Article

Effects of Polyethylene Glycol on Renal Functional Parameters in Rats

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Abstract

Background: The use of polyethylene glycol (PEG) is increasing day by day in various purposes by pharmaceutical industries as well as its anti-colonic cancer effects in clinical diseases. **Objective:** To evaluate the effects of PEG on renal functional and morphological parameters in rats. **Methods:** Six weeks age matched Sprague-Dawley rats were treated with either vehicle (2 mL of 5% dist. water in saline/kg, i.p. twice daily, n=6), or PEG 400 (2 mL of 5% PEG 400 in saline/kg, i.p. twice daily, n=6). After three weeks treatment all rats were subjected to uninephrectomy (UNX) and continued the treatment for further two weeks. Unpaired 't' test was used for statistical significance. **Results:** Before and after uninephrectomy intraperitoneal injection of PEG 400 did not alter systolic blood pressure, renal blood flow, body weight gain and twenty-four hours urine protein excretion rate compared to control rats, however, three weeks after UNX urine protein excretion rate were increased in both groups and was not significant. Intraperitoneal PEG 400 treatment significantly increased twenty-four hours water intake and urine volume compared to control rats. Histopathological evaluation of renal section by periodic acid Schiff staining, Massen's trichrom staining and desmin staining did not show any morphological changes compared to control rats. **Conclusion:** Renal functional and morphological status did not show significant alterations after intraperitoneal injection of PEG for three weeks of uninephrectomized rats. Therefore, present finding suggested that PEG 400 may not have any adverse effects on renal functional as well as morphological parameters in rat.

Key words: polyethylene glycol, renal function, rat polyethylene glycol on renal functional parameters

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Introduction

Polyethylene glycol (PEG) is a neutral, water-soluble, nontoxic polymer[1]. During the past decay the use of PEG is increasing by pharmaceutical industries due to its chemical compatibility and nontoxicity[2,3], organ preservation[1,4] as well as in clinics[5-7]. PEG is used for drug delivery to improve solubility, the pharmacokinetics and reduce the immunogenicity of therapeutic and diagnostic agents[8]. A recent study showed that PEG-based preservation solutions optimize graft quality in experimental kidney transplantation[4]. PEG prevents the activation of ischemia–reperfusion injury-induced inflammation, which can have a long-term effect on graft outcome[9]. Previous study also showed that PEG suppresses colon cancer and causes dose-dependent regression of azoxymethane-induced aberrant crypt foci in rats[10], and consistent as well as fast inhibition of colon carcinogenesis in mice and rats given various carcinogens[11]. Samsamshariat *et al*[3] showed that PEG have no effects on cardiovascular and hemodynamic except decreased effective arterial elastance (*Ea*) in rats compared to a placebo. Although some previous studies reported that the use of PEG solution for

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colonic cleansing before colonoscopy exacerbated congestive heart failure[12,13], however, recent clinical studies showed that PEG have less risk of impaired renal function after colonoscopy in patients compared to sodium phosphate solution[7,14]. Meanwhile, the effects of PEG on renal functional and morphological parameters have not yet been reported. Therefore, the aim of the present study is to evaluate the effects of PEG on renal functional as well as morphological parameters in rats.

Methods

Animals

Experiments were performed on six weeks old male Sprague-Dawley (SD) rats (SLC, Shizuoka, Japan). Rats were maintained in a temperature-controlled (24±2°C) room under a 12-hours light/dark cycle. Rats were free access to standard laboratory chow and tap water *ad libitum*. All experimental procedures were performed according to the Guidelines for the Care and Use of Animals established by Kagawa University.

Experimental Design

After one week acclimatization, male SD rats were randomly divided into two groups as follows; group I (n=6): Control (intraperitoneal injection of 2 mL of 5% dist. water in normal saline/kg, twice daily), group II (n=6): PEG 400 (intraperitoneal injection of 2 mL of 5% PEG 400 in normal saline/kg, twice daily). The dose of PEG 400 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was determined on the basis of results from previous study in rats[3]. After three weeks treatment all rats from group I and II were subjected to right uninephrectomy by flank incision under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). After seven days from surgical operation above mention treatments were again continued for further two weeks. Figure 1 illustrated the experimental layout.

