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RESEARCH PAPER

Loss of multidrug and toxin extrusion 1 (MATE1) is associated with metformin-induced lactic acidosis

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BACKGROUNDS AND PURPOSE

Lactic acidosis is a fatal adverse effect of metformin, but the risk factor remains unclear. Multidrug and toxin extrusion 1 (MATE1) is expressed in the luminal membrane of the kidney and liver. MATE1 was revealed to be responsible for the tubular and biliary secretion of metformin. Therefore, some *MATE* polymorphisms, that cause it to function abnormally, are hypothesized to induce lactic acidosis. The purpose of this study is to clarify the association between *MATE* dysfunction and metformin-induced lactic acidosis.

EXPERIMENTAL APPROACH

Blood lactate, pH and bicarbonate ion (HCO_3^-) levels were evaluated during continuous administration of 3 mg·mL^{-1} metformin in drinking water using *Mate1* knockout (–/–), heterozygous (+/–) and wild-type (+/+) mice. To determine the tissue accumulation of metformin, mice were given 400 mg·kg^{-1} metformin orally. Furthermore, blood lactate data were obtained from diabetic patients given metformin.

KEY RESULTS

Seven days after metformin administration in drinking water, significantly higher blood lactate, lower pH and HCO_3^- levels were observed in *Mate1*$^{-/-}$ mice, but not in *Mate1*$^{+/-}$ mice. The blood lactate levels were not affected in patients with the heterozygous *MATE* variant (*MATE1*-L125F, *MATE1*-G64D, *MATE2-K*-G211V). Sixty minutes after metformin administration (400 mg·kg^{-1}, p.o.) the hepatic concentration of metformin was markedly higher in *Mate1*$^{-/-}$ mice than in *Mate1*$^{+/+}$ mice.

CONCLUSION AND IMPLICATIONS

MATE1 dysfunction caused a marked elevation in the metformin concentration in the liver and led to lactic acidosis, suggesting that the homozygous *MATE1* variant could be one of the risk factors for metformin-induced lactic acidosis.

Abbreviations

ALT, alanine aminotranferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; MATE, multidrug and toxin extrusion; MPP, 1-methyl-4-phenylpyridinium; OCT, organic cation transporter

Introduction

Metformin (*N*,*N*-dimethylbiguanide) is widely used in the treatment of type II diabetes mellitus. It exhibits pharmacological effects in the liver and is almost entirely excreted in urine in an unchanged form (Scheen, 1996). Lactic acidosis is a fatal adverse effect of biguanide agents including metformin (Bailey and Turner, 1996). It was reported that metformin-induced lactic acidosis was associated with an elevation in plasma concentrations of metformin in patients

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with renal failure (Pearlman *et al*., 1996; Safadi *et al*., 1996). However, it is noteworthy that metformin-induced lactic acidosis also occurs in patients without any risk factors (Tymms and Leatherdale, 1988; al-Jebawi *et al*., 1998; Misbin *et al*., 1998). These reports suggest that some risk factors in addition to renal impairment are associated with metformin-induced lactic acidosis.

Multidrug and toxin extrusion 1 (MATE1/SLC47A1) is expressed in the luminal membranes of renal proximal tubules and bile canalicular membranes of hepatocytes and is responsible for the efflux of cationic compounds, including metformin, from the cells (Otsuka *et al*., 2005; Yonezawa and Inui, 2011). In the basolateral membranes of liver and kidney, organic cation transporters, OCT1 (SLC22A1) and OCT2 (SLC22A2), mediate metformin uptake from blood into cells respectively (Wang *et al*., 2003; Kimura *et al*., 2005). The renal clearance of metformin is five times higher than the glomerular filtration rate (Scheen, 1996), suggesting that the vectorial secretion via basolateral OCT2 and luminal MATE is important for the urinary excretion of metformin. Previously, we have demonstrated that the pharmacokinetics of metformin was significantly changed in *Mate1* knockout (*Mate1*$^{-/-}$) mice (Tsuda *et al*., 2009). It was shown that the total body clearance and renal clearance was decreased to 25% and 18% in comparison with the wild-type (*Mate1*$^{+/+}$) mice, respectively. Therefore, it is thought that MATE1 determines the pharmacokinetics of metformin.

Transporter function is associated with inter-individual variations in drug responses. A *MATE1* rs2289669G>A variant located in the intron region was reported to be related to a glucose-lowering effect after metformin treatment in patients with diabetes, although little is known about the influence of the molecular mechanism of this variant on the MATE1 transporter (Becker *et al*., 2009). In addition, it was reported that metformin elevates the blood lactate level in the presence of the MATE1 inhibitor pyrimethamine in mice, but under the conditions where the plasma concentration of metformin is much higher than those found in clinical situations (Ito *et al*., 2010). These reports suggested that MATE1 function influenced the pharmacodynamics of metformin under these limited conditions. Previously, we and other groups have identified non-synonymous *MATE* variants with reduced or negligible transport activity (Chen *et al*., 2009; Kajiwara *et al*., 2009; Meyer zu Schwabedissen *et al*., 2010). Taken together, these data indicate that functional impairment of MATE1 such as occurs with the genetic polymorphisms is involved in the lactic acidosis caused by metformin.

