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Original Research Paper

Evaluation of pharmacokinetics underlies the collaborated usage of lamivudine and oxymatrine in beagle dogs

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ABSTRACT

Combinational therapy of lamivudine and oxymatrine has been employed in the battle against hepatitis B virus in clinical setting. However, the pharmacokinetic behavior of the drug or active metabolism in intravenous/oral co-administration regime is poorly investigated. Herein, we evaluated the pharmacokinetic characteristic through a tailor-designed 3 way crossover-Latin square experiment in adult male beagle dogs. Six dogs were randomly treated by intravenous administration of lamivudine (2.5 mg/kg), oxymatrine (15 mg/kg) and combinational dosage, named as intravenous regime. Meanwhile the other six dogs were orally administrated with lamivudine (2.5 mg/kg), oxymatrine (15 mg/kg) and combinational dosage, named as oral regime. The pharmacokinetic feature in simultaneous oral treatment appeared to have no significant difference when compared with individual administration, even including matrine, the active metabolite of oxymatrine. In intravenous regime, the main pharmacokinetic parameters of simultaneous administration were nearly consistent with intravenous regime remedy. The collaborated application of lamivudine and oxymatrine contributed to non-distinctive pharmacokinetic fluctuations of beagle dogs in intravenous/oral regime, compared with individual employment, which established a vital base for the clinical co-administration against hepatitis B. Furthermore, the present study demonstrated that the determination of pharmacokinetics between combinational and individual therapy might assist in the development of drug compatibility in clinical therapy.

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1. Introduction

More than 240 million individuals around the world have suffered from chronic hepatitis B virus (HBV) infection, which is a DNA virus characterized by its reverse transcription for replication in infected hepatocytes [1–4]. At present, the therapy options mainly depend on nucleotide analogues (NA) and interferon alpha (INF-α) [5]. Lamivudine (3TC, Fig. 1A), possessing anti-HBV replicated function, was the first approved oral NA. However, 3TC-resistance phenomena, such as viral drug-resistance and dose-dependent side effects [6], will be likely to emerge in the long-term treatment when using 3TC alone due to the emergence of drug resistance mutations in polymerase protein. To deal with the challenge, co-administrated therapeutic strategy combining 3TC with other antiviral drugs appeared [7–9]. Furthermore, the collaborated employment of lamivudine and oxymatrine could significantly decrease the conversion rate of HBVDNA and hepatitis B e antigen (HBeAg) in patients when compared with mono-therapy of 3TC because of the ability of oxymatrine to inhabit the development of drug resistance to 3TC [6,10–13].

Oxymatrine (OM, Fig. 1B) is a major quinolizidine alkaloid from the Chinese herb *Sophora alopecuraides L.*, *Sophora subprostrata* and *Sophora flavescens Ait.*, and has been extensively used as liver protecting drugs in the treatment of liver ailments in traditional Chinese medicines (TCM) [14]. OM and its active metabolite matrine (M, Fig. 1C) have been proven to have an active antiviral effect on HBV infection in the clinical trials [6,8,11,15,16]. And Xiaoyan Cui reported the superiority of the combination of 3TC with oxymatrine or matrine over 3TC alone [6].

Although the single use of 3TC to treat HBV has largely been replaced by more effective combination with OM, which has been demonstrated previously in the literature, the impact of combination therapy on the pharmacokinetics of each drug or its active metabolite is largely unknown [6]. Herein, the present study is carried out to investigate the effects of OM on the pharmacokinetics of 3TC and to characterize the pharmacokinetic behavior of OM and 3TC during co-administration to beagle dogs following intravenous or oral administration. Whether or not there are significant pharmacokinetic interactions, the results of which will assist in the development of this two-drug combination in clinical therapy strategy.

2. Materials and methods

2.1. Chemicals

3TC (content >98.5%) was purchased from Hefei Scenery Chemical Co., Ltd. (Hefei, Anhui, China). Standard OM, M and famotidine (internal standard) were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Bulk OM (content >98.5%) was purchased from Ningxia Bo-er-tai-li Pharmaceutical Co. Ltd. (Yinchuan, Ningxia, China). Acetonitrile was of HPLC grade and other chemicals were of analytical grade.