Sample Collection

During the six-weeks treatment period, systolic blood pressure (SBP) was measured by tail-cuff plethysmography (BP-98A; Softron Co., Tokyo, Japan). Twenty-four hours urine samples were collected using metabolic cages. Urine samples were stored at -30°C for urinary total protein analyzed by a protein assay kit (microTP-test; Wako, Osaka, Japan). Plasma creatinine was analyzed by creatinine assay kit (microCRE-test; Wako, Osaka, Japan). For renal blood flow (RBF) measurement, a doppler flow probe (HDP 10.20R; Crystal Biotech, MA, USA) was placed around the renal artery and RBF was continuously monitored, as previously described[15]. After completing the surgical procedures with sodium pentobarbital anesthesia (50 mg/kg, i.p.), the rats were left alone for 60 minutes to stabilize SBP, HR and then RBF was continuously measured[16]. Rats were sacrificed after RBF measurement under anesthesia with over dose of sodium pento-barbital, the left kidney was perfused with chilled saline solution. Kidney sections were fixed in 10% formalin for histochemical analysis. Renal cortical tissues were snap-frozen in liquid nitrogen and stored at -80°C until processing for further analysis.

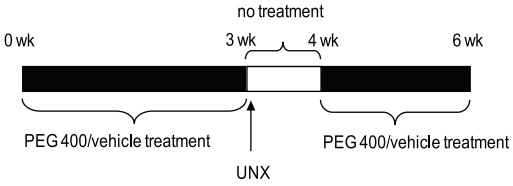

Figure 1: Schematic illustration of the experimental layout. PEG; Polyethylene glycol 400, UNX; right uninephrectomy.

Histological Examination

A slice of each kidney tissues were fixed with 10% formalin (pH 7.4), embedded in paraffin, sectioned into 4 ¼m slices, and stained with periodic acid-Schiff (PAS) reagent. Thereafter, renal morphological changes were evaluated by using light microscopy[17,18]. The diameters of the glomeruli and percentage of PAS-positive area in each experimental rat were also measured using image measurement software, WinROOF (Mitani Corp., Tokyo, Japan). A total of 25–30 glomeruli were examined for each rat. The extent of the interstitial fibrotic area was evaluated quantitatively by an automatic image analysis, which determined the area occupied by interstitial tissue positive for Masson's trichrome-staining as described

previously[17-19], and was analyzed using Image-Pro plus software (Media Cybernetics, Bethesda, MD, USA). Twenty consecutive microscopic fields were examined for each rat (X200 magnification). Immuno-histochemistry for desmin, a marker of glomerular podocyte injury was also performed, as previously described[20]. All of the morphometric measurements were performed in a blinded manner to avoid any bias.

All values are presented as the means±SEM. Statistical comparisons of the differences were performed using unpaired t-test. *P*-values below 0.05 were considered statistically significant.

Results

During the experimental period, all rats showed increased body weight and intraperitoneal injection of PEG 400 for six weeks does not have any effects on body weight gain as well as systolic blood pressure (Figure 2A and B). Before and after uninephrectomy PEG injected rats showed significantly increased water intake and twenty-four hours urine volumes (Figure 3A and B) compared to control rats. As shown in Figure 3C intraperitoneal injection of PEG 400 did not affect daily urine protein excretion rate. Although, after uninephrectomy both groups showed non-significant trends to increase daily protein excretion rate compared to baseline or three week values. Plasma creatinine levels were similar between the groups (47.35±1.35 and 48.66±2.50 µmol/L in control and PEG 400 treated group, respectively).

In addition, intraperitoneal injection of PEG 400 for six weeks did not alter RBF during anesthetized condition (Figure 3D).

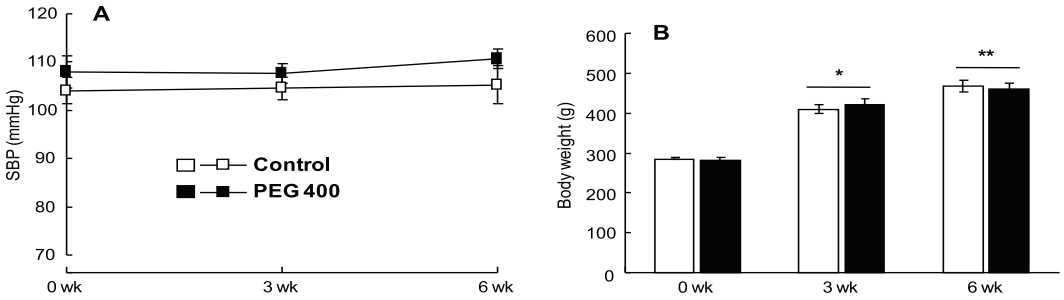

Figure 2: Comparison of systolic blood pressure (SBP; **A**) and body weight (**B**) between control and PEG 400 treated rats. $^{*}P<0.05$, $^{**}P<0.01$ when 0 week values were compared with 3rd week or 6th week values.

