Environ Health Perspect

DOI: 10.1289/EHP7612

Supplemental Material

The Kidney-Related Effects of Polystyrene Microplastics on Human Kidney Proximal Tubular Epithelial Cells HK-2 and Male C57BL/6 Mice

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Figure S4. Quantification of Western blots evaluating the phosphorylation of MAPK signaling pathway components ERK1/2, JNK, and p38 in HK-2 cells treated with PS-MPs. MAPK signaling pathway components, such as p-ERK1/2, ERK1/2, p-JNK, JNK, p-p38 and, p38, were assessed after PS-MPs treatment at a concentration of 0.8 mg/ml for 0, 5, 10, 20, 30, 60 min. The Western blotting results were graphed and statistically analyzed. (A) p-ERK1/2/ERK1/2 ratio, N=3. (B) p-JNK/JNK ratio, N=3. (C) p-p38/ p-p38 ratio, N=2. Data are presented as the mean ± SD. *P < 0.05, **P <0.01, and ***P < 0.001 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S5. Quantification of Western blots evaluating cPLA2 and COX-1 in HK-2 cells treated with PS-MPs. Inflammation-related proteins cPLA2 and COX-1, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) cPLA2/GAPDH ratio. (B) COX-1/GAPDH ratio. N=3. Data are presented as the mean ± SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S6. Quantification of Western blots evaluating the phosphorylation of mTOR and Akt in HK-2 cells treated with PS-MPs. AKT/mTOR signaling pathway components, such as p-mTOR, mTOR, p-AKT, and AKT, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 1 h. The Western blotting results were graphed and statistically analyzed. (A) p-mTOR/mTOR ratio. (B) p-AKT/AKT ratio. N=2. Data are presented as the mean ± SD. *P < 0.05 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S7. Quantification of Western blots evaluating the expression of p62, Beclin 1, and LC3 in HK-2 cells treated with PS-MPs. Autophagy-related proteins p62, Beclin 1, and LC3, were assessed after PS-MPs treatment at concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml for 24 h. The Western blotting results were graphed and statistically analyzed. (A) p62/GAPDH ratio. (B) Beclin 1/GAPDH ratio. (B) LC3-II/LC3-I ratio. N=3. Data are presented as the mean ± SD. *P < 0.05, **P <0.01, and ***P < 0.001 compared with control group as determined by one-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S8. Quantification of Western blots evaluating the expression Bad, IRE1α, p-ERK1/2, p-mTOR, and LC3-II/LC3-I ratio in HK-2 cells treated with PS-MPs alone, MitoTEMPO alone or in combination. (A) Cells were pretreated for 1 h with MitoTEMPO (100 μM) then exposed to PS-MPs (0.8 mg/ml) for 20 min. Mitochondrial-mediated apoptosis protein Bad was assessed. (B) Cells were pretreated for 1 h with MitoTEMPO and then exposed to PS-MPs for 24 h. ER stress-related protein IRE1α was assessed. (C) Cells were pretreated for 1 h with MitoTEMPO for 12 h and exposed to PS-MPs for 30 min. MAPK signaling pathway component p-ERK1/2 and ERK1/2 was assessed. (D) Cells were pretreated for 1 h with MitoTEMPO and then exposed to PS-MPs for 1 h. AKT/mTOR pathway components p-mTOR and mTOR were assessed. (E) Cells were pretreated for 1 h with MitoTEMPO for 1 h and then exposed to 0.8 mg/ml PS-MPs for 24 h. Autophagy-related protein LC3 was assessed. The Western blotting results were graphed and statistically analyzed. N=2. Data are presented as the mean ± SD. *P < 0.05 compared with control group as determined by t test. PS-MPs 0.8 mg/ml group vs MitoTEMPO (0 μM) group, (A) P=0.0462. (B) P= 0.0325. (C) P= 0.4509. (D) P= 0.0107. (E) P= 0.0475. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S9. Quantification of western blot analysis of ATG5 knockdown cells treated with PS-MPs for expression of ATG5, LC3 and COX-1. Inflammation-related proteins were evaluated after PS-MPs treatment at concentrations of 0.4 and 0.8 mg/ml for 48 h in $ATG5^{KD}$ HK-2cells. The Western blotting results were graphed and statistically analyzed. (A) ATG5/GAPDH ratio, N=3 (B) LC3-II/LC3-I ratio, N=3 (C) COX-1/GAPDH ratio, N=2. Data are presented as the mean ± SD. *P < 0.05, **P <0.01, and ***P < 0.001 compared with control group as determined by two-way ANOVA with Dunnett's multiple comparison test. The mean and SD summary data for quantification of Western blots are shown in Table S3. The actual P-values for non-statistically and statistically significant results have shown in Table S4.

Figure S10. The effects of PS-MPs on mouse muscle and grip strength. Six-week-old C57BL/6 male mice without and with 0.2 mg/day and 0.4 mg/day PS-MPs 2 times per week were examined, and the leg muscles of mice were harvested at 8 weeks. (A) Hematoxylin and eosin (H&E) staining, Masson's trichrome staining (MTS), and IHC staining of dystrophin in the muscular sections from mice with or without oral gavage of PS-MPs. Hematoxylin-stained cell nuclei were blue and eosin-stained the extracellular matrix and cytoplasm were pink. MTS-stained collagen fiber was blue and muscle fiber was red. Muscle fiber and IHC staining of dystrophin were quantified and presented % area. The mean and SD summary data for quantification are shown in Table S5. (B) Handgrip strength in a single-blind test of mice with oral gavage of 0.4 mg/day PS-MPs for 8 weeks before the mice were sacrificed. Data are presented as the mean ± SD. N=7, ***P < 0.001 compared with sham group of mice as determined by t test (Sham group vs PS-MPs group: P<0.001). The mean and SD summary data for handgrip strength are shown in Table S3. Scale bar=60 μm.

Figure S11. Protein expression in mouse urine after treatment with PS-MPs. (A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of urine from the mice was collected after oral gavage of 0.4 mg/day PS-MPs for 4 weeks. The red frame shows the difference between the groups treated with oral gavage of 0.4 mg/day PS-MPs or the sham group. Bovine serum albumin (BSA) is a serum albumin protein derived from cows. N=3. (B) Immunoblotting of urine samples from 3 different mice with albumin at 8 weeks. (C) The Western blotting results were graphed and statistically analyzed. N=3. Data are presented as the mean ± SD. ***$P < 0.001$ compared with sham group as determined by t test. $P< 0.001$. The mean and SD summary data for quantification of Western blots are shown in Table S3.