2.2. Animals

Twelve healthy adult male beagle dogs (Laboratory Animal Center of Shenyang Pharmaceutical University, Shenyang, Liaoning, China) weighing 10 ± 1.3 kg (mean ± standard error) were used for the pharmacokinetic study. The animal experimental protocols described below were approved by the Animal Care and Use Committee at Shenyang Pharmaceutical University.

2.3. Pharmacokinetic experiments

A randomized 3-way crossover-Latin square experiment with a washout period of 1 week was designed. Dogs ($n = 6$) were randomly assigned to groups to characterize the pharmacokinetics and interaction of 3TC (2.5 mg/kg) and OM (15 mg/kg) given intravenously alone and in combination. Another six dogs were used to characterize the pharmacokinetics and interaction of 3TC (5.0 mg/kg) and OM (30 mg/kg) given orally alone and in combination. The dogs were housed in standard stainless-steel cages under a 12 h light/dark cycle with access to water and standard laboratory diet. The drugs were administered to all dogs following an overnight fast, and access to food was restored 4 h after dosing. In the intravenous administration study, blood samples were collected prior to drug administration and at 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360 and 480 min after dosing through an intravenous catheter placed in the opposite foreleg vein. In the oral administration study, blood samples were collected prior to drug administration and at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 540 and 720 min after dosing through an intrave-

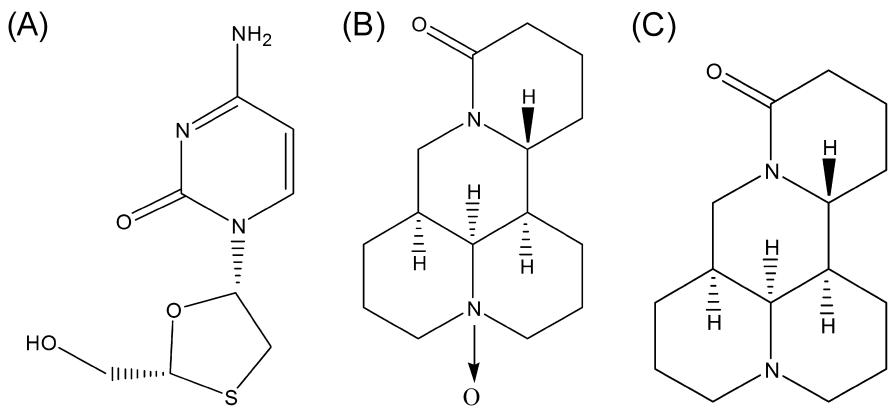

Fig. 1 – Structures of 3TC, OM and M (A-3TC; B-OM; C-M).

nous catheter placed in the foreleg vein. The blood samples were centrifuged immediately, and plasma was stored at –20 °C until analysis.

2.4. Assay of 3TC, OM and M

The 3TC, OM and M concentrations in dog plasma were determined by means of the HPLC-UV method reported. Briefly, the analytes and internal standard were simultaneously extracted from plasma samples with OASIS® HLB Extraction Cartridges (1 cc, 30 mg, Waters, Corp.), and separated on a C_{18} column with an isocratic mobile phase consisting of 13% acetonitrile in 5 mmol/l sodium heptanesulfonate with the pH adjusted to 3.2 with phosphoric acid. The UV absorbance was monitored at 220 nm, and the column temperature was maintained at 40 °C. The linear calibration curves of the analytes were obtained in the concentration range of 0.1 ~ 40 mg/l ($r^2 > 0.999$). The lower limit of quantification of the method was 0.1 mg/l for these analytes. The method was evaluated in terms of recovery, accuracy and precision. The recoveries extraction of 3TC, OM and M was ≥ 70%. Accuracy and precision for the determination fell well within the limits of acceptability (<15%).

2.5. Pharmacokinetic analysis

The maximum concentration (C_{max}) and corresponding peak time (T_{max}) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (k_e) was obtained from the least-squares fitted terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($t_{1/2}$) was calculated from $0.693/k_e$. The area under the curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal rule with extrapolation to infinity with k_e. The mean residence time from zero to infinity (MRT) was estimated by moment analysis. The total clearance from plasma (CL) was calculated as dose/$AUC_{0-\infty}$. The steady-state volume of distribution (V_{ss}) was calculated using (dose ×$AUMC_{0-\infty}$)/($AUC_{0-\infty}$)^2. The relative bioavailability (F_r) was calculated as $AUC_{co\text{-}administration}/AUC_{alone}$. The oral bioavailability (F) was calculated using ($AUC_{p.o.} \times dose_{i.v.}$)/($AUC_{i.v.} \times dose_{p.o.}$).