Here, we examined whether dysfunctional MATE1 caused metformin-induced lactic acidosis by using *Mate1* knockout mice as models of *MATE* variant carriers. In addition, we also investigated the effect of heterozygous *MATE* variants on blood lactate levels after metformin treatment both in mice and in humans. Our results indicated that MATE dysfunction is one of the risk factors for metformin-induced lactic acidosis.

Methods

Animals

Animals used in the present study were male C57BL/6 *Mate1*$^{+/+}$, *Mate1*$^{+/-}$ and *Mate1*$^{-/-}$ mice between 10 and 17 weeks of age. The methods used to generate the *Mate1* knockout mice were described previously (Tsuda *et al*., 2009). In the present study, 6–12 mice were used in each experiment. Mice were kept in a temperature-controlled environment with a 14 h light and 10 h dark cycle, and received a standard diet and water *ad libitum*. All animal care and experimental procedures were conducted in accordance with *The Guidelines for Animal Experiments of Kyoto University*. All protocols were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

Long-term oral administration of metformin in mice

Mice (*Mate1*$^{+/+}$, n = 9; *Mate1*$^{+/-}$, n = 9; *Mate1*$^{-/-}$, n = 11) were given 3 mg·mL^{-1} metformin in drinking water for 21 days. One to four mice were kept in the same cage. The total volume of drinking water in each cage was measured at days 7, 14 and 21 to calculate the mean daily dosage of metformin. For blood collection, the mice were deprived of food (fasted) for 4 h before being anaesthetized with sodium pentobarbital (50 mg·kg^{-1} i.p.). Blood samples were collected from the tail vein, under 40 min of anaesthesia, before and at 7, 14 and 21 days after the administration of metformin. Blood lactate, pH and bicarbonate ion (HCO_3^-) levels were measured by an i-STAT analyser with a CG4+ cartridge (Fuso Pharmaceutical Industries, Osaka, Japan). Body weight was also measured at the same time. At the end of this experiment, blood samples were collected from the abdominal aorta, and aspartate aminotransferase (AST), alanine aminotranferease (ALT), blood urea nitrogen (BUN) and plasma creatinine levels were determined.

Single oral administration of metformin in mice

Mice were given 5 mL·kg^{-1} water (*Mate1*$^{+/+}$, n = 6; *Mate1*$^{-/-}$, n = 7) or 400 mg·kg^{-1} metformin (*Mate1*$^{+/+}$, n = 7; *Mate1*$^{-/-}$, n = 12) via oral gavage under 40 min of anaesthesia. To determine the baseline lactate level, blood samples were obtained by tail bleeding before the oral administration of metformin. At 24 h after oral administration, blood lactate levels and plasma concentrations of metformin were determined using blood samples obtained from the tail vein. Blood lactate levels were measured by Lactate Pro LT-1710 (Arkray Inc., Kyoto, Japan). At the end of this experiment, blood samples were collected from the abdominal aorta, and biochemical parameters were evaluated by i-STAT analyser with CG4+ and CHEM8+ cartridges (Fuso). Thereafter, mice were killed, and samples of the kidney, liver and skeletal muscle were obtained.

Metformin and lactate concentration–time profile in Mate1 knockout mice

Mate1$^{-/-}$ mice (n = 8) were deprived of food for 12 h before the administration of 150 mg·kg^{-1} metformin via oral gavage. Blood samples were obtained from the tail vein as before and at 0.5, 1, 2, 4 and 8 h after the oral administration metformin to measure blood lactate levels and plasma concentrations of metformin. Blood lactate levels were measured by Lactate Pro (Arkray).

Clinical study

Blood lactate and *MATE* genotype data were collected from patients, as described previously by Toyama *et al*. (2010). Of the 48 patients, lactate data were obtained from 29 patients receiving 250 mg of metformin in the morning. All were inpatients at the Department of Diabetes and Clinical Nutrition, Kyoto University Hospital. Patients were given metformin hydrochloride tablets (Melbin®, Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan) continuously for the treatment of diabetes mellitus. Blood lactate levels were measured at 0, 4 and 9 h after the administration of metformin using Lactate Pro (Arkray). This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by Kyoto University Graduate School and the Faculty of Medicine, Ethics Committee.

Uptake experiments

The detail of the methods used for the metformin uptake experiments were as described previously (Tanihara *et al*., 2007). Briefly, HEK293 cells (American Type Culture Collection CRL-1573, Manassas, VA, USA) were transfected with mouse Mate1, mouse Oct1, and mouse Oct2 cDNAs using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA). The cells were incubated at 37°C for 1 or 2 min with incubation medium (pH = 7.4) containing [^{14}C]-metformin in the presence or absence of 5 mM 1-methyl-4-phenylpyridinium (MPP). Unlabelled metformin was added to [^{14}C]-metformin to obtain the final concentrations. The concentration-dependence of metformin transport by mouse Mate1, mouse Oct1 and mouse Oct2 was analysed by use of the Michaelis–Menten equation: $V = V_{max}[S] / (K_m + [S]) + K_d \cdot [S]$, where V is the transport rate, V_{max} is the maximal transport rate, [S] is the concentration of metformin, K_m is the Michaelis–Menten constant and K_d is a diffusion constant.

