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# Effects of cysteine on metformin pharmacokinetics in rats with protein–calorie malnutrition: partial restoration of some parameters to control levels

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## Abstract

Metformin is metabolized primarily via hepatic microsomal cytochrome P450 (CYP)2C11, CYP2D1 and CYP3A1/2 in rats. The expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 are decreased in rats with protein–calorie malnutrition (PCM), but these levels are fully or partially restored to control levels in PMC rats by oral cysteine supplementation (PCMC rats). Thus, it would be expected that the pharmacokinetic parameters of metformin in PCM rats would be returned to control levels in PCMC rats. Metformin was administered i.v. (100 mg kg$^{-1}$) and orally (100 mg kg$^{-1}$) to control, CC (control rats with oral cysteine supplementation), PCM and PCMC rats. The following pharmacokinetic parameters of metformin following i.v. administration were restored from levels in PCM rats to levels in control rats in PCMC rats: intrinsic clearance (0.0350, 0.0309, 0.0253 and 0.0316 mL min$^{-1}$ mg$^{-1}$ protein for control, CC, PCM, and PCMC rats, respectively), total area under the plasma concentration–time curve from time zero to time infinity (AUC; 4110, 4290, 5540 and 4430 $\mu$g min mL$^{-1}$, respectively), and time-averaged non-renal clearance (8.12, 7.95, 5.94 and 8.17 mL min$^{-1}$ kg$^{-1}$, respectively). AUC values following oral administration were comparable between control and PCMC rats (1520, 1480, 2290 and 1680 $\mu$g min mL$^{-1}$, respectively).

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## Introduction

Protein–calorie malnutrition (PCM) is considered to be a global problem, particularly in vulnerable children, infants and the institutionalized elderly (Denke & Wilson 1998). A number of diseases, including cancer, digestive disorders and acquired immune deficiency syndrome (AIDS), are also associated with PCM (Denke & Wilson 1998). Changes in drug metabolism in malnutrition (Buchanan 1978; Krishnaswamy 1978) and in pharmacokinetics with respect to changes in the expression and mRNA levels of hepatic cytochrome P450 (CYP) isozymes in PCM rats (Lee et al 2004) have been reported.

Cho et al (1999) reported that in male Sprague–Dawley rats with PCM (fed on 5% casein diet for 4 weeks), the expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 decreased compared with controls, but that these decreases were partially or completely returned to control levels by oral cysteine supplementation for 1 week (250 mg kg$^{-1}$ twice daily during the fourth week). This implies that some pharmacokinetic parameters of drugs in PCM rats could be completely (or partially) returned to control levels by oral cysteine supplementation, as reported in other studies (Lee et al 2004 & 2007, and references therein).

Metformin is a biguanide antiglyacemic agent that is widely used in the management of type 2 diabetes; it lowers blood glucose concentration without causing hypoglycaemia (Scheen 1996). After i.v. (0.25–1.0 g) and oral (0.5–1.5 g) administration of metformin to four healthy volunteers, the terminal half-life of metformin was 1.52–4.50 h; 78.9–99.9% of the dose was excreted in the urine via active renal tubular secretion; absorption was incomplete (20–30% of the oral dose was recovered from the faeces), possibly reflecting an active, saturable absorption process; the absolute

oral bioavailability (F) was 33–55% (Scheen 1996). Recently, Choi & Lee (2006) reported that metformin was metabolized primarily via hepatic CYP2C11, CYP2D1 and CYP3A1/2, but not via CYP1A1/2, CYP 2B1/2 or CYP2E1, in male Sprague–Dawley rats. Thus, it would be expected that the pharmacokinetics of metformin would be altered in PCM rats, and that some pharmacokinetic parameters in PCM rats would be returned to control levels by cysteine supplementation.

Morley & Perry (1991) reported that approximately 50% of individuals with type 2 diabetes are over 65 years of age, and that the dietary changes required for the treatment of diabetes can lead to severe protein–energy malnutrition, particularly in older people. Turnbull & Sinclair (2002) also reported that community-dwelling elderly patients with diabetes may have more risk factors for malnutrition than do non-diabetic individuals. Thus, metformin was selected for this study using PCM rats as an animal model.

The aim of this study was to examine metformin pharmacokinetics after i.v. and oral administration ($100\,mg\,kg^{-1}$) in control rats (given metformin only), control rats given cysteine supplementation ($250\,mg\,kg^{-1}$; twice daily during the fourth week; CC rats), PCM rats and PCM rats given oral cysteine supplementation (PCMC rats). In particular, the ability of oral cysteine administration to restore metformin pharmacokinetics was examined.

## Materials and Methods

### Chemicals

Metformin hydrochloride and ipriflavone (internal standard for the HPLC analysis of metformin) were donated by Daelim Pharmaceutical Company (Seoul, South Korea) and Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea), respectively. The reduced form of $\beta$-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), ethylendiamine tetraacetatic acid (EDTA), tri(hydroxymethyl)aminomethane (Tris)-buffer, and dextran (MW 65,000 Daltons) were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). Other chemicals were reagent or HPLC grade.

### Rats and diets

The protocol for this animal study was approved by the Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats, 5–8 weeks old and weighing 150–250 g, were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). Rats were assigned randomly to one of two diets, one containing 23% casein (control rats) and the other containing 5% casein (PCM rats), for 4 weeks. Both diets were isocaloric; the compositions of the diets are reported by Cho et al (1999).

