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Research Paper

Evaluation of Enhanced Peritoneum Permeability in Methylglyoxal-treated Rats as a Diagnostic Method for Peritoneal Damage

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Purpose. As peritoneal damage in long-term peritoneal dialysis therapy is a major problem correlated to patient prognosis, diagnosis of peritoneal damage is important. To develop a diagnostic method for peritoneal damage, we focused on hyperpermeability across the peritoneum in a pathogenic peritoneal damage condition. In this study, disposition characteristics of an intraperitoneally injected marker substance in peritoneal damaged rats were analyzed.

Materials and Methods. Peritoneal damaged rats were prepared by intraperitoneal injection of a glucose degradation product, methylglyoxal (MGO), for five or ten consecutive days. Phenolsulfonphthalein (PSP), as a marker substance, was intraperitoneally or intravenously injected into MGO-treated rats. Subsequently, the PSP disposition characteristics were pharmacokinetically analyzed.

Results. In both cases of 5 and 10 days treatment of MGO, absorption of PSP after intraperitoneal injection was significantly enhanced. Plasma concentration and urinary excretion of PSP in MGO-treated rats were also higher than those in saline-treated rats in the early phase. On the contrary, there was no significant difference in terms of the pharmacokinetic parameters of intravenously injected PSP in saline- or MGO-treated rats. These results indicated that intraperitoneally injected MGO primarily acts on the peritoneal membrane; therefore, the peritoneal permeability of the marker substance was enhanced.

Conclusion. We demonstrated that pharmacokinetic analysis of peritoneum permeability might be a potent diagnostic method for peritoneal damage in experimental animals and patients receiving peritoneal dialysis.

KEY WORDS: diagnosis of peritoneal damage; methylglyoxal; peritoneal dialysis; peritoneum permeability; phenolsulfonphthalein.

INTRODUCTION

Recently, patients with chronic renal failure have been increasing, so patients requiring renal replacement therapy such as hemodialysis and renal transplantation are also increasing every year. There were 248,166 chronic dialysis patients in Japan as of December, 2004 (1); the number has been increasing annually by tens of thousands in recent years.

Peritoneal dialysis, which was introduced in 1980, has been given to 10,000 patients at present in Japan. Peritoneal dialysis is a hemocatharsis method, by injecting dialysate into the peritoneal cavity and subsequently dialyzing bodily waste via the peritoneum. Continuous ambulatory peritoneal dialysis (CAPD) is generally performed, and automated peritoneal dialysis is also performed. In comparison with hemodialysis, CAPD has some benefits, such as free activity during dialysis with minimum restraint and less cardiac load due to a stable body condition. Furthermore, CAPD is good for maintaining remaining renal function (2–4). However, peritoneal damage is a major problem in long-term peritoneal dialysis. Prolonged peritoneal dialysis is linked to morphological changes such as fibrosis and sclerosis of the peritoneum, loss of peritoneal mesothelial cells, induction of neoangiogenesis, and fibrosis of the vascular wall; as a result, ultrafiltration failure is induced (5–7). Therefore, in many cases, it is difficult to continue peritoneal dialysis due to peritoneal damage. If peritoneum deterioration progresses further, encapsulating peritoneal sclerosis (EPS), which is a very severe condition with an unfavorable prognosis, can occur (8,9). To prevent fibrosis and tylosis of the peritoneum, the therapeutic effects of an angiotensin converting enzyme inhibitor and an angiogenesis inhibitor were investigated (10–12), but no effective treatment for EPS has been established. Therefore, it is very important to understand the symptoms of EPS and interrupt peritoneal dialysis before EPS development.

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In patients with peritoneal damage, absorption of glucose from the dialysate is increased; thus, peritoneum permeability is enhanced (13). Therefore, it may be useful to determine the changes in disposition characteristics of a marker substance to assess the degree of peritoneal damage because absorption of a marker substance in the peritoneal cavity, unlike endogenous substances such as glucose, is independently detectable and quantification of a marker substance is easy and inexpensive. We previously developed a method for application of drugs to the surface of intraperitoneal organs such as the liver (14–21), kidney (22, 23), stomach (24,25), mesenteric membrane (26), caecum (27), and intestine (20,28) and absorption of drugs from the surface of these organs was pharmacokinetically analyzed. We also reported on the absorption of drugs from the peritoneal cavity (29). As a result, we have developed a basic technique and information on the absorption of drugs from the peritoneal cavity.