Analytical methods

Metformin concentrations in plasma, liver, kidney and skeletal muscle were determined by HPLC, as described previously (Kimura *et al*., 2005). The methods used for sample pretreatment were as described in detail previously (Tsuda *et al*., 2009). AST and ALT were measured by Transaminase C II-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan) according to the manufacturer's directions. Creatinine and BUN were measured by the LabAssay creatinine and UN *B*-test Wako (Wako Pure Chemical Industries Ltd.), respectively.

Materials

Metformin hydrochloride was obtained from Wako Pure Chemical Industries Ltd. [^{14}C]-metformin hydrochloride (54 mCi·mmol^{-1}) was purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). MPP iodide was purchased from Sigma Aldrich (St. Louis, MO, USA). All other compounds used were of the highest purity available.

Statistical analysis

Data are expressed as the mean ± SEM. Data were analysed statistically using Student's unpaired *t*-test or one-way ANOVA with Dunnett's multiple comparison test. The data were analysed using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Continuous oral administration of metformin in mice

Metformin-induced lactic acidosis was evaluated using *Mate1*$^{+/+}$, *Mate1*$^{+/-}$ and *Mate1*$^{-/-}$ mice. It was confirmed that the volume of drinking water without metformin was not different amongst the three genotypes. In the presence of metformin, the volume of drinking water was decreased in *Mate1*$^{-/-}$ mice, resulting in a reduction in the mean daily dose of metformin in *Mate1*$^{-/-}$ mice (7.0 mg·day^{-1}) compared with *Mate1*$^{+/+}$ (12.0 mg·day^{-1}) and *Mate1*$^{+/-}$ mice (11.4 mg·day^{-1}) (Figure 1A). Plasma concentrations of metformin were within the range for clinical use (*Mate1*$^{+/+}$, 1.4 ± 0.2; *Mate1*$^{+/-}$, 2.1 ± 0.3; *Mate1*$^{-/-}$, 4.2 ± 0.3 μg·mL^{-1}). There was no significant difference in body weight change amongst the three genotypes (Figure 1B). At 7, 14 and 21 days after metformin treatment, a higher blood lactate level was observed in *Mate1*$^{-/-}$ mice compared with those in *Mate1*$^{+/+}$ mice (Figure 1C). pH and HCO_3^- levels were significantly lower in *Mate1*$^{-/-}$ mice (Figure 1D and E). At day 21, there were no significant differences in AST, ALT and BUN between *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice (Table 1). In *Mate1*$^{+/-}$ mice, all parameters were comparable to those in *Mate1*$^{+/+}$ mice (Figure 1C–E).

Blood lactate levels in diabetic patients

Because of the limited number of patients examined (n = 48), no homozygous *MATE* variant carrier was found in this clinical study. Heterozygous *MATE* variants have little influence on the metformin plasma concentration and oral clearance as described previously (Toyama *et al*., 2010). In the present study, the effect of metformin on blood lactate levels was examined in 29 patients receiving 250 mg of metformin. Four heterozygous *MATE*-variant carriers were found; one patient carried *MATE1*-L125F variant, two carried *MATE1*-G64D variant and one carried *MATE2-K*-G211V variant. As expected, there was no significant difference in blood lactate levels between the two groups (Figure 2).

Single oral administration of metformin in mice

Based on the present data, no functional difference in the *MATE1* heterozygous genotype in comparison with the wild-type was observed *in vivo*, and therefore, we focused on the difference between the *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice. In the long-term administration experiment, the pharmacokinetics of metformin in *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice could not be rigorously evaluated because of the different dosages between the two groups. Therefore, the blood lactate levels and the pharmacokinetics of metformin were investigated in *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice after a single oral dose. In the vehicle-treated mice, there was no significant difference between these parameters in *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice. In metformin-treated mice, two *Mate1*$^{-/-}$ mice died and blood lactate levels were significantly high in *Mate1*$^{-/-}$ mice compared with

