

Pharmacokinetic/pharmacodynamic profiles of baicalin against *Mycoplasma gallisepticum* in an in vivo infection model

Jiaxin Bao,^{*} Zhiyong Wu,^{*} Muhammad Ishfaq,^{*,†} Jian Wang,^{*} Yusong Miao,^{*} Dong Niu,^{*} Rui Li,^{*} Jichang Li,^{*,†,1} and Chunli Chen^{*,†}

^{*}College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, P. R. China; [†]Heilongjiang Key Laboratory for Animal Disease Control and Pharmaceutical Development, Harbin 150030, P. R. China.; and ¹College of Computer Science, Huanggang Normal University, Huanggang, 438000, P. R. China

ABSTRACT *Mycoplasma gallisepticum* (*M. gallisepticum*), a devastating avian pathogen that commonly causes chronic respiratory disease in chicken, is responsible for tremendous economic losses to the poultry industry. Baicalin is the main constituent of *Scutellaria baicalensis* that shows potential therapeutic effects against *M. gallisepticum*. However, the pharmacokinetic/pharmacodynamics (PK/PD) profiles of baicalin against *M. gallisepticum* are not well understood. The main objective of the present study was to determine the relationship between the PK/PD index and efficacy of baicalin in the *M. gallisepticum* infection model in chickens. The experiments were carried out on 10-day-old chickens that were challenged with *M. gallisepticum* in the bilateral air sacs. While, baicalin was orally administrated once in a day for 3 consecutive days, started from d 3 postinfection. Ultra-performance liquid

chromatography (UPLC) was used to evaluate the PK parameters of baicalin at doses of 200, 400, and 600 mg/kg in *M. gallisepticum*-infected chickens. Real-time PCR (RT-PCR) was used for the quantitative detection of *M. gallisepticum* in lungs. The PK and PD data were fitted to WinNonlin software to evaluate the PK/PD profiles of baicalin against *M. gallisepticum*. The minimum inhibitory concentration (MIC) of baicalin against *M. gallisepticum* strain R_{low} was 31.25 µg/mL. The in vivo data suggested that baicalin concentration in the lung tissues was higher than plasma (1.21–1.73 times higher). The ratios of AUC_{24h}/MIC of baicalin against bacteriostatic, bactericidal, and eradication were 0.62, 1.33, and 1.49 h, respectively. In conclusion, these results provided potential reference for future clinical dose selection of baicalin and evaluation of susceptibility breakpoints.

Key words: *Mycoplasma gallisepticum*, baicalin, PK/PD, chicken

2021 Poultry Science 100:101437

<https://doi.org/10.1016/j.psj.2021.101437>

INTRODUCTION

Mycoplasma gallisepticum (*M. gallisepticum*) is one of the serious pathogens that harm the global poultry industry. *M. gallisepticum* is considered one of the primary causative agents of chronic respiratory disease in chicken (Stanley et al., 2001; Beaudet et al., 2017). Its primary signs include shedding of tears, sneezing, increased nasal discharge, and other respiratory symptoms. The transmission route of *M. gallisepticum* includes horizontal route via aerosol and vertical route via eggs. It has been previously demonstrated that *M. gallisepticum* infection suppressed the host immune response and

increased the chances for secondary bacterial infections (Zhiyong et al., 2019; Wu et al., 2020; Wang et al., 2021b). It causes considerable economic losses in poultry industry in terms of reduced egg production, feed conversion rates and high mortality rates (Zhang et al., 2021). Currently vaccination and antibiotics are used to control *M. gallisepticum* infection (Kanci Condello et al., 2020). However, it is difficult to eradicate the rapid spread of *M. gallisepticum* infection. Therefore, there is an urgent need to find better therapeutic agents.

Baicalin, a flavonoid compound possesses potential therapeutic properties, one of the main constituents of *Scutellaria baicalensis*. Previous studies demonstrated that baicalin has a variety of biological activities, such as antimicrobial, anti-inflammatory, antioxidant, antihepatotoxic, and antitumor properties (de Oliveira et al., 2015; Gong et al., 2017; Fu et al., 2018). Baicalin has the ability to interact with various signaling pathways. Our previous study explained the preventive effects of

© 2021 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Received April 28, 2021.

Accepted August 15, 2021.

¹Corresponding authors: lijichang@neau.edu.cn

baicalin against *M. gallisepticum*-induced inflammation, oxidative stress, and apoptosis by activating the Nrf2 signaling pathway and NF- κ B signaling pathway (Ishfaq et al., 2019), and baicalin could protect the immune organs from *M. gallisepticum* infection-mediated structural and functional damage (Zhang et al., 2020; Hu et al., 2021; Ishfaq et al., 2021). However, the lower bioavailability of baicalin limits its clinical uses. Numerous studies have been conducted on the pharmacokinetic profile of baicalin to clarify its in vivo properties (Wei et al., 2016), but it is difficult to obtain the connection between the efficacy and exposure concentration in vivo. These questions may be addressed through simulations from pharmacokinetic and pharmacodynamic models.