2.6. Statistical analysis

Data are expressed as the mean ± standard error (SE). Comparisons between co-administration and single-administration groups were performed using the pairwise Student's two one-sided *t*-test, and differences were considered statistically significant when $P < 0.05$.

3. Results and discussion

3.1. Pharmacokinetics of 3TC

The plasma concentrations of 3TC were measured by the validated UPLC method as reported in our previous study. Fig. 2 shows the mean plasma concentration–time profiles for 3TC after either oral or intravenous administrations of 3TC, alone and in combination with OM. The pharmacokinetic parameters for 3TC are demonstrated in Table 1. The absorbed fractions of 3TC, a ratio of the AUCs for single oral or co-combination of OM oral administration to single intravenous administrations, were 80.5% and 73.3%, respectively. There were no statistically significant differences in the main pharmacokinetic parameters of 3TC between mono-therapy and co-administration with OM after either oral or intravenous administrations, despite the appearance of a 13.4% increase in the intravenous$AUC_{0-\infty}$ (from 6.35 ± 1.62 to 5.50 ± 0.68 μg·h/ml) ($P > 0.05$). The results indicated no pharmacokinetic changes in 3TC following oral or intravenous administration when in combination with OM to beagle dogs.

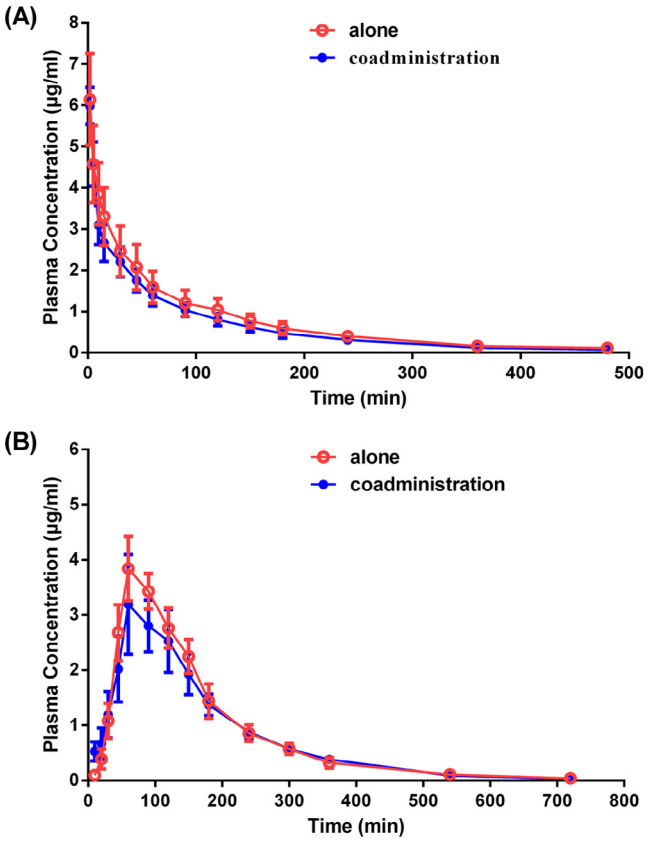

Fig. 2 – (A) Mean plasma 3TC concentration–time curves following two intravenous regimens involving a single dose of 2.5 mg/kg 3TC to beagle dogs: alone (red line) and in combination with a single dose of 15 mg/kg OM (blue line). (B) Mean plasma 3TC concentration–time curves following two oral regimens involving a single dose of 5.0 mg/kg 3TC to beagle dogs: alone (red line) and in combination with a single dose of 30 mg/kg OM (blue line). Each value represents the mean ± SE ($n = 6$).

3.2. Pharmacokinetics of OM

The plasma concentrations of OM were measured by the validated UPLC method as reported by Piao et al. [17]. The mean pharmacokinetic parameters of OM are summarized in Table 2 and the corresponding mean plasma concentration–time profiles for OM are illustrated in Fig. 3. In the case of the pharmacokinetics of OM following intravenous mono- and

Table 1 – Pharmacokinetics of 3TC following intravenous and oral administration alone and in combination with OM.

