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

Fang Liu*1,2, Hao Zhang*1, Hong Wu3, Shikun Yang1, Jun Liu1, Jianwen Wang#1

1Department of Nephropathy, Third Xiangya Hospital of Central South University, Changsha, China

2Department of Nephropathy, Zhangjiajie City People’s Hospital, Zhangjiajie, China

3Department of Anesthesiology, Third Xiangya Hospital of Central South University, Changsha, China

*Corresponding author:

Jianwen Wang

Department of Nephropathy, Third Xiangya Hospital of Central South University, Changsha 410013, Hunan Province, China

Tel:+86-13627311665

E-mail:jwwangdoc@163.com

*These authors contributed equally to this work.
Running title: Indobufen in patients on peritoneal dialysis

Number of manuscript pages: 36

Number of figures: 5

Number of tables: 5

Abstract word count: 242

Introduction word count: 285

Discussion word count: 1870

Abbreviations
ALT: aspartate aminotransferase
AST: asparagine transaminase
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
PDF: peritoneal dialysis fluid
PET: peritoneal equilibrium test
PLR: platelet-to-lymphocyte ratio
PTH: parathyroid hormone
TGF-β1: transforming growth factor-β1
TNF-α: tumor necrosis factor-α
ULN: upper limit of normal
VEGF: vascular endothelial growth factor
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 micro-inflammatory 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 (VEGF) 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, micro-inflammatory state, and biochemical indices between the two groups before treatment (P>0.05). After 6 months of treatment, platelet-to-lymphocyte ratio (PLR) 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 micro-inflammatory state and peritoneal fibrosis.

Key words: Indobufen; Continuous ambulate peritoneal dialysis; Micro-inflammation; Peritoneal fibrosis; Peritoneal transport function
Significance Statement

Micro-inflammation 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 micro-inflammatory 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 is expected to reach 8%, which is about 6%-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 micro-inflammation mainly include immunomodulatory therapy, anti-fibrosis 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, non-rheumatic 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 activation, and improves the micro-inflammatory 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 micro-inflammatory 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) 18-75 years of age, (2) stage 5 chronic kidney disease (CKD) 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 × 10^9/L, prothrombin time > 14 s, activated partial prothrombin time > 40 s), (2) severe liver dysfunction (alanine aminotransferase (ALT) or aspartate aminotransaminase (AST) > 3 upper limit of normal (ULN)), (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 6000-8000 ml/day. 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 8:00 p.m. (on the day before the examination) to 8:00 a.m., 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 × g for 10 min 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 (PTH). 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 Biological 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). Non-normally 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 (%) and compared using the chi-square 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 90-198 months were enrolled, including 31 men (51.7%) and 29 women (48.3%). They were 15-75 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 (Figure 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 (Figure 2a), cFN (Figure 2c), and VEGF (Figure 2e) (all P>0.05). The correlation coefficients of TNF-α and TGF-β1 (Figure 2b), as well as cFN (Figure 2d) and VEGF (Figure 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 (Figure 2g), cFN (Figure 2i), and VEGF (Figure 2k) in patients who were undergoing CAPD indicated that there was a significant correlation between PET results and serum levels of TGF-β1 (Figure 2g), cFN (Figure 2i), and VEGF (Figure 2k) (all P<0.05). The correlation coefficient between the serum levels of cFN and VEGF was in the range of 0.4-0.6 (Figure 2k), indicating that there was a moderate positive correlation between PET results and serum levels of cFN and VEGF. The
The correlation coefficient between PET results and serum TGF-β1 level was between 0.2 and 0.4 (Figure 2g), demonstrating a weak positive correlation.

