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Astaxanthin supplementation as a potential anti-fibrotic agent in peritoneal dialysis rats

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ABSTRACT

Introduction: Peritoneal dialysis (PD) is a recommended treatment for chronic kidney disease (CKD). Continuous exposure to dialysate solution in PD leads to peritoneal fibrosis, which is characterized by changes in morphology and function of the peritoneal membrane. Astaxanthin is considered to have potent antioxidant and anti-inflammatory properties, which has a promising anti-fibrosis effect and suppresses peritoneal thickness in peritoneal fibrosis.

Objectives: This study aimed to investigate the impact of astaxanthin supplementation on histological features among PD model rats, which determined astaxanthin as a potential anti-fibrotic agent for PD.

Materials and Methods: This study used a laboratory experimental study with a posttest-only control group design. Thirty-two male rats were divided randomly into four groups. There are two control groups and two treatment groups. Negative (NC), given intraperitoneal (IP) injection of sterilized aquadest, positive control (PC), given dialysate 4.25% injection IP. Treatment group 1 (T1) was given dialysate 4.25% injection IP and astaxanthin 0.216 mg supplementation for 14 days, and treatment group 2 (T2) was given dialysate 4.25% IP and astaxanthin 0.216 mg supplementation for 21 days. The peritoneum tissues were then collected and prepared for histological examination.

Results: Astaxanthin supplementation prevents peritoneal fibrosis development in CKD model rats ($P<0.05$). However, there was no significant difference in the mean fibrosis thickness based on astaxanthin duration ($P>0.05$).

Conclusion: Astaxanthin could reduce fibrotic thickness in PD model rats. This study was relevant to conclude that astaxanthin has a potential antifibrotic agent for PD.

Implication for health policy/practice/research/medical education:
The investigation of astaxanthin supplementation on histological peritoneal features may provide information that could improve potential adjuvant therapy to reduce fibrosis in peritoneal dialysis.

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Original

Introduction

Chronic kidney disease (CKD) is one of the non-communicable diseases (NCDs) which is currently a health problem in almost all countries in the world (1,2). The prevalence of CKD is increasing every year and is estimated to reach 13% of the global population. Global Burden of Disease (GBD) in 2015 stated that 5-10 million people die from CKD each year and predicted this number would increase along with the shifting of the health paradigm from infectious diseases to NCD (3–5). CKD

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is defined as an abnormal condition of the structure and function of the kidney that lasts for more than 3 months (6). Recommended treatment for these patients is renal replacement therapy (RRT) (1,7). Peritoneal dialysis (PD), one of the RRT modalities, is often selected because of its several advantages, such as flexibility, continuity, and it is less invasive compared to hemodialysis (8,9). Nevertheless, PD as well has a risk of developing infectious and other non-infectious complications (10,11).

One of the non-infectious complications of long-term use of PD is the development of peritoneal fibrosis, which leads to reduced integrity of the peritoneal membrane, resulting in inadequate dialysis or even treatment failure. Peritoneal fibrosis is characterized by changes in morphology and function of the peritoneal membrane caused by continuous exposure to acidic, hyperosmotic, and hyperglycemic dialysate solutions (12,13).

Peritoneal fibrosis is found in 50-80% of continuous ambulatory peritoneal dialysis (CAPD) patients within approximately 2 years of therapy. The progression of the fibrosis process varies from mild submesothelial thickening to the occurrence of encapsulating peritoneal sclerosis, a condition where there is a massive thickening of the peritoneal membrane that can cause intestinal obstruction below and can be life-threatening. Therefore, peritoneal fibrosis is a side effect that needs to be considered in the long-term use of CAPD (10,14,15).

Astaxanthin, a natural carotenoid, is considered to have potent antioxidant and anti-inflammatory properties (16,17). It has been investigated which those activities might benefit varying diseases. Studies showed that astaxanthin has a promising anti-fibrosis effect and suppresses peritoneal thickness but there is still a lack of studies using astaxanthin in PD (18,19).