	Intravenous administration			Oral administration		
	Alone	Combination	P (95% CI)	Alone	Combination	P (95% CI)
C_{max} (μg/ml)	–	–	–	4.12 ± 0.48	3.75 ± 0.82	0.64
T_{max} (h)	–	–	–	1.25 ± 0.11	1.08 ± 0.20	0.36
$t_{1/2}$ (h)	1.79 ± 0.19	1.81 ± 0.16	0.93	1.76 ± 0.19	1.58 ± 0.16	0.53
$AUC_{0-\infty}$ (μg·h/ml)	6.35 ± 1.62	5.50 ± 0.68	0.55	10.22 ± 0.99	9.31 ± 1.30	0.66
MRT (h)	2.05 ± 0.19	2.09 ± 0.16	0.91	2.63 ± 0.14	2.95 ± 0.13	0.12
CL (l/h/kg)[a]	0.57 ± 0.12	0.53 ± 0.07	0.75	0.51 ± 0.05[a]	0.59 ± 0.08[a]	0.52
V_{ss} (l/kg)[b]	1.03 ± 0.20	1.07 ± 0.14	0.86	1.35 ± 0.16[b]	1.77 ± 0.28[b]	0.29
F_r (%)		86.71			91.10	

[a] Apparent oral clearance (CL/F) was calculated as dose/ $AUC_{0-\infty}$.
[b] Apparent oral steady-state volume of distribution (V_{ss}/F) was calculated as (dose × $AUMC_{0-\infty}$)/($AUC_{0-\infty}$)^2.

co-administration with 3TC, the OM concentrations declined in a bi-exponential manner. Furthermore, the fractions of OM orally absorbed, determined by the ratio of AUCs, with and without oral 3TC, to intravenous administration alone were 30.9% and 26.3%, respectively. In spite of the approximate 10% differences illustrated in main pharmacokinetic parameters, no statistically significant pharmacokinetic interactions were observed following intravenous or oral co-administration of OM between mono- and co-administration ($P > 0.05$).

3.3. *Pharmacokinetics of M*

In the case of the active metabolite of OM, Fig. 4 shows the mean plasma concentration–time profiles of M after administration of OM alone and in combination with 3TC [18]. The pharmacokinetic parameters for M are presented in Table 3. Following intravenous administration, the concentrations of M in plasma were generally lower than those of its parent compound. After oral administration, the C_{max} (12.30 ± 4.10 and 10.06 ± 2.88 μmol/l) and $AUC_{0-\infty}$ (56.31 ± 19.13 and 50.64 ± 8.25 μmol·h/l) were approximately four-fold higher than the intravenous C_{max} (2.87 ± 0.85 and 2.59 ± 0.16 μmol/l) and $AUC_{0-\infty}$ (6.64 ± 1.59 and 7.90 ± 2.23 μmol·h/l). As illustrated in Fig. 4A, OM was immediately biotransformed into M, as the C_{max} was reached in the first collected sample. In oral regimen of OM alone or concomitantly with 3TC, the T_{max} values of M were 3.75 ± 0.36 and 4.50 ± 0.22 h, respectively, and the concentrations of M in plasma were higher than those of OM in the following time-point. The oral biotransformation rates of OM were 49.8% and 51.2% following single and combinational therapy, respectively. On the basis of the equimolecular transformation of OM into M [8,13], the absorbed total fractions of OM, calculated by the ratio of the AUCs of OM plus M for single oral and 3TC-containing oral administration to single intravenous administrations, were 57.4% and 50.3%, respectively. The difference in these pharmacokinetic parameters was evaluated to be statistically insignificant ($P > 0.05$), indicating that there were no pharmacokinetic interactions involving the metabolism of OM following both oral and intravenous administration in combination with 3TC.

3TC is not significantly metabolized and is eliminated primarily as unchanged drug via the kidneys. Approximately 5% to 10% of the parent compound is metabolized to the pharmacologically inactive trans-sulfoxide metabolites after a single oral dose [11]. However, M, the metabolite of OM, is bioactive and represents a primary reduction pathway by intestinal bacteria and liver [12,13]. So it is necessary to investigate simultaneously the pharmacokinetic behavior of M and OM after OM administration.