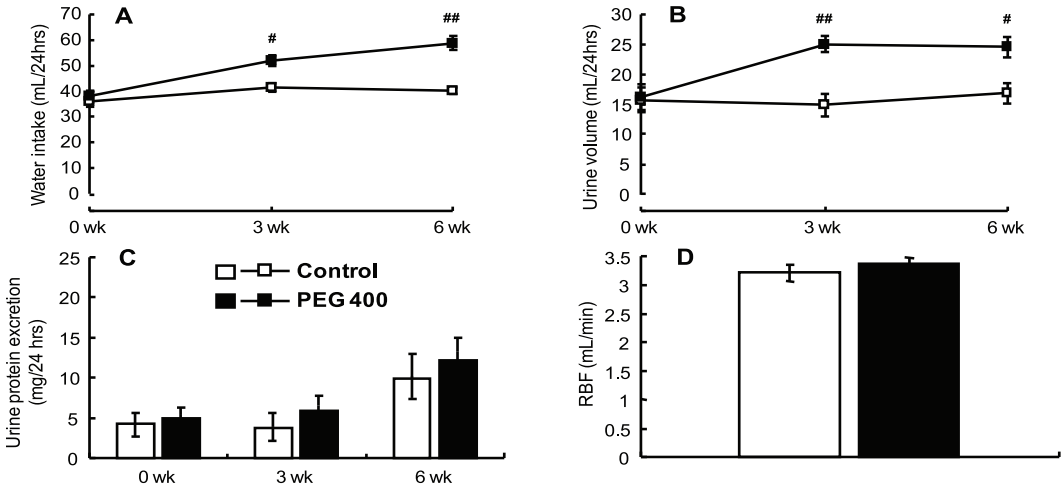

Figure 3: Effects of PEG 400 on water intake (**A**), Twenty-four hours urine volume (**B**), Twenty-four hours urine protein excretion rate (**C**) and renal blood flow (RBF; **D**) in rats. $^{\#}P<0.05$, $^{\#\#}P<0.01$ when control group values were compared with PEG 400 group values.

Histochemical analysis of renal section by periodic acid Schiff staining (Figure 4A), massen's trichrom staining (Figure 4B) and desmin staining (Figure 4C) did not show any morphological, fibrotic changes or podocyte injury compared to control rats.