Pharmacokinetic-pharmacodynamic (PK/PD) modeling is a better method to understand the underlying mechanisms of drug action and the relationship on the physiologic system compared with single analytical method. It is well-known that PK/PD models, which have been used extensively in clinical trial design and the selection of dose regimens, provides useful information in optimizing the clinical dosage, improving the therapeutic efficacy and prohibiting resistance emergence (Nielsen and Friberg, 2013). In this study, we evaluated the PK/PD profiles of baicalin using *M. gallisepticum* infection model in chickens. Furthermore, we determined the better PK/PD index that correlated with the efficacy of baicalin. It was expected that our investigation would illustrate the relationship between dosing regimens and efficacy of baicalin, and assist further clinical development of optimal dosing strategies.

MATERIALS AND METHODS

Ethical Statement

The present study was conducted under the approval of the Laboratory Animal Ethics Committee of Northeast Agricultural University (Heilongjiang province, China) by Laboratory animal-Guideline for ethical review of animal welfare (GB/T 35892-2018, National Standards of the People's Republic of China).

Mycoplasma Strains and Chemicals

The *M. gallisepticum* strain R_{low} was obtained from Harbin Institute of Veterinary Medicine, Chinese Academy of Agricultural Sciences. The culture conditions for growing *M. gallisepticum* were kept the same as mentioned in our previous study (Lu et al., 2017). Briefly, *M. gallisepticum* was grown at 37°C in modified Hayflicks medium containing 0.05% Penicillins, 0.1% Nicotinamide adenine dinucleotide (NAD), 10% freshly prepared yeast extract, 20% fetal bovine serum and 0.05% thallium acetate. At mid-exponential phase of *M. gallisepticum*, a color change was observed from phenol red to orange. Its concentration was adjusted at a density of 5×10^9 color change unit per milliliter (CCU/mL) and kept at °C for use within 2 wk.

The reference standards of baicalin with a purity of above 98%, was purchased from the National Institute for the Food and Drug Control (Beijing, China). Methanol and Acetonitrile was of CHROMASOLV gradient grade for HPLC and obtained from Merck. LC/MS-grade Concentrated phosphoric acid and triethylamine were supplied by VWR.

Susceptibility Determination

The in vitro susceptibility of baicalin against *M. gallisepticum* R_{low} was determined by the Microdilution method: 10 μ L samples of *M. gallisepticum* culture (5×10^9 CCU/mL) were inoculated onto 96-well plates containing 2-fold serial dilutions of baicalin. Growth control (*M. gallisepticum* inoculum without baicalin) and sterility control (blank medium) were also included in the MIC determination. The MIC was determined as the minimal concentration of the antibacterial agent that resulted in no growth on the 96-well plates after 7 d.

Animals and Inoculation

Three hundred 1-day-old Hyline variety of White Leghorn chickens were bought from the Harbin Yinong farming Co., Ltd. (Harbin, China). Chickens were housed in a positive-pressure fiberglass isolator, and provided with antibacterial-free balanced feed and fresh drinking water ad libitum. The antibacterial-free chicken feed was purchased from Lenong Feed Co., Ltd. (Harbin, Heilongjiang, China). The chickens were randomly divided into 4 groups: 1) A group of 50 chickens were used as negative control group; 2) 20 chickens were used as a *M. gallisepticum*-infected group and challenged with *M. gallisepticum* strain R_{low} (1×10^9 CCU/mL) in the bilateral air sacs of the thoracic region on d 10. The *M. gallisepticum*-infected group and negative control group were administered by oral gavage with 0.85% NaCl started on d 13, once daily for 3 d; (3) A group of 180 chickens were used to study the pharmacokinetics of baicalin in infected chickens (infection route is same as described above, and the baicalin treatment started on day 13 in 3 different doses for 3 d consecutively, and 60 chickens were orally administered for each dose once in a day). 4) A group of 50 chickens were used to study the pharmacodynamics of baicalin in infected chickens (the baicalin treatment was administered on d 13 in 8 different doses for 3 consecutive days, and 6 chickens were orally administered once in a day for each dose separately). At the 16th d, all chickens were humanely sacrificed to avoid pain and suffering of chickens. Plasma and lung samples in each group were collected for further analysis.

M. Gallisepticum Quantitation by Real-Time qRT-PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to identify DNA copies of *M. gallisepticum* in different samples using a Roche

LightCycler instrument (Shanghai, China) with *M. gallisepticum*-specific *mgc2* gene. The primers are: *mgc2*-F: 5'-TTGGGTTTtagggattgggatt; *mgc2*-R: 5'-CCAAGGGATTCAACCATCTT, as described in a previous study (Raviv and Kleven, 2009). Lungs were collected, homogenized in 2 mL PBS and centrifuged at 500 rpm for 5 min. An aliquot of 0.5 mL supernatant was used for DNA extraction with a bacterial DNA kit (Omega Bio-tek, Inc., Norcross, GA). The in vitro standard DNA curve was plotted by the numbers of *M. gallisepticum* derived from the culture method and cycle threshold (Ct) values obtained using qRT-PCR results as previously described (Wang et al., 2020).

Efficacy of Baicalin Against *M. Gallisepticum* in Chicken Infection Model

To evaluate the efficacy of baicalin at 24 h post 3-d infection, the infected chickens were administrated baicalin orally by gavage with either 0.85% NaCl (controls) or baicalin at 100, 150, 200, 250, 300, 400, 500, or 600 mg/kg once daily for 3 consecutive days (6 chickens/dose). After 24 h of the last drug administration, the amounts of DNA copies of *M. gallisepticum* in each chicken were calculated using the method as described above.