Before treatment, the correlation analysis of PET results and TGF-β1 level (Figure 2h), as well as cFN (Figure 2j) and VEGF (Figure 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 (Figure 2h), cFN (Figure 2j), and VEGF (Figure 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 0.6-0.7 (Figure 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 (Figure 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 0.2-0.4 (Figure 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 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 micro-inflammatory 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 $P>0.05$) (Figure 3). No significant correlations between PLR and serum and peritoneal effusion TGF-β1, cFN, and VEGF were observed (all $P>0.05$) (Figure 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 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 above-mentioned results indicated that indobufen could improve the micro-inflammatory 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 anti-fibrosis 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 $P>0.05$) (Figure 4). Taken together, indobufen could improve peritoneal transport function. After treatment, a negative correlation was observed between peritoneal effusion TNF-α and VEGF ($r=-0.547$, $P<0.01$) (Figure 5f). There were no significant correlations between PET and serum and peritoneal effusion TGF-β1, cFN, and VEGF (all $P>0.05$) (Figure 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 were 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 micro-inflammation, peritoneal fibrosis, and angiogenesis.

Systemic micro-inflammation in patients undergoing peritoneal dialysis is associated with uremic toxins, while local micro-inflammation during peritoneal dialysis can be associated with chronic non-specific inflammatory diseases caused by the peritoneal dialysis catheters (Li et al., 2017b). Micro-inflammation is involved in the pathophysiological changes of patients with atherosclerosis, malnutrition, left ventricular hypertrophy, heart failure, peritoneal fibrosis, angiogenesis, etc., 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 micro-inflammation 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 micro-inflammation 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 micro-inflammation in 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 micro-inflammation 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 (Elioglu 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 (MCAO/R) (Li et al., 2021b). Besides, 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 above-mentioned results suggested that indobufen could delay the progression of peritoneal fibrosis by regulating micro-inflammation 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 CKD, and they also demonstrated that the mechanism of indobufen in improving renal fibrosis could be 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 (PMCs) 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 micro-inflammation 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., 2021a). 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 micro-inflammatory 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 h, 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 12.5-14.9 mg/L 2 h 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 (AUC) 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 1/2β) was 6-7 h. The proportion of the administered dose discharged from the urine within 48 h after administration is 70%-80%, most of which are discharged through the kidney in the form of a glucuronic acid conjugate, and 11%-13% are 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 b.i.d., lasting 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 h. 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 six healthy individuals was 1.3 L/h (ClCr >6 L/h). T1/2 in patients with moderate to severe renal impairment was also prolonged (15-48 h). 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 T1/2 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 T1/2 of indobufen in patients with renal insufficiency is prolonged (Savazzi et al., 1984), which might increase the risk of bleeding and advocating caution regarding dosage. Hence, the dose of indobufen should 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% (Glowka 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).
The limitations of the study should be pointed out. Firstly, a peritoneal biopsy was not performed, preventing clarifying the inhibitory effects of indobufen on peritoneal fibrosis. Secondly, the small sample size restricted the generalization of the results. Thirdly, the long-term effects of indobufen on patients undergoing CAPD were not followed up. Fourthly, 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 above-mentioned 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 micro-inflammatory state and peritoneal fibrosis in patients undergoing CAPD, providing a new idea for improving the prognosis of such patients.
Acknowledgments

None.

Authorship Contributions

Participated in research design: Jianwen Wang and Hao Zhang; Conducted experiments: Fang Liu; Contributed new reagents or analytic tools: Hong Wu and Jun Liu; Performed data analysis: Fang Liu, Shikun Yang, and Jianwen Wang; Wrote or contributed to the writing of the manuscript: Fang Liu and Jianwen Wang.
REFERENCES


Fuccella LM, Corvi G, Moro E, Pogliani E, Tamassia V and Tosolini G (1979) Pharmacokinetic, bioavailability and pharmacodynamic study of indobufen (K


Li F, Xu D, Hou K, Gou X, Lv N, Fang W and Li Y (2021a) Pretreatment of Indobufen and Aspirin and their Combinations with Clopidogrel or Ticagrelor


FOOTNOTES

Funding

This study was supported by Natural Science Foundation of Hunan Province [2020JJ8108] and [2017JJ2342].

