culture medium (control), .9% NaCl, Hanks solution, Earles solution, new peritoneal dialysis fluid with low glucose degradation products (GDP) concentration (PDF), and peritoneal dialysis fluid with high concentration of GDP (PDF-GDP). Afterwards, morphology of the cells as well as leakage of lactate dehydrogenase (LDH) from their cytosol were evaluated. During the next 24 hours when the cells were cultured in standard medium synthesis of interleukin-6 (IL-6), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) were studied.

Results: Mesothelial monolayers exposed to .9% NaCl or to PDF-GDP showed destruction of their morphology after 6 hours incubation and in case of PDF-GDP release of LDH from the cytosol was increased by 275% versus the control ($P < .05$). During subsequent culture of all cells in standard medium, the release of IL-6 was decreased in cases of cells pretreated with .9% NaCl (−58%, $P < .05$) or PDF-GDP (−93%, $P < .001$). The release of t-PA was also reduced from cells pretreated with .9% NaCl (−71%, $P < .01$) or with PDF-GDP (−74%, $P < .01$) but increased after exposure of these cells to PDF (+35%, $P < .05$). Statistically significant decrease of PAI-1 synthesis was observed in cells preexposed to .9% NaCl (−69%, $P < .01$) or to PDF-GDP (−82%, $P < .05$). When changes in the PAI-1/t-PA ratio were calculated, a strong tendency for increase of that value was seen in cells pretreated with .9% NaCl or Earles salts solution but not with PDF. However, in cases of Hanks solution, a significant increase in the PAI-1/t-PA ratio was observed (+104%, $P < .01$).

Conclusion: Exposure of the peritoneal mesothelial cells to .9% NaCl, Hanks, Earles salts solution, or PDF-GDP results either in reduction of their viability or in loss of their fibrinolytic activity. Peritoneal dialysis fluid with a low content of glucose degradation products appears to be the optimal solution causing the least damage to mesothelial cells and therefore may be the ideal solution for rinsing the abdominal cavity with low risk of inducing deterioration of the mesothelial cells fibrinolytic activity and formation of adhesions. We postulate therefore that such hypertonic peritoneal dialysis fluids should be used not only during peritoneal dialysis but also for rinsing the abdominal cavity during any surgical procedures.

Keywords: Mesothelium; Fibrinolytic activity; Dialysis fluid; Peritoneal adhesions

Peritoneal mesothelial cells lining the abdominal cavity play an important role in physiological conditions, providing a slippery surface that allows free movement of the intraabdominal organs. Loss of these properties, which happens especially after surgical interventions within the abdominal cavity, results in formation of adhesions that may lead to fatal complications [1]. Also, during chronic peritoneal dialysis when repeatedly large volumes of unphysiological dialysis fluids are infused into the peritoneal cavity, damage to the mesothelial cells occurs [2]. In extreme situations, encapsulating peritoneal sclerosis occurs that may lead to death [3]. Fibrinolytic activity of the mesothelial cells that

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produce both activators of fibrinolysis, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) as well as inhibitors of that process such as plasminogen activator inhibitors (PAI-1 and PAI-2), is the main mechanism preventing formation of the peritoneal adhesions [4]. An imbalance between intraperitoneal fibrin deposition and fibrinolysis is the initiating step leading to formation of adhesions [5]. It was shown that activity of t-PA within the peritoneum decreases during surgery in humans even in the absence of infection [6]. However, in experimental studies in rats, increased intraperitoneal synthesis of t-PA was observed during the days after surgery, which was interpreted as a compensatory reaction to increased deposition of fibrin [7]. In the presence of inflammatory cytokines, the balance between plasminogen activators and inhibitors moves in favor of the latter, which may predispose to deposition of fibrin and formation of adhesions [8–10]. An imbalance between intraperitoneal coagulation and fibrinolysis in patients treated with peritoneal dialysis with acute peritonitis may lead in the long term to damage of the peritoneum as a dialysis membrane [11,12].