After mono-administration in this study, 3TC was rapidly eliminated after both intravenous and oral administration, and had excellent oral bioavailability (T_{max} = 1.25 h, F = 80.5%). However, compared with the findings of Hussey et al. (CL = 0.36 l/h/kg) [10], the higher values observed in our study (0.57 ± 0.12 l/h/kg) are probably due to the exclusion of samples taken 8 h after intravenous dosing alone. Furthermore, the CL and V_{ss} fol-

Table 2 – Pharmacokinetics of OM following intravenous and oral administration alone and in combination with 3TC.

	Intravenous administration			Oral administration		
	Alone	Combination	P (95% CI)	Alone	Combination	P (95% CI)
C_{max} (μmol/l)	–	–	–	21.64 ± 2.70	23.46 ± 3.87	0.69
T_{max} (h)	–	–	–	1.25 ± 0.17	1.08 ± 0.14	0.52
$t_{1/2}$ (h)	1.51 ± 0.15	1.35 ± 0.16	0.48	2.18 ± 0.31	2.14 ± 0.26	0.91
$AUC_{0-\infty}$ (μmol·h/l)	91.85 ± 22.49	82.42 ± 10.53	0.54	56.82 ± 8.92	48.36 ± 8.36	0.30
MRT (h)	2.19 ± 0.29	1.92 ± 0.18	0.16	2.85 ± 0.19	2.61 ± 0.13	0.32
CL (l/h/kg)	0.76 ± 0.15	0.74 ± 0.09	0.86	2.28 ± 0.38[a]	2.64 ± 0.35[a]	0.38
V_{ss} (l/kg)	1.57 ± 0.27	1.37 ± 0.12	0.52	6.65 ± 1.25[b]	6.97 ± 1.08[b]	0.84
F_r (%)		89.73			85.11	

[a] Apparent oral clearance (CL/F) was calculated as dose/ $AUC_{0-\infty}$.
[b] Apparent oral steady-state volume of distribution (V_{ss}/F) was calculated as (dose × $AUMC_{0-\infty}$)/($AUC_{0-\infty}$)^2.

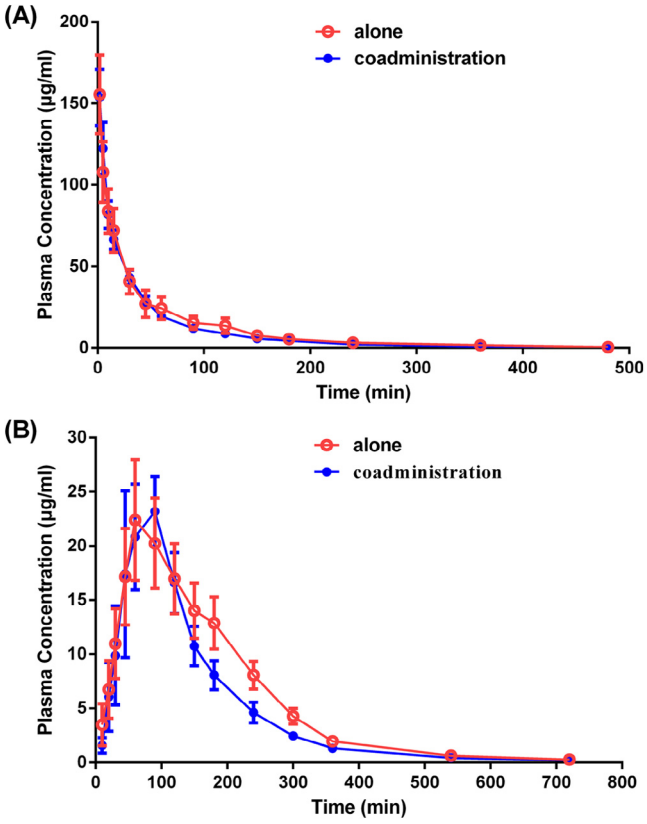

Fig. 3 – (A) Mean plasma OM concentration–time curves following two intravenous regimens involving a single dose of 15 mg/kg OM to beagle dogs: alone (red line) and in combination with a single dose of 2.5 mg/kg 3TC (blue line). (B) Mean plasma OM concentration–time curves following two oral regimens involving a single dose of 30 mg/kg to beagle dogs: alone (red line) and in combination with a single dose of 5.0 mg/kg 3TC (blue line). Each value represents the mean ± SE (n = 6).

