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EFFECT OF VITAMIN E ON PEROXIDATION AND PERMEABILITY OF THE PERITONEUM

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Because of the evidence that peritoneal macrophages are activated during peritoneal dialysis, we hypothesised that the injury of the peritoneum is, at least in part, dependent on the intraperitoneal generation of free radicals. The aim of the study was to evaluate the effect of vitamin E on the peroxidation and permeability of the peritoneum during chronic peritoneal dialysis in rats. Supplementation of the intraperitoneally infused saline with vitamin E decreased the peroxidation of peritoneum estimated as the malondialdehyde (MDA) level in rats’ omentum. However the permeability of the peritoneum to glucose and protein in vitamin E treated rats was increased. In in vitro study we have found that vitamin E is cytotoxic to human mesothelial cells (HMC) as measured by inhibition of their proliferation and this effect was irreversible. We conclude that vitamin E, despite its antioxidant effect, causes the changes of the peritoneum permeability which could decrease the effectiveness of peritoneal dialysis.

Key words: free radicals, vitamin E, mesothelial cells, peritoneal permeability

INTRODUCTION

Continuous ambulatory peritoneal dialysis (CAPD) is an accepted form of the treatment of patient with end-stage renal failure. However, long-term peritoneal dialysis leads in some patient to loss of ultrafiltration (1). It is accompanied by changes in the structure of mesothelial cells and stroma. Peritoneal biopsy from CAPD patients demonstrate a reduction of quantity of microvilli on the mesothelial cells surface, reduplication of their basal membrane and edema with a signs of sclerosis in the subjacent tissue (2, 3). The earliest, such changes may appear already several days after start of CAPD (2).
Moreover, it is believed that peritoneal dialysis fluid repeatedly introduced into the peritoneal cavity induces a chronic sterile inflammatory process and phagocytic cells obtained from the dialysate of peritonitis-free patients are activated (4—6). Activation of these cells is connected with the respiratory burst and increased generation of free radicals (7, 8).

Free radicals production has an essential role for bacterial killing by phagocytes (9, 10). But on the other hand the production of such reactive species can be injurious to the mesothelial cells and stroma unless it is tightly controlled. The increased generation of free radicals may cause lipid peroxidation, protein oxidation, DNA damage as well as depolymerization of hyaluronic acid and gelatination of collagen (11, 12).

Vitamin E is a part of the antioxidant system, which protects organisms against free radicals injury. Oral administration of vitamin E decreases the malondialdehyde level (MDA) — one of the end product of lipid peroxidation — in the plasma and red blood cells in human (13, 14). In in vitro study vitamin E protects the endothelial cells against injury caused by immune triggered granulocytes (15). Its antioxidant effect is also a reason of the increased survival of rats with experimental sepsis (16). It is well known that MDA level in serum and erythrocytes of CAPD patients is increased (17, 18). However, no difference in serum level of vitamin E between CAPD patient and healthy control is reported (19, 20). But it is noticeable that the MDA serum level may not reflect its tissue level and metabolism. In CAPD patients with normal serum level of vitamin E, its concentration in low density lipoproteins (LDL) is decreased what increases their susceptibility to oxidation (20).

In this study we estimated the role of intraperitoneal injection of vitamin E on the peroxidation and permeability of the peritoneum in rat.

MATERIAL AND METHODS

In vivo study

The effect of intraperitoneal infusions of saline on peroxidation and permeability of the peritoneum

The experiment was done on male, Wistar rats weighing between 250 and 350 g. Daily, under ether anaesthesia, the animals were infused intraperitoneally with 15 ml of 0.9% NaCl (Polfa, Poland) and thereafter the rats were kept free given food and water ad libitum. This procedure was repeated for 6 days. Non-infused rats were used as the control group. In all animals, on the 7th day.
4 hour peritoneal dialysis with 2.5% Dianel (Baxter, St. Louis, USA) was performed. At the end of the dialysis, blood samples were taken from the heart and the animals were sacrificed by bleeding. Then the peritoneal cavity was opened and the residual dialysate was collected to determine the net ultrafiltration (volume drained — volume infused). Later, a piece of omentum was cut off (200—300 mg) and the level of malondialdehyde (MDA) in tissue was estimated using the thiobarbituric acid test procedure (21). End dwell dialysate glucose was measured with the enzymatic test (Sigma, St. Louis, USA) and albumin concentration in serum and dialysate with bromcresol method (Sigma, St. Louis, USA) to calculate the D/P ratio.

The effect of intraperitoneal infusions of vitamin E on peroxidation and permeability of the peritoneum

To evaluate the effect of vitamin E on lipid peroxidation and permeability changes of the peritoneum caused by saline, Wistar male rats weighing 250—350 g were infused intraperitoneally with 15 ml of 0.9% NaCl containing vitamin E (0.1 g/dl) for 6 days. Saline-infused animals were used as the control group. The outline of the experiment was similar to the presented above.