Nakayama *et al.* reported that intraperitoneal injection of high dose methylglyoxal (MGO) induced peritoneal damage in rats (30). In this study, we prepared MGO-induced peritoneal damaged rats and pharmacokinetically analyzed the changes in disposition of phenolsulfonphthalein (PSP) as a marker substance. PSP has several merits as follows; (1) clear disposition characteristics in normal rats (14,29); (2) clinically applicable; (3) easy quantification; (4) low cost.

MATERIALS AND METHODS

Materials

MGO and PSP were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of the highest purity available.

Animals

Male Wistar rats (250–300 g) were housed in a cage in an air-conditioned room and maintained on a standard laboratory diet (MF, Oriental Yeast, Co., Ltd., Tokyo, Japan) and water ad libitum. All animal experiments in the present study conformed to the Guidelines for Animal Experimentation of Nagasaki University.

Preparation of Peritoneal Damaged Rats

Rats were anesthetized with ether and subsequently 20 ml of MGO (20 mM) in saline per day was injected intraperitoneally for five or ten consecutive days (30). In a control experiment, saline was intraperitoneally injected in the same fashion.

Changes in PSP Disposition in Peritoneal Damaged Rats

Peritoneal damaged rats were anesthetized with sodium pentobarbital (60 mg/kg intramuscular injection for intraperitoneal injection of PSP; 50 mg/kg intraperitoneal injection for intravenous injection of PSP). The right femoral artery was catheterized with heparin (Mochida Pharmaceutical Co., Ltd., Tokyo)-filled polyethylene tubing (i.d. 0.5 mm, o.d. 0.8 mm; Natsume Seisakusho Co., Ltd., Tokyo) for blood sampling. The bile duct was catheterized with polyethylene tubing (i.d. 0.28 mm, o.d. 0.61 mm; Becton Dickinson & Co., MD, USA). PSP was dissolved in an isotonic phosphate buffer (pH 7.4) and administered as follows.

Intraperitoneal injection: The skin was cut and the abdominal wall exposed. One milligram of PSP (100 μg/ml) was injected at 3 cm below the ensiform cartilage using a syringe with a 21 G×1/2″ needle (Top Co., Tokyo). The syringe was removed and the abdominal wall was closed with surgical adhesive (Aron Alpha A, Sankyo Co., Ltd., Tokyo) to prevent leakage.

Intravenous injection: Zero point two five milligrams of PSP (2.5 mg/ml) was quickly injected via the jugular vein using syringe with a 26 G×1/2″ needle (JMS Co., Ltd., Hiroshima, Japan).

Drug solution was recovered from the peritoneal cavity 15, 30, 60, 120 or 180 min after intraperitoneal injection, and residual solution was recovered by washing with saline. Urine was collected from the urinary bladder by syringe. Blood was collected from the right femoral artery catheter at the indicated time point up to 360 min. For intravenous injection, bile and blood were collected up to 240 min and urine was collected at the end of the experiment. Each blood sample was immediately centrifuged at 12,500 $\times g$ for 5 min, and the supernatant (plasma) was obtained and assayed for PSP quantification. The residual solution in the peritoneal cavity was centrifuged at 1,500 $\times g$ for 15 min. Bile and urine were centrifuged at 1,500 $\times g$ for 10 min. Each supernatant was assayed for PSP quantification.

PSP Quantification

PSP in the sample solution was quantified according to the method reported by Hart and Schanker (31). Plasma, residual solution in the peritoneal cavity and bile (0.1 ml) were diluted with 2 ml of 1 M NaOH, and immediately absorbance at 560 nm was measured. Urine was appropriately diluted by saline before PSP quantification.

Pharmacokinetic Analysis

The plasma profiles were analyzed by statistical moment analysis. Briefly, the area under the plasma concentration-time curve (AUC_p) and mean residence time (MRT_p) were calculated as follows:

$$AUC_p = \int_0^\infty C_p dt \tag{1}$$

$$MRT_p = \int_0^\infty tC_p dt / AUC_p \tag{2}$$

where t is the time and C_p is the plasma concentration of PSP. The moment parameters can be calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (32). Mean absorption time (MAT) was calculated by subtracting the mean value of MRT_P for intravenous injection from that for intraperitoneal injection.

Statistical Analysis

Animal experiments were performed at least five times, and the mean and standard error were calculated. Statistical comparisons were performed by unpaired student's *t* test.