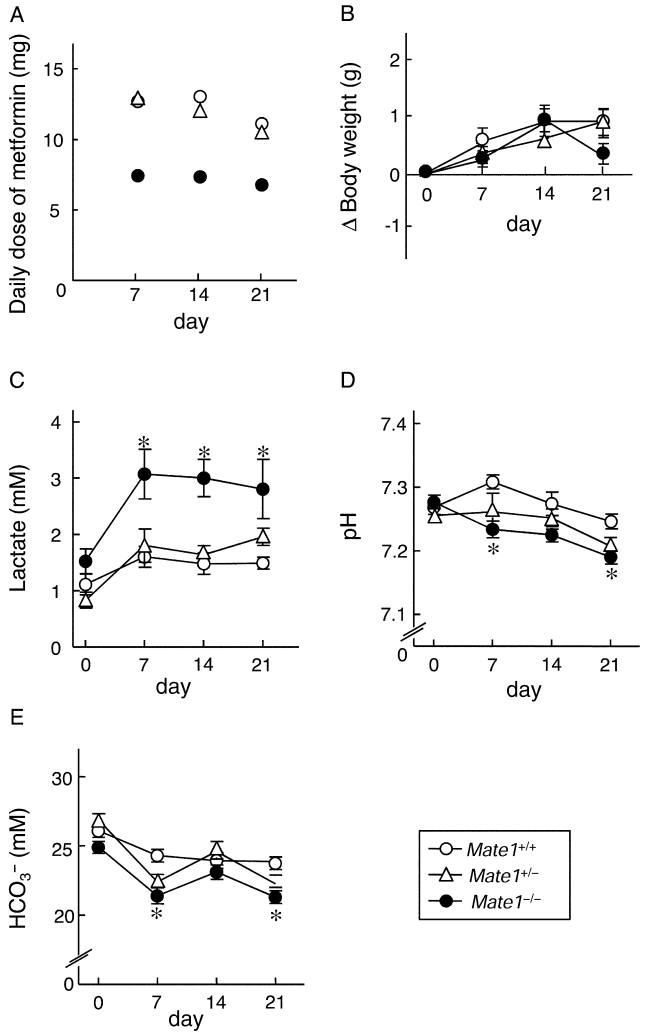

Figure 1

Long-term toxicity of metformin in *Mate1*$^{+/+}$, *Mate1*$^{+/-}$ and *Mate1*$^{-/-}$ mice. *Mate1*$^{+/+}$ (n = 9), *Mate1*$^{+/-}$ (n = 9) and *Mate1*$^{-/-}$ (n = 11) mice were given 3 mg·mL^{-1} metformin in drinking water for 21 days. The mean daily dose of each 7 days (A), body weight change from baseline (B), blood lactate level (C), pH (D) and HCO_3^- levels (E) were determined at 0, 7, 14 and 21 days after metformin treatment. Blood samples were collected under anaesthesia in 4 h-fasted mice. Blood lactate, pH and HCO_3^- levels were measured by i-STAT. Each point represents the mean ± SEM. *P < 0.05, significantly different from *Mate1*$^{+/+}$ mice at each day.

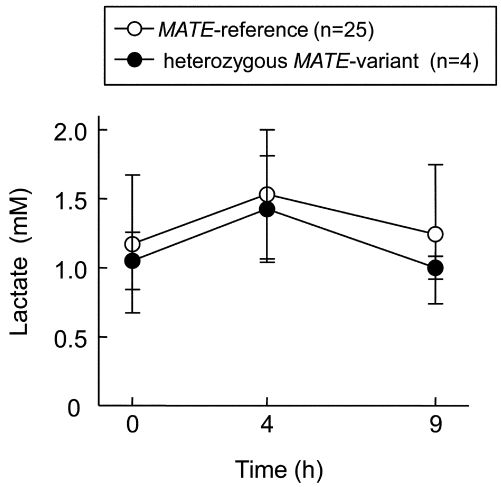

Figure 2

Lactate concentration–time profile in diabetic patients. Metformin was administered to patients in the *MATE*-reference group (n = 25) and heterozygous *MATE*-variant group (n = 4). Blood lactate levels were measured by Lactate Pro at 0, 4 and 9 h after the oral administration of metformin. One *MATE1*-L125F variant carrier, two *MATE1*-G64D variant carriers and one *MATE2-K*-G211V variant carriers were found in this study. Each point represents the mean ± SD.

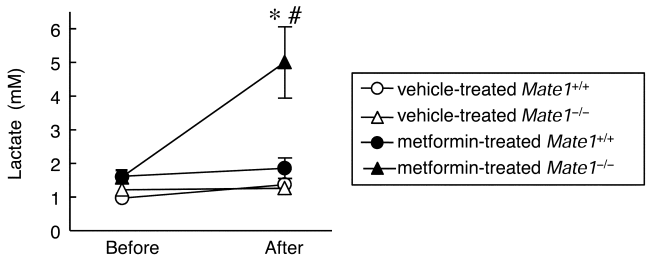

Figure 3

Blood lactate level in vehicle- or metformin-treated *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice after a single dose of metformin. All mice were given 400 mg·kg^{-1} metformin via oral gavage. Blood lactate levels were determined before and 24 h after the oral administration of metformin in vehicle-treated *Mate1*$^{+/+}$ mice (n = 6), vehicle-treated *Mate1*$^{-/-}$ mice (n = 7), metformin-treated *Mate1*$^{+/+}$ mice (n = 7) and metformin-treated *Mate1*$^{-/-}$ mice (n = 10). Each point represents the mean ± SEM. *P < 0.05, significantly different from vehicle-treated mice with the same genotype. #P < 0.05, significantly different from metformin-treated *Mate1*$^{+/+}$ mice.

Mate1$^{+/+}$ mice (Figure 3). Several other biochemical parameters were also evaluated (Table 2). The pH and HCO_3^- levels were lower in metformin-treated *Mate1*$^{-/-}$ mice than in metformin-treated *Mate1*$^{+/+}$ mice. In addition, the base excess of extracellular fluid as an indicator of metabolic acidosis was also significantly decreased in metformin-treated *Mate1*$^{-/-}$ mice compared with metformin-treated *Mate1*$^{+/+}$ mice.