Baicalin Pharmacokinetics in In Vivo Infection Model

Groups of infected chickens were orally administrated baicalin by gavage at a dose of 200 mg/kg, 400 mg/kg, 600 mg/kg once daily for 3 d, and they were euthanized at 0.17, 0.25, 0.5, 0.75, 1, 2, 3, 6, 8, 12, and 24 h after the first oral gavage of baicalin. Blood and lung tissues were collected from 5 chickens at each sampling time point per treatment group. Blood samples were centrifuged at $3,000 \times g$ for 10 min at $^{\circ}\text{C}$, and then plasma was collected. The samples of plasma and lung tissues were stored at -20°C until analyzed by UPLC within 2 wk.

Baicalin concentrations in plasma and lung tissues were determined by Ultra-Performance Liquid Chromatography (UPLC) (Waters Technologies, Shanghai, China). The UPLC was equipped with a Waters BEH-C18 column (2.1 mm \times 50 mm, 1.7 μm) using a mobile phase of triethylamine phosphate (pH 2.4): Methanol (47:53, v/v) and a flow rate of 0.8 mL/min. Injection volume was 6 μL . The calibration curve was established with seven baicalin concentrations in plasma and lung tissues respectively.

Plasma and lung tissues were treated with three times the volume of methanol-acetonitrile mixed solution, vortexed for 2 min and incubated at 45°C in a water bath for 10 min to precipitate the proteins. Then, the samples were centrifuged at $12,000 g$ for 10 min. The supernatant was collected and the residue was extracted again. The extracts were combined and evaporated to dryness under a gentle stream of nitrogen at 40°C . The residue

was dissolved in 200- μL mobile phase, and filtered through a 0.22 μm syringe filter prior to UPLC analysis. The recovery and precision were calculated by analysis of spiked samples at 3 concentration levels (5 replicates of each concentration).

Pharmacokinetics and Pharmacodynamics Analysis

The PK profiles of baicalin were analyzed by the noncompartmental model with uniform weighting using the WinNonlin software (version 6.1; Pharsight, CA). The surrogate index of antibacterial activity, $\text{AUC}_{24\text{h}}/\text{MIC}$ and $\text{C}_{\text{max}}/\text{MIC}$ were calculated using in vitro MIC value and PK parameters obtained from 3 doses of baicalin. The bacterial load for each animal was calculated based on Ct values and the in vitro standard DNA curve. The efficacy of baicalin was evaluated by the reduction of *M. gallisepticum* load after treatment compared with the initial bacterial count before treatment. The PK/PD relationship of baicalin against *M. gallisepticum* was described by WinNonlin software (version 6.1; Pharsight). Linear, E_{max} and Sigmoid E_{max} models were chosen as candidate models. A dose-response relationship for baicalin was detected by multiple contrast tests, and then we choose the Sigmoid E_{max} model as the best-fit model in terms with the following equation:

$$E = E_0 + \frac{E_{\text{max}} \times C_e^N}{EC_{50}^N + C_e^N}$$

where E is the change in Log_{10} CCU/mL for different dosage regimens, E_0 is the change in Log_{10} CCU/mL in the control sample (absence of baicalin), E_{max} is the difference in effect between the greatest amount of growth (as seen for the growth control, E_0) and the greatest amount of kill, C_e is the $\text{AUC}_{24\text{h}}/\text{MIC}$ in the effect compartment, EC_{50} is the $\text{AUC}_{24\text{h}}/\text{MIC}$ value producing a 50% reduction in bacterial counts, and N is the Hill coefficient that describes the steepness of the curve.

RESULTS

Susceptibility Testing

The MIC of baicalin against the studied strain was 31.25 $\mu\text{g}/\text{mL}$.

***M. Gallisepticum* Infection Model**

The model was successfully developed after 3 d of infection. These signs were observed including coughing, sneezing, nasal exudation and tracheitis in infected birds. Necropsy examination revealed cloudiness of air sacs and inflammation of the lungs. On the 13th day, the morbidity and mortality rates were 96 and 20%, respectively. While, the clinical signs of disease or *M. gallisepticum*-induced antibody were not observed in the negative control group.