Objectives

This study aimed to investigate the impact of astaxanthin supplementation on histological features among PD model rats which determine astaxanthin as a potential anti-fibrotic agent for PD.

Materials and Methods

Study design

Preparation of astaxanthin

Astaxanthin, which is used in this study, was natural astaxanthin, manufactured and purchased from SOHO Global Health (Jakarta, Indonesia). The available preparation produced in Indonesia is astaxanthin with a dosage of 12 mg. Furthermore, this dosage was converted to animal equivalent dose based on rats' body surface area (BSA) and the calculation results obtained a dose of 0.216 mg. Astaxanthin was given per oral once daily.

Animals and experimental design

Thirty-two male rats (3-4 months old, 200-300 g body weight *Rattus novergicus*) were divided randomly into 4 groups. Rats in all groups were housed in standard cage under temperature of 23 °C and a 12-hour light/dark cycle with free access to laboratory meals and water (*ad libitum*) adjusted to their body weight. All subjects were induced by a unilateral ureteral obstruction model (UUO) procedure to form the CKD model. In brief, this procedure induce rats with anesthesia, then the abdomen was incised and one side of the ureter was ligated with silk 3.0 at two locations for 7 days (20).

After the induction of UUO, all groups were intervened with PD. The subjects were anesthetized by ketamine adjusted to their body weights, then incised 3 cm lengths vertically at the inferior midline of the xiphoid process. Furthermore, the peritoneal membrane was incised until the peritoneal cavity was exposed. The peritoneal catheter was inserted and fixated with suture. Moreover, 10 mL of dialysate solution was injected into the catheter and flowed up to the cavity (21).

Group 1, the negative control group (NC), was only given sterilized aquadest intra-peritoneal (IP). The other three groups were given dialysate solution 4.25% in the amount of 10 ml into their peritoneal cavity every day. Group 2, the positive control group (PC), was given dialysate solution 4.25% IP. Group 3, the treatment group 1 (T1), was given dialysate solution 4.25% and oral astaxanthin 0.216 mg for 14 days, whereas Group 4, the treatment group 2 (T2), was supplemented with astaxanthin 0.216 mg for 21 days.

After treatment was completed, the rats underwent a cervical dislocation procedure and the peritoneum tissues were collected and prepared for histological examination.

Histological examination

Tissue incision deparaffinization is performed to remove paraffin from the tissue using standard laboratory methods, namely gradually for a certain time adding the preparations into liquid acetone, xylol, 100% alcohol, 90% alcohol, 80% alcohol, 70% alcohol, and water.

Deparaffinization was carried out using standard laboratory methods, samples were gradually added with acetone, xylol, 100% alcohol, 90% alcohol, 80% alcohol, 70% alcohol, and water for a certain time. Then the tissue was stained with Masson's trichrome. The tissue was washed with flowing water and covered with deck glass. Sample examination was carried out by researchers and pathologists using the Optilab Viewer and Image raster at the pathology laboratory, faculty of medicine, Sebelas Maret university. Fibrosis thickness was measured based on the percentage of fibroblasts in the entire visual field.

Statistical analysis

All data are presented as mean ± standard deviation using one-way ANOVA, then followed by Bonferroni multiple comparisons. Data were analyzed by Statistical Package for the Social Sciences (SPSS) for Windows version 25.0.

P value < 0.05 was considered to indicate a statistically significant difference.

Results

In this study, fibrosis thickness is measured to assess peritoneal fibrosis. The result showed that astaxanthin measurement of fibrosis in the peritoneal tissue in the group with astaxanthin supplementation obtained a lower mean result than the positive control group ($P < 0.05$; Table 1). These results suggest that astaxanthin administration can prevent the development of peritoneal fibrosis in CKD rats model.

Further statistical tests were carried out with Post Hoc Bonferroni to determine the differences in the mean of the two independent groups in each group.