Plasma concentrations of metformin were 15-fold higher in *Mate1*$^{-/-}$ mice than in *Mate1*$^{+/+}$ mice (Figure 4A). Metformin concentrations in the kidney, liver and skeletal muscle were also 21-, 69- and 44-fold higher in *Mate1*$^{-/-}$ than those in *Mate1*$^{+/+}$ mice, respectively (Figure 4B, D and F). In *Mate1*$^{-/-}$ mice, the apparent tissue-to-plasma concentration ratio (K_p) was higher in the liver and skeletal muscle, but not in the kidney (Figure 4C, E and G).

Metformin and lactate concentration–time profile in mice

To clarify the association between the PK/PD profile, we examined the metformin and lactate concentration–time profile in *Mate1*$^{-/-}$ mice. The blood lactate level increased later than the plasma concentration of metformin; the plasma

Table 1

Biochemical parameters in *Mate1*$^{+/+}$, *Mate1*$^{+/-}$ and *Mate1*$^{-/-}$ mice 21 days after metformin treatment

	Mate1$^{+/+}$	*Mate1*$^{+/-}$	*Mate1*$^{-/-}$
Hepatic function			
AST (IU·L^{-1})	24 ± 3	21 ± 2	21 ± 2
ALT (IU·L^{-1})	33 ± 4	23 ± 1	20 ± 1
Renal function			
Creatinine (mg·mL^{-1})	0.0035 ± 0.0001	0.0038 ± 0.0001	0.0044 ± 0.0001*
BUN (mg·mL^{-1})	0.22 ± 0.02	0.23 ± 0.02	0.22 ± 0.01
Acid-base balance			
Lactate (mM)	1.5 ± 0.1	2.0 ± 0.2	2.8 ± 0.6*
pH	7.24 ± 0.01	7.21 ± 0.02	7.19 ± 0.01*
HCO_3^- (mM)	24 ± 1	22 ± 1	21 ± 0*

Mate1$^{+/+}$ (n = 9), *Mate1*$^{+/-}$ (n = 9) and *Mate1*$^{-/-}$ mice (n = 11) were used.
*P < 0.05, significantly different from *Mate1*$^{+/+}$ mice.

Table 2

Biochemical parameters and body weight in vehicle- or metformin-treated *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice.

	Vehicle		Metformin	
	Mate1$^{+/+}$	*Mate1*$^{-/-}$	*Mate1*$^{+/+}$	*Mate1*$^{-/-}$
BW (g)	30 ± 1	27 ± 1	29 ± 1	27 ± 0
pH	7.32 ± 0.02	7.28 ± 0.03	7.29 ± 0.02	7.03 ± 0.10**$^{\#\#}$
pCO_2 (mmHg)	50 ± 3	51 ± 4	48 ± 3	39 ± 3*
pO_2 (mmHg)	109 ± 17	114 ± 14	121 ± 4.1	127 ± 12
BE_{ecf} (mM)	−1 ± 1	−3 ± 1	−4 ± 1	−16 ± 2***$^{\#\#\#}$
HCO_3^- (mM)	25 ± 1	24 ± 1	23 ± 1	12 ± 2***$^{\#\#\#}$
TCO_2 (mM)	27 ± 1	25 ± 1	24 ± 1	15 ± 2***$^{\#\#\#}$
sO_2 (%)	96 ± 2	97 ± 1	98 ± 0	95 ± 1
Na (mM)	148 ± 1	151 ± 1	148 ± 1	153 ± 1$^{\#\#}$
K (mM)	5.5 ± 0.5	4.7 ± 0.2	4.9 ± 0.2	5.2 ± 0.2
Cl (mM)	116 ± 1	120 ± 1	117 ± 1	131 ± 0***$^{\#\#\#}$
iCa (mM)	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.1
Glu (mg·mL^{-1})	2.03 ± 0.16	1.78 ± 0.13	1.76 ± 0.09	1.44 ± 0.10*$^{\#}$
Hct (%PCV)	43 ± 2	42 ± 1	39 ± 1	42 ± 1
Hb (g·mL^{-1})	0.15 ± 0.01	0.14 ± 0.00	0.13 ± 0.00	0.14 ± 0.00
AnGap (mM)	15 ± 1	15 ± 0	15 ± 1	16 ± 1
BUN (mg·mL^{-1})	0.24 ± 0.04	0.24 ± 0.01	0.19 ± 0.05	0.45 ± 0.11
Creatinine (mg·mL^{-1})	0.0034 ± 0.0010	0.0036 ± 0.0002	0.0030 ± 0.0001	0.0054 ± 0.0008

Vehicle-treated mice (*Mate1*$^{+/+}$, n = 6; *Mate1*$^{-/-}$, n = 7), metformin-treated mice (*Mate1*$^{+/+}$, n = 7; *Mate1*$^{-/-}$, n = 10).
*P < 0.05, **P < 0.01, ***P < 0.001, significantly different from vehicle-treated mice with the same genotype; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001, significantly different from *Mate1*$^{+/+}$ mice.
BW, body weight; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; BE_{ecf}, base excess of extracellular fluid; TCO_2, total carbon dioxide; sO_2, saturation oxygen; iCa, ionized calcium; Glu, glucose; Hct, haematocrit; Hb, haemoglobin; AnGap, anion gap.