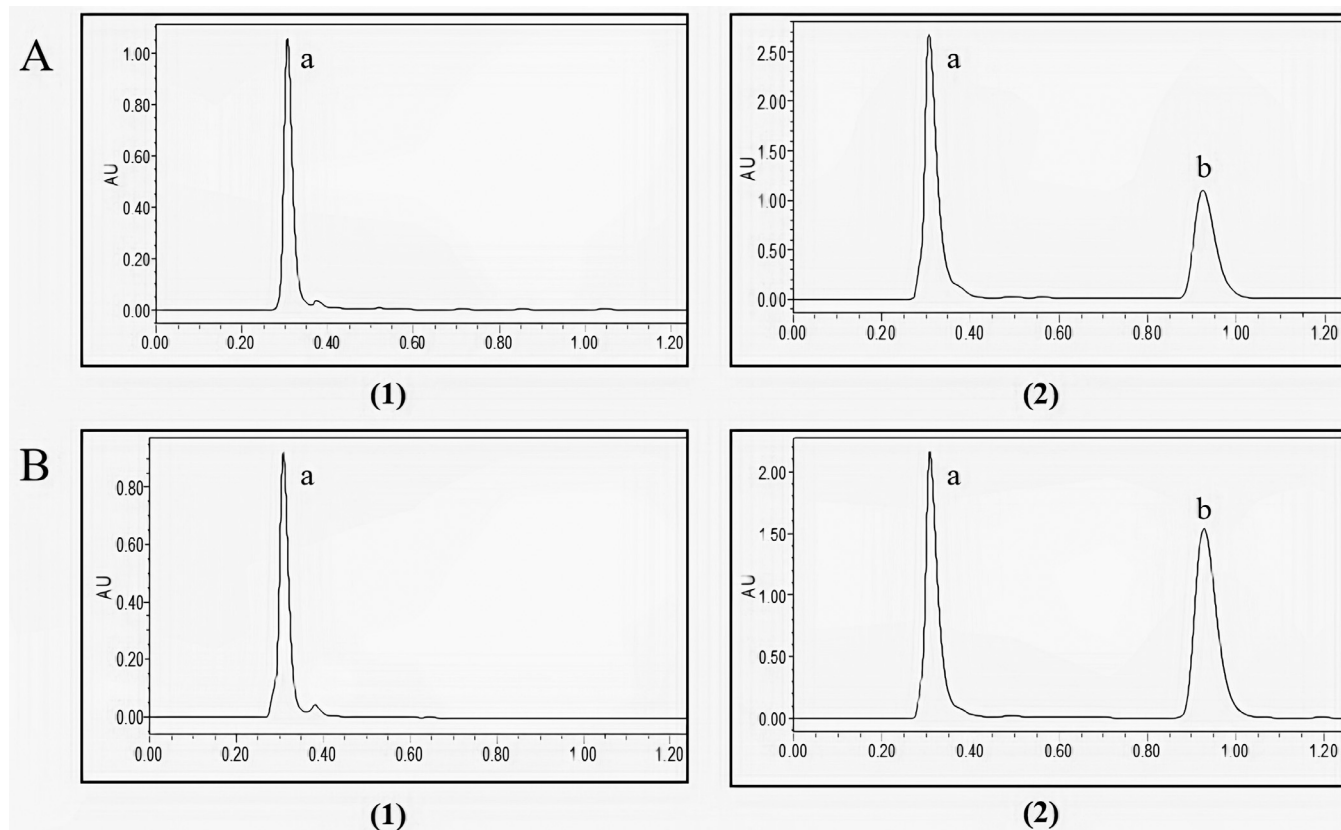


Figure 1. Typical chromatograms obtained in samples: (A) Plasma; (B) lung; (1) blank samples; (2) samples with addition of standard reference of baicalin; (a) the peak of endogenous substance; (b) the peak of baicalin.

UPLC Analyses

The selectivity of the method was obtained by comparing the control samples before administration with the samples after addition of the baicalin standard. Figure 1 shows that UPLC method had a good selectivity. The linear regression equation is showed in Table 1, and baicalin in the plasma and lung homogenates all had good linearity within the concentration range. The intra and interday precision and accuracy were showed in Table 2. The precision of baicalin calculated as the relative standard deviation (RSD) at various concentrations. It has been noted that RSD was lower than 14% for intra- and interday experiment. The results showed that the precision was acceptable. The recovery of baicalin was higher than 85%, the LOD was 0.2 $\mu\text{g}/\text{mL}$ and the LOQ was 0.05 $\mu\text{g}/\text{mL}$ in the samples. These results suggested that excellent sensitivity and reproducibility were achieved under the above condition.

Table 1. The standard curve of baicalin in plasma and lungs.

Samples	Regression equations	Correlation (r)	Linearity range ($\mu\text{g}/\text{mL}$)
Plasma	$Y = 37930X - 3125.2$	0.9993	0.049–25.00
Lung	$Y = 27173X - 49727$	0.9997	0.024–25.00

PK Profiles of Baicalin in Infection Model

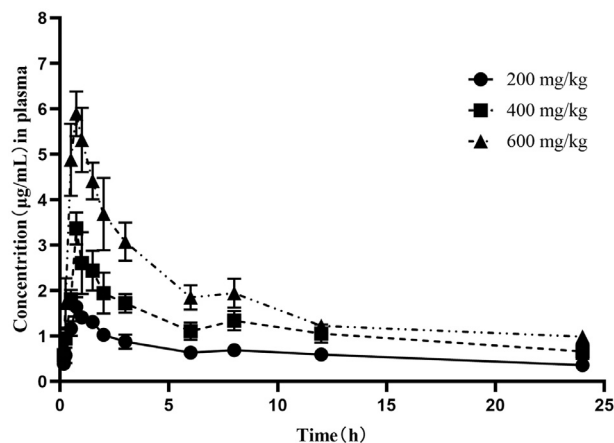
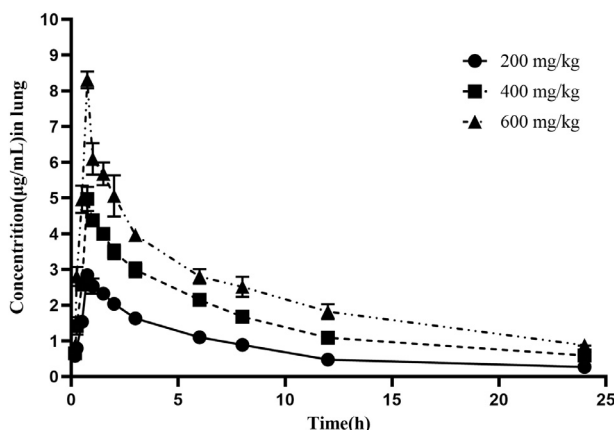
The time-concentration curves of baicalin in plasma and lung tissues after 3 oral administrations at a dose of 200, 400, and 600 mg/kg are shown in Figures 2 and 3, respectively. The main PK parameters obtained from plasma and lung tissues are presented in Tables 3 and 4, respectively. The T_{max} was 0.75 h in plasma and lung tissues for 3 different doses. A second peak was observed for all the doses administered at 7 to 9 h in plasma. The mean half-life ($T_{1/2}$) was 14.45 ± 3.44 h in plasma and 8.75 ± 1.93 h in lung tissues. There were significant differences in $T_{1/2}$ between lung and plasma. Besides, the PK parameters were dose-dependent, a significant correlation between dose and $\text{AUC}_{24\text{h}}$ or C_{max} was observed ($R^2 = 0.996$ and 0.997 for dose-to- $\text{AUC}_{24\text{h}}$ and dose-to- C_{max} ratios, respectively; Figures 4). The $\text{AUC}_{24\text{h}}$ values at different doses were calculated according to the linear relationship within the dose range from 100 to 600 mg/kg.

