Supplemental Materials

Journal of Pharmacology and Experimental Therapeutics

TP0463518, a novel prolyl hydroxylase inhibitor, specifically induces erythropoietin production in the liver

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Supplemental Methods

Dimethyloxaloylglycine (DMOG) was purchased from Tokyo Chemical Industry Co., Ltd. Seven-week-old SD rats (Japan SLC, Hamamatsu, Shizuoka, Japan) received intraperitoneal DMOG injection dissolved in saline. The rats were euthanized at 4 hours post administration, and the kidneys and liver were removed. The right kidney of the SD rats was divided into the inferior half and superior half. The inferior half (whole kidney) was cut into 3-mm-thick slices. All of the sliced whole kidney, specimens of the left kidney cortex, and specimens of the liver were immersed in RNAlater solution and stored at 4°C until the mRNA extraction.
Supplemental Figure 1

DMOG scarcely increases the expressions of EPO mRNA in the kidney cortex, whereas it markedly increases the expressions of EPO mRNA in the liver. (A) EPO mRNA expression in the kidney cortex at 4 hours post administration slightly, but significantly increased at 600 mg/kg. (B) DMOG caused a dramatic increase of the EPO mRNA expression in the liver at 4 hours post administration at 60 mg/kg or more. Data are represented as the means ± S.E.M. n = 6. Dunnett’s multiple comparison test was used to compare the DMOG-treated groups and the corresponding vehicle-treated groups. ***P < 0.001.
Supplemental Figure 2

Total EPO mRNA expression levels in the whole liver are higher than those in the whole kidney. Relative EPO mRNA expression at 4 hours post DMOG administration was multiplied by the organ weight and the total mRNA amount, to determine the total EPO mRNA expression levels in the whole kidney (gray bar) and whole liver (black bar). The total EPO mRNA expression level in each organ was normalized by the renal EPO mRNA expression level in the vehicle-treated group. The total EPO mRNA expression in the whole liver was dramatically increased, whereas that in the whole kidney was scarcely increased. Data are represented as the means ± S.E.M. n = 6. Dunnett’s multiple comparison test was used to compare the DMOG-treated groups and the vehicle-treated groups. **P < 0.01; ***P < 0.001.