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The Effects of Indobufen on Micro-Inflammation and Peritoneal Transport Function in Patients Undergoing Continuous Ambulate Peritoneal Dialysis: A Prospective Randomized Controlled Study

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ABSTRACT

Indobufen possesses anticoagulant and antithrombotic effects that can improve micro-inflammation and renal function. This study aimed to examine whether indobufen could improve the microinflammatory state in patients on continuous ambulatory peritoneal dialysis (CAPD) and explore its therapeutic effects on peritoneal transport function. A total of 60 patients undergoing CAPD from October 2019 to October 2020 were selected and randomized to the control and indobufen groups. All patients received conventional treatments. Blood routine and the serum and peritoneal effusion levels of tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), cellular fibronectin (cFN), and vascular endothelial growth factor were determined before and after 6 months of treatment. The peritoneal equilibrium test (PET) was used to evaluate peritoneal transport function. There were no significant differences in PET results, microinflammatory state, and biochemical indices between the two groups before treatment ($P > 0.05$). After 6 months of treatment, platelet-to-lymphocyte ratio and serum and peritoneal effusion TNF-α levels in the indobufen group were decreased compared with the control group ($P < 0.05$). Serum and peritoneal effusion TGF-β1 and cFN levels in the indobufen group were reduced compared with the control group ($P < 0.05$). PET results in the indobufen group were decreased compared with baseline ($P < 0.05$). The difference in PET results between the two groups before and after treatment was statistically significant ($P < 0.05$). Indobufen could improve the peritoneal transport function in patients undergoing CAPD. The underlying mechanism might be related to the improvement of the microinflammatory state and peritoneal fibrosis.

SIGNIFICANCE STATEMENT

Microinflammation and peritoneal fibrosis can lead to peritoneal failure in CAPD. Indobufen is a novel antiplatelet drug that can alleviate renal fibrosis and improve renal function in patients with diabetic nephropathy. Indobufen can improve the peritoneal transport function in patients undergoing CAPD. The mechanism of indobufen improving the peritoneal function might be related to the improvement of the microinflammatory state and peritoneal fibrosis.

Introduction

Peritoneal dialysis is the most prevalent therapeutic strategy for patients with end-stage renal disease (ESRD) worldwide. The annual growth rate of peritoneal dialysis (PD) is expected to reach 8%, which is about 6% to 7% higher than that of hemodialysis (Li et al., 2017a). Structural changes in the peritoneum can appear after long durations of peritoneal dialysis, eventually leading to peritoneal fibrosis, peritoneal angiogenesis, and inflammatory bowel disease (Yáñez-Mó et al., 2003; Zhou et al., 2016; Balzer, 2020).

The continuous exposure to peritoneal dialysis fluid (PDF) to low or poor biocompatibility can destroy the normal structure and function of the peritoneum, leading to peritoneal dialysis termination. At present, the treatment methods for microinflammation mainly include immunomodulatory therapy, antifibrosis therapy, traditional peritoneal dialysis catheterization, and protection of the intraperitoneal mesenchymal stem cells (Huddam et al., 2015; Li et al., 2015; Wakabayashi et al., 2015; Raby et al., 2018; Wu et al., 2018; Chiu et al., 2019). Still, further effective and safe drugs need to be developed.

As a new generation of antiplatelet drugs, indobufen can be clinically used in ischemic stroke, nonrheumatic atrial fibrillation, myocardial infarction, thrombosis, and peripheral vascular disease (Wiseman et al., 1992; Bhana and McClellan, 2001; Patrono et al., 2008). Indobufen possesses anticoagulant and antithrombotic effects, reduces fibrinogen

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ABBREVIATIONS: CAPD, continuous ambulatory peritoneal dialysis; cFN, cellular fibronectin; ESRD, end-stage renal disease; MPR, mean platelet volume-to-platelet ratio; NLR, neutrophil-to-lymphocyte ratio; PD, peritoneal dialysis; PDF, peritoneal dialysis fluid; PET, peritoneal equilibrium test; PLR, platelet-to-lymphocyte ratio; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

activation, and improves the microinflammatory state (Bhana and McClellan, 2001; Patrono et al., 2008). Indobufen can improve renal function in diabetic nephropathy, possesses renal protective effects, and can alleviate renal fibrosis (Shestakova et al., 1996; Lou et al., 2019).

To date, no study focused on the effects of indobufen on patients undergoing peritoneal dialysis. Therefore, the present study investigated whether indobufen could improve the microinflammatory state and peritoneal transport function in patients on continuous ambulatory peritoneal dialysis (CAPD).

Materials and Methods

Study Design and Participants. This randomized controlled trial recruited patients who were undergoing CAPD at the Third Xiangya Hospital of Central South University (Changsha, China) between January 2017 and January 2019 (Department of Nephrology). The study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (Approval No. 21106). All participants were fully informed about the aims of the study and signed the informed consent form prior to any study procedure.

Inclusion and Exclusion Criteria. The inclusion criteria were (1) 18 to 75 years of age, (2) stage 5 chronic kidney disease according to the guidelines of the National Kidney Foundation, (3) CAPD for ≥ 3 months, and (4) regularly undergoing CAPD with clinical stability. The exclusion criteria were (1) abnormal coagulation indices (fibrinogen level < 2 g/L, platelet count $< 100 \times 10^9$/L, prothrombin time $>$ 14 seconds, activated partial prothrombin time > 40 seconds); (2) severe liver dysfunction (alanine aminotransferase or aspartate aminotransaminase > 3 upper limit of normal); (3) history of active ulcer, hemorrhagic diseases, or menorrhagia (> 80 mL) within half a year before enrollment; (4) history of idiopathic thrombocytopenic purpura, hemophilia, or aplastic anemia; (5) history of major trauma and undergoing surgery within 3 months before enrollment; (6) received antiplatelet drugs for 4 weeks; (7) diagnosis of peritonitis within 1 month before enrollment; (8) allergic to the study drugs; (9) pregnant or lactating women; or (10) previously underwent renal transplantation and hemodialysis.

Randomization and Blinding. The participants were randomized to the indobufen and control groups. The statistician was blinded to grouping during data analysis, but the participants undergoing CAPD and physicians were aware of the grouping. A standardized process of CAPD was conducted in our hospital, and a closed dialysate input-output system was implemented by trained staff in our dialysis unit.

Interventions. The participants in the indobufen group were orally given indobufen (100 mg each time, twice/day; Zhongmei Huadong Pharmaceutical Co., Ltd., Hangzhou, China) for 6 consecutive months and received no other anticoagulants during the study. The participants in the control group did not receive indobufen or a placebo. The participants in the two groups received conventional treatments, including drugs aiming to manage blood pressure, calcium-phosphate metabolism, and anemia. CAPD was conducted using a 1.5% or 2.5% intraperitoneal dialysis solution (Baxter International Inc., Deerfield, IL, USA) and a twin-bag system at 6000 to 8000 ml/d The dialysate was the same for the two groups.

Follow-Up. During the 6-month treatment period, the patients were followed up by telephone, SMS, or the WeChat social app. The patients were also required to come to the hospital regularly for follow-up and complete the relevant examinations. At the same time, we monitored the treatment of the patients through the information data platform. After the end of the study, follow-up was continued for 6 months according to the follow-up requirements of the PD center.