RESULTS

Changes in Disposition of PSP after Intraperitoneal Injection

The effect of peritoneal damage on disposition of PSP after intraperitoneal injection was examined. In addition, dosing days of MGO were varied for comparison. Figure 1 shows semi-logarithmic plots for the time course of the remaining PSP amount in the peritoneal cavity of saline- or MGO-treated (five consecutive days) rats. At all indicated time points in Fig. 1, the remaining PSP amount in MGO-treated rats was significantly decreased compared to saline-treated rats. For the 10-day treatment, similar to the 5-day treatment, the remaining PSP amount in MGO-treated rats was significantly decreased compared to saline-treated rats (data not shown). Therefore, peritoneal permeability was enhanced in MGO-treated rats.

Plasma concentration profiles after intraperitoneal injection of PSP in saline- or MGO-treated rats for five consecutive days were examined (Fig. 2). Plasma concentration of PSP in MGO-treated rats increased more quickly and the maximum concentration was higher than that in saline-treated rats. This result may be due to enhanced peritoneum permeability as shown in Fig. 1; therefore, PSP is rapidly transported into the blood. For the 10-day treatment, the results in saline- and MGO-treated rats were similar to the 5-day treatment (data not shown). Moment analysis was performed to quantitatively describe the plasma concentration-time curve (Table I). MRT_P in MGO-treated rats for five consecutive days was significantly lower than that in saline-treated rats, reflecting the rapid absorption of PSP in MGO-treated rats. In contrast, there was no significant difference in AUC_p of saline- and MGO-treated rats, indicating that the difference in the early phase of plasma profiles was little reflected.

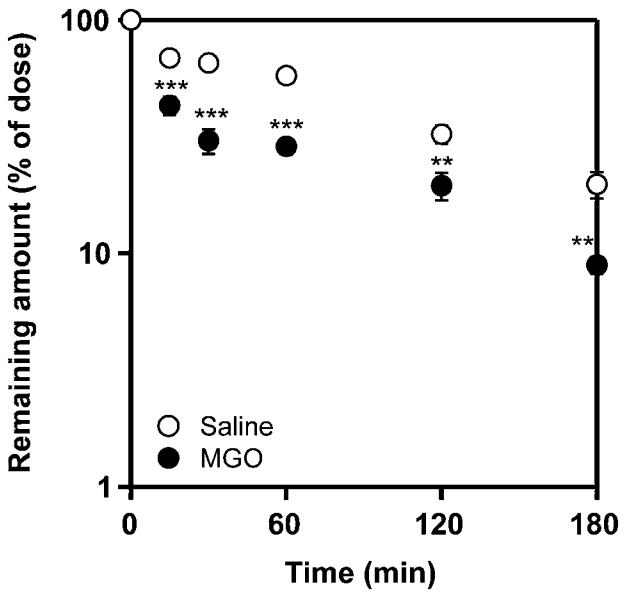

Fig. 1. Semi-logarithmic plot of the remaining PSP amount in the peritoneal cavity after intraperitoneal injection at a dose of 1 mg in saline- or MGO-treated rats for five consecutive days. Each *point* represents the mean±S.E. of at least six experiments. $^{**}p<0.01$, $^{***}p<0.001$, significantly different from saline-treated rats.

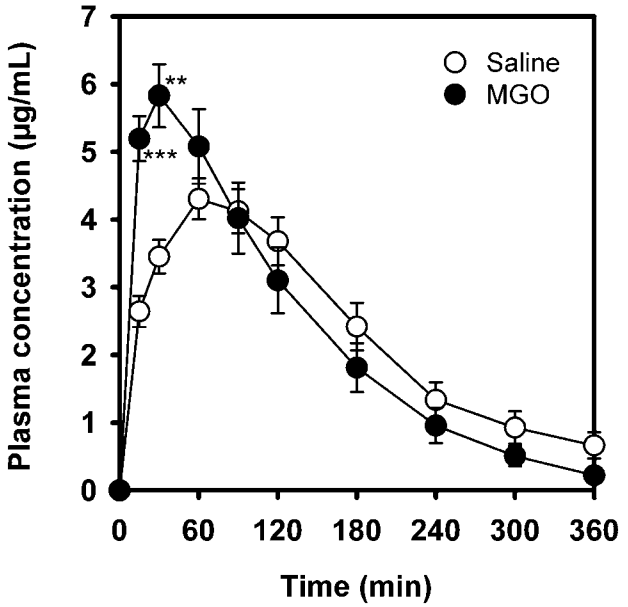

Fig. 2. Plasma concentration profiles of PSP after intraperitoneal injection at a dose of 1 mg in saline- or MGO-treated rats for five consecutive days. Each *point* represents the mean±S.E. of at least six experiments. $^{**}p<0.01$, $^{***}p<0.001$, significantly different from saline-treated rats.