Conflict of interests

None.
LEGEND FOR FIGURES

**Figure 1.** Study flowchart. 30 and 29 patients were allocated to the indobufen group and the control group, respectively.

**Figure 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.

**Figure 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.

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

**Figure 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.
Table 1. Baseline demographic and clinical characteristics of the study participants

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

Note: Data are mean ± SD, n or median (interquartile range). Abbreviations: PD, peritoneal dialysis.

*Other primary diseases included ischemic nephropathy, obstructive nephropathy, lupus nephritis, gouty nephropathy, and systemic vasculitis and renal damage.
Table 2 Blood biochemical indexes in the two groups of patients at baseline and after 6 months of indobufen

<table>
<thead>
<tr>
<th>Index</th>
<th>Control group (n=29)</th>
<th>Indobufen group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 months</td>
<td>6 months</td>
</tr>
<tr>
<td>HDL-C (mmol/L, x± s)</td>
<td>1.23±0.32</td>
<td>1.24±0.29</td>
</tr>
<tr>
<td>LDL-C (mmol/L, x± s)</td>
<td>2.48±0.66</td>
<td>2.77±0.86</td>
</tr>
<tr>
<td>TC (mmol/L, x± s)</td>
<td>4.64±1.02</td>
<td>5.00±1.23</td>
</tr>
<tr>
<td>TG (mmol/L, x± s)</td>
<td>1.88±1.15</td>
<td>2.11±1.42</td>
</tr>
<tr>
<td>Ca (mmol/L, x± s)</td>
<td>2.26±0.26</td>
<td>2.26±0.26</td>
</tr>
<tr>
<td>P (mmol/L, x± s)</td>
<td>1.61±0.46</td>
<td>1.64±0.45</td>
</tr>
<tr>
<td>PTH [pg/mL, Md (P25, P75)]</td>
<td>194.35</td>
<td>285.27</td>
</tr>
<tr>
<td></td>
<td>(111.16,439.04)</td>
<td>(181.77,397.41)</td>
</tr>
<tr>
<td>BUN (mmol/L, x± s)</td>
<td>19.81±6.41</td>
<td>21.19±5.65</td>
</tr>
<tr>
<td>Scr (μmol/L, x± s)</td>
<td>959.31±299.08</td>
<td>1026.31±297.82*</td>
</tr>
<tr>
<td>UA (mmol/L, x± s)</td>
<td>410.41±106.30</td>
<td>420.46±82.04</td>
</tr>
<tr>
<td>TF (g/L, x± s)</td>
<td>1.89±0.52</td>
<td>1.99±0.10</td>
</tr>
<tr>
<td>SI [μmol/L, Md (P25, P75)]</td>
<td>10.90</td>
<td>14.80 (10.6,18.25)</td>
</tr>
<tr>
<td></td>
<td>(7.95,13.38)</td>
<td>*</td>
</tr>
<tr>
<td>Ferr [ng/mL, Md (P25, P75)]</td>
<td>322.50</td>
<td>346.90</td>
</tr>
<tr>
<td></td>
<td>(135.50,517.65)</td>
<td>(195.50,599.15)</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD or median (interquartile range). Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; BUN, blood urea nitrogen; Scr, serum creatinine; UA, uric acid; TF, transferrin; SI, serum iron; Ferr, ferritin.

*P < 0.05 vs. same group at month 0 (before the start of treatment); #P < 0.05 vs. control group at month 6.
Table 3 Comparison of the micro-inflammatory indicators between the two groups of patients at baseline and after 6 months of indobufen