The analysis of the mean difference of each independent sample with the post hoc test (Table 2) showed that the test between the NC and PC groups obtained significant results ($P < 0.05$) whereas in the PC group the level of fibrosis thickness was higher. Between NC and T1 and T2, it was not obtained significant results ($P > 0.05$). Furthermore, the mean peritoneal fibrosis of the PC group when compared with T1 and T2 showed significant results ($P < 0.05$), meanwhile the administration of astaxanthin could reduce the development of fibrotic tissue in the peritoneum of rats in T1 and T2 groups. However, between T1 and T2, there was no significant difference in the mean thickness of fibrosis ($P > 0.05$). These results indicated that astaxanthin supplementation can significantly reduce peritoneal fibrosis.

Discussion

Peritoneal fibrosis is common in patients on PD and manifests as peritoneal thickening. In this study, peritoneal fibrosis was evaluated by measuring the percentage of fibroblasts. Based on previous studies, histopathological examination of the biopsy results of PD model mice showed a prominent cellular proliferation on the peritoneal surface with collagen fiber deposition, interstitial edema, and neoangiogenesis. In addition, pathological changes were found in the form of a widening of the submesothelial compact zone and loss of the mesothelial cell layer. In contrast, in the normal group, the peritoneal surface consists of only one layer of mesothelial cells (Figures 1 and 2) (22,23).

Histopathological examination in this study showed the thickening of the peritoneal mesothelial tissue in the PC

Table 1. Differences in the mean thickness of fibrosis (μm)

Group	Mean ± Standard deviation	*P* value
NC (n=8)	17.47 ± 8.39	< 0.006
PC (n=8)	43.09 ± 17.80	
T1 (n=8)	21.79 ± 9.44	
T2 (n=8)	21.47 ± 17.12	

NC: Negative control; PC: Positive control; T1: Treatment group 1; T2: Treatment group 2.

Table 2. Comparison of two differences in mean fibrosis thickness (μm)

Group	Mean differences ± Standard error	*P* value
NC and PC	-25.63 ± 7.01	0.007*
NC and T1	-4.33 ± 6.77	1.000
NC and T2	-4.01 ± 7.01	1.000
PC and T1	21.30 ± 7.01	0.032*
PC and T2	21.62 ± 7.24	0.037*
T1 and T2	0.31 ± 7.01	1.1

NC: Negative control; PC: Positive control; T1: Treatment group 1; T2: Treatment group 2. *Significance $P < 0.05$.

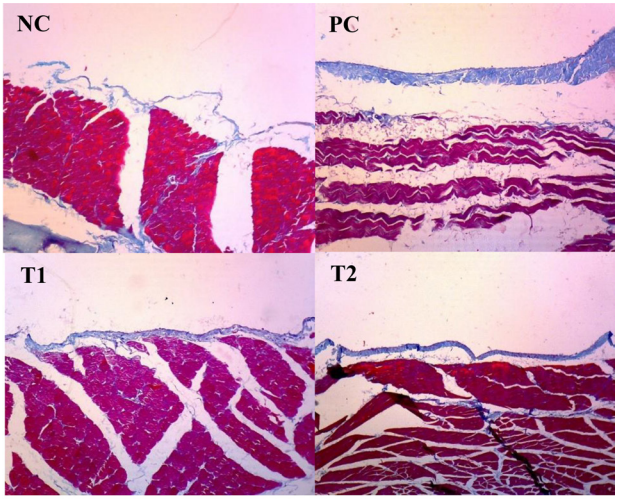

Figure 1. Peritoneal Fibrosis of Each Group at 100× magnification. An overview of peritoneal fibrosis with Masson's Trichrome staining at 100× magnification with an Olympus BX-50 Model BX-50F-3 Pentax Optio 230 Digital Camera 2.0 Megapixel. NC, Negative control; PC, Positive control; T1, Treatment group 1; T2, Treatment group 2.