The urinary excretion rate was also measured after intraperitoneal injection of PSP (Fig. 3). Sixty minutes after injection, the urinary excretion rate in MGO-treated rats for five consecutive days was significantly higher than that in saline-treated rats. For the 10-day treatment, similar to the 5-day treatment, the urinary excretion rate in MGO-treated rats was significantly higher than that in saline-treated rats 60 min after injection (data not shown). These results are due to the high plasma concentration of PSP in MGO-treated rats up to 60 min.

Disposition of PSP after Intravenous Injection

To evaluate the systemic action of MGO, disposition of PSP after intravenous injection in MGO-treated rats was examined. Plasma concentration profile of PSP after intravenous injection in MGO-treated rats for five consecutive

Table I. Moment Parameters for Plasma Concentration Profiles of PSP after Intraperitoneal Injection at a Dose of 1 mg in Saline- or MGO-treated Rats for Five Consecutive Days

Parameters	Saline	MGO
AUC_p (μg·min/ml)	943.5±115.3	858.0±121.2
MRT_p (min)	168.1±20.8	110.4±9.5*

Each value represents the mean±S.E. of at least six experiments. $^{*}p<0.05$, significantly different from saline-treated rats.

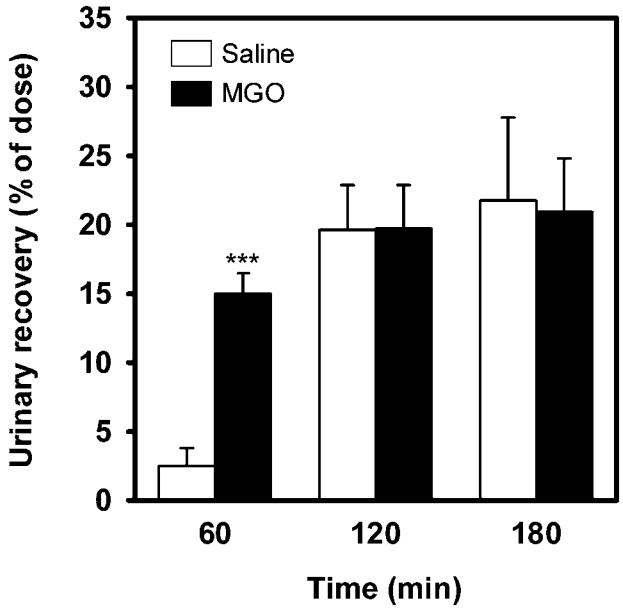

Fig. 3. Urinary recovery of PSP after intraperitoneal injection at a dose of 1 mg in saline- or MGO-treated rats for five consecutive days. Each *bar* represents the mean+S.E. of at least five experiments. ***$p<0.001$, significantly different from saline-treated rats.

days was similar to saline-treated rats (data not shown). Moment analysis revealed that there was no significant difference in AUC_P (288.8±38.5 and 216.4±25.7 μg·min/ml for saline- and MGO-treated rats) and MRT_P (79.9±12.7 and 61.9±7.7 min for saline- and MGO-treated rats). Biliary excretion rate profiles in saline- or MGO-treated rats were almost the same after intravenous injection of PSP (data not shown). In addition, there was no significant difference in biliary clearance and renal clearance. (data not shown). Thus, the disposition characteristics of PSP in saline- or MGO-treated rats after intravenous injection were quite similar.

DISCUSSION

Glucose and glucose degradation products (GDPs) in the dialysate are one of the risk factors for peritoneal damage during long-term peritoneal dialysis. Glucose is an osmotic substance for dialysate and is autoclaved for sterilization; consequently, GDPs are produced. It is known that both GDPs and a high concentration of glucose have a cytotoxic effect on peritoneal mesothelial cells (33–35). On the other hand, GDPs induce production of advanced glycation end products (36,37), which are thought to be related to interstitial fibrosis and vascular sclerosis in long-term CAPD patients. MGO is a GDP and its existence in dialysate has been confirmed (38). Therefore, the MGO-induced peritoneal damage model is similar to the clinical condition. In investigations using peritoneal mesothelial cells, MGO treatment preceded production of vascular endothelial growth factor (VEGF) (39) and harmed tight junctions (40). In addition, VEGF concentration in blood and dialysate was high in solute transport-enhanced CAPD patients, and there was negative correlation between dialysate VEGF and excretion volume during dialysis (41,42). Therefore, peritoneal permeability is thought to be enhanced by these effects of MGO. The present study clearly showed that enhanced peritoneal permeability caused by MGO treatment could be evaluated by intraperitoneal injection of a marker substance PSP. Rapid absorption of PSP in MGO-treated rats was supported by remaining amount in peritoneal cavity (Fig. 1), plasma concentration profiles (Fig. 2) and urinary recovery (Fig. 3). MAT for saline- and MGO-treated rats were 88.2 and 48.5 min, also supporting rapid absorption of PSP in MGO-treated rats.

