Arch Pharm Res Vol 25, No 5, 709-717, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Effects of Acute Moderate Hypoxemia on Kinetics of Metoclopramide and its Metabolites in Chronically Instrumented Sheep

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(Received August 10, 2002)

Hypoxemia is known to induce various physiological changes which can result in alteration in drug pharmacokinetics. To examine the effect of acute moderate hypoxemia on metoclopramide (MCP) pharmacokinetics, a continuous 14-hour infusion of MCP during a normoxemic, hypoxemic and subsequent normoxemic period was conducted in eight adult sheep. Arterial blood and urine samples were collected to examine the effects on the pharmacokinetics of MCP and its deethylated metabolites. MCP and its mono- and di-deethylated metabolites were quantitated using a GC/MS method. Steady-state concentrations of MCP were achieved in each of the three periods. During hypoxemia, MCP plasma steady-state concentration increased significantly from 50.72 ± 1.06 to 63.62 ± 1.79 ng/mL, and later decreased to 55.83 ± 1.15 ng/mL during the post-hypoxemic recovery period. Total body clearance (CL_{TB}) of MCP was significantly decreased from 274.2 ± 48.0 L/h to 205.40 ± 28.2 L/h during hypoxemia, and later restored to 245.8 ± 44.2 L/h during the post-hypoxemic period. Plasma mono-deethylated MCP concentration (32.78 ± 1.73 ng/mL) also increased, compared to the control group (21.20 ± 1.39 ng/mL), during hypoxemia and subsequent normoxemic period. Renal excretion of MCP and its metabolites was also decreased during hypoxemia, while urine flow was increased with a concomitant decrease in urine osmolality. Thus, the results indicate that acute moderate hypoxemia affects MCP pharmacokinetics.

Key words: Metoclopromide, Hypoxemia, Pharmacokinetics, GC/MS

INTRODUCTION

Metoclopramide (MCP) is a procainamide analogue with central and peripheral dopamine antagonist activities (Harrington *et al.*, 1983). It is a potent antiemetic and gastric motility modifier used in the treatment of nausea and vomiting of various etiologies (Desmond *et al.*, 1986). MCP is rapidly cleared from the blood by the liver (Desmond *et al.*, 1986) and undergoes N-deethylation to generate N-monodeethyl MCP [mdMCP] and N,N-didethyl MCP [ddMCP] (Teng *et al.*, 1977). MCP and its metabolites may undergo further glucuronide- and sulphate-conjugations in humans and some species (Cowan *et al.*, 1977).

Moderate hypoxemia (HO) is known to reduce the clearance of theophylline (Letarte *et al.*, 1984), phenytoin (du Souich *et al.*, 1986) and lidocaine (Marleau *et al.*, 1987). It has been suggested that a hypoxemia-induced reduction in drug clearance may be associated with a decrease in the activity of hepatic cytochrome P-450 (Kennedy *et al.*, 1998; Ng *et al.*, 2000). Since hypoxemia affects the deethylation of lidocaine (Marleau *et al.*, 1987) and the deethylation of MCP shares similar chemical characteristics with that of lidocaine, it was hypothesized that moderate hypoxemia would also affect the kinetics of MCP.

The aims of the present study were to examine the effect of acute moderate hypoxemia on the plasma and renal pharmacokinetics of MCP and its metabolites and on various physiological parameters in chronically instrumented sheep.

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MATERIALS AND METHODS

Animals and Materials

The studies described here were approved by the

Committee on Animal Care of the University of British Columbia and conformed to the regulations of the Canadian Council on Animal Care. Eight ewes (Dorset, Suffolk, or crossbred) with body weights of 53.1-69.5 Kg (mean 61.4 ± 2.0 Kg) were used in the study. The ewes were brought into the research unit about one week prior to the surgery to allow them to become accustomed to the environment. Animals were fasted overnight before surgery and each received a 3 mg intravenous injection of atropine sulphate to reduce salivation (Astra Pharmaceuticals Inc., Mississauga, ON, Canada) 10-15 min prior to induction of anaesthesia with pentothal (15 mg/kg, Abbott Laboratories, Montreal, QC, Canada). Following endotracheal intubation, anaesthesia was maintained by ventilating the ewes throughout the surgery with 1.0-1.5% halothane (Ayerst Laboratory, Montreal, QC, Canada) and 60-70% N2O in oxygen. Aseptic techniques were employed throughout surgery. *Via* an incision in the groin, sterile silicone rubber catheters (Dow Corning, Corning, NY, USA) filled with heparinized (12 U/mL) 0.9% saline, were implanted in the femoral vein and artery. The catheters were tunneled subcutaneously, and exteriorized through a small incision in the right flank of the ewe where they were stored in a cloth pouch. A catheter for nitrogen infusion (10.0 mm o.d.) was then implanted in the trachea, bet- ween adjacent tracheal rings, 5-6 cm below the larynx, and inserted for 4-5 cm; this catheter did not affect normal breathing by the ewe (Gleed *et al.*, 1986). Immediately following surgery, intramuscular doses of ampicillin (500 mg; Novopharm Ltd., Toronto, ON, Canada) and genta- micin (40 mg; Schering Ltd., Pointe Claire, QC, Canada) were administered to the ewe. Following surgery, the ewes were kept in holding pens with other sheep and given free access to food and water. Intramuscular ampicillin (500 mg) and gentamicin (40 mg) were also given prophylactically to the ewe for 3 days following surgery. All animals were allowed to recover for a mini-mum of 3 days before experiments.