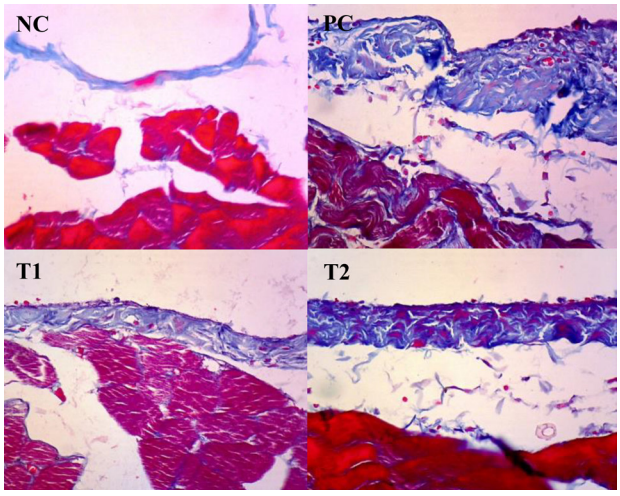

Figure 2. Peritoneal Fibrosis of Each Group at 400× magnification. An overview of peritoneal fibrosis with Masson's Trichrome staining at 400× magnification with an Olympus BX-50 Model BX-50F-3 Pentax Optio 230 Digital Camera 2.0 Megapixel. NC, Negative control; PC, Positive control; T1, Treatment group 1; T2, Treatment group 2.

group compared to the NC group. The administration of astaxanthin supplementation in the PD rat group, namely T1 and T2, was able to reduce the thickening of the peritoneal mesothelial tissue. Statistical analysis showed a significant difference in the mean thickness of fibrosis between the four groups ($P < 0.05$). However, there was no significant difference with increasing the frequency of astaxanthin administration to 21 days ($P > 0.05$).

The results of the two-group difference test between NC and T1 also NC and T2 did not show a significant difference ($P > 0.005$). It is because the NC group was not injected with intraperitoneal dialysate, therefore the peritoneum of the NC group rats were not exposed to this bioincompatible fluid. Thus, the mesothelial surface thickness in the NC group did not increase because it was not related to PD.

A previous study showed morphological changes in chlorhexidine gluconate (CG)-induced rats, where the administration of astaxanthin can reduce peritoneal thickening along with the expression of inflammatory and neovascularization factors in peritoneal tissue such as cells that are positive for monocyte chemoattractant protein-1 (MCP-1), collagen type 3, tumour necrosis factor α (TNFα), and α-smooth muscle actin (α-SMA) (18,24).

Research on the protective effect of astaxanthin on the peritoneum on dialysis has not been conducted, however there are studies on other drugs such as the use of angiotensin-converting enzyme-inhibitor (ACE-I) agents, diuretics, vitamin C, vitamin E, selenium, zinc, glutathione, and others have been done. Consequently it is necessary to do further comparing astaxanthin with other anti-oxidant and anti-inflammatory agents (25–27).

This study showed similar results that astaxanthin reduces fibrosis thickness in rats exposed to the dialysate. The administration of astaxanthin in addition, alleviates inflammatory response in peritoneal tissue, thus potentially preventing the formation of fibrosis on PD.

Conclusion

Based on this study, the results showed that astaxanthin could reduce fibrotic thickness in PD model rats. This study was relevant to conclude that astaxanthin has a potential antifibrotic agent for PD.

Limitations of the study

Our study has some limitations; Only one dose was used, so it was not possible to compare the effect of increasing or decreasing the astaxanthin dose on the outcome of peritoneal fibrosis. Further research is still needed regarding the safety and side effects of drugs in experimental animals with various doses and frequency of administration.

Authors' contribution

Conceptualization: Ratih Tri Kusuma Dewi, Bambang Purwanto, Maia Thalia Giani, Mahatma Chakra Wardana.
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Writing–original draft: Ratih Tri Kusuma Dewi, Mahatma Chakra Wardana.
Writing–review and editing: Maia Thalia Giani, Indah Saigitaisna Putri.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The animals were treated in corresponding with the National Institutes of Health Guide for the Care and Use of Laboratory Animals guideline. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Sebelas Maret University, Surakarta, Indonesia. The research was approved by the Ethics Committee of Dr Moewardi General Hospital (Ethical code No. 1.277/XII/HREC/2019). The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

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