Animals were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at 20–23°C with a 12 h light–dark cycle (07:00–19:00 and 19:00–07:00) and relative humidity of 50 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered pathogen-free air and with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water available ad libitum. Food intake and body weight were measured at least once a week.

From the start of the fourth week, control and PCM rats were divided randomly into two further groups. One group was treated with oral cysteine, $250\,mg\,kg^{-1}$ twice daily (cysteine was dissolved in tap water to produce a concentration of $100\,mg\,mL^{-1}$) – CC and PCMC rats; the other (control) groups were given the same volume of tap water.

### Preliminary study

This preliminary study was performed after 4 weeks on each diet (n = 5 in each group) to measure liver and kidney function. An 18 h urine sample was collected for the measurement of urine output and creatinine levels. A plasma sample was collected for the measurement of total protein, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and creatinine levels (analysed by Green Cross Reference Lab., Seoul, South Korea). Whole kidneys and livers were excised from each rat, rinsed with 0.9% saline, blotted dry with tissue paper and weighed. Small portions of each organ were fixed in 10% neutral phosphate-buffered formalin and then processed for routine histological examination with haematoxylin and eosin (H&E) staining.

### Measurement of metformin plasma protein binding

Protein binding of metformin was measured in fresh plasma from control, CC, PCM and PCMC rats (n = 5 in each group) using equilibrium dialysis (Choi et al 2006) at a metformin concentration of $1\,\mu g\,mL^{-1}$. Plasma (1 mL) was dialysed against 1 mL isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran in a 1 mL dialysis cell (Spectrum Medical Industries, Los Angeles, CA, USA) using a Spectra/Por 4 membrane (MW cut-off 12 000–14 000 Daltons; Spectrum Medical Industries). After 24 h incubation, two 50 $\mu$L aliquots were removed from each compartment and stored at −70°C prior to HPLC analysis of metformin (Hale et al 2002).

### Measurement of enzyme kinetics in microsomal fractions

The procedures used were similar to previously reported methods (Lee et al 2007). The livers of control, CC, PCM and PCMC rats (n = 4 or 5 for each group) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in ice-cold buffer (0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA, pH 7.4). The homogenate was centrifuged at 9000 *g* for 30 min, and the supernatant centrifuged at 100 000 *g* for 90 min. The protein concentration was measured using the method described by Bradford (1976).

Aliquots of the microsomal fraction (equivalent to 0.5 mg protein) were incubated with 5 $\mu$L 0.9% saline containing 1, 2.5, 5, 7.5, 10, 50, 100 or 200 $\mu$M metformin base, and 50 $\mu$L 0.1 M phosphate buffer (pH 7.4) containing 1 mM

NADPH, made up to a final volume of 0.5 mL with 0.1 M phosphate buffer (pH 7.4). Incubations were done in a shaking waterbath (Jeio-tech, Seoul, South Korea; 50 oscillations $min^{-1}$) at 37°C. Disappearance of metformin was linear under these conditions. The reaction was terminated after 15 min by addition of 1 mL acetonitrile containing 2 $\mu g\,mL^{-1}$ ipriflavone (internal standard). Metformin was measured by an HPLC method described by Hale et al 2002.

The maximum velocity ($V_{max}$), apparent Michaelis–Menten constant ($K_m$) and the concentration at which the rate is 50% of $V_{max}$ for the disappearance of metformin were calculated using a non-linear regression method (Duggleby 1995). The intrinsic clearance ($Cl_{int}$) for the disappearance of metformin was calculated by dividing $V_{max}$ by $K_m$.

### Intravenous and oral administration of metformin

Procedures used for the pretreatment of rats, including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for i.v. drug administration) were similar to reported methods (Kim et al 1993). Both cannulae were exteriorized to the dorsal side of the neck, terminated with a long silastic tube (Dow Corning, Midland, MI, USA) and inserted into a wire sheath to allow free movement of the rat. Rats were housed individually in metabolic cages (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from light ether anesthesia for 4–5 h before beginning the experiment. Rats were not restrained during the study.

For the i.v. study, metformin (metformin hydrochloride dissolved in 0.9% saline), at a dose equivalent to 100 $mg\,kg^{-1}$ metformin base, was infused in a total volume of 2 $mL\,kg^{-1}$ via the jugular vein over 1 min to control, CC, PCM (all n = 9) and PCMC (n = 10) rats. Blood samples (approximately 0.12 mL) were taken from the carotid artery at 0 (control), 1 (at the end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min after the start of the metformin infusion. Blood samples were centrifuged immediately and 50 $\mu$L aliquots of plasma samples stored at −70°C for subsequent HPLC analysis of metformin (Hale et al 2002). At the end of the experiment (24 h), each metabolic cage was rinsed with 20 mL distilled water and the rinsings combined with the 24 h urine sample. The exact volume of the combined urine sample was measured and two 50 $\mu$L aliquots were stored at −70°C for subsequent HPLC analysis of metformin.

At 24 h, each rat was exsanguinated and killed by cervical dislocation. The abdomen was opened and the entire gastrointestinal (GI) tract (including its contents and faeces) was removed, transferred into a beaker containing 100 mL methanol (to facilitate the extraction of metformin) and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 50 $\mu$L aliquots of the supernatant were collected from each beaker and stored at −70°C for subsequent HPLC analysis of metformin.