<table>
<thead>
<tr>
<th>Index</th>
<th>Control group (n=29)</th>
<th>Indobufen group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 months</td>
<td>6 months</td>
</tr>
<tr>
<td>NLR</td>
<td>4.45 (3.03,5.54)</td>
<td>4.06 (3.04,5.86)</td>
</tr>
<tr>
<td>PLR</td>
<td>196.43±84.10</td>
<td>204.03±81.87</td>
</tr>
<tr>
<td>MPR</td>
<td>0.05±0.02</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>Serum TNF-α</td>
<td>47.37 (31.34,91.48)</td>
<td>55.45 (43.18,84.31)</td>
</tr>
<tr>
<td>Effusion TNF-α</td>
<td>36.39 (27.69,73.35)</td>
<td>56.81 (38.67,92.49)</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD or median (interquartile range). Abbreviations: NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MPR, mean platelet volume and platelet ratio; TNF-α, tumor necrosis factor -α

*P < 0.05 vs. same group at month 0 (before the start of treatment); #P < 0.05 vs. control group at month 6.
Table 4 Comparison of the peritoneal fibrosis indexes between the two groups of patients at baseline and after 6 months of indobufen

<table>
<thead>
<tr>
<th>Index</th>
<th>Control group (n=29)</th>
<th>Indobufen group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 months</td>
<td>6 months</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β1 (ng/ml)</td>
<td>9.04±3.25</td>
<td>10.27±2.78*</td>
</tr>
<tr>
<td>cFN (ng/ml)</td>
<td>30735.89±4398.11</td>
<td>31631.37±5132.02</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>540.44±173.46</td>
<td>558.35±165.26</td>
</tr>
<tr>
<td>Effusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β1 (ng/ml)</td>
<td>8.02±2.75</td>
<td>8.75±2.52</td>
</tr>
<tr>
<td>cFN (ng/ml)</td>
<td>312.54</td>
<td>331.72</td>
</tr>
<tr>
<td></td>
<td>(279.95,345.13)</td>
<td>(304.10,399.28) *</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>522.08±229.29</td>
<td>532.41±244.82</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD or median (interquartile range). Abbreviations: TGF-β1, transforming growth factor -β1; cFN, cell fibronectin; VEGF, vascular endothelial cell growth factor.

*P < 0.05 vs. same group at month 0 (before the start of treatment); #P < 0.05 vs. 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

<table>
<thead>
<tr>
<th>Time</th>
<th>Control group (n=29)</th>
<th>Indobufen group (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>0.704±0.15</td>
<td>0.728±0.09</td>
<td>0.471</td>
</tr>
<tr>
<td>6 months</td>
<td>0.708±0.15</td>
<td>0.673±0.10*</td>
<td>0.303</td>
</tr>
<tr>
<td>Difference of PET</td>
<td>-0.004±0.98</td>
<td>0.055±0.12#</td>
<td>0.046</td>
</tr>
<tr>
<td>P</td>
<td>0.831</td>
<td>0.019</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: Data are mean ± SD. Abbreviations: PET=4h peritoneal effusion corrected creatinine value /2h blood creatinine value.

*P < 0.05 vs. same group at month 0 (before the start of treatment); #P < 0.05 vs. control group at month 6.
Figure 1

Enrollment

Assessed for eligibility (n=82)
- Excluded (n=22)
  - Not meeting inclusion criteria
  - Combined hemodialysis
  - Declined to participate
  - Other reasons

Randomized (n=60)

Allocated to control group (n=30)
- Lost to follow-up (renal transplantation) (n=1)

Allocated to indobufen group (n=30)
- Lost to follow-up (give reasons) (n=0)

Follow-up

Analysis

Final analysis

Final analysis
Figure 2
Figure 3

(a) $r = 0.147, P = 0.266$

(b) $r = 0.058, P = 0.662$

(c) $r = 0.194, P = 0.141$

(d) $r = 0.153, P = 0.247$

(e) $r = 0.074, P = 0.575$

(f) $r = 0.015, P = 0.908$
Figure 4

(a) Correlation between PET and serum TNF-α (pg/ml) with $r=0.098$, $P=0.462$.

(b) Correlation between PET and TNF-α in peritoneal effusion (pg/ml) with $r=0.131$, $P=0.321$.

(c) Correlation between PET and PLR with $r=-0.158$, $P=0.233$. 