PD of Baicalin in Infected Chicken model

The effects of baicalin against *M. gallisepticum* in lung tissues with different regimens are shown in Figures 5. The data indicated that as the baicalin concentration increased from 100 to 600 mg/kg results in decrease in the bacterial load, implying that antimycoplasmal activity of baicalin was increased.

Table 2. Precision and recovery of the baicalin in plasma and tissues in *M. gallisepticum* infection model.

Samples	Concentration ($\mu\text{g}/\text{mL}$)	Recovery (%)	RSD (%)	
			Intraday (n = 5)	Interday (n = 3)
Plasma	1.5	95.47 ± 2.12	2.22	7.14
	12.5	89.41 ± 1.82	2.03	6.13
	50	97.96 ± 2.60	2.65	7.30
Lung	2.5	98.84 ± 0.95	1.13	11.70
	25	85.01 ± 2.08	2.80	9.74
	100	88.83 ± 2.29	2.64	8.47

**Figure 2.** The time-concentration curves of baicalin in plasma after three oral administrations doses of 200, 400, and 600 mg/kg in *M. gallisepticum* infection model (n = 5/time point).**Figure 3.** The time-concentration curves of baicalin in lung after three oral administrations doses of 200, 400, and 600 mg/kg in *M. gallisepticum* infection model (n = 5/time point).

PK and PD Analysis

The PK/PD indices $\text{AUC}_{24\text{h}}/\text{MIC}$ and $C_{\text{max}}/\text{MIC}$ were integrated using the PK parameters, dose proportionality, and MIC data. The effect (**E**) was calculated as the reduction of *M. gallisepticum* using the unit of

Log_{10} CCU/mL. The relationship between the ratio of $\text{AUC}_{24\text{h}}/\text{MIC}$ and the ratio of $C_{\text{max}}/\text{MIC}$ with efficacy was described using the Sigmoid E_{max} model (Figure 6). The efficacy of baicalin correlated best with the $\text{AUC}_{24\text{h}}/\text{MIC}$ ratio with an R^2 value of 0.959, followed by the $C_{\text{max}}/\text{MIC}$ ($R^2 = 0.898$). The model parameters of Hill coefficient, N , E_{max} and $\text{AUC}_{24\text{h}}/\text{MIC}$ are presented in Table 5. The values of the $\text{AUC}_{24\text{h}}/\text{MIC}$ ratio required for bacteriostatic activity ($E = 0$), bactericidal activity ($E = -2$), and bacterial elimination ($E = -3$) were 0.62, 1.33, and 1.49 h, respectively.

DISCUSSION

M. gallisepticum is one of the primary pathogens that cause respiratory diseases in poultry, and severely impacts the development of commercial poultry production. Drug resistance to *M. gallisepticum* has emerged in recent years, due to long-term improper use of antibacterial agents (Morrow et al., 2020). Therefore, there is an urgent need to further explore new treatments and control methods. Our previous study demonstrated that the preventive effects of baicalin against *M. gallisepticum*-induced inflammation and apoptosis in the lungs of chicken (Wu et al., 2019; Ishfaq et al., 2020). In the current study, we established an in vivo PK/PD profile of baicalin and evaluated the efficacy of baicalin against *M. gallisepticum* infection model.