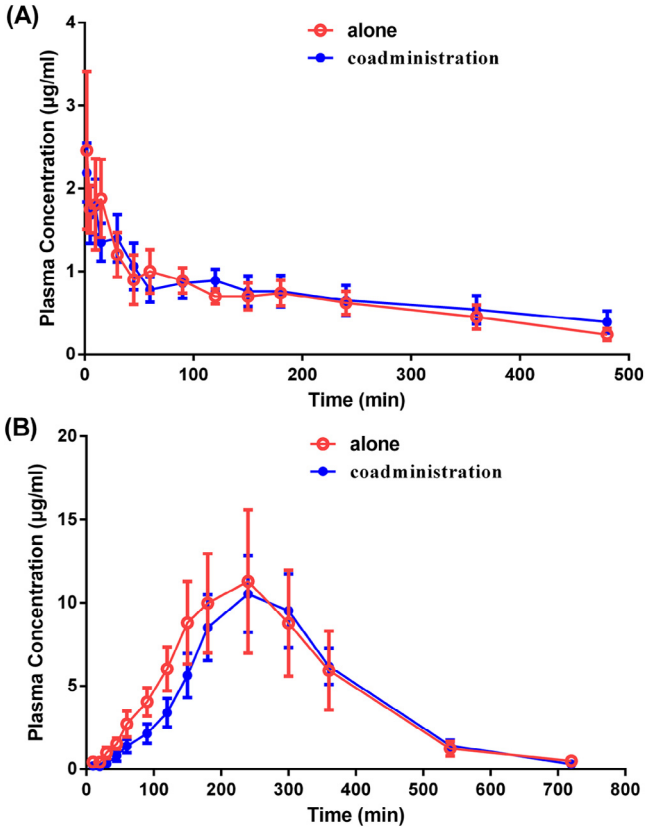

Fig. 4 – (A) Mean plasma M concentration–time curves following two intravenous regimens involving a single dose of 15 mg/kg OM to beagle dogs: alone (red line) and in combination with a single dose of 2.5 mg/kg 3TC (blue line). (B) Mean plasma M concentration–time curves following two oral regimens involving a single dose of 30 mg/kg to beagle dogs: alone (red line) and in combination with a single dose of 5.0 mg/kg 3TC (blue line). Each value represents the mean ± SE (n = 6).

lowing intravenous mono-administration of 3TC were consistent with the allometric estimation according to CL, V_{ss} and species body weight (W): $CL = 0.74 \times W^{0.76}$, $V_{ss} = 1.09 \times W^{0.94}$. The estimates of CL and V_{ss} (0.48 l/h/kg and 0.95 l/kg, respectively) were similar to the observed values for dogs in this study (0.57 ± 0.12 l/h/kg and 1.03 ± 0.20 l/kg, respectively).

In our study, following intravenous administration of OM alone, the V_{ss} of 1.57 ± 0.27 l/kg is slightly greater than the total body fluid of dogs, and larger than the value of 3TC (1.03 l/kg) in dogs. This suggests that OM may undergo intracellular distribution and freely penetrate tissues beyond the systemic circulation, and that the concentration of OM distributing in tissues might be higher than that of blood vessels. Meanwhile, the phenomenon of biotransformation from OM to M was also observed in our investigation, which was consistent with previous studies showing that OM and its metabolite M, after intramuscular injection, undergo wide tissue distribution in mice or rats, including the kidney, liver, lung, bone marrow, spleen, and heart [12]. Additionally, OM is rapidly eliminated ($t_{1/2}$ = 1.51 ± 0.15 h) after intravenous administration and the oral bioavailability of OM plus M for single oral administration to single intravenous administration was about 57.4% in our investigation.

It was seen in the oral regimen that the estimate of OM transformed into M (49.8%) was in conformity with that obtained previously from a single-dose study with dogs (56.5%) [18]. In addition, the bioconversion of M after intravenous administration of OM was less than 10%. The ratio of the AUC of M after intravenous dosing to oral dosing is roughly regarded as reflection of the metabolism by liver compared to the sum of the metabolism by liver and gastrointestinal bacteria. Therefore, the results suggested that the reduction metabolism of OM by gastrointestinal bacteria was four times higher than that of liver and gastrointestinal bacteria is the main pathway of OM metabolism. In this study, it was also indicated that the relatively large individual variance of pharmacokinetic parameters of OM and that of M emerged. This may be attributable to the facts that OM is metabolized via hepatic and intestinal bacteria pathways, and that the hepatic function and the flora distribution are individualized.