Data Collection. From 8 PM (on the day before the examination) to 8 AM, the patients fasted, and blood samples were taken on an empty stomach. During peritoneal dialysis, 2000 mL dialysis fluid (called dialysate) was infused into the abdominal cavity overnight on the night before the examination, and peritoneal dialysis was performed the next day. Sex, age, duration of peritoneal dialysis, peritoneal dialysis regimen, and primary disease were collected. Blood samples (10 mL) were obtained before and 6 months after starting treatment. The blood samples were centrifuged at $3000 \times g$ for 10 minutes to collect the serum. The samples were stored at -80°C. The peritoneal dialysate was retained and processed to collect the serum. The following biochemical indices were measured using standard clinical methods: routine blood test, renal function test, serum lipid profile, serum iron, ferritin, transferrin, serum calcium, serum phosphate, and parathyroid hormone. The serum and dialysate levels of tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), cellular fibronectin (cFN), and vascular endothelial growth factor (VEGF) were measured using an enzyme-linked immunosorbent assay kit (Huamei Biologic Engineering Co., Ltd., Wuhan, China), following the manufacturer's instructions. The peritoneal equilibration test (PET) was performed before and 6 months after starting treatment. The adverse reactions in the two groups were monitored during treatment.

Statistical Analysis. Statistical analysis was performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were expressed as means ± standard deviation and compared using the paired t test (before/after comparisons) or the independent-samples t test (between-group comparisons). Nonnormally distributed continuous variables were presented as median (interquartile range) and compared using the Wilcoxon signed-rank test (intragroup comparisons) or the Mann–Whitney U test (intergroup comparisons). Categorical variables were expressed as n (percentage) and compared using the χ^2 test. The Pearson correlation analysis was used for analyzing normally distributed continuous data; otherwise, Spearman correlation analysis was adopted. A two-sided $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics of the Patients. Sixty patients who had been undergoing CAPD for 90 to 198 months were enrolled, including 31 men (51.7%) and 29 women (48.3%). They were 15 to 75 years old (mean age, 55.59 ± 11.17 years old). In the present study, 30 and 29 patients were allocated to the indobufen and control groups, respectively (Fig. 1). There were no significant differences in age, duration of peritoneal dialysis, sex, and diseases between the two groups (all $P > 0.05$) (Table 1).

Correlation Analysis of Inflammatory Factors and Peritoneal Fibrosis in Patients Who Underwent CAPD. Before treatment, there were no significant linear correlations between serum TNF-α levels and serum levels of TGF-β1 (Fig. 2a), cFN (Fig. 2c), and VEGF (Fig. 2e) (all $P > 0.05$). The correlation coefficients of TNF-α and TGF-β1 (Fig. 2b), as well as cFN (Fig. 2d) and VEGF (Fig. 2f) in peritoneal effusion, ranged from 0.2 to 0.4, and there was a positive correlation between them (all $P < 0.05$).

Correlation Analysis of peritoneal transport function and Peritoneal Fibrosis in Patients Who Underwent CAPD. Before treatment, the correlation analysis between PET results and serum levels of TGF-β1 (Fig. 2g), cFN (Fig. 2i), and VEGF (Fig. 2k) in patients who were undergoing CAPD indicated that there was a significant correlation between PET results and serum levels of TGF-β1 (Fig. 2g), cFN (Fig. 2i), and VEGF (Fig. 2k) (all $P < 0.05$). The correlation coefficient between the serum levels of cFN and VEGF was in the range of 0.4 to 0.6 (Fig. 2k),

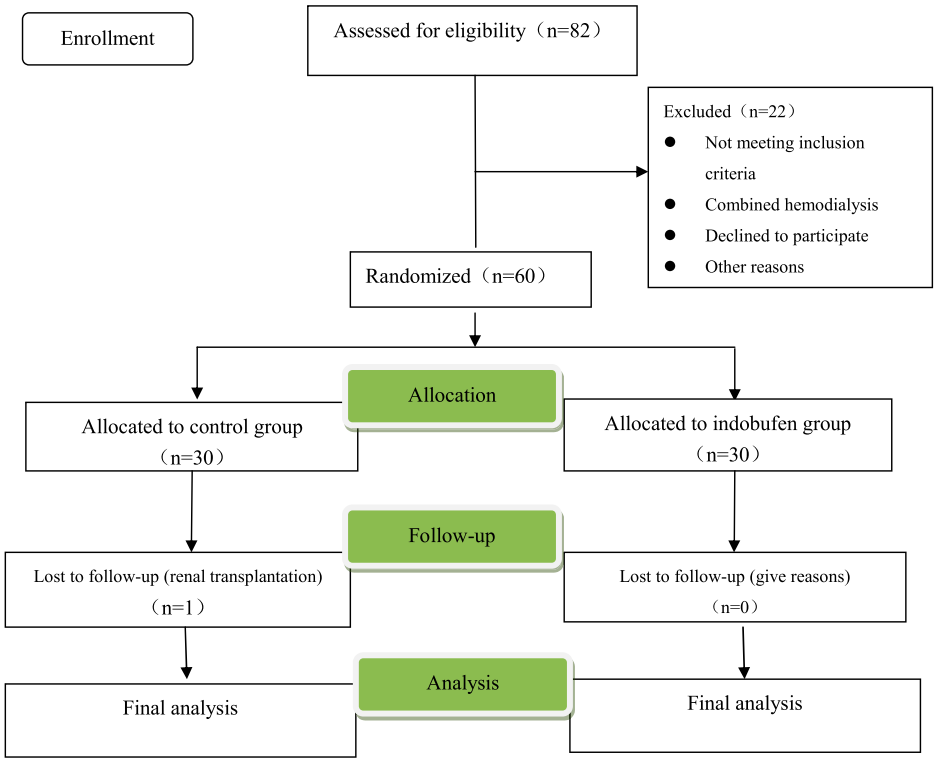

Fig. 1. Study flowchart. Thirty and 29 patients were allocated to the indobufen group and the control group, respectively.

indicating that there was a moderate positive correlation between PET results and serum levels of cFN and VEGF. The correlation coefficient between PET results and serum TGF-β1 level was between 0.2 and 0.4 (Fig. 2g), demonstrating a weak positive correlation.

Before treatment, the correlation analysis of PET results and TGF-β1 level (Fig. 2h), as well as cFN (Fig. 2j) and VEGF (Fig. 2l) levels in the peritoneal effusion of patients who were undergoing CAPD, revealed that there were significant correlations between PET results and peritoneal effusion levels of TGF-β1 (Fig. 2h), cFN (Fig. 2j), and VEGF (Fig. 2l) (all $P < 0.05$). In addition, the correlation coefficient between PET results and peritoneal effusion TGF-β1 levels in peritoneal effusion was in the range of 0.6 to 0.7 (Fig. 2h), indicating that PET results were strongly positively correlated with TGF-β1 levels in peritoneal effusion. The correlation coefficient between PET results and cFN levels in peritoneal effusion was between 0.4 and 0.6 (Fig. 2j), highlighting that there was a moderate positive correlation between PET results and cFN levels in peritoneal effusion. The correlation coefficient between PET results and VEGF levels in peritoneal effusion was in the range of 0.2 to 0.4 (Fig. 2l), demonstrating a weak positive correlation.

The Effects of Indobufen on Biochemical Indices. Before treatment, there were no significant differences in the levels of biochemical blood indices between the two groups ($P > 0.05$). The ferritin levels were decreased in the indobufen group after 6 months of treatment compared with the control group (U = 274.00, $P = 0.015$), while there were no significant differences in the levels of the other biochemical blood indices after 6 months of treatment (all $P > 0.05$) (Table 2).

With the prolongation of dialysis time, serum creatinine levels in both groups noticeably increased ($P < 0.05$). The serum iron levels in the control group significantly increased compared with before treatment (Z = 2.606, $P = 0.009$), while

TABLE 1
Baseline demographic and clinical characteristics of the study participants
Data are mean ± S.D., n, or median (interquartile range).