Experimental Protocol

Treatment Group

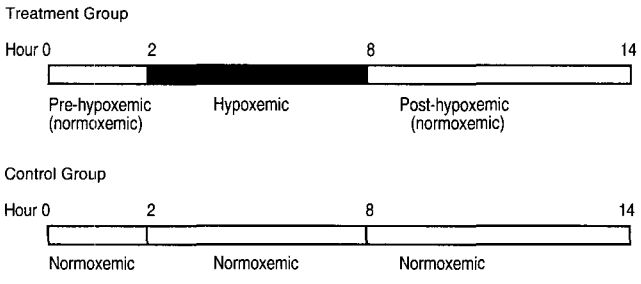
On study days, the ewe was placed in a monitoring cage adjacent to the holding pen in full view of companion ewes and with free access to food and water. A Foley® catheter (Bard Urological Div., CR Bard Inc., Conington, GA, USA) was inserted into the bladder via the urethra for continuous urine collection. Metoclo- Pramide HCl (Reglan® injectable, 5 mg/mL; A.H. Robins, Montreal, QC, Canada) was diluted with sterile isotonic saline to 3 mg/mL. A continuous infusion at the rate of 0.21 mg/min (0.07 mL/min) for 14 hours, employing a Harvard infusion pump (model 944, Harvard Apparatus, Millis, MA, USA), was preceded by a loading dose of 15 mg (5 mL) given *via* the femoral vein. After reaching MCP steady-state during normoxemia (2 hours), hypoxemia was induced by giving 7 L/min of nitrogen through the implanted tracheal catheter. The blood gas status was monitored by taking arterial blood samples at given intervals and the nitrogen flow rate was adjusted to maintain the hypoxemia at the desired level of PO2 (i.e., about 60 mm Hg). The hypoxemic period was continued for 6 hours. Then, the nitrogen flow was stopped and normal air breathing was resumed. The MCP infusion was continued for another 6 hours (See Scheme 1). The total infusion time of MCP was 14 hours. Arterial blood (2.5 mL) samples for MCP determination were taken over the 14 hour period (-0:05, 0:05, 0:15, 0:30, 0:45, 1:00, 1:15, 1:30, 2:00 [pre-HO]; 2:05, 3:00, 4:00, 5:00, 6:00, 7:00, 7:15, 7:30, 8:00 [HO]; 8:05, 9:00, 10:00, 11:00, 12:00, 13:00, 13:15, 13:30, 14:00 hr [post-HO]). The blood samples for drug assay were transferred immediately to a hepar- inized Vacutainer® (Becton-Dickinson, Rutherford, NJ, USA) and centrifuged at 3500 rpm for 10 min. Cumulative urine samples were collected every hour. Urine volume and pH were measured with a graduated cylinder and a Fisher Accumet® pH meter model 620 (Fisher Scientific Inc., Cambridge, MA, USA), respectively. Plasma and urine samples were transferred to disposable borosilicate glass culture tubes (Corning Glass Works, Corning, NY, USA) with Teflon-lined screw caps and stored at -20°C until the time of assay.

Control Group

Control experiments were also carried out, using the same MCP infusion and sampling protocol, but without the induction of hypoxemia as shown below.

Recording Procedures and Blood Gas Analysis

For 24 hours before, during and 24 hours following MCP infusion, arterial blood pressure was measured using a disposable DTX pressure transducer (Spectramed, Oxnard, CA, USA). Heart rate was measured from the arterial pulse pressure using a cardio-tachometer (Model 9857, Sensormedic Corp., Anaheim, CA, USA). These variables were recorded on a polygraph recorder (Beckman R612 recorder, Beckman, Schiller Park, IL or Gould TA4000 thermal array recorder, Gould Inc., Valley View, OH, USA). The analog signals of arterial pressure and heart rate were converted simultaneously to digital data