For the oral study, the same solution of metformin as used in the i.v. study was administered orally using a feeding tube, at a dose equivalent to 100 $mg\,kg^{-1}$ metformin base (total volume 5 $ml\,kg^{-1}$) to control (n = 8), CC and PCM (n = 10) and PCMC (n = 12) rats. Blood samples (0.12 mL) were taken at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960, 1200 and 1440 min after oral administration of metformin. Other procedures were similar to those for the i.v. study described above

### HPLC analysis of metformin

Concentrations of metformin in the samples described above were determined using a reported HPLC method (Hale et al 2002), but using ipriflavone instead of hydrocodeine as the internal standard. Briefly, each 50 $\mu$L aliquot of biological sample was deproteinized with 100 $\mu$L acetonitrile, and 50 $\mu$L methanol containing 10 $\mu g\,mL^{-1}$ ipriflavone (internal standard) added. After vortex mixing and centrifugation (16 000 *g* for 10 min), a 50 $\mu$L aliquot was injected directly onto a reverse-phase ($C_{18}$) HPLC column (Lichrosorb RP-18, Merck, NJ, USA). The mobile phase (pH = 6) was 10 mM $KH_2PO_4$ and acetonitrile, at a ratio of 47.8:52.2 (v/v) for plasma and GI tract samples and 28:72 (v/v) for urine samples, delivered at a flow rate of 1.5 $mL\,min^{-1}$. The column eluent was monitored using a UV detector at 235 nm at room temperature. The retention times of metformin and ipriflavone (internal standard) were approximately 4 and 6.5 min, respectively, in rat plasma and GI tract samples, and approximately 12 and 6 min, respectively, in urine samples. The quantitation limits of metformin in rat plasma and urine samples were 0.05 and 0.1 $\mu g\,mL^{-1}$, respectively. The inter- and intra-day coefficients of variation for plasma and urine samples in the concentration ranges 0.05–5000 $\mu g\,mL^{-1}$ and 0.1–1000 $\mu g\,mL^{-1}$ were below 9.91 and 8.35%, respectively.

### Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated using the trapezoidal rule with extrapolation to infinity (Chiou 1978). The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters using non-compartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA, USA): time-averaged total body clearance (CL), renal clearance ($CL_R$), non-renal clearance ($CL_{NR}$), terminal half-life, first moment of AUC, mean residence time (MRT), apparent volume of distribution at steady state, and F (Kim et al 1993). The peak plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($T_{max}$) were read directly from the extrapolated data.

Glomerular filtration rate (GFR) was estimated from the creatinine clearance ($CL_{CR}$) assuming that kidney function was stable during the experimental period. $CL_{CR}$ was measured by dividing the total amount of unchanged creatinine excreted in the 18 h urine by the AUC of the 0–18 h concentration–time curve of creatinine in plasma.

### Statistical analysis

A *P* value below 0.05 was deemed to be statistically significant using Duncan's multiple range test (SPSS Inc., Chicago,

IL, USA) a posteriori analysis of variance among the four means for the unpaired data. All data are expressed as mean ± s.d., except for $T_{max}$, which is given as median (range).

## Results and Discussion

### Effects of cysteine

Body weight, food, protein and calorie intakes, 18 h urine output, plasma chemistry data, $CL_{CR}$ and relative liver and kidney weights in control, CC, PCM and PCMC rats are given in Table 1. Final body weight (body weight gain), and food, protein and calorie intakes were significantly less, and plasma levels of total protein and albumin were significantly lower in PCM and PCMC rats than in control and CC rats. These parameters were not significantly different between the control and CC rats, or between PCM and PCMC rats, suggesting that cysteine supplementation does not affect the aforementioned parameters in the control and PCM rats.

Protein deprivation for 4 weeks (5% casein diet; PCM rats) significantly reduced body weight gain (from 207 to 328 g in the controls vs from 214 to 165 g in PCM rats) and food consumption compared with controls. PCM rats consumed approximately 70% less food than the control rats on the higher-protein diet, despite ad libitum supply of food. As a result, the protein and calorie intakes of PCM rats were 90.9% and 58.2% lower, respectively, than in control rats ($P < 0.05$). Since both protein and calorie intakes were decreased significantly in PCM rats, these rats suffered both protein and calorie deficiencies. Thus, any changes in pharmacokinetics of metformin in PCM rats should be attributed to both protein and calorie deficiencies and not solely to protein deficiency.

Plasma levels of total protein and albumin were significantly lower in PCM and PCMC rats than in control and CC rats, but the values were in the reported ranges for normal rats (Mitruka & Rawnsley 1981). Plasma levels of urea nitrogen, GOT and GPT, plasma protein binding of metformin, $CL_{CR}$ and relative liver and kidney weights listed in Table 1 were not significantly differ between the four groups of rats. These findings indicate that kidney function was not seriously impaired in PCM rats. Consistent with the kidney histology, no significant findings were detected in the kidneys of all four groups of rats, as reported in another study (Krishnaswamy 1978). However, moderate pericentral hydropic changes and mild fatty changes were observed in the livers of PCM and PCMC rats, but no significant findings were observed in the livers of control and CC rats based on liver histology. These findings suggest that cysteine supplementation in PCM rats (PCMC rats) could not improve hepatic impairment (based on the liver histology) induced in PCM rats.