Characteristic	Control group (n = 29)	Indobufen group (n = 30)	*P*
Age (years)	53.75 ± 9.25	57.43 ± 13.09	0.217
Male	16	15	0.691
PD duration (months)	34.13 (19.82, 49.82)	37.73 (20.82, 55.55)	0.693
Primary diseases			0.500
Chronic glomerulonephritis	18	16	
Diabetic nephropathy	1	5	
Hypertensive nephropathy	3	4	
Polycystic kidney	2	1	
Others[a]	5	4	

PD, peritoneal dialysis.
[a]Other primary diseases included ischemic nephropathy, obstructive nephropathy, lupus nephritis, gouty nephropathy, and systemic vasculitis and renal damage.

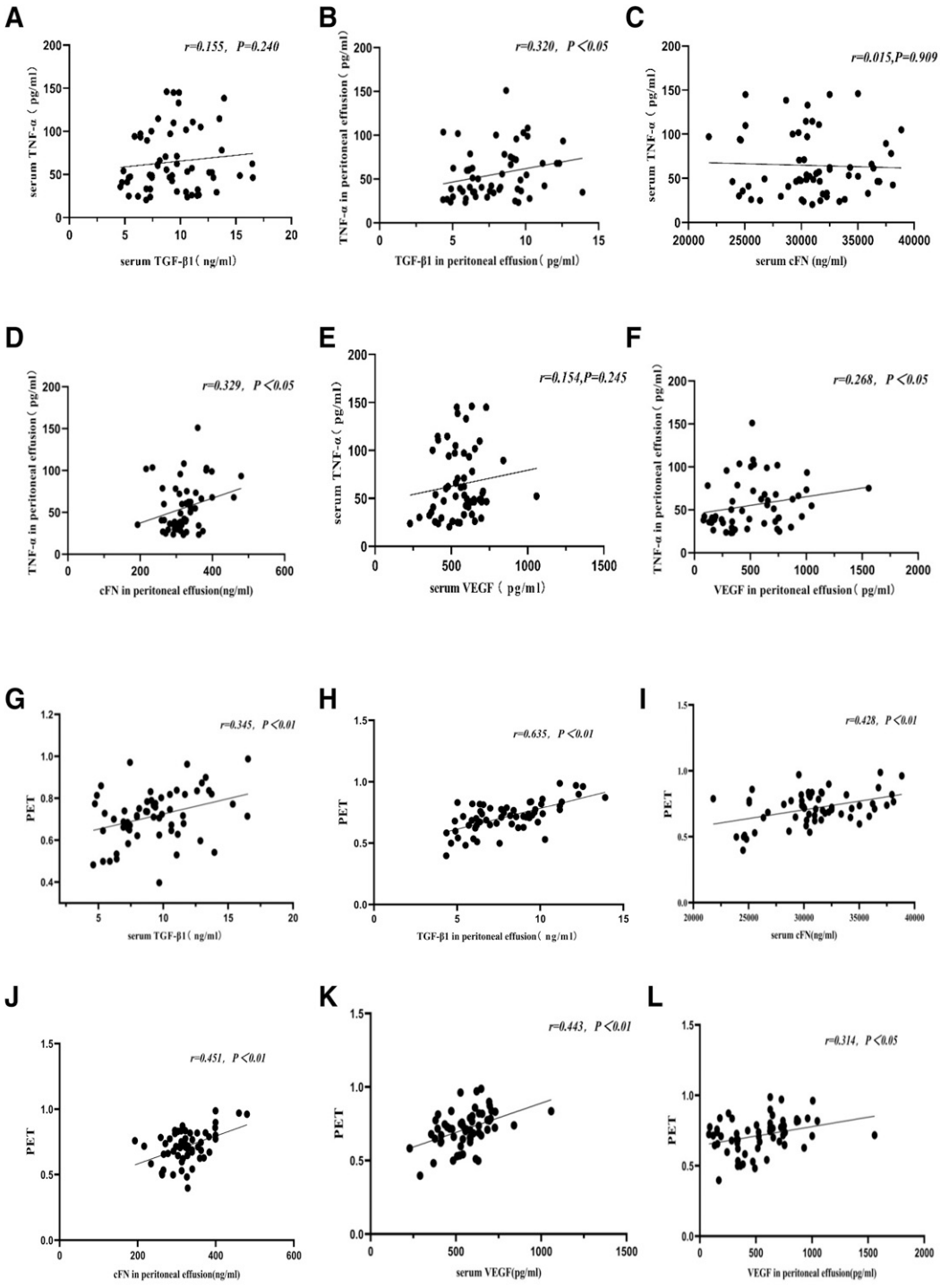

Fig. 2. Correlation analysis between TNF-α and TGF-β1, cFN and VEGF in serum (a, c, and e) and peritoneal effusion (b, d, and f) of CAPD patients at baseline. Correlation analysis between PET and TGF-β1, cFN and VEGF in serum (j, i, and k) and peritoneal effusion (h, j, and l) of CAPD patients at baseline.

there were no significant differences in the other biochemical blood indices between the two groups before and after treatment (all $P > 0.05$) (Table 2).

Indobufen Could Improve the Microinflammatory State of Patients Undergoing CAPD. There were no significant differences in TNF-α levels in serum and peritoneal effusion and neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume-to-platelet ratio (MPR) between the two groups before treatment (all $P > 0.05$) (Fig. 3). No significant correlations between PLR and serum and peritoneal effusion TGF-β1, cFN, and VEGF were observed (all $P > 0.05$) (Fig. 3). After 6 months of treatment, PLR and TNF-α levels in serum and peritoneal effusion in the indobufen group were significantly decreased compared with the control group ($P < 0.05$) (Table 3). After 6 months of treatment, NLR and

TABLE 2
Blood biochemical indexes in the two groups of patients at baseline and after 6 months of indobufen
Data are mean ± S.D. or median (interquartile range).

	Control group (n = 29)		Indobufen group (n = 30)	
Index	0 months	6 months	0 months	6 months
HDL-C (mmol/L, $\bar{x}$± s)	1.23 ± 0.32	1.24 ± 0.29	1.13 ± 0.29	1.19 ± 0.24
LDL-C (mmol/L, $\bar{x}$± s)	2.48 ± 0.66	2.77 ± 0.86	2.36 ± 1.03	2.78 ± 0.84
TC (mmol/L, $\bar{x}$± s)	4.64 ± 1.02	5.00 ± 1.23	4.59 ± 1.50	4.84 ± 1.51
TG (mmol/L, $\bar{x}$± s)	1.88 ± 1.15	2.11 ± 1.42	1.93 ± 1.70	2.06 ± 1.31
Ca (mmol/L, $\bar{x}$± s)	2.26 ± 0.26	2.26 ± 0.26	2.12 ± 0.25	2.19 ± 0.29
P (mmol/L, $\bar{x}$± s)	1.61 ± 0.46	1.64 ± 0.45	1.62 ± 0.55	1.63 ± 0.40
PTH [pg/mL, Md (P_{25}, P_{75})]	194.35 (111.16, 439.04)	285.27 (181.77, 397.41)	172.52 (81.75, 407.64)	216.34 (98.12, 343.46)
BUN (mmol/L, $\bar{x}$± s)	19.81 ± 6.41	21.19 ± 5.65	20.04 ± 7.35	19.67 ± 6.98
Scr (μmol/L, $\bar{x}$± s)	959.31 ± 299.08	1026.31 ± 297.82*	967.00 ± 320.62	1049.17 ± 335.14*
UA (mmol/L, $\bar{x}$± s)	410.41 ± 106.30	420.46 ± 82.04	397.83 ± 83.54	392.17 ± 78.53
TF (g/L, $\bar{x}$± s)	1.89 ± 0.52	1.99 ± 0.10	1.69 ± 0.41	1.85 ± 0.56
SI [μmol/L, Md (P_{25}, P_{75})]	10.90 (7.95, 13.38)	14.80 (10.6, 18.25)*	9.75 (6.53, 13.08)	13.00 (6.65,18.08)
Ferr [ng/mL, Md (P_{25}, P_{75})]	322.50 (135.50, 517.65)	346.90 (195.50, 599.15)	256.75 (135.20, 395.10)	240.30 (92.55, 411.13)#

BUN, blood urea nitrogen; Ca, calcium; Ferr, ferritin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; P, phosphorus; PTH, parathyroid hormone; Scr, serum creatinine; SI, serum iron; TC, total cholesterol; TF, transferrin; TG, triglycerides; UA, uric acid.
*$P < 0.05$ versus same group at month 0 (before the start of treatment); #$P < 0.05$ versus control group at month 6.