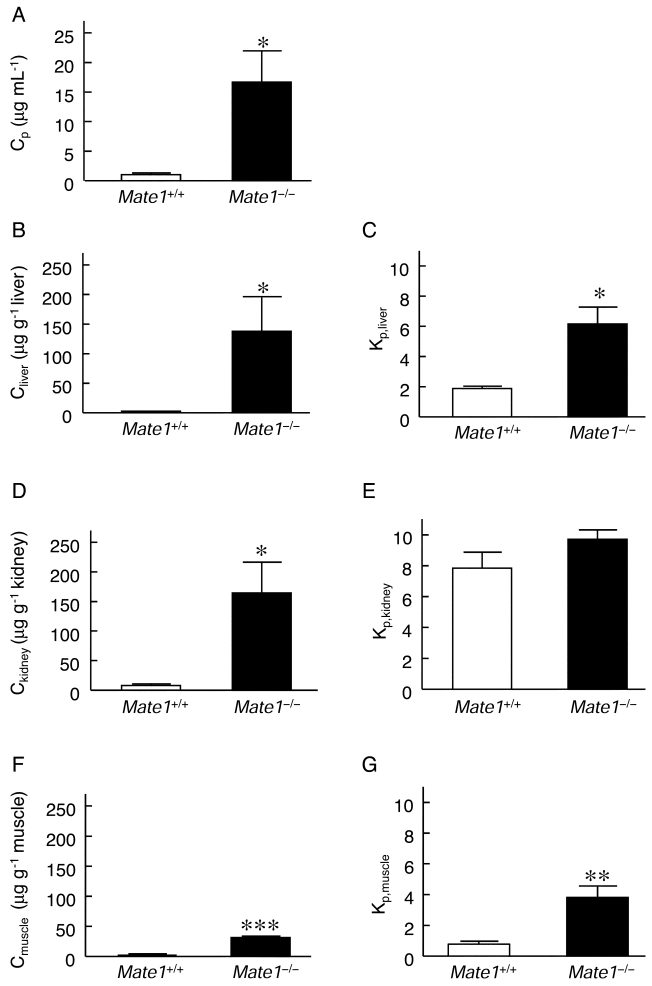

Figure 4

Pharmacokinetics of metformin in *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice after a single dose of metformin. In the same mice as shown in Figure 3, metformin concentrations in plasma (A), liver (B), kidney (D) and skeletal muscle (F) were determined by HPLC. K_p values in the liver (C), kidney (E) and skeletal muscle (G) were calculated by dividing the tissue concentration by the plasma concentration of metformin. Data represent mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from *Mate1*$^{+/+}$ mice.

concentration of metformin and blood lactate level peaked at 2 and 4 h, respectively, after the oral administration metformin in these mice (Figure 5).

Concentration-dependence of [^{14}C]-metformin uptake by mouse Mate1, mouse Oct1 and mouse Oct2

We examined the characteristics of metformin transport by mouse Mate1, mouse Oct1 and mouse Oct2. The uptake of [^{14}C]-metformin by mouse Mate1, mouse Oct1 and mouse Oct2 was found to be concentration-dependent (Figure 6). The apparent K_m values of metformin transport by mouse Mate1-, mouse Oct1- and mouse Oct2-expressing cells were 0.3 ± 0.0, 1.8 ± 0.1 and 2.9 ± 0.4 mM, respectively. The V_{max}

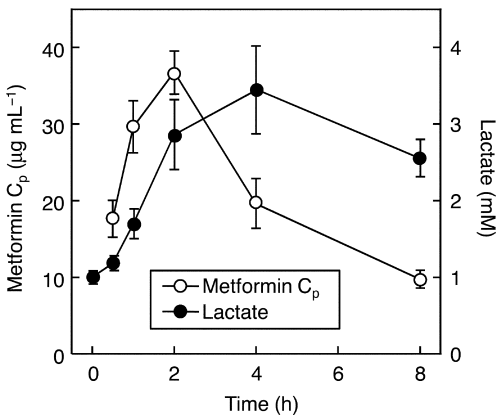

Figure 5

Lactate and metformin concentration–time profiles in *Mate1*$^{-/-}$ mice. Overnight-fasted mice were given 150 mg·kg^{-1} metformin (n = 8). Plasma concentration of metformin and blood lactate levels ere measured by HPLC and Lactate Pro respectively. Data represent mean ± SEM.

values were 5.3 ± 0.7 nmol·mg^{-1} protein·min^{-1}, 11.5 ± 2.3 nmol·mg^{-1} protein·2 min^{-1} and 18.5 ± 2.0 nmol·mg^{-1} protein·2 min^{-1} respectively.