There is a general understanding that both peritoneal dialysis fluids and other solutions that are infused during or at the end of abdominal surgery into the peritoneal cavity for washing out the residual tissues debris must be more biocompatible and less injurious to mesothelial cells. In recent years, a new generation of dialysis fluids with a neutral pH and low content of glucose degradation products (GDPs) was introduced [13]. On the other hand, there is no general consensus as to which solution is the most suitable for washing the abdominal cavity during surgery. In this article, we present results from in vitro experiments on primary cultures of human peritoneal mesothelial cells in which the effect of different solutions on the viability and fibrinolytic activity of these cells was evaluated.

Material and Methods

Experiments were performed on primary in vitro cultures of human peritoneal mesothelial cells. Cells were harvested from a sample of omentum tissue obtained during abdominal surgery in consenting patients and cultured as described previously [14]. Peritoneal mesothelial cells were identified by their positive immunostaining for cytokeratins. Mesothelial cells from the first or second passages were used during the study. Medium M199 supplemented with antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL) and with 10% fetal calf serum (FCS) (Life Science Technologies, GIBCO, Germany) was used for in vitro culture. Unless otherwise described, all chemicals used for cell culture and during experiments were purchased from Sigma (St Louis, MO). Disposable plastic equipment for tissue culture was purchased from Nunc (Roskilde, Denmark). Confluent mesothelial monolayers in 75-cm^2 culture flasks were harvested with trypsin .05% EDTA .02% solution in Hanks, resuspended in fresh medium, and seeded into 24-well clusters. Experiments were performed on mesothelial monolayers in 24-well clusters.

During the study, we tested the effect of the 6-hour exposure of the mesothelial monolayers in in vitro culture on their viability, synthesis of t-PA, and interleukin (IL)-6. Cells were exposed to the following solutions (in all fluids pH was originally 7.4 or its value was adjusted to 7.4 with 1 N NaOH):

1. Medium M199 + .1%FCS (292 mOsm/kg H_2O) (Sigma)
2. .9% NaCl (304 mOsm/kg H_2O) (Baxter-Terpol, Poland)
3. Hanks solution (285 mOsm/kg H_2O) (Sigma)
4. Earle's salts solution (288 mOsm/kg H_2O) (Sigma)
5. Peritoneal dialysis fluid Gambrosol-Trio 10 (Gambro, Lund, Sweden) of the following composition in mmol/L: Na = 133, Ca = 1.79, Mg = .26, Cl = 96.2, lactate 41, and glucose 85 (357 mOsm/kg H_2O), which contains low concentration of glucose degradation products (PDF)
6. Peritoneal dialysis fluid Gambrosol-Trio 10 with the same composition and osmolality as above but additionally heat autoclaved (120°C, 30 minutes) to generate glucose degradation products (PDF-GDP).

At the end of the 6 hours of exposure, supernatant was collected from the wells and its lactate dehydrogenase (LDH) activity was measured enzymatically with a commercially available kit (Sigma). Morphology of the mesothelial monolayers at that stage of the experiment was documented with photography of the cells observed in an inverted microscope. Afterwards, the cells monolayers were washed with standard culture medium M199 + 10% FCS and fresh culture medium M199 + 10% FCS was added to the wells. After that 24 hours, incubation was started in air atmosphere with 5% CO_2 and at 37°C. At the end of this incubation period, supernatant was collected for further biochemical analysis and stored at −80°C. Cells in the wells were lysed with distilled water, and the lysates were stored at −80°C. The concentration of protein in the cells lysates was measured with the Bradford method available in a kit (Bio-Rad Laboratories, Munich, Germany).

In the supernatants collected after 24 hours of incubation, concentration of IL-6 was measured with enzyme-linked immunosorbent assay (DuoSet; R&D Systems Europe, Oxon, United Kingdom) as well as the concentration of t-PA (commercially available enzyme-linked immunosorbent assay kit from Bender MedSystems GmbH, Vienna, Austria) and PAI-1 (commercially available kit from Technoclone GmbH, Vienna, Austria). Synthesis of these molecules was recalculated per amount of the cell protein.

Statistical analysis

Results are presented as mean ± standard error of the mean. All results come from 8 independent experiments, performed on different primary mesothelial cells cultures.