PLR in the indobufen group were remarkably reduced compared with the control group (all $P < 0.05$) (Table 3). Moreover, TNF-α levels in peritoneal effusion in the control group were elevated compared with the indobufen group ($Z = 2.606$, $P = 0.009$) (Table 3). The aforementioned results indicated that indobufen could improve the microinflammatory state of patients undergoing CAPD.

The Effects of Indobufen on Peritoneal Fibrosis. Before treatment, there were no statistically significant differences in the levels of TGF-β1, cFN, and VEGF in peritoneal effusion and serum between the two groups ($P > 0.05$) (Table 4). After 6 months of treatment, the levels of TGF-β1 and cFN in peritoneal effusion and serum in the indobufen group were significantly reduced compared with the control group (all $P < 0.05$) (Table 4). After 6 months of treatment, there were no significant differences in VEGF levels in serum and peritoneal effusion between the two groups (all $P > 0.05$) (Table 4). These results demonstrated that indobufen possesses antifibrosis effects.

Indobufen Could Improve Peritoneal Transport Function. After 6 months of treatment, PET results significantly changed in the indobufen group compared with before treatment ($t = 2.485$, $P = 0.019$), while there were no significant differences in PET results in the control group before and after treatment ($P > 0.05$) (Table 5). The indobufen group showed remarkably greater changes in PET results before and after treatment compared with the control group ($t = 2.044$, $P = 0.046$) (Table 5). There were no correlations between PET and serum TNF-α, peritoneal effusion TNF-α, and PLR (all $P > 0.05$) (Fig. 4). Taken together, indobufen could improve peritoneal transport function. After treatment, a negative correlation was observed between peritoneal effusion TNF-α and VEGF ($r = -0.547$, $P < 0.01$) (Fig. 5f). There were no significant correlations between PET and serum and peritoneal effusion TGF-β1, cFN, and VEGF (all $P > 0.05$) (Fig. 5g-l).

Adverse Reactions. No cardiovascular and cerebrovascular events occurred in the indobufen group. In the control group, two cases of unstable angina pectoris and one case of posterior circulation ischemic stroke were identified. In the indobufen group, there was one case of hematuria and one case of gastrointestinal infection. In the control group, one case of hematuria and one case of gastrointestinal disease were observed.

Discussion

Peritoneal dialysis is a modality of choice for renal replacement therapy for patients with acute kidney injury because of its advantages compared with hemodialysis (Li et al., 2017a; Himmelfarb et al., 2020; Cullis et al., 2021). A long duration of peritoneal dialysis induces structural and functional changes in the peritoneum, resulting in microinflammation, peritoneal fibrosis, and angiogenesis.

Systemic microinflammation in patients undergoing peritoneal dialysis is associated with uremic toxins, while local microinflammation during peritoneal dialysis can be associated with chronic nonspecific inflammatory diseases caused by the peritoneal dialysis catheters (Li et al., 2017b). Microinflammation is involved in the pathophysiological changes of patients with atherosclerosis, malnutrition, left ventricular hypertrophy, heart failure, peritoneal fibrosis, angiogenesis, and so on, which is one of the risk factors for cardiovascular and cerebrovascular events in patients undergoing peritoneal dialysis (Lai et al., 2015). Studies have shown that microinflammation is a major promoter of peritoneal fibrosis, and inhibition of inflammation can reduce the inflammatory response in vivo and partially mitigate the progression of peritoneal fibrosis (Li et al., 2017b; Balzer, 2020). The results of the present study showed that TNF-α levels in peritoneal effusion were positively correlated with the levels of TGF-β1, cFN, and VEGF in peritoneal effusion, suggesting that inflammation can promote the development of peritoneal fibrosis.

The status of microinflammation is mainly determined by detecting the levels of inflammatory markers. In recent years, NLR, PLR, and MPR have been widely studied. Scholars reported that PLR was positively correlated with NLR, IL-6, and TNF-α levels in patients undergoing peritoneal dialysis (Turkmen et al., 2013). Turkmen et al. also found that NLR was closely associated with inflammatory responses in patients on peritoneal dialysis and hemodialysis (Turkmen et al., 2012). These studies suggested that PLR and NLR could reflect the status of microinflammation in