### Plasma protein binding of metformin

Protein binding of metformin to fresh plasma from control, CC, PCM and PCMC rats was 13.4 ± 4.80, 14.3 ± 6.70, 12.7 ± 3.12, and 12.7 ± 6.30%, respectively; the values were not significantly different between the four groups of rat. Sirtori et al (1978) and Tucker et al (1981) reported that binding of metformin to human plasma proteins does not occur. Adsorption of metformin to the equilibrium dialysis apparatus, which included the semi-permeable membrane, was low: 88.3–113% of the spiked amount of metformin

**Table 1** Body weight, food, protein and calorie intakes, 18 h urine output, plasma chemistry data, plasma protein binding of metformin, $CL_{CR}$, and relative liver and kidney weights in control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively)

| Parameter | Control rats | CC rats | PCM rats | PCMC rats |
|---|---|---|---|---|
| Body weight (g) | | | | |
| Initial | 207 ± 35.6 | 196 ± 11.4 | 214 ± 11.4 | 198 ± 13.0 |
| Final* | 328 ± 36.5 | 304 ± 19.2 | 165 ± 19.0 | 153 ± 15.7 |
| Food intake (g $day^{-1}$ per rat)* | 18.5 ± 2.28 | 18.7 ± 1.22 | 5.55 ± 0.778 | 7.93 ± 0.916 |
| Protein intake (g $day^{-1}$ per rat)* | 4.25 ± 0.526 | 4.30 ± 0.281 | 0.386 ± 0.0389 | 0.396 ± 0.0458 |
| Calorie intake (kcal $day^{-1}$ per rat)* | 74.7 ± 9.23 | 75.6 ± 4.93 | 31.2 ± 3.14 | 32.0 ± 3.70 |
| Urine output (mL in 18 h)** | 18.4 ± 10.2 | 13.5 ± 5.55 | 8.80 ± 1.25 | 9.50 ± 3.12 |
| Plasma | | | | |
| Total protein (g $dL^{-1}$)* | 5.96 ± 0.439 | 5.66 ± 0.1954 | 4.80 ± 0.1874 | 5.04 ± 0.385 |
| Albumin (g $dL^{-1}$)* | 3.82 ± 0.217 | 3.62 ± 0.110 | 3.04 ± 0.207 | 3.12 ± 0.303 |
| Urea nitrogen (mg $dL^{-1}$) | 12.5 ± 4.78 | 11.7 ± 6.76 | 20.3 ± 4.68 | 11.5 ± 9.30 |
| GOT (IU $L^{-1}$) | 72.0 ± 14.74 | 62.4 ± 9.45 | 78.2 ± 25.0 | 95.4 ± 68.8 |
| GPT (IU $L^{-1}$) | 27.0 ± 12.9 | 23.4 ± 5.41 | 27.4 ± 9.71 | 31.0 ± 7.97 |
| Protein binding of metformin (%) | 13.4 ± 4.80 | 14.3 ± 6.70 | 12.7 ± 3.12 | 12.7 ± 6.30 |
| $CL_{CR}$ (mL $min^{-1}$ $kg^{-1}$) | 3.27 ± 1.20 | 3.03 ± 0.975 | 2.19 ± 0.871 | 2.17 ± 0.600 |
| Liver weight (% body weight) | 4.09 ± 0.649 | 3.82 ± 0.275 | 3.41 ± 0.213 | 4.04 ± 0.723 |
| Kidney weight (% body weight) | 0.871 ± 0.133 | 0.858 ± 0.0921 | 0.915 ± 0.0935 | 1.00 ± 0.166 |

Values are mean ± s.d. (n = 5) *PCM and PCMC groups were significantly different from control and CC groups ($P < 0.05$); **PCM and PCMC groups were significantly different ($P < 0.05$) from control and CC groups, but PCMC and CC groups were comparable. $CL_{CR}$, creatinine clearance; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; IU, international unit.

were recovered from both plasma and buffer compartments. Choi et al (2006) reported that the binding of metformin to 4% human serum albumin was independent of metformin concentration in the range 1–200 $\mu g\,mL^{-1}$; the mean value was 10.1%. Thus, a metformin concentration of 10 $\mu g\,mL^{-1}$ was arbitrarily chosen for the plasma protein binding study.

### Kinetics of metformin disappearance in hepatic microsomal fractions

The $V_{max}$, $K_m$, and $CL_{int}$ for the disappearance of metformin in hepatic microsomal fractions from the four groups of rats are listed in Table 2. $V_{max}$ was significantly slower in PCM rats than in controls (27.4% decrease), suggesting that the maximum velocity for the disappearance (primarily metabolism) of metformin was significantly slower in PCM rats than in the controls. However, $K_m$ was comparable between PCM rats and controls, suggesting that the affinity of metformin for the enzyme(s) did not alter in PCM rats. As a result, $CL_{int}$ was significantly slower in PCM rats than in the other three groups (27.7, 19.9 and 18.1% decreases compared with control, CC and PCMC rats, respectively); these values did not differ significantly between control, CC and PCMC rats. These data suggest that the metabolism of metformin is decreased significantly in PCM rats compared with control rats but was returned to control levels by administration of cysteine (PCMC rats).

