INFLUENCE OF VITAMIN E GIVEN INTRAPERITONEALLY TO PREVENT PERITONEAL ADHESIONS IN RATS

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The aim of the study was to evaluate the influence of vitamin E administered intraperitoneally on the prevention of peritoneal adhesion formation in rats on the basis of macroscopic and microscopic assessment of the adhesions.

Material and methods. Experimental studies were performed on 50 Sprague-Dawley male rats, which were randomly divided into 5 groups, 10 rats in a group. Experimental group I (EI) included 10 rats which had peritoneal adhesions provoked by scraping of the wall of cecum and parietal peritoneum followed by intraperitoneal administration of vitamin E in the dose of 100 mg/kg body weight. Experimental group II (EII) included 10 rats which had peritoneal adhesions provoked by surgery, without administration of vitamin E. Control Group I (CI) included 10 rats which had the abdominal cavity opened without provoking peritoneal adhesions, and vitamin E was administered. Control Group II (CII) included 10 rats which had peritoneal adhesions provoked by surgery, and then lipid based solution was administered intraperitoneally. Control Group III (CIII) included 10 rats which had the abdominal cavity only opened and closed.

Groups EI, CI and CII were the subject of the drugs intraperitoneal re-injection in first, second and third day after surgery. The animals were killed during the 8th postoperative day. Macroscopic examination of peritoneal adhesions using the classification reported by Nair was performed and samples for microscopic examination were excised.

Results. In group EI peritoneal adhesions were formed in 60% rats (40% weak and 20% solid). In group EII peritoneal adhesions were found in all animals (30% weak and 70% solid). Reduction of the inflammatory response and less severe fibrosis were observed in animals with intraperitoneal administration of vitamin E.

Conclusion. In the study, vitamin E administered intraperitoneally to rats decreased the intensity and extensiveness of peritoneal adhesions, which was confirmed by macroscopic and microscopic examinations.

Key words: peritoneal adhesions, free radicals, vitamin E, rats

Peritoneal adhesions are formed as a result of surgeries or inflammatory complications within abdominal cavity and pelvis minor. Peritoneal adhesions can cause intestinal obstruction, chronic pain, infertility and complications in the subsequent surgeries (1). One of the participants of peritoneal adhesions formation are free oxygen radicals. Each surgery intervention causes an injury within peritoneal cavity. The injury causes a release of free oxygen radicals as well as activation of antioxidative defense system. Free radicals are released as a response to ischaemia in the course of the ischaemia/reperfusion syndrome. Another source of free oxygen radicals are neutrophils which produce them in the process.
called “oxygen outbreak” (2-5). There has been not even one effective method so far which would prevent peritoneal adhesions. Antiadhesive properties of various antioxidative compounds, such as methylene blue, lazaroids, antioxidative enzymes and vitamins A and C were proved (6-9).

Vitamin E is also proved to be an antioxidative compound. It was shown that feeding the operated animals with a vitamin E enriched feed results in decrease of intensification and extension of peritoneal adhesions (10). Vitamin E administered subcutaneously or intraperitoneally in the course of the faecal peritonitis in rats prevents lipid peroxidation and tissue damage (11, 12, 13). The anti-adhesive function of vitamin E after its intramuscular administration was not proved, however, when administered intraperitoneally it was inhibiting the process of peritoneal adhesions formation (14, 15, 16).

The aim of the study was to evaluate the influence of vitamin E administered intraperitoneally on the prevention of peritoneal adhesion formation in rats on the basis of macroscopic and microscopic assessment of the adhesions. However positive effect of vitamin E given intramuscularly on the intestinal anastomotic wound healing was observed (17).

MATERIAL AND METHODS

Experimental studies were performed on 50 Sprague-Dawley male rats, with body mass of 320 to 420 g. The studies were carried out after the permission number 63a/04 was granted by the Local Ethical Commission for Experiments on Animals, Silesian Medical University in Katowice. The animals were kept in specially marked cages, five per cage. They were fed with standard granulated feed, with water access ad libitum. The animals were kept in normal environment conditions. They were randomly divided into 5 groups, 10 rats in a group.

Experimental group I (EI) included 10 rats which had peritoneal adhesions provoked by surgery, and then vitamin E was administered intraperitoneally. Experimental group II (EIi) included 10 rats which had peritoneal adhesions provoked by surgery. Control group I (CI) included 10 rats which had the abdominal cavity opened without provoking peritoneal adhesions, and vitamin E was administered. Control group II (CII) included 10 rats which had peritoneal adhesions provoked by surgery, and then lipid based solution was administered intraperitoneally. Control group III (CIII) included 10 rats which had the abdominal cavity only opened and closed.

The animals were operated under general anesthesia after intramuscular ketamin hydrochloride administration (Bioketan, Vetoquinol Biovet.) in a dose of 20 mg/100 g body mass. Abdominal cavity was opened with the central incision 4 cm long. Peritoneal adhesions were formed with a scalpel. The caecum wall and adjacent peritoneal wall were scarified within the surface area of 100 mm². Homeostasis was not used in the places of scarification.