Discussion

Genetic variants of drug transporters affect the pharmacokinetics of substrates and are involved in serious clinical outcomes. The organic anion transporting polypeptide 1B1 *(OATP1B1/SLCO1B1)* variant has been shown to increase plasma concentrations of simvastatin acid and is associated with statin-induced myopathy (Pasanen *et al.*, 2006; Link *et al.*, 2008). Our recent report suggested that the breast cancer resistant protein *(BCRP/ABCG2)* 421C>A variant causes the plasma concentration of sunitinib to increase and is associated with severe adverse effects to this drug such as skin reactions in the hands and feet (Mizuno *et al.*, 2010). In the case of metformin, a recent report demonstrated that MATE is involved in the excretion of metformin in humans by using the MATE inhibitor pyrimethamine (Kusuhara *et al.*, 2011). In addition, it was previously reported that the administration of metformin in the presence of pyrimethamine resulted in plasma concentrations of metformin 50 times higher than the therapeutic concentrations and also led to an increased blood lactate level (Ito *et al.*, 2010). In the present study, we focused on the clinical risk of metformin-induced lactic acidosis and carried out two experiments to examine the influence of *MATE* dysfunction on metformin-induced lactic acidosis in mice. On the continuous administration of metformin in drinking water, the mean plasma concentration of metformin in *Mate1*$^{+/+}$ mice was 1.4 μg·mL^{-1}, which is consistent with the clinical plasma concentration (Scheen, 1996; Toyama *et al.*, 2010). In *Mate1*$^{-/-}$ mice, the blood lactate level was significantly increased despite a lower dosage of metformin than in the *Mate1*$^{+/+}$ mice (Figure 1). After the same dose of metformin,

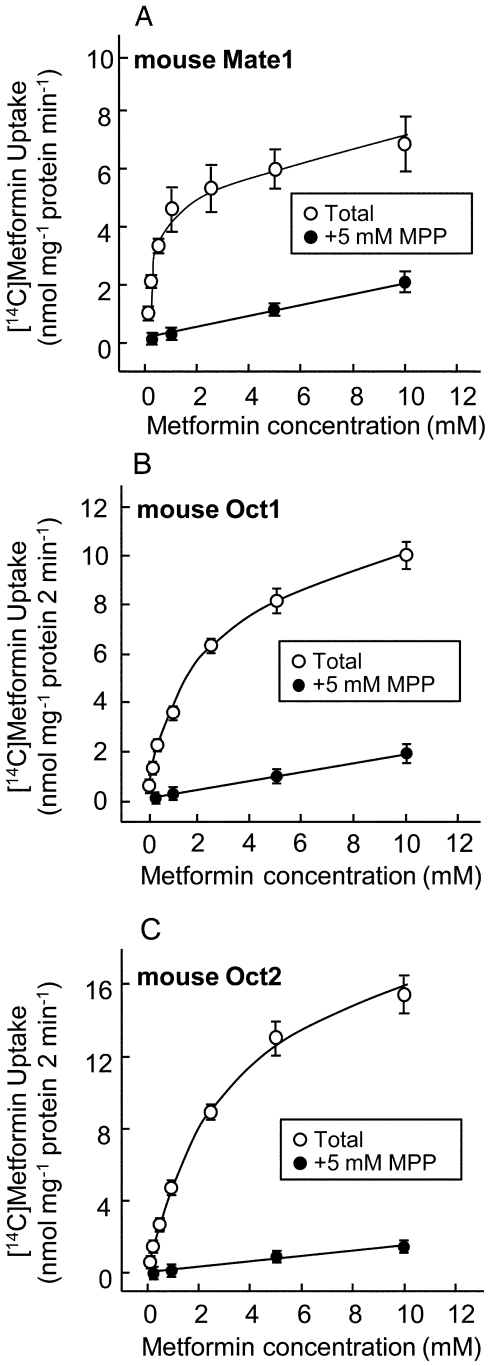

Figure 6

Concentration dependence of [^{14}C]-metformin uptake by mouse Mate1-, mouse Oct1- or mouse Oct2-expressing cells. The cells were incubated with [^{14}C]-metformin in the presence or absence of 5 mM MPP at 37°C for 1 min in mouse Mate1-expressing cells (A) and for 2 min in mouse Oct1- (B) or mouse Oct2-expressing cells (C). Data represent mean ± SEM.

administered as a bolus p.o., in *Mate1*$^{+/+}$ and *Mate1*$^{-/-}$ mice, severe lactic acidosis was observed in *Mate1*$^{-/-}$ mice, as shown by the levels of lactate, pH, HCO_3^- and several electrolytes, but not in the *Mate1*$^{+/+}$ mice (Figure 3 and Table 2). In addition, two of the *Mate1*$^{-/-}$ mice died after this acute metformin treatment, and the plasma creatinine concentration was also elevated in these metformin-treated *Mate1*$^{-/-}$ mice (Tables 1 and 2). These phenomena were reported in the patients with metformin-induced lactic acidosis (Misbin *et al.*, 1998; Tymms and Leatherdale, 1988; al-Jebawi *et al.*, 1998; Stades *et al.*, 2004). It was shown that the peak of blood lactate level followed the elevation of metformin concentration, and the high blood lactate level was maintained even though the metformin concentration was reduced immediately (Figure 5). These results suggest that metformin-induced lactic acidosis is caused by a completely dysfunctional MATE even when plasma concentration of metformin was within therapeutic range in *MATE*-reference carriers. Taken together, the data indicate that a dysfunctional *MATE* mutation could be a high risk factor for metformin-induced lactic acidosis.