### Pharmacokinetics of metformin after i.v. administration

Figure 1 shows the mean arterial plasma concentration–time profiles in the four groups of rats following i.v. administration of metformin, 100 $mg\,kg^{-1}$; relevant pharmacokinetic parameters are listed in Table 3. The AUC was significantly greater in PCM rats (34.8, 29.1 and 25.1% increases compared with control, CC and PCMC rats, respectively), MRT was significantly longer (108, 149 and 76.5% increases, respectively), and CL and $CL_{NR}$ significantly lower (24.3, 26.1 and 21.4% decrease, respectively, for CL; 26.8, 25.3 and 27.3% decreases, respectively, for $CL_{NR}$). These values did not differ significantly between control, CC and PCMC rats. $CL_R$ was significantly slower in PCM rats than in control and CC rats (22.9 and 26.4% decreases, respectively) but did not significantly differ between control and CC rats. Note that body weight gain, food, protein and calorie intakes and 24 h urine output were also significantly lower in PCM and PCMC rats than in control and CC rats, but values did not differ significantly between control and CC rats, or between PCM and PCMC rats.

### Pharmacokinetics of metformin after oral administration

Figure 2 shows the mean arterial plasma concentration–time profiles in the four groups of rats following oral administration of metformin, 100 $mg\,kg^{-1}$; relevant pharmacokinetic parameters are listed in Table 4.

Absorption of the drug from the GI tract was rapid: metformin was detected in plasma at 15 or 30 min in all rats. The AUC of metformin was significantly greater in PCM rats than in control, CC and PCMC rats (50.7, 54.7 and 36.3% increases, respectively); these values did not differ significantly between control, CC and PCMC rats. $C_{max}$ was significantly lower in PCMC rats (42.6, 36.0 and 43.8% decreases, respectively) and $T_{max}$ significantly longer (300, 50.0 and 50.0% increases, respectively) than in control, CC and PCM rats, but values did not differ significantly between the latter three groups. The 24-h urine output was significantly lower in PCMC rats than in controls (45.0% decrease). Body weight gain, and food, protein and calorie intakes were significantly lower in PCM and PCMC rats than in control and CC rats, but values did not differ significantly between control and CC rats, or between PCM and PCMC rats.

## General discussion

Choi et al (2006) reported that the AUC for metformin is proportional to dose after both i.v. and oral administration in the dose range studied (50, 100, and 200 $mg\,kg^{-1}$). Thus, a dose of 100 $mg\,kg^{-1}$ was chosen for the study.

The contribution of $CL_R$ to CL of metformin was considerable: values were greater than 64.4% for all four groups of rat (Table 3), indicating that metformin was eliminated mainly via renal excretion. The contribution of GI (including

**Table 2** Kinetics of metformin disappearance in hepatic microsomes of control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively)

| Parameter | Control rats | CC rats | PCM rats | PCMC rats |
|---|---|---|---|---|
| $V_{max}$ (nmoL $min^{-1}$ (mg $protein^{-1}$))* | 4.05 ± 0.742 | 3.47 ± 2.07 | 2.94 ± 0.361 | 4.19 ± 1.15 |
| $K_m$ (mM) | 118 ± 31.0 | 120 ± 90.9 | 117 ± 15.9 | 135 ± 44.9 |
| $CL_{int}$ (mL $min^{-1}$ $kg^{-1}$)** | 0.0350 ± 0.00487 | 0.0309 ± 0.00323 | 0.0253 ± 0.00260 | 0.0316 ± 0.00437 |

Values are mean ± s.d. (n = 4 for control, PCM and PCMC groups; n = 5 for CC group).
*PCM group significantly different from control group ($P < 0.05$); **PCM group significantly different from control, CC and PCMC groups ($P < 0.05$).
$V_{max}$, maximum velocity; $K_m$, apparent Michaelis–Menten constant; $CL_{int}$, intrinsic clearance ($V_{max}/K_m$).

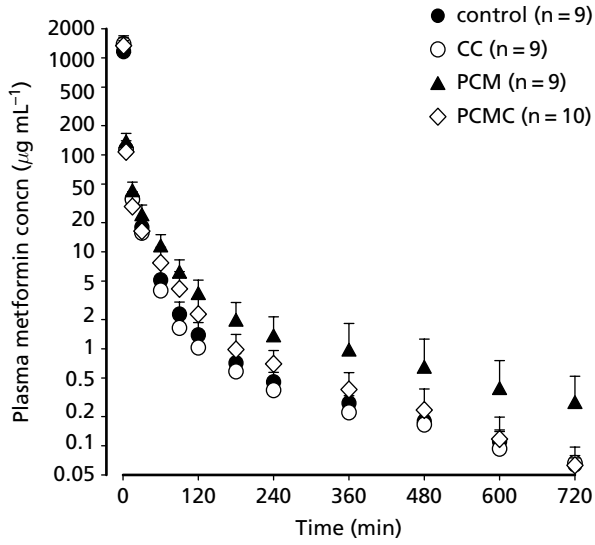


**Figure 1** Mean arterial plasma concentration–time profiles of metformin following intravenous administration of 100 mg kg$^{-1}$ (given over 1 min) to control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively). Vertical bars represent s.d.

biliary) excretion of unchanged metformin to $CL_{NR}$ was negligible: the proportion of the i.v. dose of metformin recovered from the GI tract (including its contents and faeces) at 24 h was 1.38, 2.32, 4.75 and 2.74% for control, CC, PCM and PCMC rats, respectively (Table 3). These negligible values are not likely to be due to chemical or enzymatic degradation of metformin in gastric fluids. Choi et al (2006) reported that metformin was stable for up to 48 h when incubated in various buffer solutions with pH values ranging from 1 to 12, and for up to 24 h when incubated in rat gastric juices (pH values of 2.5 and 4.5). Choi et al (2006) also reported that biliary excretion of unchanged metformin following an i.v. dose of 100 mg kg$^{-1}$ to bile-duct-cannulated rats was negligible (0.343%). Thus, the $CL_{NR}$ of metformin given in Table 3 could represent the metabolic clearance of the drug, and changes in the $CL_{NR}$ of metformin could represent changes in metabolism of the drug in rats.