using an on-line computer system consisting of an Apple IIe computer, Interactive systems A-D converter (Daisy Electronics, Newton Square, PA, USA) and clock card (Mountain Software, Scott's Valley, CA, USA) with a sampling rate of 15 Hz. The one- minute average values of arterial pressure and heart rate were stored on floppy diskettes. Samples (ca. 1 mL) for blood gas analysis and glucose/lactate measurement were taken simultaneously with those for drug analysis. Arterial PaO_2, $PaCO_2$, base excess and pH were measured with an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy) set at a temperature of 37.0°C and corrected to 39.0°C (normal body temperature in sheep). Oxygen satura- tion and hemoglobin concentration were measured, in duplicate, using an OSM2 Hemoximeter® (Radiometer, Copenhagen, Denmark). Blood glucose and lactate con- centrations were measured, in triplicate, using a glucose/lactate 2306 STAT plus analyzer (YSI Inc., Yellow Spring, OH, USA). Urine osmolality was measured by the freezing-point depression method using an Advanced DigiMatic® Osmometer, Model 3D2 (Advanced Instru-ments, Norwood, MA, USA).

Assay Procedure

The measurement of metoclopramide and its metabolites, mdMCP and ddMCP, was accomplished using a capillary gas chromatography-mass spectrometer (GC/MS), in electron impact ionization mode with selective ion monitoring (SIM) previously developed in our laboratory (Riggs *et al.*, 1994). This method is capable of measuring metoclopramide, mdMCP and ddMCP in plasma, urine and other biological fluids over a standard curve range from 1-40 ng/mL, with an average CV < 10%, using small volumes (0.01-0.5 mL) of these fluids.

Data Analysis

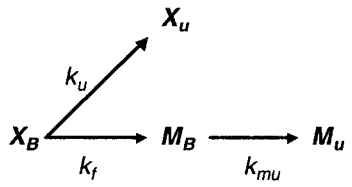
Total body clearance (CLTB) was estimated as:

$$CL_{TB} = k_o/C_{pss}$$

where k_o is the infusion rate and C_{pss} is the apparent arterial steady-state concentration. Renal clearance values of MCP and mdMCP were calculated by a) dividing the accumulated drug (or metabolite) recovered in urine (D_u) by the partial area under the plasma drug (or metabolite) concentration vs. time curve (pAUC) during the hypoxemic and normoxemic periods [$D_{u(t2-t1)}/pAUC_{(t2-t1)}$] and b) using the slope of the accumulated drug (or metabolite) in urine (D_u) versus AUC curve. The fractional renal excretion rate constant of MCP ($fu = k_u/K_E$) was calculated by dividing the slope of the asymptote of the accumulated MCP in urine vs. time curve with the infusion rate, k_o.

$$D_u = (k_u * k_o/K_E)\, t - (k_u * k_o/K_E^2)$$

Slope of the asymptote = $(k_u * k_o)/K_E$



where X_B and M_B represent the amount of drug and metabolite in the body, respectively X_u and M_u represent the drug and metabolite excreted in urine, respectively. k_u and k_f are renal excretion and metabolite elimination rate constants and the sum of these two rate constants is equal to K_E, the apparent first order drug elimination rate constant. k_{mu} represents renal elimination rate constant of the metabolite.

Since k_o is a known parameter, the ratio (k_u/K_E) can be calculated. The fractional renal metabolite excretion constants for mdMCP and ddMCP were also calculated in the study. These parameters are the product of the two fractional constants, $f_{m(metabolite)}$ and $f_{mu(metabolite)}$. The fractional metabolite formation rate constant $f_{m(metabolite)}$ is the fraction of $k_{f(metabolite)}/K_{E(parent\ drug)}$, which represents the proportion of metabolic elimination of a specific metabolite to the total parent drug elimination. The second fractional constant, the fractional renal metabolite elimination rate constant $f_{mu(metabolite)}$, is the fraction of $k_{mu(metabolite)}/K_{m(metabolite)}$ which represents the renal excretion proportion of the total metabolite elimination. Therefore, the composite fractional constant, a fractional renal metabolite excretion rate constant $f_{u(metabolite)} = f_{m(metabolite)} * f_{mu(metabolite)}$, represents the proportion of renal excretion of a specific metabolite to the total drug elimination.

Statistical evaluations were performed on various pharmacokinetic and physiological parameters using either the Student's t-test (paired or unpaired) or ANOVA/Tukey test. The level of significance was chosen to be $p < 0.05$. The mean values in the text and tables are presented as the mean ± standard error of the mean (SEM).

The attainment of steady-state plasma MCP concentration was evaluated according to the following procedures and criteria:

1. Visual inspection: the plot of plasma concentration vs. time was visually inspected for a plateau portion.
2. Coefficient of variance: the coefficient of variation for the data set of plasma MCP concentrations in the plateau portion was calculated. A maximum criterion of CV = 10% was used.
3. Student's t-test/Analysis of Variance (ANOVA) for the regression: the slope of linear regression line from the data set of plasma MCP concentration in the plateau portion was analyzed using a two-tailed t-test and ANOVA with the null hypothesis (H_o): slope = 0 against the alternative hypothesis (H_A): slope $\neq 0$ with $\alpha = 0.05$. In these tests, the rejection of H_o suggests that the plasma drug concentration tends to either increase

or decrease in a given period (*i.e.*, a steady-state was not achieved).