The effects of MGO treatment on remaining amount in peritoneal cavity, plasma concentration profiles and urinary recovery were similar between 5- and 10-day treatments, indicating validity of the result in the present study. Longer treatment of MGO than 5 days showed presumably no effect on these disposition characteristics, thus 5-day treatment of MGO would be enough for development of peritoneal damage in rats. MGO may have effects on body functions other than the peritoneum. However, MGO treatment did not change plasma concentration profiles, biliary excretion rate profiles, and biliary and renal clearance after intravenous injection of PSP. These results suggest that MGO-treatment might not affect body function except for the peritoneum, and that changes in pharmacokinetics after intraperitoneal injection of PSP in MGO-treated rats are induced by increased permeability of the peritoneum.

In this study, PSP (molecular weight 354) was used as a marker substance. It was suggested that PSP was useful to evaluate enhanced peritoneum permeability in MGO-treated rats. PSP has several merits as described in introduction section; however, more suitable compounds including higher molecular weight substances may be developed for future clinical diagnosis of peritoneal damage. On the other hand, determination of remaining amount in the peritoneal cavity requires recovery of whole amount because concentration is variable by volume change. Thus, in clinical application, it might be difficult to monitor remaining amount in the peritoneal cavity. To resolve this problem, it would be necessary to use a reference substance for measurement of volume change. Since remaining PSP amounts in the peritoneal cavity were significantly lower at all tested time points in MGO-treated rats than in saline-treated rats, once-sampling of fluid in the peritoneal cavity would be enough to diagnose peritoneum hyperpermeability; thus it might be not a burden for patients.

As mention to moment analysis for plasma concentration profiles after intraperitoneal injection of PSP, AUC_p values in saline- and MGO-treated rats were similar, and MRT_p values of MGO-treated rats was slightly decreased compared with saline-treated rats. Although early phase of MGO-treated rats markedly differed from saline-treated rats, this difference was little reflected in AUC_p and MRT_p values. AUC_p and MRT_p values were calculated as infinite time and terminal phases between saline- and MGO-treated rats were similar profiles; as a consequence differences in early phase poorly affected AUC_p and MRT_p. In addition, repeated sampling of blood for moment analysis may be the troublesome procedure for patients. Thus, both AUC_p and MRT_p values were not suitable for diagnostic method of peritoneal damage. In contrast, plasma concentrations of PSP until 30 min after intraperitoneal injection in MGO-treated rats were markedly high compared with saline-treated rats. Once-

sampling of blood might be enough for diagnosis of peritoneum hyperpermeability. In addition, it might be possible to perform the diagnosis simultaneously with other blood test. Thus it would not be a burden for patients.

Although PSP is already used as a diagnostic drug for human, further study is needed for clinical usage of evaluation of enhanced peritoneum permeability using PSP as a diagnostic method for peritoneal damage. Essential frequency to monitor peritoneum permeability is unclear; it should be determined in future clinical trials. In this study, MGO concentration was set to 20 mM as previously reported (30). There is a possibility that peritoneum permeability of a marker substance may correlate to MGO concentration; it should be tested prior to future clinical application. On the other hand, it is necessary to develop drugs to prevent peritoneal damage and EPS. To prevent fibrosis and tylosis of the peritoneum, the therapeutic effects of an angiotensin converting enzyme inhibitor and an angiogenesis inhibitor were investigated (10–12). Prophylactic usage of these drugs may be effective to prevent generation of EPS. Dialysate composition not including glucose should be also investigated for safety usage of peritoneal dialysis. Evaluation of enhanced peritoneum permeability in MGO-treated rats would be useful for development and screening of the drugs and the safe dialysate.

In conclusion, we analyzed the pharmacokinetics of PSP as a marker substance after intraperitoneal injection in peritoneal damaged rats. The remaining amount in the peritoneal cavity, plasma profile and urinary excretion rate of PSP were significantly affected by MGO treatment, indicating enhanced peritoneum PSP permeability in MGO-treated rats. Monitoring disposition of PSP after intraperitoneal injection would be useful for diagnosing peritoneal damage in long-term peritoneal dialysis patients because it is a simple, easy and safe procedure. Furthermore, it would also be useful for developing drugs to treat peritoneal damage in experimental animals. This study will contribute to safety usage and popularization of peritoneal dialysis.

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