The significantly greater AUC in PCM rats after i.v. administration of metformin could have been due to significantly slower CL than in the controls (Table 3). The slower CL was attributable to significantly slower $CL_R$ and $CL_{NR}$ in PCM rats (Table 3). Since the percentage of the dose excreted in the 24 h urine sample ($Ae_{0-24\,h}$) values were comparable between control and PCM rats, the significantly slower $CL_R$ could have been due to significantly greater AUC than in the controls. The significantly slower $CL_{NR}$ in PCM rats (Table 3) could have been due to decreased expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 (Cho et al 1999), since metformin is metabolized via hepatic CYP2C11, CYP2D1 and CYP3A1/2 in rats (Choi & Lee 2006). Changes in the expression and mRNA level of hepatic CYP2D1 were not investigated in PCM and PCMC rats; thus, more studies on the effect of hepatic CYP2D1 on the pharmacokinetics

**Table 3** Pharmacokinetic parameters of metformin after intravenous infusion (over 1 min) at a dose of 100 mg kg$^{-1}$ to control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively)

| Parameter | Control rats | CC rats | PCM rats | PCMC rats |
|---|---|---|---|---|
| Body weight (g) | | | | |
| Initial | 191 ± 9.28 | 188 ± 6.67 | 184 ± 5.37 | 186 ± 5.16 |
| Final* | 349 ± 32.5 | 356 ± 32.8 | 168 ± 10.6 | 175 ± 14.5 |
| Food intake (g day$^{-1}$ per rat)* | 20.2 ± 1.34 | 20.6 ± 1.52 | 8.66 ± 0.939 | 8.06 ± 0.625 |
| Protein intake (g day$^{-1}$ per rat)* | 4.64 ± 0.308 | 4.73 ± 0.349 | 0.433 ± 0.0469 | 0.403 ± 0.0313 |
| Calorie intake (kcal day$^{-1}$ per rat)* | 81.5 ± 5.40 | 83.1 ± 6.13 | 35.0 ± 3.79 | 32.6 ± 2.53 |
| 24 h urine volume (mL)* | 33.1 ± 8.57 | 34.8 ± 10.9 | 23.3 ± 6.26 | 26.4 ± 7.21 |
| AUC (μg min mL$^{-1}$)** | 4110 ± 558 | 4290 ± 637 | 5540 ± 1160 | 4430 ± 463 |
| Terminal half-life (min) | 168 ± 26.4 | 173 ± 18.7 | 190 ± 69.1 | 162 ± 47.4 |
| MRT (min)** | 27.8 ± 3.31 | 23.2 ± 5.32 | 57.7 ± 26.8 | 32.7 ± 14.5 |
| CL (mL min$^{-1}$ kg$^{-1}$)** | 24.7 ± 3.26 | 25.3 ± 2.38 | 18.7 ± 3.85 | 23.8 ± 1.98 |
| $CL_R$ (mL min$^{-1}$ kg$^{-1}$)*** | 16.6 ± 3.76 | 17.4 ± 3.41 | 12.8 ± 3.87 | 15.6 ± 2.78 |
| $CL_{NR}$ (mL min$^{-1}$ kg$^{-1}$)** | 8.12 ± 2.19 | 7.95 ± 1.95 | 5.94 ± 1.83 | 8.17 ± 2.06 |
| $V_{ss}$ (mL kg$^{-1}$) | 690 ± 138 | 652 ± 166 | 916 ± 316 | 726 ± 244 |
| $Ae_{0-24\,h}$ (% of dose) | 66.8 ± 9.66 | 68.2 ± 8.35 | 67.5 ± 10.1 | 65.5 ± 8.59 |
| $GI_{24\,h}$ (% of dose) | 1.38 ± 0.710 | 2.32 ± 1.78 | 4.75 ± 4.93 | 2.74 ± 3.66 |

Values are mean ± s.d. (n = 9 for control, CC and PCM rats; n = 10 for PCMC rats.

*PCM and PCMC groups significantly different from control and CC groups; **PCM group significantly different from control, CC and PCMC groups; ***PCM group significantly different from control and CC groups (all $P < 0.05$).

AUC, area under the plasma concentration–time curve for time zero to time infinity; MRT, mean residence time; CL, clearance; $CL_R$, renal clearance; $CL_{NR}$, non-renal clearance; $V_{ss}$, volume of distribution at steady state; $Ae_{0-24\,h}$, percentage of the dose excreted in the 24 h urine sample; $GI_{0-24\,h}$, percentage of the dose excreted in the gastrointestinal contents (including feces) at 24 h.

