Supporting Information for

**Comparison of hepatic metabolism of triazolam in wild type and Cyp3a-knockout mice for understanding CYP3A-mediated metabolism in CYP3A-humanized mice *in vivo***

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Contents

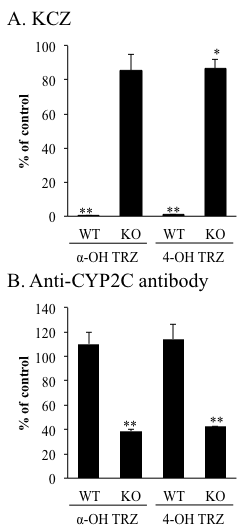
**Figure S1.** Effects of ketoconazole and an antibody against CYP2C on TRZ α- and 4-hydroxylation activities in liver microsomes of WT and Cyp3a-KO mice treated with PCN.

**Figure S2.** Effect of PCN treatment on plasma concentrations of TRZ, α-OH TRZ, 4-OH TRZ in CYP3A-humanized mice.

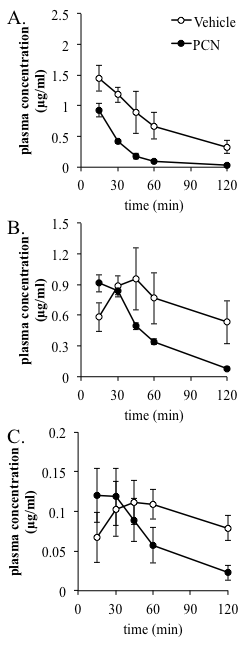
**Table S1.** Oligonucleotide primers used in real-time PCR

**Table S2.** Incubation conditions in microsomal reactions

**Table S3.** Pharmacokinetic parameters of TRZ and its metabolites in CYP3A-humanized mice *in vivo*



**Figure. S1. Effects of ketoconazole and an antibody against CYP2C on TRZ α- and 4-hydroxylation activities in liver microsomes of WT and Cyp3a-KO mice treated with PCN.** TRZ (4 μM) was incubated with pooled liver microsomes (each four mice) in the presence or absence of 1 µM ketoconazole (a) or an antibody against CYP2C (b). TRZ α- and 4-hydroxylation activities in the liver microsomes of mice treated with PCN were analyzed by LC-MS/MS. Data are shown as mean with S.D. of triplicate incubations. \*p < 0.05 and \*\*p < 0.01 versus control values in the absence of ketoconazole or anti-CYP2C antibody. Statistical analysis was performed by Welch’s *t* test.



**Figure S2. Effect of PCN treatment on plasma concentrations of TRZ, α-OH TRZ, 4-OH TRZ in CYP3A-humanized mice.** Plasma concentration versus time curves of TRZ (a), α-OH TRZ (b) and 4-OH TRZ (b) after intravenous TRZ administration (2 mg/kg) in CYP3A-humanized mice. Mice were pretreated with vehicle (corn oil) or PCN (100 mg/kg, i. p) for three days. Data are shown as mean with S.D. of four to five mice.

**Table S1 Oligonucleotide primers used in real-time PCR**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Direction | Sequence (5’ to 3’) | References |
| Cyp2c29 | Forward | CCAGAGATTCATCGACCTCCT | 27 |
|  | Reverse | TAGTTCCCTTGGGGATGAGGTAT |
|  |  |  |  |
| Cyp2c39 | Forward | TTGTGTGAATAAAAAGGGTTTCCA | 27 |
|  | Reverse | TTGAGAAATTGGTTAAGGATTGGCT |
|  |  |  |  |
| Cyp2c55 | Forward | AGGAAGCTCTGGATGACCTTGG | 28 |
|  | Reverse | TTGAGAAGCGCCGAAGCTCCTT |
|  |  |  |  |
| Cyp2c70 | Forward | TGGCTTTCTCAGCAGGAAGAA | 27 |
|  | Reverse | AACTGGCTTGGTGTCGATGT |
|  |  |  |  |
| Gapdh | Forward | AAGCCCATCACCATCTTCCAGG | 29 |
|  | Reverse | GGTTCACACCCATCACAAACAT |

**Table S2 Incubation conditions in microsomal reactions**

|  |  |  |
| --- | --- | --- |
| Liver microsome | Concentration  (mg protein/mL) | Incubation time (min) |
| WT, Vehicle | 0.2 | 60 |
| WT, PCN | 0.05 | 30 |
| KO, Vehicle | 0.025 | 30 |
| KO, PCN | 0.01 | 30 |

**Table S3 Pharmacokinetic parameters of TRZ and its metabolites in CYP3A-humanized mice *in vivo***

|  |  |  |
| --- | --- | --- |
|  | CYP3A-humanized | |
|  | Vehicle | PCN |
| Triazolam |  |  |
| AUC0-120  (µg/mL\*min) | 101 ± 21 | 35 ± 3\*\* |
| AUC0-∞  (µg/mL\*min) | 125 ± 30 | 36 ± 3\*\* |
| total clearance  (mL/min/g) | 17 ± 5 | 56 ± 5\*\* |
| half-life  (min) | 48.9 ± 10.6 | 22.8 ± 2.1\*\* |
| **α**-OH triazolam |  |  |
| AUC0-120  (µg/mL\*min) | 81 ± 21 | 49 ± 2\*\* |
| 4-OH triazolam |  |  |
| AUC0-120  (µg/mL\*min) | 10.7 ± 2.2 | 7.7 ± 2.5 |

\*\*p < 0.01 versus CYP3A-humanized mice treated with the vehicle.