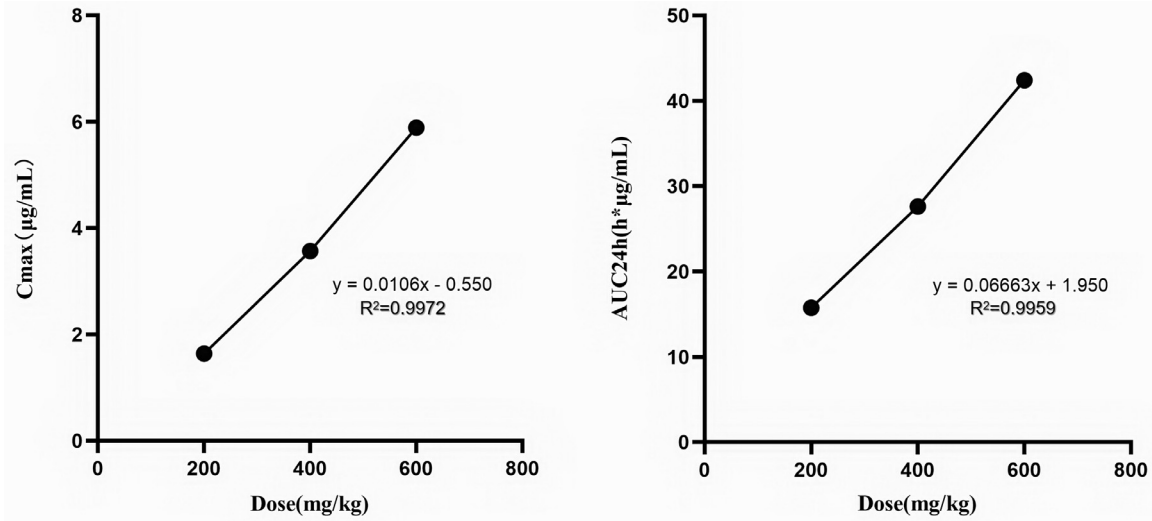
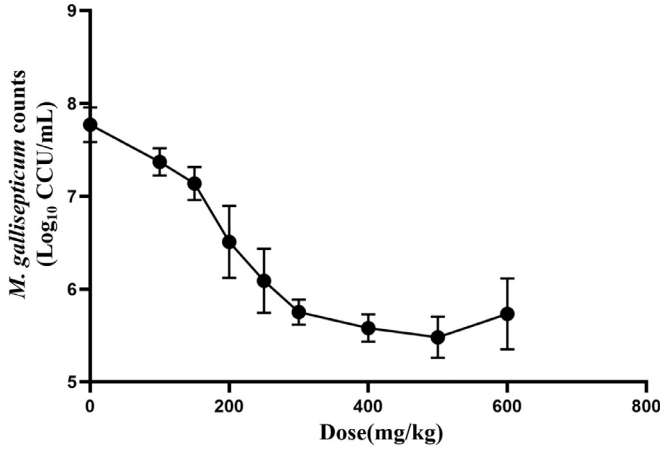
Our choice for inoculation routes and the concentration of *M. gallisepticum* were based on previous studies. We compared four different *M. gallisepticum* infection models and found that the direct air sac injection is the faster and more effective method (Bao et al., 2020). The concentration of *M. gallisepticum* for pretest infection was the same as before, 5×10^9 CCU/mL. Additionally, we considered the selection of the PDs calculation method. It is well understood that the isolation rate of *M. gallisepticum* is low, due to its high requirements on culture medium conditions and susceptibility to external contamination (Garcia et al., 1995). So, it may not be an effective method to evaluate the PD by isolation count.

Table 3. Pharmacokinetic parameters of baicalin in plasma tissues following oral administrations of various doses in *M. gallisepticum*-infected chickens.

Dose (mg/kg)	T_{max} (h)	C_{max} ($\mu\text{g}/\text{mL}$)	$\text{AUC}_{24\text{h}}$ ($\text{h} \times \mu\text{g}/\text{mL}$)	$T_{1/2}$ (h)	V/F (mL)	CL/F (L/h/kg)	MRT (h)
200	0.75	1.64	16.19	17.80	208.17	8.10	9.55
400	0.75	3.37	28.24	14.63	207.48	9.83	9.30
600	0.75	5.89	58.81	10.93	162.99	10.34	8.63

Table 4. Pharmacokinetic parameters of baicalin in lung tissues following oral administrations of various doses in *M. gallisepticum*-infected chickens.

Dose (mg/kg)	T _{max} (h)	C _{max} (μg/mL)	AUC _{24h} (h × μg/mL)	T _{1/2} (h)	V/F (mL)	CL/F (L/h/kg)	MRT (h)
200	0.75	2.84	19.02	6.86	91.77	9.26	7.24
400	0.75	4.84	36.99	8.67	112.72	9.02	7.86
600	0.75	8.31	55.09	10.72	135.11	8.73	8.06

**Figure 4.** Linear regression plots between administered dose and C_{max} values, and between administered dose and AUC_{24h} values.**Figure 5.** Calculated in vivo *M. gallisepticum* counts after baicalin treatment (n = 6/dose).

Studies have confirmed that it is more convenient and efficient to use real-time PCR methodology to detect *M. gallisepticum* quantitatively, and it can be used to evaluate the PD parameters (Xiao et al., 2015; Xiao et al., 2016). Thus, in this study, we choose to detect *M. gallisepticum* quantitation by qRT-PCR separately before and after administration, which may provide a more accurate estimation of the difference in PDs.