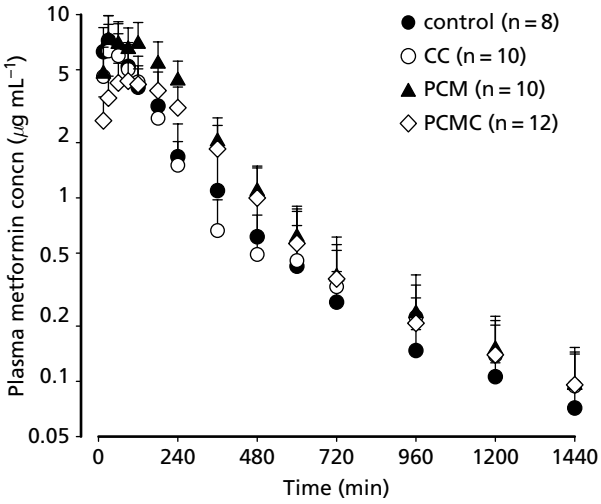


**Figure 2** Mean arterial plasma concentration–time profiles of metformin after oral administration of 100 mg kg$^{-1}$ to control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively). Vertical bars represent s.d.

of metformin in PCM and PCMC rats are required. Choi et al (2006) reported that the hepatic first-pass metabolism of metformin was approximately 27.1% in rats. This low hepatic extraction ratio (Wilkinson & Shand 1975) indicates that the hepatic clearance of metformin in rats depends more on $CL_{int}$ than on hepatic blood flow. The significantly slower $CL_{NR}$ of metformin in PCM rats (Table 3) could be supported by significantly slower $CL_{int}$ in-vitro for the disappearance of the drug than in the controls (Table 2). The contribution of free (unbound to plasma protein) fraction of metformin in plasma to slower $CL_{NR}$ of the drug in PCM rats (Table 3) seems negligible, as plasma protein binding values were comparable between control and PCM rats (Table 1).

As mentioned above, Cho et al (1999) have reported that decreased expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 in PCM rats returned partially to control levels by supplementation with oral cysteine (PCMC rats). Thus, one would expect that the pharmacokinetics of metformin in PCMC rats would at least partly return to control levels compared with PCM rats. $CL_{int}$ (Table 1), AUC, MRT, CL and $CL_{NR}$ of metformin following i.v. administration (Table 3), and AUC of metformin following oral administration (Table 4), were indeed comparable between control and PCMC rats.

Changes in the expression and mRNA levels of some CYP isozymes in the livers of PCM rats (Cho et al 1999) could result from adaptive responses elicited by limitation of protein and energy sources, as explained in our previous paper (Lee et al 2007). However, considering that it was not enough to replace protein and calorie insufficiency in PCM rats with cysteine (PCMC rats; Tables 1, 3 and 4), the restorative effects of cysteine on the expression and mRNA levels of some CYP isozymes in PCMC rats (Cho et al 1999) might be due at least partially to decreased oxidative stress in PCM status (Lee et al 2007). PCM causes oxidative stress in humans and rats (Khaled 1994; Sodhi et al 1998) and increased oxidative stress induced by rifampicin and isoniazid (Sodhi et al 1998). Pahan et al (1997) reported that oxidative stress downregulated the expression of CYP isozymes such

**Table 4** Pharmacokinetic parameters of metformin after oral administration at a dose of 100 mg kg$^{-1}$ to control rats (given metformin only), rats with protein–calorie malnutrition (PCM rats) and control and PCM rats given oral cysteine supplements (CC and PCMC rats, respectively)

| Parameter | Control rats | CC rats | PCM rats | PCMC rats |
|---|---|---|---|---|
| Body weight (g) | | | | |
| Initial | 206 ± 10.6 | 198 ± 9.19 | 197 ± 10.6 | 202 ± 9.37 |
| Final* | 340 ± 15.6 | 318 ± 33.0 | 154 ± 13.9 | 160 ± 16.9 |
| Food intake (g day$^{-1}$ per rat)* | 19.2 ± 0.426 | 18.8 ± 0.170 | 7.86 ± 0.733 | 8.03 ± 0.367 |
| Protein intake (g day$^{-1}$ per rat)* | 4.30 ± 0.141 | 4.32 ± 0.0392 | 0.393 ± 0.0366 | 0.401 ± 0.0183 |
| Calorie intake (kcal day$^{-1}$ per rat)* | 77.2 ± 1.75 | 76.0 ± 0.687 | 31.8 ± 2.96 | 32.4 ± 1.48 |
| 24 h urine volume (mL)** | 33.1 ± 17.2 | 26.1 ± 3.78 | 18.2 ± 6.02 | 24.0 ± 14.1 |
| AUC ($\mu$g min mL$^{-1}$)*** | 1520 ± 440 | 1480 ± 305 | 2290 ± 590 | 1680 ± 444 |
| Terminal half-life (min) | 433 ± 123 | 448 ± 151 | 400 ± 103 | 395 ± 136 |
| $C_{max}$ ($\mu$g mL$^{-1}$)† | 8.26 ± 2.31 | 7.41 ± 2.88 | 8.43 ± 2.48 | 4.74 ± 1.08 |
| $T_{max}$ (min)† | 22.5 (15.0–60.0) | 60.0 (15.0–120) | 60.0 (30.0–120) | 90.0 (30.0–180) |
| $GI_{24h}$ (% of dose) | 8.89 ± 5.56 | 8.33 ± 7.11 | 6.69 ± 6.01 | 9.56 ± 3.05 |
| F (%) | 37.0 | 34.5 | 41.3 | 37.9 |

Values are mean ± s.d. (n = 8 for control rats; n = 10 for CC and PCM rats; n = 12 for PCMC rats).
*PCM and PCMC groups significantly different from control and CC groups ($P < 0.05$);**PCM group significantly different from control group;***PCM group significantly different from control, CC and PCMC groups; †PCMC group significantly different from control, CC and PCM groups (all $P < 0.05$).
AUC, area under the plasma concentration–time curve for time zero to time infinity; $C_{max}$, maximum plasma concentration; $T_{max}$, time of $C_{max}$; $GI_{24h}$, percentage of the dose excreted in the gastrointestinal contents (including faeces) at 24 h; F, absolute oral bioavailability.