RESULTS

Blood gas status and pH

Body weight averaged 61.4 ± 3.5 Kg (n = 4) within the treatment group and 61.3 ± 2.1 Kg (n = 4) in the control group. The initiation of nitrogen infusion through the intratracheal catheter in the treatment group decreased arterial PaO_2 within 5 min. During the hypoxemic period, arterial PaO_2, $PaCO_2$, and O_2 saturation were significantly reduced (Table 1), whereas no changes were observed in the control group. In two of the animals, the nitrogen flow was increased to about 9-11 L/min from 7 L/min to maintain PaO_2 at levels close to 60 mmHg. After cessation of the nitrogen infusion, arterial PaO_2, $PaCO_2$, and O_2 saturation were restored during the post-hypoxemic period in the treatment group (Table 1). Unlike the blood gas variables, arterial blood pH did not change during the hypoxemic period, although a trend toward alkalosis was observed. In contrast, blood pH was significantly decreased during the post-hypoxemic period in the treatment group. Arterial blood lactate concentration was increased (ca. 60%) during the late hypoxemic (0.85 ± 0.04 mmol/L) and early post-hypoxemic periods (0.88 ± 0.05 mmol/L) from the baseline level (0.56 ± 0.02 mmol/L). Similarly, arterial blood glucose concentration was increased (ca. 10%) during the late hypoxemic (2.9 ± 0.1 mmol/L) and post-hypoxemic period (3.03 ± 0.04 mmol/L) compared to that of pre-hypoxemic period (2.7 ± 0.1 mmol/L).

Table 1. Mean (± SEM) arterial blood pH, gas partial pressure ($PaCO_2$ and PaO_2) and O_2 saturation.

	pre-hypoxemia	hypoxemia	post-hypoxemia
pH			
Treatment	7.424 ± 0.005	7.442 ± 0.006	7.409 ± 0.005 [c]
Control	7.501 ± 0.008	7.495 ± 0.007	7.492 ± 0.006
$PaCO_2$ (mm Hg)			
Treatment	41.3 ± 0.39	37.4 ± 0.48 [a]	40.8 ± 0.58
Control	37.1 ± 0.41	37.7 ± 0.65	37.2 ± 0.50
PaO_2 (mm Hg)			
Treatment	116.9 ± 1.15	60.7 ± 1.57 [a,b]	116.5 ± 0.71
Control	117.7 ± 1.90	116.1 ± 1.88	120.0 ± 1.91
O_2 saturation (%)			
Treatment	100.2 ± 0.40	82.8 ± 1.53 [a,b]	100.2 ± 0.20
Control	97.1 ± 0.17	96.6 ± 0.22	96.6 ± 0.14

[a] $p < 0.05$ [ANOVA/Tukey] from pre-HO and post-HO.
[b] $p < 0.05$ [paired *t*-test] from the control group.
[c] $p < 0.05$ [ANOVA/Tukey] from pre-HO and HO.

Mean arterial blood pressure (MAP) and heart rate (HR)

MAP and HR were also significantly increased from 90.3 ± 10.6 mmHg and 109.0 ± 6.6 beats/min to 97.0 ± 10.3 mmHg and 122.5 ± 9.2 beats/min, respectively, during the hypoxemic period in the treatment group. Later, they were restored to the pre-hypoxemic levels ($p > 0.05$) during the post-hypoxemic period. In the control group, no significant changes in MAP and HR were observed through the entire study period.

Urine flow and osmolality

Hypoxemia-induced diuresis was observed in the early to mid hypoxemic period (urine flow: 132 ± 20 mL/hr [HO] from 80 ± 13 mL/hr [pre-HO]) and it returned to normal during the post-hypoxemic period (78 ± 8 mL/hr). During the diuresis, decreased urine pH (7.468 ± 0.011 from 7.532 ± 0.021) and low urine osmolality (808 ± 93 from 1141 ± 38 mOsmol/Kg) were also observed. There was a negative correlation (r = -0.80) between urine osmolality and urine flow. In addition, the renal osmolal excretion rate constant, the product of urine osmolality and urine flow, was also significantly decreased from 88.2 ± 6.4 mOsmol/h in the pre-hypoxemic period to 61.3 ± 4.6 mOsmol/h during the hypoxemic period, and later restored to 89.4 ± 8.6 mOsmol/h in the post-hypoxemic period. In the control group, no changes in urine flow, osmolality and pH were observed though the entire study period.

