Supporting Information for

In vivo evaluation of intestinal human CYP3A inhibition by macrolide antibiotics in CYP3A-humanized mice

Contents

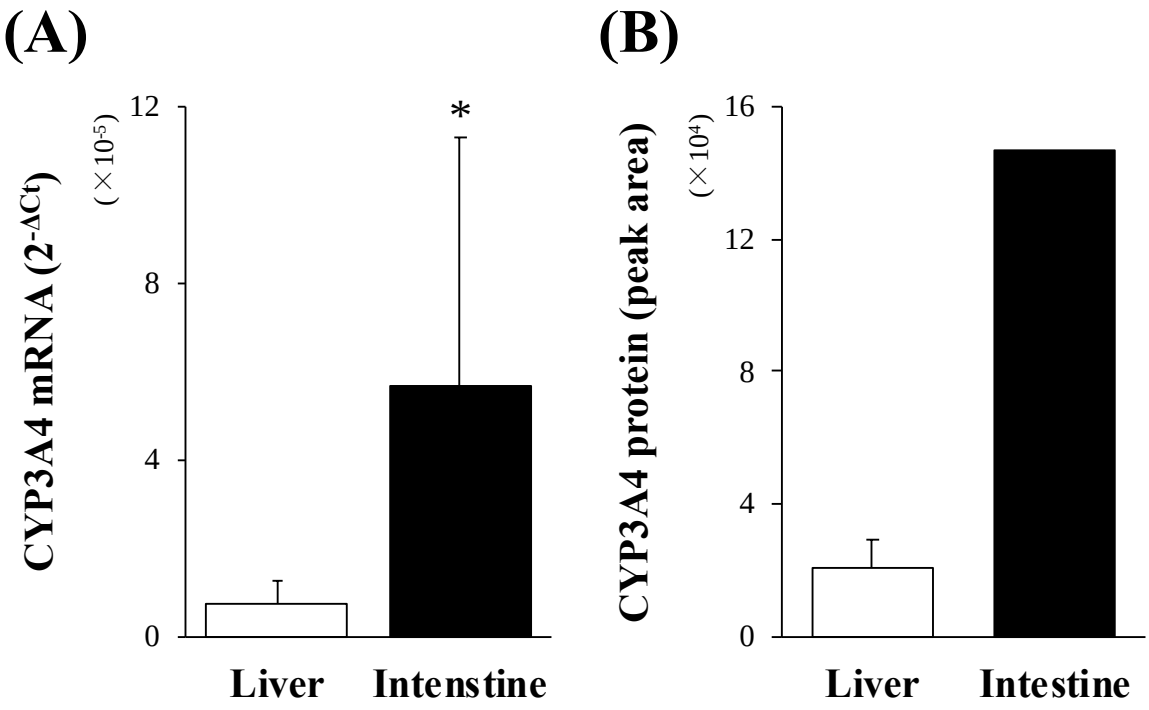

Figure S1. Expression levels of CYP3A4 in the liver and intestine of hCYP3A-MAC mice. (A) The mRNA expression levels of CYP3A4 in liver and intestine were normalized by the expression levels of 18S rRNA. Data are shown as mean with S.D. of four mice. *$p < 0.05$ versus liver. Statistical analysis was performed by U-test. (B) The protein expression levels of CYP3A4 in liver microsomes and intestine microsomes were determined by peak counts of digested peptide ion. Data for liver are shown as mean with S.D. of four mice. Data for intestine are mean of duplicate determination for pooled microsomes of four mice.