Vitamin E (dl-α-tocopherol, DSM Nutritional Products) dissolved in lipid base (Intralipid 10%, Fresenius Kabi) in a dose of 100 mg/kg body mass and lipid base (Intralipid 10%, Fresenius Kabi) were given intraperitoneally during the surgery and in the form of intraperitoneal injections on the side opposite to the site of adhesion formation on the first, second and third day after the operation.

On the eighth day after the operation all animals were euthanized with intramuscular injection of hydrochloride ketamin (Bioketan, Vetoquinol Biovet.). Next, the central incision of 4 cm was done in the laparotomy wound, which was elongated to the sides on the lower and upper poles of the wound, in order to evaluate precisely peritoneal adhesions. Peritoneal adhesions were evaluated according to the five-grade Nair scale (0 – no adhesions, 1 – one adhesion between viscera or between viscera and parietal peritoneum, 2 – two adhesions between viscera or between viscera and parietal peritoneum, 3 – more than two adhesions between viscera or between viscera and parietal peritoneum, 4 – solid direct viscera adhesions with parietal peritoneum) and backed up with photographs. Grade 1 adhesions were assumed to be weak, while adhesions 2, 3 and 4 grade were defined as solid adhesions. Histopathological specimens were taken from the adhesion’s site. The preparations were routinely stained with hematoxin and eosin. Moreover, additional staining for collagen fibre presence was made by Van Gieson’s method and with the use of Masson trichrom. Next, they were
observed in light microscope in the enlargement from 40 to 400x and backed up with photographs.

Statistic analysis of the results was done with STATISTICA Pl v. 6.0 program. Significance level was assumed α=0.05.

The influence of vitamin E or lipid base on a degree of peritoneal adhesion intensification was assessed with the non-parametric ANOVA rank Kruskal-Wallis’ test. Rejecting the zero hypothesis about equality of adhesion intensification degree compared in groups allowed applying U Mann-Whitney’s test in order to perform more detailed comparisons for each pair among three groups.

RESULTS

In group EI, where peritoneal adhesions were induced, and next, vitamin E was given, peritoneal adhesions were formed in six animals (60%) and they were weak in four rats (40%) and solid in two rats (20%). Macroscopic evaluation of peritoneal adhesion intensification during post mortem examination showed peritoneal adhesions in all the rats from group EII, where caecum wall and adjacent parietal peritoneum were scarified without further administration of vitamin E. In this group, the adhesions were weak in three animals (30%), while in seven rats (70%) they were solid (fig. 1).

In groups CI and CIV no adhesions in peritoneal cavity were found. However, in group CII peritoneal adhesions were formed in six animals, and in group CIII in two rats. The frequency of occurrence of peritoneal adhesions in each rat group is shown in tab. 1 and fig. 2. The comparison of the intensification degree of peritoneal adhesions according to Nair’s scale in each group of rats with the use of ANOVA

![Fig. 1. Solid direct adhesions 4º of viscus with scarification site of parietal peritoneum and suture line in rat no 1 from group EII](image)

Table 1. Macroscopic evaluation of peritoneal adhesions intensification according to Nair scale in groups of rats groups (EI – experimental group I, EII – experimental group II, CI – control group I, CII – control group II, CIII – control group III).

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Intensification and extension of peritoneal adhesions in Nair scale</th>
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<tbody>
<tr>
<td></td>
<td>0º</td>
</tr>
<tr>
<td>DI / EI</td>
<td>4</td>
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<tr>
<td>DII / EII</td>
<td>0</td>
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<tr>
<td>KI / CI</td>
<td>10</td>
</tr>
<tr>
<td>KII / CII</td>
<td>1</td>
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<tr>
<td>KIII / CIII</td>
<td>8</td>
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</tbody>
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![Fig. 2. Frequency of peritoneal adhesions occurrence in the rat groups (EI – experimental group I, EII – experimental group II, CI – control group I, CII – control group II, CIII – control group III)](image)
rang Kruskal-Wallis’s test and U Mann-Whitney’s test are presented in fig. 3.

Microscopic observation of the adhesions in group EI showed the features of chronic inflammatory process with a presence of lymphocytes multinuclear giant cells with coccus reaction, noncaseatous, non-specific on the surface of caecum serosa. A presence of collagen was found in a small quantity, only individual fibres were visible. In group EII, on the surface of caecum serosa, the features of chronic inflammation with a presence of lymphocytes and neutrophils were visible focally. The observed inflammatory changes were more intensive than in group EI. Moreover, a fibrosis of slight degree was observed. In group CI, histopathology showed a normal weaving of the intestine with the thickened muscular tissue. In the rats from group CII, the features of chronic inflammation with a presence of lymphocytes were visible on the surface of caecum serosa focally. The slight fibrosis was found, however, with a bigger number of fibroblasts than in group EII. Microscopic examination of the samples from the rats of group CIII showed a normal intestine and muscular tissue weaving with a slight inflammatory reaction in serosa and slight amount of fibrous tissue present focally.