To obtain a satisfactory analytical method, chromatographic conditions including the composition, pH of mobile phase, and apparatus were all optimized after several trials and referred to some previous studies (Xu et al., 2020; Zhu et al., 2021). We compared the methods and found that the obtained baicalin target peak response value was higher when using 0.4% (v/v) phosphoric acid (A) and methanol (B) as the mobile

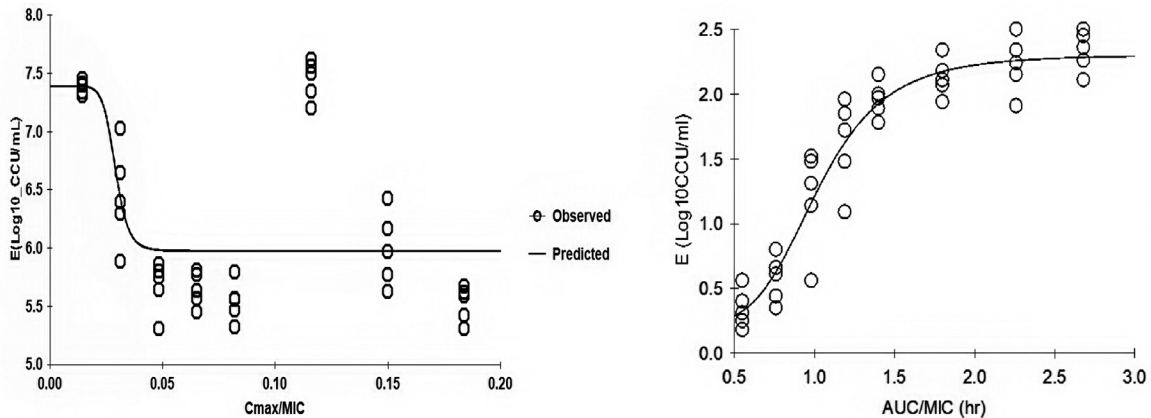
**Figure 6.** Sigmoid E_{max} relationships between antimycoplasmal effect (E, Log₁₀ CCU/mL) and in vivo C_{max}/MIC and AUC_{24h}/MIC ratio against *M. gallisepticum* in the lung tissues of chickens.

Table 5. PK/PD analysis of baicalin in *M. gallisepticum* infection model.

Parameters	Value
E_{\max} (Log ₁₀ CCU/mL)	2.30
E_0 (Log ₁₀ CCU/mL)	1.03
EC ₅₀ (h)	1.03
AUC _{24h} /MIC for 0 Log ₁₀ CCU/mL	0.62
AUC _{24h} /MIC for 2 Log ₁₀ CCU/mL	1.33
AUC _{24h} /MIC for 3 Log ₁₀ CCU/mL	1.49
Slope (N)	4.78

E_0 is the change in Log₁₀ CCU/mL after 24 h incubation in the control sample (absence of drug) compared with the initial inoculum. E_{\max} is the difference in effect of the greatest amount of kill. EC₅₀ is the AUC_{24h}/MIC value producing a 50% reduction in bacterial counts from the initial inoculum. AUC_{24h}/MIC is the 24 h area under concentration-time curve/minimum inhibitory concentration ratios. N is the Hill coefficient that describes the steepness of the AUC_{24h}/MIC-effect curve.

phase method. It may be due to the weak acidity of baicalin. A certain amount of phosphoric acid can increase the resolution of baicalin and inhibit its dissociation. Meanwhile, we found that adding triethylamine in the mobile phase can prevent the target peak from tailing and improve the peak shape. In addition, the determination method by HPLC usually took too much time (>40 min), and was not sensitive enough. Taking into account the high protein binding rate of baicalin in vivo, we switched to UPLC for baicalin quantification. It is found that on the basis of the same mobile phase conditions, using UPLC has higher sensitivity and shorter retention time.

To the best of our knowledge, this was the first report about the PK/PD of baicalin in *M. gallisepticum*-infected chickens. In the present investigation, PK profiles of baicalin ranged from 200 mg/kg to 600 mg/kg has been described using baicalin concentration in the plasma and lung tissues in infected chickens. Baicalin was rapidly absorbed with the peak concentrations achieved at 0.75 h. The half-life was in the range of 10.93 to 17.80 h in plasma and in the range of 6.86 to 10.72 h in the lung, which was lower than the value of previously reported studies (Wei et al., 2017; Li et al., 2018). Hence, it indicated that the elimination rate of baicalin was significantly different among species. The PK profiles revealed that the average baicalin concentration in lung tissues was higher (1.21–1.73 times higher) than the corresponding concentration in plasma. It is consistent with the previously published article that higher concentrations of baicalin in lung tissues of rabbits and rats were noted compared to plasma (Zhu et al., 2013; Wei et al., 2016). The difference in the concentration of baicalin in the lung tissues may be due to transporters (Kalapos-Kovács et al., 2015). Baicalin can be transferred to the alveolar space as a substrate through a variety of transport proteins.

In this experiment, baicalin was rapidly absorbed in the body, the first absorption peak appeared at 0.75 h, and the second absorption peak appeared at about 8 h. Many studies have given multiple mechanisms to explain this bimodal phenomenon. The previous study showed after oral or intravenous injection of baicalin, it

undergoes a glucuronidation reaction in rats, and the hepatocentric circulation has a great influence on the absorption and metabolism of baicalin (Xing et al., 2005). Some researchers have also proposed that absorption sites and intestinal flora may also be responsible for the multipeak absorption of baicalin. Experiments have shown that the absorption of baicalin was segment dependent (Fong et al., 2012). In addition, the biotransformation and the mechanism of action of baicalin in vivo is closely related to the intestinal flora that produces metabolic enzymes (Noh et al., 2016; Wang et al., 2021a).