In vitro study

Human mesothelial cells (HMC) culture

The experiments were done on HMC obtained from a piece of omentum which was taken during laparotomy. The details of our tissue culture procedure was carried out according to the previously described method (22).

The effect of vitamin E of HMC proliferation

HMC were seeded into 24 well plates (10⁴/well) and cells were counted in a hemocytometer the first time 24 hours after seeding (the beginning of the experiment). At the same time the medium in the remaining wells was exchanged for the plain medium in control group and medium containing vitamin E (0.05 g/dl, 0.25 g/dl or 0.5 g/dl) in experimental groups. HMC were counted in 6 wells in each group at 24, 72 and 120 hours of the experiment.

We also estimated whether the inhibitory effect of vitamin E on HMC proliferation was reversible. After 72 hours incubation of the cells in control medium or medium containing vitamin E (0.05 g/dl, 0.25 g/dl or 0.5 g/dl) the number of cells in the individual groups was counted. Thereafter, medium was exchanged in all groups to the plain medium. HMC were counted in all groups 24, 48 and 72 hours after the medium exchange.

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. Data analysis was done with a non-parametric Mann-Whitney test. A p value less than 0.05 was considered significant.
RESULTS

In vivo study

Effect of intraperitoneal infusion of saline and saline supplemented with vitamin E on peroxidation and permeability of peritoneum

Intraperitoneal infusion of saline over 6 days caused an augmentation of MDA level in the peritoneum (p < 0.05) vs. control — non infused rats (100% ± 6% and 140% ± 13%, respectively; Fig. 1). No difference was found in dialysate/plasma ratio (D/P) for albumin. But the end dwell glucose concentration was significantly higher in control group (p < 0.01 — 414 mg/dl ± 13 mg/dl and 299 mg/dl ± 21 mg/dl); as well as the net ultrafiltration (p < 0.001 — 2.3 ml ± 0.3 ml and −0.2 ml ± 0.3 ml).

1. MDA level in omentum

![Graph](image1)

2. Net ultrafiltration

![Graph](image2)

3. End dwell glucose

![Graph](image3)

4. Permeability to protein

![Graph](image4)

**Fig. 1.** Peroxidation and 4-hour peritoneal dialysis parameters in rats infused intraperitoneally over 6 days with saline (Group 1 — control — non infused rats; group — 2 rats injected intraperitoneally over 6 days with 15 ml of saline).

The supplementation of the intraperitoneally infused saline with vitamin E (0.1 g/dl) decreased MDA level in omentum when compared with saline infused rats (p < 0.001 — 100% ± 10% and 48% ± 3%; Fig. 2). There was
no significant difference in net ultrafiltration between the groups although the tendency for aggravation of the ultrafiltration failure in vitamin E treated animals was observed (Fig. 2). This tendency was accompanied by higher permeability to glucose (p < 0.001 — 381 mg/dl ± 12 mg/dl and 309 mg/dl ± 14 mg/dl; Fig. 2) and albumin (p < 0.02 — D/P 0.109 ± 0.004 and 0.121 ± 0.004).

Fig. 2. Effect of vitamin E on peroxidation and 4-hour peritoneal dialysis parameters in rats infused intraperitoneally with saline (Group 1 — control — rats injected intraperitoneally over 6 days with 15 ml of NaCl; group 2 — rats injected intraperitoneally over 6 days with 15 of 0.9% NaCl + vitamin E (0.1%)).

In vitro study

Effect of vitamin E on proliferation of HMC

Vitamin E inhibited proliferation of HMC in a dose- and time-dependent manner (Tab. 1). Growth of HMC incubated over 72 hours in the presence of vitamin E (0.25 g/dl or 0.5 g/dl or 0.5 g/dl) was still slower during the following 72 hours when no more vitamin E was present in the medium (Table 2).
Table 1. Effect of vitamin E on HMC proliferation: mean amount of cells per well ± SEM (Statistical analysis was done vs. control group).

<table>
<thead>
<tr>
<th>Time: 0 h</th>
<th>CULTURE MEDIUM</th>
<th>Time: 24 h</th>
<th>Time: 72 h</th>
<th>Time 120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plain culture medium (Control)</td>
<td>1653 ± 222</td>
<td>12639 ± 745</td>
<td>17972 ± 1665</td>
</tr>
<tr>
<td>1927 ± 383</td>
<td>Vit. E containing culture medium (0.05 g/dl)</td>
<td>1695 ± 159</td>
<td>11736 ± 686</td>
<td>11834 ± 1805 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>Vit. E containing culture medium (0.25 g/dl)</td>
<td>1542 ± 115</td>
<td>8542 ± 1427 (p &lt; 0.01)</td>
<td>8528 ± 2119 (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Vit. E containing culture medium (0.5 g/dl)</td>
<td>2222 ± 185</td>
<td>4814 ± 600 (p &lt; 0.01)</td>
<td>2306 ± 475 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

Table 2. Reversibility of the toxic effect of vitamin E on HMC proliferation: mean amount of cells per well ± SEM (Statistical analysis was done vs. control group).