Plasma MCP and mdMCP kinetics

Semi-logarithmic plots of mean (± SEM) plasma MCP and mdMCP concentration versus time profiles obtained following the initial intravenous loading dose and 14-hour infusion are shown in Fig. 1 for the treatment group and Fig. 2 for the control group. The steady-state concentration of MCP was attained by 30-45 min after the initiation of the infusion in 3 of the 8 ewes, and by 1-1.25 hours in the remaining ewes. Mean plasma MCP steady-state concentrations were estimated using two different methods: a) calculation from the set of individual steady-state concentrations and b) assessment of the mean plasma MCP concentrations over the duration of the experiment. Both estimations appear to be in good agreement (Table 2). During the hypoxemic period, the mean MCP steady-state concentration increased significantly in the treatment group (Table 2) from the pre-hypoxemic period and from the control group. During the post-hypoxemic period, the plasma MCP steady-state concentration decreased to a concentration close to that seen in the pre-hypoxemic group (Table 2). Total body clearance of MCP was also significantly decreased during hypoxemia, and later returned to the control value in the post-hypoxemic period (Table 2). The mean MCP steady-state concentration and

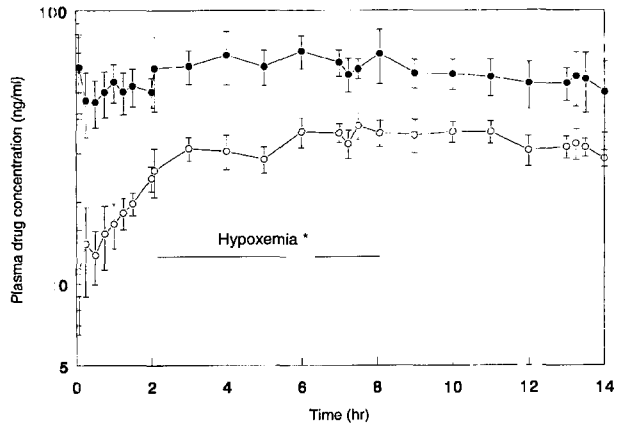


Fig. 1. Mean (± SEM) plasma MCP and mdMCP concentration (ng/mL) over the duration of the study [treatment group]. The period of hypoxemia is indicated by the solid line. (●: MCP; ○: mdMCP).

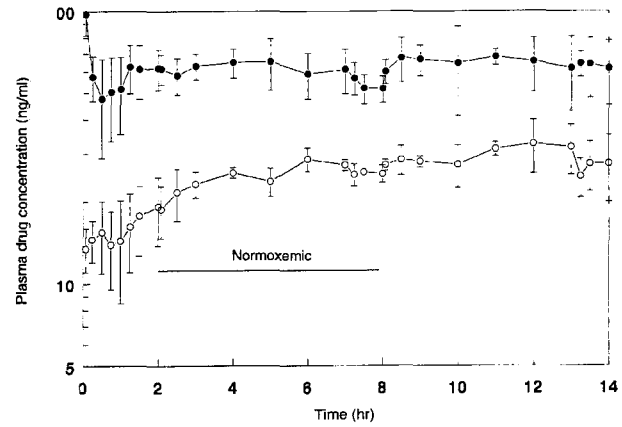


Fig. 2. Mean (± SEM) plasma MCP and mdMCP concentration (ng/mL) over the duration of the study [control group]. The period of normoxemia is indicated by the solid line. (●: MCP; ○: mdMCP).

total body clearance in the control group remained the same throughout the entire study period.

Visual inspection of the plasma mdMCP concentration-time profiles (Figs. 1 and 2) suggested that the plasma mdMCP concentrations also reached an apparent steady-state however, the time to an apparent steady-state was longer than for MCP, ranging from 4 to 5 hours. The plasma mdMCP concentrations were not significantly changed during the hypoxemic (34.01 ± 3.28 ng/mL) and post-hypoxemic (32.72 ± 2.50 ng/mL) periods in the treatment group. However, these values were significantly higher than the corresponding values in the control group (20.78 ± 2.82 and 23.31 ± 2.36 ng/mL for the hypoxemic and post-hypoxemic periods, respectively), suggesting a higher plasma mdMCP accumulation. No detectable concentration of ddMCP was observed in plasma samples.

Renal MCP and metabolite kinetics

The renal clearance of MCP and mdMCP were calculated by a) dividing the amount recovered (D_u for MCP; M_u for mdMCP) by the partial area under drug plasma concentration curve (pAUC) as a function of time during the hypoxemic and normoxemic periods [$D_{u(t2\text{-}t1)}/pAUC_{(t2\text{-}t1)MCP}$] and [$M_{u(t2\text{-}t1)}/pAUC_{(t2\text{-}t1)mdMCP}$] and b) using the slope of the accumulated drug or metabolite in urine (D_u or M_u) vs. (AUC_{MCP} or AUC_{mdMCP}). Due to the short duration of the pre-hypoxemic period, renal clearance during this time period could not be accurately estimated. Therefore, renal clearances calculated during hypoxemia were only compared to those estimated in post-hypoxemic period. Using both methods, the renal clearance of MCP was observed to be 33-75% lower (mean 64 ± 9%) during hypoxemia