as CYP2E1. Cysteine can reduce oxidative stress through a mechanism involving elevation of intracellular glutathione levels. In other words, part of cysteine can be converted to intracellular glutathione in the liver, which plays a critical role in the detoxification of reactive intermediates of oxidative metabolism (Miura et al 1992; Stipanuk et al 1992). A previous study has shown that the cysteine concentration in the liver was significantly lower in PCM rats than in controls (79% decrease), and the value in PCMC rats was significantly higher than in PCM rats (238% increase) and lower than in control rats (29.1% decrease) (Kim et al 2003). The glutathione concentration in the livers of PCM rats was also significantly lower than in controls (84.5% decrease) and the value in PCMC rats was significantly higher than in PCM rats (680% increase) and control rats (20.7% increase) (Kim et al 2003). Thus, changes in the expression and mRNA levels of some CYP isozymes in PCM and PCMC rats (Cho et al 1999) seem to be affected, at least partially, by cysteine and glutathione contents in the liver (Kim et al 2003).

The $CL_R$ of metformin in the four groups of rats was estimated from the free fraction of drug in plasma based on the $CL_R$ (Table 3) and plasma protein binding of metformin (Table 1). Estimated values were 19.2, 20.3, 14.7 and 17.9 mL min $kg^{-1}$ for control, CC, PCM and PCMC rats, respectively, which are all considerably faster than the GFR, as estimated by $CL_{CR}$ (Table 1), indicating that metformin is primarily secreted in renal tubules in all groups of rat. Scheen (1996) also reported the active renal excretion of metformin in humans.

The AUC of metformin was also significantly greater in PCM rats than in control rats following oral administration (Table 4). However, this was unlikely to be due to increased absorption of metformin from the GI tract in PCM rats. After oral administration of metformin, only 7–10% of the oral dose was collected in the GI contents (Table 4). This low proportion of unchanged metformin might be partly attributed to the GI (including biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics (Choi et al 2006), the mean 'true' fraction of the oral dose unabsorbed ($F_{unabs}$) in this study could be estimated by following equations (Lee & Chiou 1983): $0.0889 = F_{unabs} + (0.370 \times 0.0138)$ for control rats; $0.0833 = F_{unabs} + (0.345 \times 0.0232)$ for CC rats; $0.0669 = F_{unabs} + (0.413 \times 0.0475)$ for PCM rats and $0.0956 = F_{unabs} + (0.379 \times 0.0274)$ for PCMC rats, in which the first value in parentheses in each equation is F (Table 4) and the second value is the $GI_{24h}$ of metformin following i.v. administration (Table 3). Values for $F_{unabs}$ were thus estimated to be 8.38, 7.53, 4.73 and 8.52% for control, CC, PCM and PCMC rats, respectively. Thus, more than 90% of the oral dose of metformin was absorbed from the GI tract in all groups of rats. Reasons for the higher AUC of metformin in PCM rats following oral administration are likely to be the same as given for the i.v. study. Following oral administration of metformin, the AUC was significantly greater in PCM rats than in controls (50.7% increase) (Table 4); this 50.7% increase is considerably greater than the 34.8% increase after i.v. administration (Table 3). Thus, decreased expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 in PCM rats cannot entirely explain the considerably greater difference in the AUC of metformin after oral administration to PCM rats (Table 4). Inhibition of the metabolism of metformin in the intestines of PCM rats could explain the difference, as the GI first-pass effect of metformin was considerable in rats: 53.8% of the oral dose at 100 mg $kg^{-1}$ (Chio et al 2006). It has been reported (Kaminsky & Fasco 1991; Kaminsky & Zhang 2003; Martignoni et al 2006) that the CYP enzymes expressed in rat intestine are mostly of the CYP3A, and expression of CYP2C11 and CYP2D isozymes is very low. Studies into changes in CYP2C11, CYP2D1 and CYP3A1/2 in the intestines of PCM rats are required.

The aforementioned effects of cysteine on metformin pharmacokinetics in PCM rats (PCMC rats) have been 'indirect' mechanisms via changes in hepatic CYP enzymes. However, cysteine may also affect metformin pharmacokinetics through a 'direct' interaction between cysteine and metformin. We tested this by administering metformin both i.v. and orally to control rats (CC rats). Cho et al (1999) reported that cysteine does not affect hepatic CYP isozymes in control rats. We observed no differences in the pharmacokinetics of metformin after either i.v. or oral administration between control and CC rats (Tables 3 and 4), thus excluding the likelihood of a 'direct' interaction between cysteine and metformin.

## Conclusion

Following i.v. administration of metformin, the $CL_{NR}$ was significantly slower in PCM rats than in controls (Table 3), probably due to decreased expression and mRNA levels of hepatic CYP2C11 and CYP3A1/2 in PCM rats (Cho et al 1999). The slower $CL_{NR}$ of metformin in PCM rats is supported by significantly slower $CL_{int}$ for the disappearance of metformin. Interestingly, changes in $CL_{int}$, AUC, MRT, CL and $CL_{NR}$ of metformin following i.v. administration, and AUC following oral administration, in PCM rats were returned to control levels by oral cysteine supplementation, presumably because levels of the above CYP isozymes were completely or partially returned to control levels (Cho et al 1999).

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