<table>
<thead>
<tr>
<th>Time: 0 h</th>
<th>CULTURE MEDIUM</th>
<th>Time: 72 h</th>
<th>CULTURE MEDIUM</th>
<th>Time: 96 h</th>
<th>Time: 120 h</th>
<th>Time: 144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plain culture medium (Control)</td>
<td>12055 ± 873</td>
<td>Plain culture medium</td>
<td>14945 ± 763</td>
<td>31690 ± 871</td>
<td>48473 ± 1204</td>
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<tr>
<td>3676 ± 289</td>
<td>Vit. E containing culture medium (0.05 g/dl)</td>
<td>11556 ± 655</td>
<td>Plain culture medium</td>
<td>14500 ± 738</td>
<td>30070 ± 1071</td>
<td>47014 ± 2332</td>
</tr>
<tr>
<td></td>
<td>Vit. E containing culture medium (0.25 g/dl)</td>
<td>8889 ± 353 (p &lt; 0.02)</td>
<td>Plain culture medium</td>
<td>12889 ± 619 (p &lt; 0.05)</td>
<td>22431 ± 1343 (p &lt; 0.01)</td>
<td>35903 ± 1060 (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Vit. E containing culture medium (0.5 g/dl)</td>
<td>7500 ± 646 (p &lt; 0.01)</td>
<td>Plain culture medium</td>
<td>9667 ± 284 (p &lt; 0.01)</td>
<td>14167 ± 416 (p &lt; 0.01)</td>
<td>25278 ± 2073 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

DISCUSSION

Peritoneal macrophages are activated during exposure of the peritoneal cavity to the dialysis fluid (4—6). It was shown by Bos et al (4) that even single intraperitoneal infusion of saline causes stimulation of the peritoneal phagocytic cells. Activated phagocytes generate augmented amount of free radicals during the respiratory burst; part of them is released extracellularly (7—9). Rozga et al demonstrated that saline strongly stimulate free radicals generation by peritoneal macrophages in vitro (23). Results of our study show
that concentration of MDA in the peritoneum increases after repeated intraperitoneal infusion of saline what suggests indirectly that free radicals are generated within the peritoneal cavity (Fig. 1). These changes were accompanied with lower net ultrafiltration due to the increased peritoneal permeability to glucose. The supplementation of the intraperitoneally infused saline with vitamin E decreased the peroxidation of peritoneum (Fig. 2). This protective effect of vitamin E could be related to scavenging of free radicals (24). Previously, we have shown that vitamin E protects human peritoneal mesothelial cells in vitro culture against free radicals injury (21). On the other hand, Sakamoto et al. (25) reported that peritoneal macrophages isolated from rats intraperitoneally infused with vitamin E had reduced ability to generate free radicals. That means that vitamin E can act not only as free radical scavenger but also as a suppressor of free radical generation and release from phagocytic cells.

Despite its antioxidant effect vitamin E given intraperitoneally in saline further increased the peritoneal permeability to glucose and albumin (Fig. 2). Lipid soluble vitamin E can be incorporated into the membrane because of vitamin E incorporation enhances the cell membrane permeability (26). Additionally, in our in vitro study vitamin E was cytotoxic to HMC as shown by inhibition of HMC proliferation (Tab. 1). According to Gotloib (27), chronic peritoneal dialysis is connected with continuous mesothelial cells injury followed by their regeneration. The inhibition of HMC proliferation may impede that process and cause the ultrafiltration failure (28).

In conclusion, our results shown that repeated exposure of the peritoneal cavity to saline increases peroxidation of the peritoneum with its concomitant augmented permeability and loss of ultrafiltration. In our previous study we found that acute intraperitoneal generation of free radicals by xanthine and xanthine oxidase system caused increased permeability of the peritoneum with ultrafiltration failure (21), what suggest that free radicals can be responsible for injury of the peritoneum during peritoneal dialysis. Lack of the protective effect of vitamin E, despite its antioxidant effect, on permeability changes of the peritoneum caused by saline might suggest that increased free radicals generation within the peritoneal cavity is not the only cause of the peritoneal damage and ultrafiltration failure. These observations suggest that it is necessary to supply dialysis fluid with antioxidant but vitamin E because of its negative side effects related to toxicity to HMC is not suitable for this purpose. Further studies should be performed to estimate the effect of other physiologic free radicals scavengers as the substances preventing the peroxidation and enhanced permeability of the peritoneum during peritoneal dialysis.

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REFERENCES


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