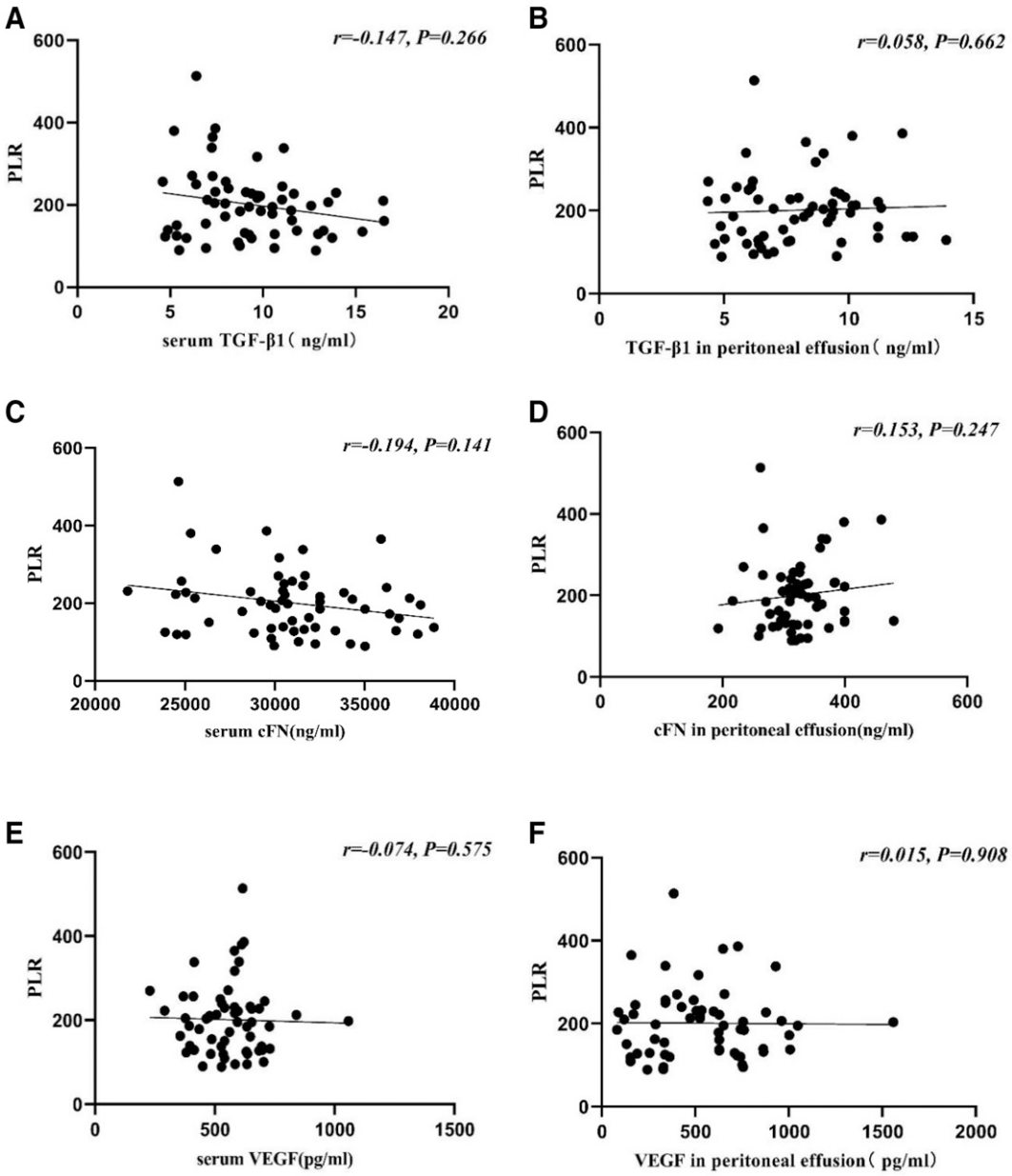

Fig. 3. Correlation analysis between PLR and TGFβ1, cFN, and VEGF in serum (a, c, and e) and peritoneal effusion (b, d, and f) of CAPD patients at baseline.

patients undergoing peritoneal dialysis. The results of the present study showed that PLR and NLR decreased in patients undergoing CAPD after indobufen treatment, and TNF-α levels in serum and peritoneal effusion decreased simultaneously, demonstrating that indobufen can reduce systemic and local microinflammation in patients undergoing CAPD. After peritoneal dialysis, activated factor VII activates the other coagulation factors (IX and X), triggering the clotting pathway, and thrombin also induces the production of IL-6 and IL-8 in endothelial cells (Levi et al., 2004; Ma et al., 2019). Previous research reported that indobufen could dose-dependably downregulate the levels of tissue factors in monocytes and reduce the activation of inflammatory factors (Eligini et al., 2006). In addition, a number of scholars pointed out that indobufen or aspirin combined with clopidogrel or ticagrelor could reduce pyroptosis mediated by inflammasomes and alleviate inflammatory responses through the NF-κB/NLRP3 signaling pathway, thereby attenuating ischemia-reperfusion injury in a rat model of middle cerebral artery occlusion/reperfusion (Li et al., 2021). In addition, TGFβ1 interacts with VEGF to promote peritoneal injury through the TGFβ1-VEGF-A pathway (Kariya et al., 2018). The results of the present study showed that the serum levels of TGF-β1, cFN, and TNF-α increased with the prolongation of dialysis duration in patients undergoing CAPD. After 6 months of indobufen treatment, the levels of TGF-β1, cFN, and TNF-α in serum and peritoneal effusion decreased. The aforementioned results suggested that indobufen could delay the progression of peritoneal fibrosis by regulating microinflammation in patients undergoing CAPD. Lou et al. (Lou et al., 2019) showed that indobufen could reduce serum TGF-β levels and alleviate renal fibrosis in a rat model of chronic kidney disease, and they also demonstrated that the mechanism of indobufen in improving renal fibrosis could be

TABLE 3
Comparison of the micro-inflammatory indicators between the two groups of patients at baseline and after 6 months of indobufen
Data are mean ± S.D. or median (interquartile range).

	Control group (n = 29)		Indobufen group (n = 30)	
Index	0 months	6 months	0 months	6 months
NLR	4.45 (3.03, 5.54)	4.06 (3.04, 5.86)	4.54 (3.11, 5.62)	3.53 (2.33, 4.70)*
PLR	196.43 ± 84.10	204.03 ± 81.87	205.91 ± 85.95	165.10 ± 63.08#*
MPR	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.06
Serum TNF-α (pg/ml)	47.37 (31.34, 91.48)	55.45 (43.18, 84.31)	58.91 (42.27, 100.69)	46.30 (33.85, 58.59)#*
Effusion TNF-α (pg/ml)	36.39 (27.69, 73.35)	56.81 (38.67, 92.49)*	55.32 (40.69, 68.92)	39.38 (31.89, 54.68)#*

MPR, mean platelet volume and platelet ratio; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; TNF-α, tumor necrosis factor-α.
*$P < 0.05$ versus same group at month 0 (before the start of treatment); #$P < 0.05$ versus control group at month 6.

correlated with the upregulation of 6-keto-prostaglandin F1α/thromboxane B2 in renal tissues. Another study revealed that inflammatory factors could induce pleural mesothelial cells to increase the expression of plasminogen activators through TGF-β1 and change the fibrinolytic state of mesenchymal cells, resulting in hypercoagulability (Nagy, 1996). Indobufen can reduce the levels of coagulation factors I, II, IV, VIII, and X, and it possesses anticoagulant and antithrombotic effects (Liu et al., 2018). Therefore, indobufen could delay the progression of peritoneal fibrosis in patients undergoing CAPD by improving intraperitoneal microinflammation and hypercoagulability, reducing TGF-β1 production and FN expression.

A long duration of peritoneal dialysis can lead to a progressive loss of ultrafiltration capability caused by structural and functional changes. The present study showed that PET results in patients undergoing CAPD were positively correlated with the levels of TGF-β1, cFN, and VEGF in serum and peritoneal effusion to varying degrees, suggesting that peritoneal fibrosis could affect the peritoneal transport function. Peritoneal fibrosis has also been reported to cause changes in the peritoneal transport function (Kariya et al., 2018). This study showed that the PET results significantly changed after 6 months of indobufen treatment, while they did not significantly change in the control group. Moreover, the differences in PET results between the two groups before and after treatment were statistically significant. Peritoneal transport can be divided into four types: low transport, low average transport, high average transport, and high transport. Long-term peritoneal dialysis can cause changes in the intraperitoneal environment and then lead to changes in the structure and function of the peritoneum. Structurally, peritoneal neovascularization and peritoneal fibrosis can occur; functionally, it can cause an increase in peritoneal solute transport function and a decrease in ultrafiltration function (Crabtree and Chow, 2017; Kariya et al., 2018). The increase in peritoneal solute transport function is an independent factor affecting the effect of peritoneal dialysis and is related to high technical failure and mortality rates (Shi et al., 2018). The results of this study showed that, after 6 months of treatment, the PET value of the indobufen group was lower than that of the control group, and the difference between the two groups before and after treatment was statistically significant, suggesting that indobufen can delay the rise of solute transport rate and better maintain the balance between toxin clearance capacity and ultrafiltration capacity. On the one hand, indobufen has antiplatelet, anticoagulant, and antithrombotic effects, as well as anti-inflammatory effects (Bhana and McClellan, 2001; Liu et al., 2018; Li et al., 2021). In addition, indobufen can reduce TGF-β1 in renal tissue and can alleviate renal fibrosis, and its mechanism may be related to the upregulation of 6-keto-PGI2/TXB2 in renal tissue (Lou et al., 2019). Combined with the present study, the available results suggest that indobufen can improve the microinflammatory state of peritoneal dialysis patients, thus playing an antifibrosis role, which might partially improve the high transport state of peritoneal solute.