Table 2. Mean (± SEM) steady-state plasma MCP concentrations ($C_{pss(MCP)}$) and plasma MCP total body clearance ($CL_{TB(MCP)}$) during a MCP infusion.

	pre-hypoxemia	hypoxemia	post-hypoxemia
$C_{pss(MCP)}$ (ng/mL)			
Treatment	49.9 ± 7.8 [b]	64.3 ± 7.2 [d]	55.6 ± 8.1
	50.7 ± 1.1 [c]	63.6 ± 1.8 [e,f]	55.8 ± 1.2
Control	49.9 ± 7.1	54.1 ± 8.8	54.8 ± 8.1
	50.7 ± 1.0	54.2 ± 2.2	55.8 ± 1.2
$CL_{TB(MCP)}$ [a]			
Treatment	274 ± 48	205 ± 28 [e]	246 ± 44
	(4.47 ± 1.04)	(3.33 ± 0.66) [e]	(4.00 ± 0.96)
Control	270 ± 42	250 ± 36	245 ± 36
	(4.37 ± 0.58)	(4.05 ± 0.52)	(3.96 ± 0.47)

[a] *unit: L/h and values in parentheses normalized to body weight (L/h/Kg).*
[b] calculated from the individual steady-state concentrations.
[c] calculated from mean plasma concentration *vs.* time curve.
[d] $p < 0.05$ [ANOVA/Tukey] compared to pre-HO and HO.
[e] $p < 0.05$ [paired *t*-test] compared to pre-HO and HO.
[f] $p < 0.05$ [Students *t*-test] compared to the control group.

Table 3. Mean (± SEM) renal clearance (CL_{renal}) of MCP and mdMCP during a continuous MCP infusion.

CL_{renal} (L/h)	hypoxemia	post-hypoxemia
MCP		
Treatment	2.89 ± 0.28 [a,c,d]	7.36 ± 1.69
	2.75 ± 0.22 [b,c,d]	8.15 ± 1.86
Control	6.72 ± 0.64	7.04 ± 0.47
	7.01 ± 0.72	6.73 ± 0.59
mdMCP		
Treatment	14.4 ± 2.5 [a,c,d]	35.5 ± 7.4
	14.6 ± 2.6 [b,c,d]	38.6 ± 9.1
Control	44.2 ± 7.8	42.6 ± 8.5
	43.9 ± 9.4	38.4 ± 7.1

[a,b] calculated according to method a and b, respectively, in data analysis section.
[c] $p < 0.05$ [paired *t*-test] compared to post-HO.
[d] $p < 0.05$ [Students *t*-test] compared to the control group.

Table 4. Mean (± SEM) fractional renal excretion rate constants (f_u) of MCP and its metabolite during a continuous MCP infusion.

f_u		hypoxemia	post-hypoxemia
MCP	Treatment	0.014 ± 0.002 [a,b]	0.033 ± 0.005
	Control	0.030 ± 0.003	0.028 ± 0.004
mdMCP	Treatment	0.038 ± 0.010 [a,b]	0.108 ± 0.034
	Control	0.072 ± 0.009	0.071 ± 0.013
ddMCP	Treatment	0.022 ± 0.006 [a]	0.056 ± 0.015
	Control	0.026 ± 0.005	0.026 ± 0.005

[a] $p < 0.05$ [paired *t*-test] compared to post-HO.
[b] $p < 0.05$ [Students *t*-test] compared to the control group.

compared to the post-hypoxemic period in the treatment group (Table 3). This is in contrast to the control group, where MCP renal clearance remained constant (Table 3). Moreover, the renal clearance of MCP was significantly lower during hypoxemia in the treatment group than in the control group. Similarly, the renal clearance of mdMCP was also lower (59 ± 7%; range 39-69%) during hypoxemia (Table 3). The renal clearance of ddMCP also appeared to be decreased, but could not be accurately assessed due to plasma concentrations below the limit of quantation. The fractional renal excretion constant for MCP, mdMCP and ddMCP, another indicator of renal drug excretion used in the study, were also significantly affected by hypoxemia (Table 4), suggesting impaired renal excretion of MCP and its deethylated metabolites.

DISCUSSION

The treatment conditions in the present study induced a moderately low PaO_2, which remained stable throughout the hypoxemic period. The nitrogen was mixed with the air normally inhaled by the animal, resulting in a lowering of inspired O_2 concentration. In addition, intra-tracheal nitrogen infusion caused a statistically significant hypocapnia likely due to hyperventilation, and an apparent trend towards respiratory alkalosis was observed, though it did not reach statistical significance.