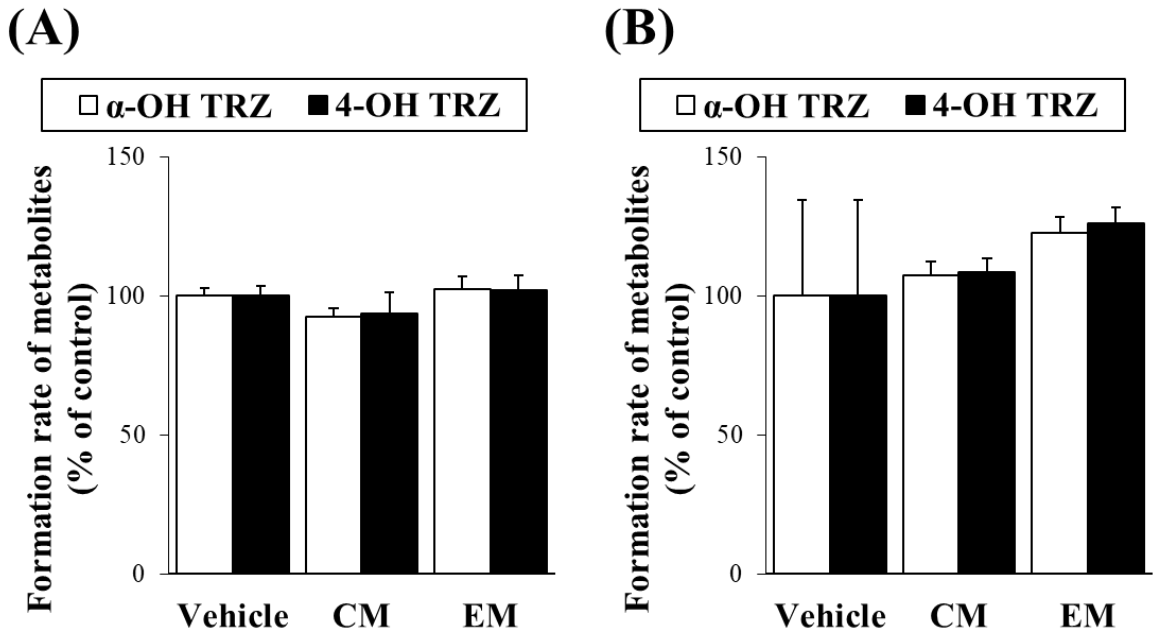

Figure. S2. Effects of macrolide antibiotics on TRZ metabolism in the liver (A) and intestine (B) microsomes of WT mice. TRZ was incubated with pre-incubated liver (1 mg/mL) or intestine (0.25 mg/mL) microsomes in the presence or absence of 100 μM CM or EM. Pooled microsomes prepared from four mice were used. Data are shown as mean with S.D. of triplicate incubations. Statistical analysis was performed by Dunnett's test.

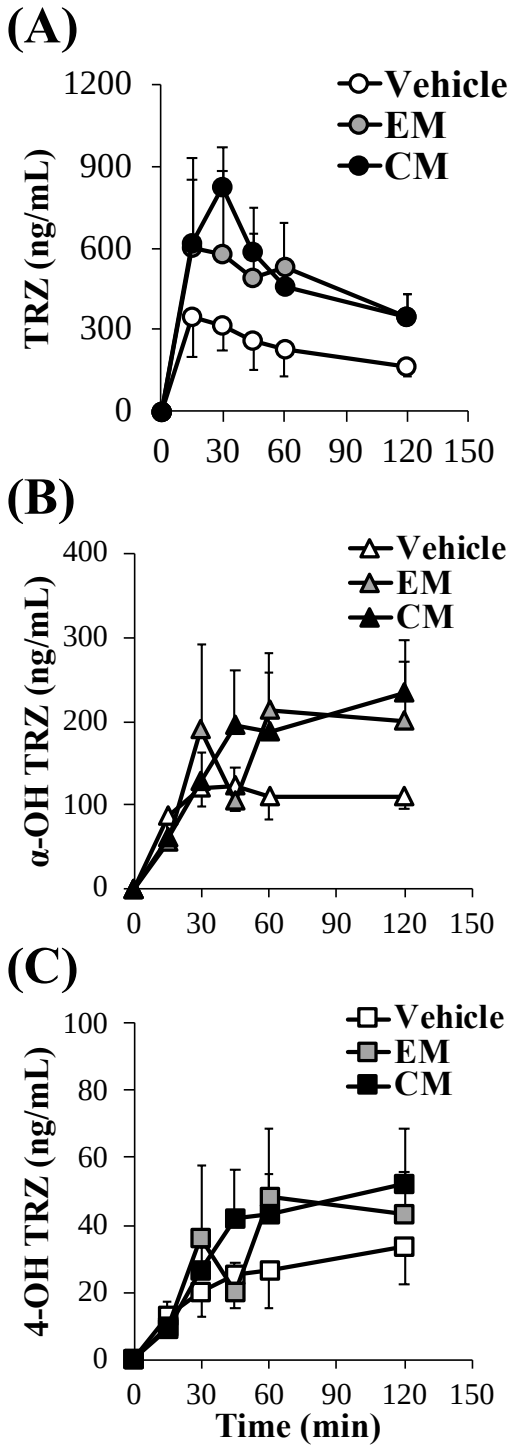

Figure S3. Effects of repeated administration of macrolide antibiotics on pharmacokinetics of TRZ and its metabolites in hCYP3A-MAC mice. Plasma concentration versus time curves of TRZ (A), α-OH TRZ (B) and 4-OH TRZ (C) after oral TRZ administration (1 mg/kg) in hCYP3A-MAC mice treated with 50 mg/kg CM, 50 mg/kg EM or 0.5% methylcellulose (vehicle) twice a day for 5 days. Data are shown as mean with S.D. of 5 or 6 mice.