In healthy volunteers, the time (T_{max}) for rapid and complete absorption of indobufen to reach the peak plasma concentration was about 2 hours, and there was a linear relationship between the dose and plasma concentration (Fuccella et al., 1979; Tamassia et al., 1979). The peak plasma concentration reached 12.5 to 14.9 mg/L 2 hour after a single dose of 100 mg. After oral administration of indobufen 100 and 200 mg, bid for 7.5 days and 5 days, the steady-state peak plasma concentrations (C_{max}) reached 16.7 and 29.2 mg/l. C_{max} and the area under the curve

TABLE 4
Comparison of the peritoneal fibrosis indexes between the two groups of patients at baseline and after 6 months of indobufen
Data are mean ± SD or median (interquartile range).

	Control group (n = 29)		Indobufen group (n = 30)	
Index	0 months	6 months	0 months	6 months
Serum				
TGF-β1 (ng/ml)	9.04 ± 3.25	10.27 ± 2.78*	9.62 ± 2.63	8.38 ± 2.64#
cFN (ng/ml)	30735.89 ± 4398.11	31631.37 ± 5132.02	30974.98 ± 3665.27	28479.23 ± 4206.84#*
VEGF (pg/ml)	540.44 ± 173.46	558.35 ± 165.26	581.23 ± 90.38	581.14 ± 264.09
Effusion				
TGF-β1 (ng/ml)	8.02 ± 2.75	8.75 ± 2.52	8.01 ± 1.85	6.81 ± 1.59#*
cFN (ng/ml)	312.54 (279.95, 345.13)	331.72 (304.10, 399.28)*	328.27 (309.70, 355.59)	308.05 (290.41, 318.11)#*
VEGF (pg/ml)	522.08 ± 229.29	532.41 ± 244.82	542.65 ± 353.37	523.86 ± 347.50

cFN, cell fibronectin; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial cell growth factor.
*$P < 0.05$ versus same group at month 0 (before the start of treatment); #$P < 0.05$ versus control group at month 6.

TABLE 5
Comparison of the peritoneal transport function indexes between the two groups of patients at baseline and after 6 months of indobufen
Data are mean ± S.D. PET is 4-hour peritoneal effusion corrected creatinine value/2-hour blood creatinine value.

Time	Control group (n = 29)	Indobufen group (n = 30)	*P*
0 months	0.704 ± 0.15	0.728 ± 0.09	0.471
6 months	0.708 ± 0.15	0.673 ± 0.10*	0.303
Difference of PET	−0.004 ± 0.98	0.055 ± 0.12#	0.046
P	0.831	0.019	—

*$P < 0.05$ versus same group at month 0 (before the start of treatment); #$P < 0.05$ versus control group at month 6.

of plasma concentration with time decreased by 30% and 14%, respectively, compared with the fasting value. The lower apparent distribution volume (average of 15 L) of indobufen in healthy volunteers might be due to its high affinity (> 99%) for plasma proteins (Fuccella et al., 1979; Tamassia et al., 1979). Indobufen elimination from plasma was biphasic, and the end-stage elimination half-life ($T_{1/2}\beta$) was 6 to 7 hours. The proportion of the administered dose discharged from the urine within 48 hours after administration is 70% to 80%, most of which is discharged through the kidney in the form of a glucuronic acid conjugate; 11% to 13% is discharged in the form of unchanged drug. It was reported in the literature (Savazzi et al., 1986) that after a single oral dose (200 mg) and the last repeated oral plan (200 mg twice a day for 5 days), in a stable state, the plasma level of indobufen was about twice as high as that after a single dose, while the plasma level distribution was similar; the C_{max} was 32.6±9.3 mg/L, and the $t_{1/2}$ was 12.8±4.4 hour. The excretion rate is not affected by the route of administration (oral or intravenous), and the renal clearance rate is not affected by food (Fuccella et al., 1979; Tamassia et al., 1979).

In patients with renal disease, the elimination of indobufen is related to the degree of renal insufficiency (Savazzi et al., 1984). The Cl (creatinine clearance rate ClCr < 1.2 L/h) of 11 patients with moderate to severe renal insufficiency was 0.43 L/h, while that of 6 healthy individuals was 1.3 L/h (ClCr > 6 L/h). $T_{1/2}$ in patients with moderate to severe renal impairment was also prolonged (15–48 hours). The renal clearance of drugs mainly depends on the glomerular filtration function and renal tubular transport function under the condition of renal injury. The amount of drug filtered through the glomerulus is related to the plasma concentration of a drug, the degree of drug binding to plasma protein, and the glomerular filtration rate. The decrease in the amounts of a drug filtered through the glomerulus during renal failure is mainly the direct result of the injury to many nephrons and the reduction in the glomerular filtration rate. Generally, when the creatinine clearance rate is >30 mL/min, the plasma half-life of a drug changes relatively slowly, but when the creatinine clearance rate is <30 mL/min, the $T_{1/2}$ can be significantly prolonged, which can lead to enhanced drug effect or increased toxicity. About 75% of indobufen is excreted in the urine in the form of glucuronic acid conjugates (Fuccella et al., 1979; Tamassia et al., 1979), and the $T_{1/2}$ of indobufen in patients with renal insufficiency is prolonged (Savazzi et al., 1984), which might increase the risk of bleeding and advocates caution regarding dosage. Hence, the dose of indobufen should

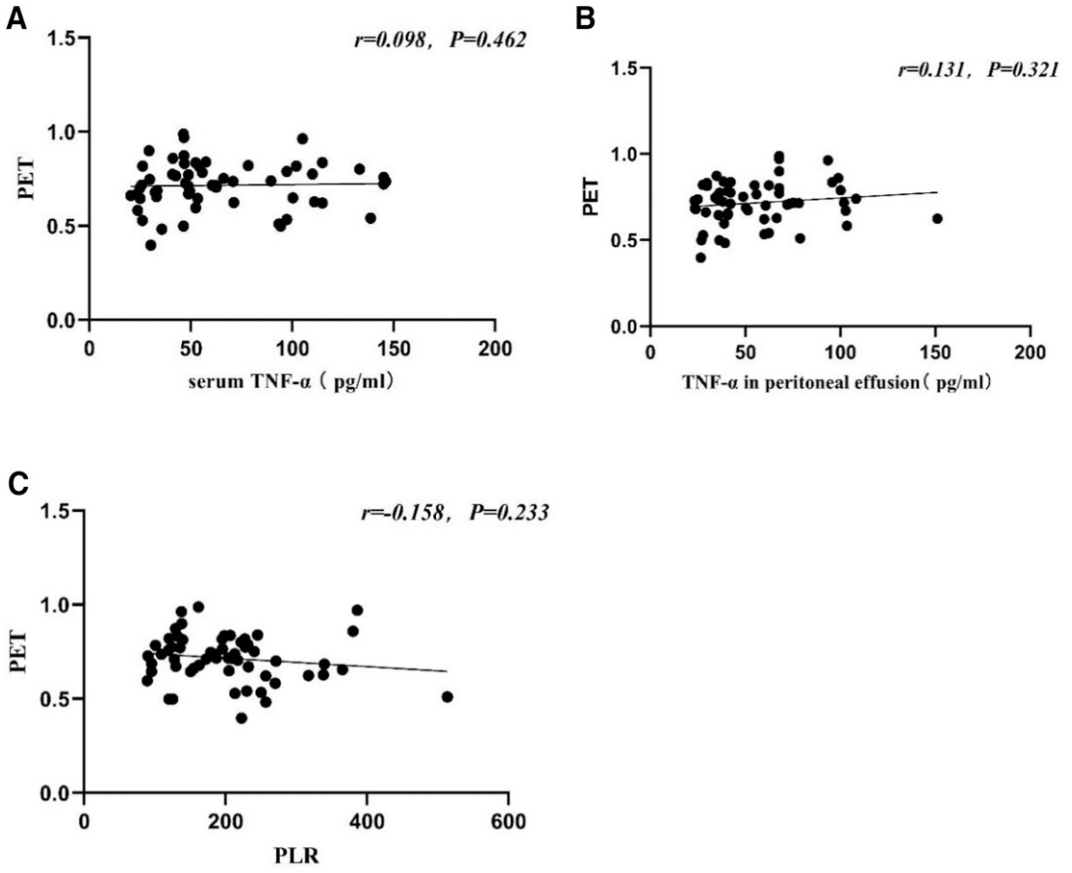

Fig. 4. Correlation analysis between PET and PLR, PET and TNF-α in serum and peritoneal effusion of CAPD patients at baseline.