DISCUSSION

The researchers around the world have been interested in adhesion prevention for years. It was proved that, between others, vitamin E due to its antioxidative properties reduces peritoneal adhesions. Oral, subcutaneous and intraperitoneal administration of vitamin E during perioperational period reduced peritoneal adhesions in 10-60% (3, 9-16). In experiment peritoneal adhesions developed in all the rats where scarification of caecum wall and parietal peritoneum was made without subsequent intraperitoneal administration of vitamin E, in most rats the adhesions were solid (70%). However, in rats which were given vitamin E intraperitoneally during surgery and for the following three days the adhesions developed in 60% animals and they were less extensive and weaker. Similar experiment were carried out by de la Portilla et al. (14).

The authors evaluated the influence of vitamin E on prevention of peritoneal adhesions in rats after intraperitoneal and intramuscular administration in a dose of 10 mg. No antiadhesive activity of vitamin E after intramuscular administration was observed. However, a decrease of extensiveness and intensity of peritoneal adhesions after intraperitoneal administration of vitamin E was shown – in this group of adhesions developed in 75% of the rats and they were: 1 grade in 55% of animals, 2 grade in 10% and 3 grade in 10% of rats. In my study, no adhesions of 3 and 4 grade were observed in the group of rats where vitamin E was applied. Lower frequency as well as intensity of peritoneal adhesions in the experiment could be a result of the fact that vitamin E was applied in one bigger dose, and also the vitamin E application was repeated three times after the operation. The increase of antiadhesive effect together with the increase of vitamin E dose have been already observed by Kalfarentzos et al. (11) – in the study group which received vitamin E before and after the surgery, adhesions were found in 40% animals, and increased vitamin E dose strengthened the antiadhesive effect; adhesions developed only in 10% of rats. Furthermore Konukoglu et al. (12) proved that antioxidative activity of α-tocopherol in the course of peritonitis increases in cases of its multiple administration. Kagoma et al (10) observed that the frequency of peritoneal adhesions was decreased after oral administration of vitamin E. In the control group, adhesions developed
in 97% animals, while the mice fed with feed enriched with vitamin E developed adhesions only in 58% animals. Even smaller percentage of animals, in which adhesions developed after oral administration of vitamin E, was shown by Kalfarentzos et al. (11) and Hemadeh et al. (9). Efficiency of vitamin E in reduction of postoperative peritoneal adhesions after intraperitoneal administration was proved by Corraless et al. (15) – vitamin E reduced its formation by 80%. Experimental study carried out by Yetkin et al. (16) also revealed that intraperitoneal vitamin E treatment was effective in peritoneal adhesion prevention. In my study, having given vitamin E intraperitoneally, it was expected to observe an increase of its antiadhesive effect in comparison to oral administration as a result of direct impact on the injured caecum wall and parietal peritoneum.

However, the obtained results are similar to those observed during oral administration, which can be related to the fact that vitamin E was given orally before the operation and that the time of administration after the operation was longer. On the other hand, it could be meaningful that intraperitoneal administration can be connected with an intraperitoneal injection, which can additionally intensify the process of adhesion formation.

Histopathological evaluation of peritoneal adhesions in the group of animals given vitamin E in the perioperational period showed features of chronic inflammation, which was proved in earlier experiments on adhesion prevention (4, 9, 16). Moreover, the group given vitamin E intraperitoneally had a low number of collagen, only individual fibres were visible. However, in the group of rats where peritoneal adhesions were induced without subsequent administration of vitamin E, the observed changes of chronic inflammation were more intensive and the degree of fibrosis was bigger. Intraperitoneal administration of vitamin E caused a decrease of inflammatory reaction in the course of peritoneal adhesions formation and less intensive fibrosis. Vitamin E acting as chain-breaking antioxidant and free radicals scavenger prevents peritoneal adhesion formation in response to the damage to the peritoneum due to surgical procedures, inflammation, ischaemia and reperfusion results in release of free oxygen radicals.

The source of free oxygen radicals are also neutrophils which are a defense mechanism against infection and produce them during the respiratory burst (2, 3, 4, 5, 18). Vitamin E is also acting as an anti-inflammatory agent due to inhibition of cyclooxygenase and conversion of the arachadonic acid in proinflammatory cytokines and as an anticoagulant agent due to inhibition of the aggregation of platelets and maintaining their stability (9, 15, 18). Reduction of the inflammatory response in the process of peritoneal adhesion formation and less severe fibrosis in rats fed with feed containing vitamin E during the perioperational period was observed by Sanfilippo et al. (4) and after intraperitoneal administration by Yetkin et al. (16).

CONCLUSIONS

In the study, vitamin E administered intraperitoneally decreased the intensity and extensiveness of peritoneal adhesions in rats, which was confirmed by macroscopic and microscopic examinations.

REFERENCES