Table S1. Tryptic digested peptides used for detection of CYP proteins in mouse microsomes.

CYP isoforms	Peptide Sequence	Theoretical MW	Theoretical m/z	z
mCyp1a2	NSIQDITSALFK	1335.7035	668.8590	2
mCyp1a2	LSDRPQLPYLEAF	1547.7983	774.9065	2
mCyp2a4/5	IQEEAGFLIDSFR	1523.7620	762.8883	2
mCyp2a12	DVYSSITQLQEHYGPVFTIHLGPR	2756.3923	690.1053	4
mCyp2b10	DFIDIYLLR	1166.6335	584.3240	2
mCyp2b19	NLQEILDYIGHSVEK	1756.8995	586.6404	3
mCyp2c29	FIDLLPTSLPHAVTCDIK	2028.0424	677.0214	3
mCyp2c70	FDYNDQTFQDFMENFHR	2252.9221	751.9813	3
mCyp2d9	FEYEDPFLIR	1327.6449	664.8297	2
mCyp2d10	SLLAIVENLLTENR	1583.8883	792.9514	2
mCyp2d26	DLTDAFLAEVEK	1349.6715	675.8430	2
mCyp2e1	FSLSILR	834.4963	418.2554	2
mCyp2f2	LLTIIHFINDNFK	1586.8821	529.9680	3
mCyp2j5	LPFVGNFFQIDTK	1524.7976	763.4061	2
mCyp2j6	EWATPDVFNPEHFLENGQFK	2404.1123	802.3781	3
hCYP3A4	LSLGGLLQPEKPVVLK	1690.0393	564.3537	3
mCyp3a11	TWGLFDGQTPL	1233.6030	617.8088	2
mCyp3a13	FPIINQFTDVLVR	1560.8665	781.4405	2
mCyp4a12a	NFPSACPQWLWGSK	1665.7432	833.8789	2
mCyp4a14	AVEDLNNLTFFR	1437.7252	719.8699	2

Table S2. m/z of TRZ and its metabolites used for MS/MS quantification.

Compound	Precursor ion (m/z)	Product ion (m/z)
LCMS8050		
α-OH triazolam	359.3	176.1
4-OH triazolam	359.3	314.0
Propranolol (IS)	260.4	116.2
QTRAP4500		
Triazolam	343.0	308.1
α-OH triazolam	359.2	175.9
4-OH triazolam	359.0	313.9
Propranolol (IS)	260.1	116.0

Table S3. Effects of repeated administration of macrolide antibiotics on AUCs of TRZ and its metabolites in hCYP3A-MAC mice.

Compounds	Vehicle	CM	EM
TRZ (μg/mL·min)	28 ± 10	55 ± 18**	58 ± 11*
α-OH TRZ (μg/mL·min)	12 ± 2.2	19 ± 6.5	20 ± 5.2
4-OH TRZ (μg/mL·min)	2.9 ± 1.0	4.1 ± 1.6	4.4 ± 0.94

Data are shown as mean with S.D. *$p < 0.05$; **$p < 0.01$ versus values in mice treated with the vehicle (Dunnett's test or Steel test).

Table S4. Effects of single administration of CM on AUCs and AUCRs of TRZ and its metabolites in hCYP3A-MAC mice.

Compounds	Parameters	Vehicle	CM
TRZ	**AUC (μg/mL·min)**	19 ± 7	14 ± 2
α-OH TRZ	**AUC (μg/mL·min)**	8.7 ± 2.0	7.2 ± 0.5
	$AUCR_{\alpha\text{-OH}}$	0.48 ± 0.09	0.51 ± 0.11
4-OH TRZ	**AUC (μg/mL·min)**	3.3 ± 0.9	2.0 ± 0.5
	$AUCR_{4\text{-OH}}$	0.19 ± 0.04	0.14 ± 0.02

Data are shown as mean with S.D. Statistical analysis was performed by Student's *t*-test.

Table S5. Effects of repeated administration of CM on plasma concentrations of TRZ and its metabolites in portal blood in hCYP3A-MAC mice.

Compounds	Vehicle	CM
TRZ (ng/mL)	231 ± 83	357 ± 14
α-OH TRZ (ng/mL)	41 ± 13	27 ± 2
4-OH TRZ (ng/mL)	14 ± 7	15 ± 3

Data are shown as mean with S.D. Statistical analysis was performed by Welch's *t*-test.