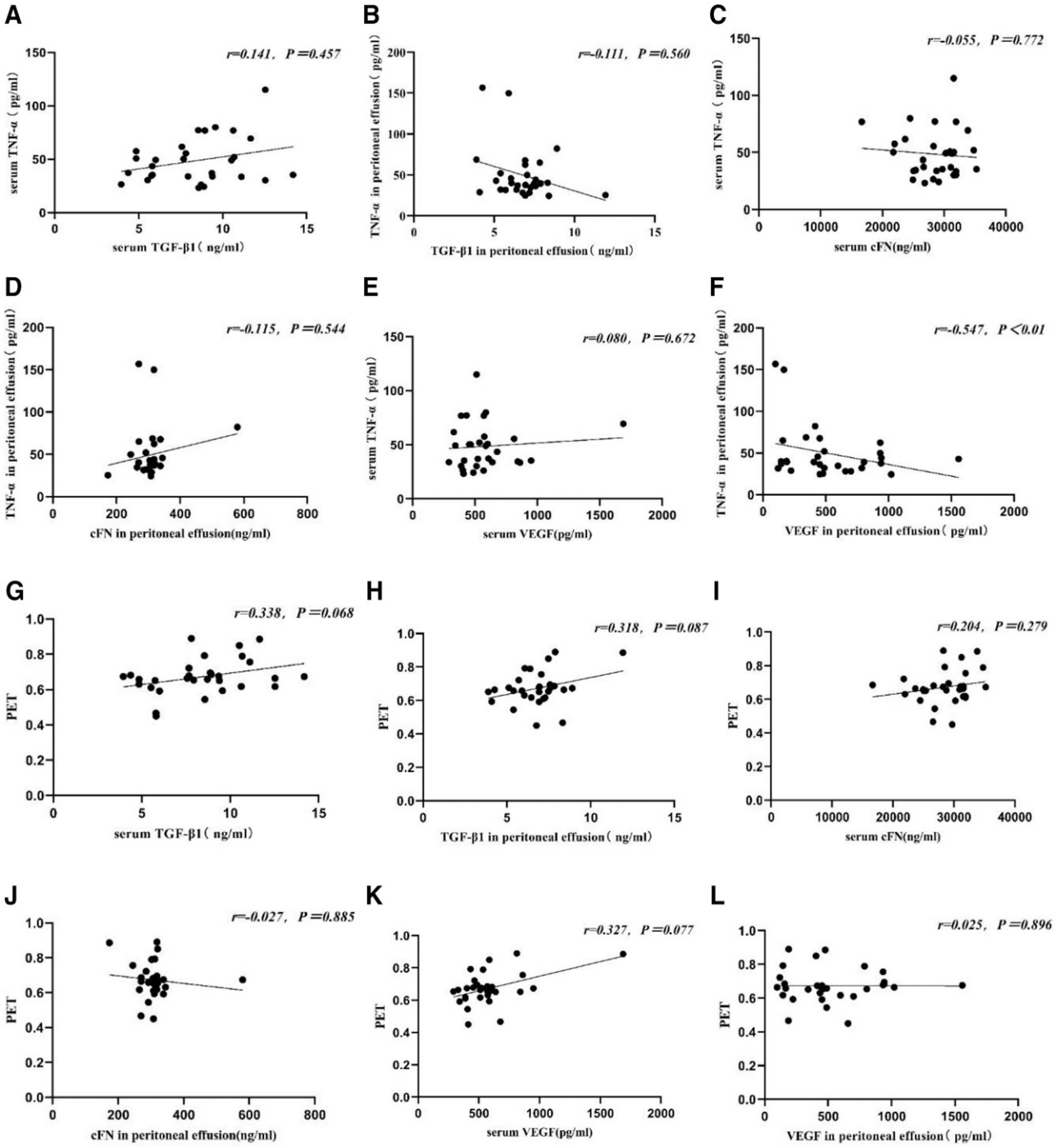

Fig. 5. Correlation analysis between TNF-α and TGFβ1, cFN and VEGF in serum (a, c, and e) and peritoneal effusion (b, d, and f) of CAPD patients in the indobufen group after 6 months of indobufen treatment. Correlation analysis between PET and TGFβ1, cFN and VEGF in serum (j, i, and k) and peritoneal effusion (h, j, and l) of CAPD patients the indobufen group after 6 months of indobufen treatment.

be reduced in patients with renal insufficiency. However, the subjects in the present study were patients undergoing CAPD who have microinflammation and hypercoagulability. In addition, the protein binding rate of indobufen is as high as 99% (Główka and Caldwell, 2002), and PD has a certain clearance effect on drugs with a high protein binding rate (Churchwell et al., 2009). Therefore, there is usually no bleeding and other toxicity because the excess drug is removed by PD, but the dose should be reduced for patients with renal insufficiency without PD.

During the study period, no noticeable indobufen-related adverse reactions were recorded. Such a favorable safety profile has been observed previously (Bhana and McClellan, 2001; Liu et al., 2018; Lou et al., 2019).

There are some limitations to this study. First, a peritoneal biopsy was not performed, preventing clarifying the inhibitory effects of indobufen on peritoneal fibrosis. Second, the small sample size restricted the generalization of the results. Third, the long-term effects of indobufen on patients undergoing CAPD were not followed up. Fourth, other inflammatory

markers, such as IL-6 and peritoneal effusion cell counts, were not collected. Hence, additional research needs to be conducted to eliminate the aforementioned deficiencies and to confirm our findings.

In conclusion, the present study revealed that indobufen could improve the peritoneal transport function in patients undergoing CAPD, and the underlying mechanism may be related to the improvement of microinflammatory state and peritoneal fibrosis in patients undergoing CAPD, providing a new idea for improving the prognosis of such patients.

Authorship Contributions

Participated in research design: Zhang, Wang.
Conducted experiments: Liu.
Contributed new reagents or analytic tools: Liu, Wu.
Performed data analysis: Liu, Yang, Wang.
Wrote or contributed to the writing of the manuscript: Liu, Wang.

Note Added in Proof: An incorrect running title was included in the Fast Forward version published November 10, 2022. The running title has now been corrected.