In accord with our previous report on the pharmacokinetics of metformin, we found that in humans, the heterozygous *MATE* variant did not affect blood lactate levels (Figure 2) (Toyama *et al.*, 2010). In addition, the same results were obtained in *Mate1*$^{+/-}$ mice (Figure 1). This suggests that metformin-induced lactic acidosis is not just caused by the heterozygous *MATE* variant. On the other hand, there is no information about the blood lactate level in human homozygous *MATE* variant carriers due to low allelic frequencies of *MATE* variants with a loss of function (Chen *et al.*, 2009; Kajiwara *et al.*, 2009; Meyer zu Schwabedissen *et al.*, 2010). Metformin-induced lactic acidosis is also quite rare with 3 cases per 100 000 patient-years, but severe with a mortality up to 50% (Bailey and Turner, 1996; Misbin *et al.*, 1998). If possible, the nucleotide sequences of MATE should be determined in patients suffering from lactic acidosis after receiving metformin treatment.

The liver is a major pharmacological target organ for the action of metformin. In a previous report it was shown that the blood lactate level was elevated in *Oct1*$^{+/+}$ mice, but not in *Oct1*$^{-/-}$ mice, after metformin treatment (Wang *et al.*, 2003). Also hepatic concentrations of metformin were significantly lower in *Oct1*$^{-/-}$ mice than in *Oct1*$^{+/+}$ mice at similar plasma concentrations. Therefore, it was suggested that liver is the key organ responsible for metformin-induced lactic acidosis. In the present study we demonstrated that the hepatic concentration of metformin was 69-fold higher in *Mate1*$^{-/-}$ mice than in *Mate1*$^{+/+}$ mice, in spite of a 15-fold increase in the plasma concentration (Figure 4A and B). In *Mate1*$^{-/-}$ mice, the renal clearance of metformin was 18% of that in *Mate1*$^{+/+}$ mice, and the plasma concentration of metformin was higher (Tsuda *et al.*, 2009). In addition, it was reported that the MATE1 inhibitor pyrimethamine causes a 53 % reduction in hepatic clearance of metformin with respect to the liver concentration of metformin (Ito *et al.*, 2010). Taken together, these data indicate that a MATE1 dysfunction synergistically increased metformin accumulation in the liver due to the loss of urinary and biliary efflux of metformin, resulting in the development of lactic acidosis.

In addition to its effects in the liver, metformin, by activation of AMP-activated protein kinase (AMPK), also has a pharmacological effect in skeletal muscle (Zhou *et al.*, 2001). The AMPK activator AICAR has been reported to increase significantly the rate of lactate release within skeletal muscle

cells (Young *et al.*, 1996). A recent report showed that metformin was transported into skeletal muscle cells by OCT3 (Chen *et al.*, 2010). MATE1 is also expressed in skeletal muscle (Otsuka *et al.*, 2005) and in the present study it was found that the K_p value for metformin was increased in muscle from *Mate1*$^{-/-}$ mice (Figure 4G). Therefore, it is likely that MATE1 is responsible for metformin efflux from the skeletal muscle as well as in the kidney and liver.

Species differences exist in the MATE family (Yonezawa and Inui, 2011). The tissue distribution of the MATE family is reported to differ between humans and mice. MATE1 and MATE2-K are expressed in human kidney, whereas MATE2-K is not expressed in mice (Masuda *et al.*, 2006; Tsuda *et al.*, 2009). Therefore, *Mate1* knockout mice could represent a model of both MATE1 and MATE2-K deficiency in humans, because of similar affinities in mice and humans (Figure 6) (Tanihara *et al.*, 2007). The renal clearance of metformin in *Mate1*$^{+/-}$ mice was comparable with that in *Mate1*$^{+/+}$ mice despite the reduction of MATE1 expression (Toyama *et al.*, 2010). Taken together with the similar transport characteristics of MATE1 and MATE2-K (Tanihara *et al.*, 2007), it is assumed that the renal excretion of metformin was not affected in either homozygous *MATE1* or homozygous *MATE2-K* variant carriers. In the pharmacological targets such as liver and skeletal muscle, MATE1 was found but not MATE2-K (Yonezawa and Inui, 2011). In *Mate1*$^{-/-}$ mice, the hepatic and skeletal muscular K_p values for metformin were higher than those in the wild-type mice (Figure 4C and G). Therefore, it is anticipated that only the homozygous *MATE1* variant causes the elevation in metformin concentrations in the liver and skeletal muscle, even though renal excretion is not changed. In fact, in a previous case report, metformin-induced lactic acidosis was found to occur within the therapeutic concentration of metformin (Tymms and Leatherdale, 1988; al-Jebawi *et al.*, 1998). MATE1 is expressed in several tissues that are associated with metformin action, but MATE2-K is expressed only in the kidney (Yonezawa and Inui, 2011). Therefore, only the homozygous *MATE1* variant would affect the pharmacodynamics of metformin without a reduction in renal clearance.

In the present study, a dysfunction in MATE1 caused lactic acidosis after metformin treatment, accompanied by a reduction in the renal clearance of metformin and increase in hepatic accumulation. These findings indicate that MATE1 plays an important role in the pharmacodynamics of metformin. In conclusion, the homozygous *MATE* variant is one of the risk factors for metformin-induced lactic acidosis. These observations may provide new insights into the causes of metformin-induced lactic acidosis.

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Conflicts of interest

None.

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