Blood lactate/glucose and hypoxemia

An increased blood lactate concentration was observed in the late hypoxemic period and it remained relatively high through the early and middle post-hypoxemic periods. However, this accumulation of lactate is not likely the result of inadequate oxygen supply to the tissues, since O_2 saturation during hypoxemia remained at about 70-80%. Rather, it is likely related to hypocapnia and the resulting changes in acid-base balance, elevated glucose levels and hyperventilation (Huckabee, 1958; Zborowska *et al.*, 1967) during hypoxemia and/or subsequent recovery periods. In addition, increased sympathetic activity is likely a contributing factor in the increased blood lactate and glucose levels (Baum *et al.*, 1980; Rizza *et al.*, 1980) as well as in the elevated heart rate and mean arterial blood pressure during hypoxemia.

Urine flow/osmolality and hypoxemia

In the present study, urine flow was significantly increased and urine osmolality was significantly decreased during hypoxemia. Hypoxemia-induced diuresis, either hypocapnic or isocapnic, has been reported in the conscious dog (Walker, 1982) and rat (Colice *et al.*, 1991). Unlike these previous studies which showed no change in urine osmolality, there was a significant fall in urine osmolality during hypoxemia in the present study. The renal osmolal excretion rate was also significantly decreased. Therefore, the diuresis in the present study may be due to enhanced water excretion (*i.e.*, decreased water re-absorption) independent of renal electrolyte excretion.

Hypoxemia and MCP kinetics

The present study shows that acute moderate hypoxemia does affect plasma MCP pharmacokinetics in the chronically instrumented conscious ewes. A moderate but statistically significant ($p < 0.05$) decrease in total body clearance of MCP (*i.e.*, higher plasma MCP steady-state concentration) was observed during the hypoxemic period. A reduction in total body drug clearance (CL_{TB}) during acute hypoxemia has been observed with a number of drugs such as theophylline (Saunier *et al.*, 1987), furosemide (Babini *et al.*, 1986) and propofol (Audibert *et al.*, 1992). Drugs such as hexobarbital and antipyrine, which undergo oxidative biotransformation by the hepatic mixed oxygenases, are directly affected by reduced oxygen tension (Jones, 1981). In addition, it was reported that acute hypoxemia decreases the activity of several isoenzymes of cytochrome P-450 (Kennedy *et al.*, 1998; Ng *et al.*, 2000). There are also observations that moderate hypoxia could promote the formation of oxygen free-radicals (Proulx *et al.*, 1990; Brass *et al.*, 1991) that are able to diminish the activity of certain isozymes of cytochrome P-450. These reports suggest that acute hypoxemia reduces drug elimination processes in general, which is in agreement with the findings of the present study.

Hypoxemia and MCP metabolism

No studies on the enzymatic pathway for the oxidative N-deethylation of MCP to mdMCP and ddMCP have been reported. However, the N-deethylation of a structurally similar compound, lidocaine, appears to be mediated by hepatic cytochrome P-450 (CYP) enzymes (Bargetzi *et al.*, 1989) and there are many reports that N-deakylation reactions such as demethylation, deethylation are controlled by the same system. In addition, the liver is thought

to be the major metabolic site for MCP (Kapil *et al.*, 1984). Therefore, it is probable that the N-deethylation of MCP is also mediated by the CYP enzyme system. *In vitro* studies have demonstrated that a low partial pressure of oxygen reduced the K_m value for the demethylation of ethylmorphine (Holtzman *et al.*, 1983; Erickson *et al.*, 1982) and for the hydroxylation of phenytoin (Tsuru *et al.*, 1982), indicating that adequate oxygen pressure may be essential for the selected route of biotransformation. In conscious rabbits, hypoxemia reduced the demethylation and/or the hydroxylation of theophylline (Letarte *et al.*, 1984) and the hydroxylation of phenytoin (du Souich *et al.*, 1986). In addition, acute hypoxemia appears to decrease CYP activity in humans (Jurgens *et al.*, 2002). These studies suggested that hypoxemia may affect several CYP isozymes and thus may explain, in part, the accumulation in plasma of MCP and mdMCP due to reduced metabolic elimination of the drug. The effect of hypoxemia on plasma ddMCP concentration was not determined due to the low plasma concentrations of this metabolite. Alternatively, changes in hepatic blood flow during acute hypoxemia-hypocapnia could also explain the reduction of MCP clearance. Moderate hypoxemia alone appears to exert only minimal effect on total hepatic blood flow (du Souich *et al.*, 1992). However, hypoxemia in combination with either hypocapnia or hypercapnia may reduce total hepatic blood flow more than hypoxemia alone (Mathie *et al.*, 1983). Since hepatic elimination of MCP is essentially modulated by the blood flow to the liver [flow-limited] (Bateman *et al.*, 1980), the effect of acute hypoxemia-hypocapnia on MCP clearance may also be explained by a reduction in total hepatic blood flow.