The pharmacokinetic parameters of 3TC obtained from co-administration in this study with beagle dogs are consistent

Table 3 – Pharmacokinetics of M following OM intravenous and oral administration alone and in combination with 3TC.

	Intravenous administration			Oral administration		
	Alone	Combination	P (95% CI)	Alone	Combination	P (95% CI)
C_{max} (μmol/l)	2.87 ± 0.85	2.59 ± 0.16	0.77	12.30 ± 4.10	10.06 ± 2.88	0.60
T_{max} (h)	0.09 ± 0.03	0.06 ± 0.02	0.54	3.75 ± 0.36	4.50 ± 0.22	0.12
$t_{1/2}$ (h)	3.07 ± 0.56	2.93 ± 0.54	0.86	3.53 ± 0.16	3.39 ± 0.12	0.52
$AUC_{0-\infty}$ (μmol·h/l)	6.64 ± 1.59	7.90 ± 2.23	0.42	56.31 ± 19.13	50.64 ± 8.25	0.73
MRT (h)	4.59 ± 0.75	4.83 ± 0.98	0.85	4.59 ± 0.28	4.99 ± 0.19	0.11
F_r (%)		118.91			89.93	

with those obtained from mono-administration ($P > 0.05$). Changes in certain issues, such as protein binding, absorption, distribution, metabolism and excretion, are the basis of many drug interactions. Changes in protein binding and metabolism would not significantly affect the distribution and elimination of 3TC, because its protein binding is low (<36%) and metabolism represents only a minor route of elimination. Approximately 5% to 10% of the parent compound is metabolized to the pharmacologically inactive metabolite [11,19]. 3TC is a highly soluble and permeable drug with a rapid dissolution rate. As a consequence, oral doses are rapidly absorbed by passive diffusion across the intestinal membrane [14]. Furthermore, its relatively low molecular weight (229 D) and low plasma protein binding result in a wide distribution and free penetration into tissues beyond the systemic circulation [11]. OM and M are unlikely to be able to affect these passive and free penetration processes. Moreover, 3TC is eliminated primarily unchanged drug via the kidneys by filtration and active renal tubular secretion, in part via the renal organic cation transport system [11]. Although OM and M are eliminated partially via the kidney, this does not significantly affect either the extent of absorption or the elimination of 3TC, as indicated by an absence of significant changes in the $AUC_{0-\infty}$ and $t_{1/2}$ of 3TC ($P > 0.05$). These findings agree with published results of drug interaction studies involving 3TC [11,12].

Furthermore, the pharmacokinetics of OM is not significantly affected by co-administration of 3TC. The low potential of OM for drug–drug interactions is similar to that of 3TC, because the properties of OM are fairly similar to those of 3TC, such as a low protein binding (29.36 ± 4.17%), relatively low molecular weight (264 D), highly water soluble (more than 150 mg/ml) character. In our laboratory, it has been shown that OM and M mainly undergo passive diffusion across Caco-2 cell membrane. Hence, it is little likely that there will be an interaction involving interference with the process of absorption. This assumption is also demonstrated by the T_{max} values of OM and 3TC (1.25 h for the single-regimen and 1.08 h for the co-regimen, respectively) after oral administration. 3TC did not markedly affect either the extent of distribution or the elimination of OM, as shown by a lack of any significant change in the V_{ss} and CL of OM, respectively ($P > 0.05$).

4. Conclusion

There is no pharmacokinetic interaction when 3TC and OM are co-administrated, either intravenously or orally, and the potential for 3TC to interact with its metabolite M has been examined to be low. And this study focused on the pharmacokinetic interactions in dogs to account for the collaborated employment of lamivudine and oxymatrine. However, the clinical pharmacokinetics remains to be studied for confirming this conclusion. Notwithstanding this limitation, the study does provides the *in vivo* pharmacokinetic basis for supporting the recent clinical practices that no dosage adjustment is needed and serious adverse events are absent when lamivudine and oxymatrine are co-administrated, which demonstrated that the determination of pharmacokinetics between combinational and individual therapy might assist the development of drug compatibility in clinical therapies.

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