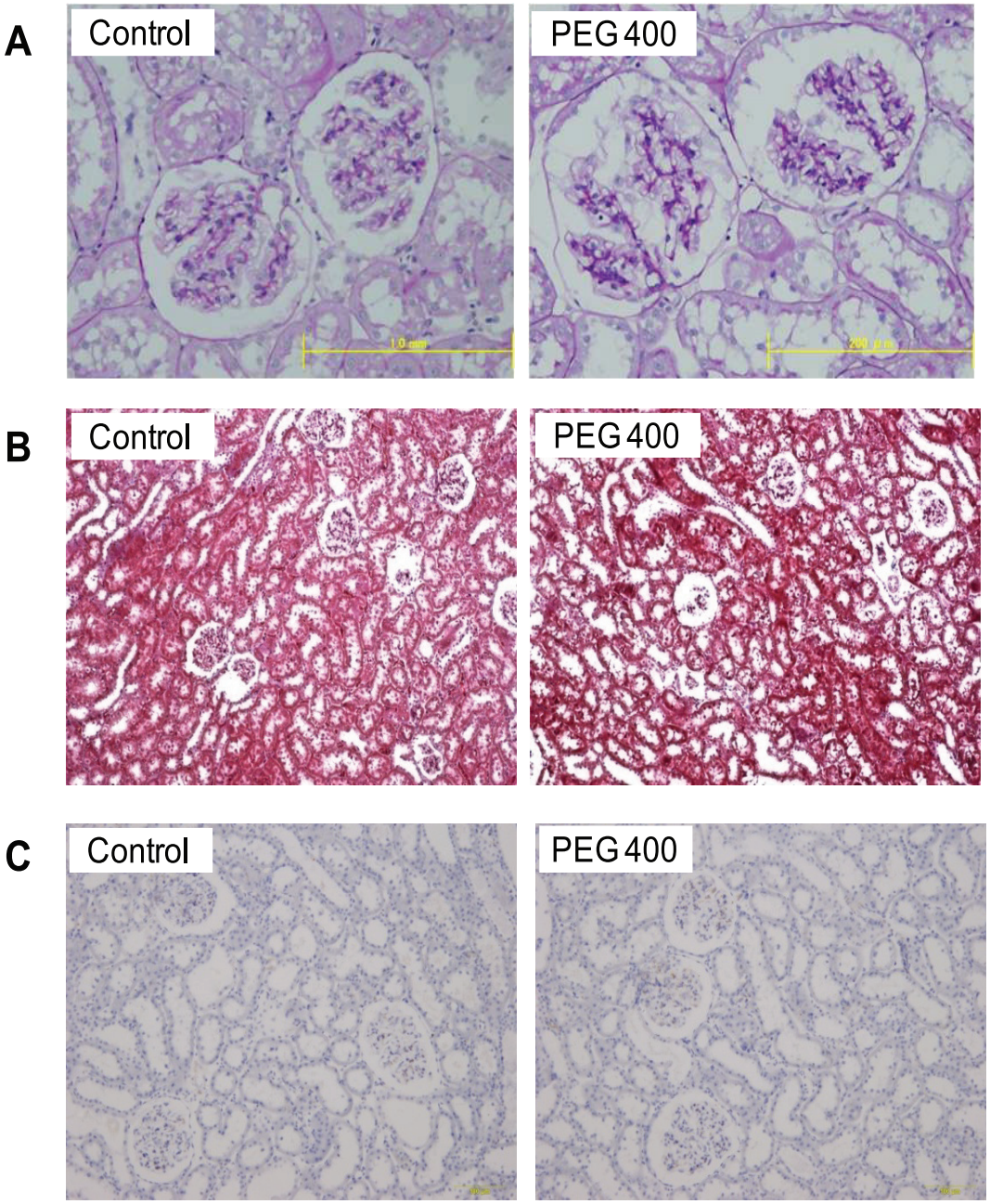

Figure 4: Representative renal cortical section stained with periodic acid Schiff (**A**) for renal morphology study, massen's trichrom staining (**B**) for renal fibrosis, and desmin staining (**C**) for podocyte injury study. Scale bar represent the value.

Discussion

To evaluate the effect of PEG on renal functional and morphological parameters, we use PEG 400 in intact rats as well as after uninephrectomy. In the present study, we found that PEG 400 did not have any effects on SBP, RBF, renal morphology and daily urine protein excretion rate, except water intake and urine volume.

Previous clinical study reported that use of PEG for bowel preparation before colonoscopy resulted in a small decrease in serum creatinine levels[14]. Another preliminary randomized study reported that oral sodium phosphate solution resulted in a slight increase in the serum creatinine level one week after colonoscopy compared with essentially no change with PEG[5]. In agreement with the previous reports, in our present study PEG did not alter serum creatinine level even after uninephrectomy.

In this present study daily urine protein excretion rate were non-significantly increased at week 6 compared to at week 3 or baseline values in both group. We believed that this changes were due uninephrectomy which causes mild renal injury[21]. This result also suggested that PEG have no effects on preexisting mild renal injury due to uninephrectomy. A Cohort study showed that in clinics about 56% of patients with preexisting renal disease recommended for the use of PEG who underwent colonoscopy[7]. This higher use of PEG in preexisting renal disease patients in clinics also highlighted the renal safety of PEG.

The clinical significant of our present findings are limited, however, due to widespread use of PEG in clinics and research laboratories the present study findings give an idea about the renal safety of PEG to the renal pharmacology researcher.

Conclusion

In conclusion, renal functional and morphological status did not show significant alterations after intraperitoneal injection of PEG for three weeks of uninephrectomized rats. Therefore, present finding suggested that PEG 400 may not have any adverse effects on renal functional as well as morphological parameters in rat.

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