References

Balzer MS (2020) Molecular pathways in peritoneal fibrosis. *Cell Signal* **75:**109778.
Bhana N and McClellan KJ (2001) Indobufen: an updated review of its use in the management of atherothrombosis. *Drugs Aging* **18:**369–388.
Chiu CW, Tsai TH, Lin CL, Yen TH, Wang IK, and Li CY (2019) Icodextrin is associated with a lower risk of stroke in peritoneal dialysis patients. *Nephron* **141:**112–118.
Churchwell MD, Pasko DA, Smoyer WE, and Mueller BA (2009) Enhanced clearance of highly protein-bound drugs by albumin-supplemented dialysate during modeled continuous hemodialysis. *Nephrol Dial Transplant* **24:**231–238.
Crabtree JH and Chow KM (2017) Peritoneal dialysis catheter insertion. *Semin Nephrol* **37:**17–29.
Cullis B, Al-Hwiesh A, Kilonzo K, McCulloch M, Niang A, Nourse P, Parapiboon W, Ponce D, and Finkelstein FO (2021) ISPD guidelines for peritoneal dialysis in acute kidney injury: 2020 update (adults). *Perit Dial Int* **41:**15–31.
Eligini S, Violi F, Banfi C, Barbieri SS, Brambilla M, Saliola M, Tremoli E, and Colli S (2006) Indobufen inhibits tissue factor in human monocytes through a thromboxane-mediated mechanism. *Cardiovasc Res* **69:**218–226.
Fuccella LM, Corvi G, Moro E, Pogliani E, Tamassia V, and Tosolini G (1979) Pharmacokinetic, bioavailability and pharmacodynamic study of indobufen (K 3920), an inhibitor of platelet aggregation, after a single dose in man. *Eur J Clin Pharmacol* **15:**323–327.
Główka FK and Caldwell J (2002) Protein binding of indobufen enantiomers: pharmacokinetics of free fraction-studies after single or multiple doses of rac-indobufen. *Chirality* **14:**736–741.
Himmelfarb J, Vanholder R, Mehrotra R, and Tonelli M (2020) The current and future landscape of dialysis. *Nat Rev Nephrol* **16:**573–585.
Huddam B, Başaran M, Koçak G, Azak A, Yalçõn F, Reyhan NH, and Duranay M (2015) The use of mycophenolate mofetil in experimental encapsulating peritoneal sclerosis. *Int Urol Nephrol* **47:**1423–1428.
Kariya T, Nishimura H, Mizuno M, Suzuki Y, Matsukawa Y, Sakata F, Maruyama S, Takei Y, and Ito Y (2018) TGF-β1-VEGF-A pathway induces neoangiogenesis with peritoneal fibrosis in patients undergoing peritoneal dialysis. *Am J Physiol Renal Physiol* **314:**F167–F180.
Lai S, Molfino A, Russo GE, Testorio M, Galani A, Innico G, Frassetti N, Pistolesi V, Morabito S, and Rossi Fanelli F (2015) Cardiac, inflammatory and metabolic parameters: hemodialysis versus peritoneal dialysis. *Cardiorenal Med* **5:**20–30.
Levi M, van der Poll T, and Büller HR (2004) Bidirectional relation between inflammation and coagulation. *Circulation* **109:**2698–2704.
Li F, Xu D, Hou K, Gou X, Lv N, Fang W, and Li Y (2021) Pretreatment of indobufen and aspirin and their combinations with clopidogrel or ticagrelor alleviates inflammasome mediated pyroptosis via inhibiting NF-κB/NLRP3 pathway in ischemic stroke. *J Neuroimmune Pharmacol* **16:**835–853.
Li J, Guo ZY, Gao XH, Bian Q, Jia M, Lai XL, Wang TY, Bian XL, and Wang HY (2015) Low molecular weight heparin (LMWH) improves peritoneal function and inhibits peritoneal fibrosis possibly through suppression of HIF-1α, VEGF and TGF-β1. *PLoS One* **10:**e0118481.
Li PK, Chow KM, Van de Luijtgaarden MW, Johnson DW, Jager KJ, Mehrotra R, Naicker S, Pecoits-Filho R, Yu XQ, and Lameire N (2017a) Changes in the worldwide epidemiology of peritoneal dialysis. *Nat Rev Nephrol* **13:**90–103.
Li PK, Ng JK, and Mcintyre CW (2017b) Inflammation and peritoneal dialysis. *Semin Nephrol* **37:**54–65.
Liu J, Xu D, Xia N, Hou K, Chen S, Wang Y, and Li Y (2018) Anticoagulant activities of indobufen, an antiplatelet drug. *Molecules* **23:**1452.
Lou X, Jin J, Gong J, Zhao L, Li Y, and He Q (2019) Comparison of the effects of indobufen and warfarin in a rat model of adenine-induced chronic kidney disease. *Med Sci Monit* **25:**3566–3572.
Ma Y, Zhou Y, Wu F, Ji W, Zhang J, and Wang X (2019) The bidirectional interactions between inflammation and coagulation in fracture hematoma. *Tissue Eng Part B Rev* **25:**46–54.
Nagy JA (1996) Peritoneal membrane morphology and function. *Kidney Int Suppl* **56:** S2–S11.
Patrono C, Baigent C, Hirsh J, and Roth G (2008) Antiplatelet drugs: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). *Chest* **133:**199s-233s.
Raby AC, González-Mateo GT, Williams A, Topley N, Fraser D, López-Cabrera M, and Labéta MO (2018) Targeting Toll-like receptors with soluble Toll-like receptor 2 prevents peritoneal dialysis solution-induced fibrosis. *Kidney Int* **94:**346–362.
Savazzi G, Castiglioni A, and Cavatorta A (1984) Effect of renal insufficiency on the pharmacokinetics of indobufen. *Curr Ther Res Clin Exp* **36:**119–125.
Savazzi GM, Castiglioni A, Cavatorta A, Garini G, Montanari M, and Borghetti A (1986) Effect of age on the pharmacokinetics of indobufen. *Int J Clin Pharmacol Ther Toxicol* **24:**265–269.
Shestakova MV, Vykhristiuk SG, Milen'kaia TM, Tokmakova AIu, and Dedov II (1996) [The thromboxane-synthesis inhibitor ibustrin in the treatment of diabetic angiopathies]. *Ter Arkh* **68:**18–22.
Shi Y, Yan H, Yuan J, Zhang H, Huang J, Ni Z, Qian J, and Fang W (2018) Different patterns of inflammatory and angiogenic factors are associated with peritoneal small solute transport and peritoneal protein clearance in peritoneal dialysis patients. *BMC Nephrol* **19:**119.
Tamassia V, Corvi G, Fuccella LM, Moro E, Tosolini G, and Tremoli E (1979) Indobufen (K 3920), a new inhibitor of platelet aggregation: effect of food on bioavailability, pharmacokinetic and pharmacodynamic study during repeated oral administration to man. *Eur J Clin Pharmacol* **15:**329–333.
Turkmen K, Erdur FM, Ozcicek F, Ozcicek A, Akbas EM, Ozbicer A, Demirtas L, Turk S, and Tonbul HZ (2013) Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-to-lymphocyte ratio in end-stage renal disease patients. *Hemodial Int* **17:**391–396.
Turkmen K, Guney I, Yerlikaya FH, and Tonbul HZ (2012) The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. *Ren Fail* **34:**155–159.
Wakabayashi K, Hamada C, Kanda R, Nakano T, Io H, Horikoshi S, and Tomino Y (2015) Oral astaxanthin supplementation prevents peritoneal fibrosis in rats. *Perit Dial Int* **35:**506–516.
Wiseman LR, Fitton A, and Buckley MM (1992) Indobufen. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in cerebral, peripheral and coronary vascular disease. *Drugs* **44:**445–464.
Wu J, Xing C, Zhang L, Mao H, Chen X, Liang M, Wang F, Ren H, Cui H, Jiang A et al. (2018) Autophagy promotes fibrosis and apoptosis in the peritoneum during long-term peritoneal dialysis. *J Cell Mol Med* **22:**1190–1201.
Yáñez-Mó M, Lara-Pezzi E, Selgas R, Ramírez-Huesca M, Domínguez-Jiménez C, Jiménez-Heffernan JA, Aguilera A, Sánchez-Tomero JA, Bajo MA, Alvarez V et al. (2003) Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. *N Engl J Med* **348:**403–413.
Zhou Q, Bajo MA, Del Peso G, Yu X, and Selgas R (2016) Preventing peritoneal membrane fibrosis in peritoneal dialysis patients. *Kidney Int* **90:**515–524.

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