Hypoxemia and mdMCP kinetics

Plasma mdMCP concentration also appeared to reach an apparent steady-state in both treatment and control groups. However, it is not possible to confirm that steady-state in a conventional sense, was achieved in the study, since the input rate constant of mdMCP (*i.e.*, a product of infusion rate of MCP and k_f (MCP → mdMCP)) could not be evaluated in the current infusion design. Plasma mdMCP concentrations were significantly higher in the treatment group as compared to the control group. The increased mdMCP concentration in the treatment group appears to be related to the induction of hypoxemia, however, mdMCP concentration did not decrease during the post-hypoxemic period. The interpretation of metabolite kinetics, mainly mdMCP in this study, is more complicated than the parent drug, since plasma mdMCP concentrations depend on: a) the rate of N-deethylation of MCP that yields mdMCP; b) renal elimination rate of mdMCP and c) biotransformation rates of mdMCP. As previously mentioned, the input rate of mdMCP (the rate of N-deethylation of MCP) could not be directly determined in the present study. However, it is highly unlikely to have been increased during hypoxemia, since the total body clearance of MCP was decreased and, as noted above, there are reports that the activity of the CYP enzyme, which mediates N-deethylation reactions appears to be reduced during acute hypoxemia. Therefore, one or more of the rate processes involved in mdMCP elimination may be diminished, such as renal excretion and subsequent metabolism of mdMCP, during hypoxemia.

Hypoxemia and renal excretion of MCP and its metabolites

The renal excretion of MCP and its metabolites was examined in the present study. Since the infusion protocol was used, the conventional methods of determining urinary excretion parameters such as the plots of A.R.E. (amount remained to be excreted) vs. time or urinary excretion rate vs. time may not be applicable. In addition, alteration in drug elimination during hypoxemia may complicate the estimation of renal drug excretion parameters. Therefore, two modified methods (see Materials and Methods) of determining urinary drug excretion during the phased infusion were developed and applied in the study.

Previous studies on renal clearance during acute hypoxemia have reported various responses. No changes in renal clearance during acute hypoxemia were observed with many drugs such as theophylline (Letarte *et al.*, 1984), furosemide (Babini *et al.*, 1986) and lidocaine (du Souich *et al.*, 1992). The renal clearance of phenytoin decreased during acute hypoxemia (from 0.2 to 0.03 mL/min/Kg), but this apparently was not statistically significant (du Souich *et al.*, 1986). Alternatively, renal clearance of sulfamethazine appears to be increased, but not significantly, during acute hypoxemia (du Souich *et al.*, 1984). The renal clearance of digoxin was significantly increased during acute hypoxemia (du Souich *et al.*, 1985). In one of the studies, renal clearance of metabolites was measured, and no significant changes in renal clearances of lidocaine metabolites, monoethylglycinexylidide and glycinexylidide, were observed (du Souich *et al.*, 1992).

There was a significant reduction in the renal clearance of MCP and mdMCP during the hypoxemic period in the treatment group. The reduction in MCP renal clearance during hypoxemia (2.75 L/h during hypoxemia and 8.15 L/h during normoxemia) are much more extensive compared to the reduction in total body clearance (205 L/h during hypoxemia and 245 L/h during normoxemia). Although the renal clearance of MCP accounts for only about 5% of total body clearance during normoxemia (about 8 L/h out of 245 L/h), the reduction in renal clearance during hypoxemia accounts for about 15% of the change in total body clearance (about 5.5 L/h out of 40 L/h). Therefore,

the reduction in renal clearance of MCP may have a larger impact on the decrease in total body clearance of MCP during hypoxemia, than is suggested by the contribution of renal drug excretion to MCP elimination during normoxemia. Similarly, the renal clearance of mdMCP was significantly affected by hypoxemia (14.6 L/h during hypoxemia and 38.6 L/h during normoxemia). However, the total body clearance of mdMCP could not be determined in this study, thus quantitative assessment of the contribution of the mdMCP renal elimination to total body clearance of MCP could not be evaluated. The renal clearance of mdMCP (~40 L/h, 11 mL/min/Kg) was much higher than the reported GFR (2.4 mL/min/Kg) (Hill *et al.*, 1988) in adult sheep, suggesting active tubular renal secretion (Roland *et al.*, 1989) of mdMCP. It has been reported that specific active renal secretion pathways exist for both basic and acidic compounds (Ganong, 1985). Therefore, the impairment of these secretory mechanisms during hypoxemia may contribute to the reduced renal clearance of mdMCP. The renal elimination of ddMCP was also significantly affected by acute hypoxemia. Therefore, acute hypoxemia appears to reduce the renal elimination of MCP and its deethylated metabolites.

Overall, the present study of the pharmacokinetics of MCP and its deethylated metabolites, mdMCP and ddMCP, during acute moderate hypoxemia demonstrates that there is a small (ca. 20%) but statistically significant reduction in the elimination of MCP and its metabolites, and that this reduction appears to be associated with reduced renal elimination of the parent drug and its metabolites.

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