The parameters of baicalin (AUC_{24h}) showed dose proportionality in the range of 200 to 600 mg/kg following administration allowed us to calculate the AUC_{24h} for other dosage administrations. We choose AUC_{24h}/MIC as the PK/PD index of baicalin, since the data from the present multiple dosage studies confirmed the conclusion that baicalin is a concentration-dependent drug (Cheng et al., 2014). Our data showed a stronger correlation between the AUC_{24h}/MIC and the in vivo antibacterial effects of baicalin against *M. gallisepticum* ($R^2 = 0.959$) than C_{\max} /MIC ($R^2 = 0.898$). The AUC_{24h}/MIC ratios for *M. gallisepticum* (a reduction of 0 Log₁₀ CCU/mL), a reduction of 2 Log₁₀ CCU/mL, and a reduction of 3 Log₁₀ CCU/mL were 0.62 h, 1.33 h, and 1.49 h, respectively. In addition, a PK population clearance of baicalin should be calculated and the MIC distribution of *M. gallisepticum* to baicalin should be evaluated. In the clinic, the parameters of this study can be used to formulate dosing schedules in combination with the different immune functions condition.

In conclusion, the present study successfully constructed a synchronous PK/PD model of baicalin in chickens infected with *M. gallisepticum*. The in vivo data suggested that baicalin concentration in lung tissues was higher than plasma in *M. gallisepticum*-infected chickens (1.21–1.73 times higher). Taken together, baicalin showed therapeutic potential against *M. gallisepticum* infection in chickens, and the values of AUC_{24h}/MIC of baicalin against bacteriostatic, bactericidal, and eradication were 0.62, 1.33, and 1.49 h, respectively.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (31772801) and Post-doctoral Research Foundation in Heilongjiang Province (LBH-Z19006)

DISCLOSURES

The authors declare that there are no potential conflicts of interests.

REFERENCES

Bao, J., Z. Wu, M. Ishfaq, Y. Miao, R. Li, A. C. Clifton, L. Ding, and J. Li. 2020. Comparison of experimental infection of normal and

- immunosuppressed chickens with *Mycoplasma gallisepticum*. *J. Comp. Pathol.* 175:5–12.
- Beaudet, J., E. R. Tulman, K. Pflaum, X. Liao, G. F. Kutish, S. M. Szczepanek, L. K. Silbart, and S. J. Geary. 2017. Transcriptional profiling of the chicken tracheal response to virulent *Mycoplasma gallisepticum* strain R_{low}. *Infect. Immun.* 85:e00343–17.
- Cheng, Z. Y., X. Tian, J. Gao, H. M. Li, L. J. Jia, and H. L. Qiao. 2014. Contribution of baicalin on the plasma protein binding displacement and CYP3A activity inhibition to the pharmacokinetic changes of nifedipine in rats *in vivo* and *in vitro*. *PLoS One.* 9:e87234.
- de Oliveira, M., S. Nabavi, S. Habtemariam, I. Erdogan Orhan, M. Daglia, and S. J. P. r. Nabavi. 2015. The effects of baicalin and baicalin on mitochondrial function and dynamics: a review. 100:296-308.
- Fong, Y. K., C. R. Li, S. K. Wo, S. Wang, L. Zhou, L. Zhang, G. Lin, and Z. Zuo. 2012. *In vitro* and *in situ* evaluation of herb-drug interactions during intestinal metabolism and absorption of baicalin. *J. Ethnopharmacol.* 141:742–753.
- Fu, S., H. Liu, L. Xu, Y. Qiu, Y. Liu, Z. Wu, C. Ye, Y. Hou, and C. J. S. r. Hu. 2018. Baicalin modulates NF- κ B and NLRP3 inflammatory signaling in porcine aortic vascular endothelial cells Infected by Haemophilus parasuis causing Glässer's disease. 8:807
- Garcia, M., M. W. Jackwood, S. Levisohn, and S. H. Kleven. 1995. Detection of *Mycoplasma gallisepticum*, *M. synoviae*, and *M. iowae* by multi-species polymerase chain reaction and restriction fragment length polymorphism. *Avian Dis.* 39:606–616.
- Gong, W. -Y., Z. -X. Zhao, B.- J. Liu, L.- W. Lu, and J. -C. Dong. 2017. Exploring the chemopreventive properties and perspectives of baicalin and its aglycone baicalein in solid tumors. *Eur J Med Chem* 126:844–852.
- Hu, W., W. Zhang, S. W. A. Shah, M. Ishfaq, and J. Li. 2021. *Mycoplasma gallisepticum* infection triggered histopathological changes, oxidative stress and apoptosis in chicken thymus and spleen. *Dev. Comp. Immunol.* 114:103832.
- Ishfaq, M., C. Chen, J. Bao, W. Zhang, Z. Wu, J. Wang, Y. Liu, E. Tian, S. Hamid, R. Li, L. Ding, and J. Li. 2019. Baicalin ameliorates oxidative stress and apoptosis by restoring mitochondrial dynamics in the spleen of chickens via the opposite modulation of NF- κ B and Nrf2/HO-1 signaling pathway during *Mycoplasma gallisepticum* infection. *Poult. Sci.* 98:6296–6310.
- Ishfaq, M., W. Zhang, S. W. Ali Shah, Z. Wu, J. Wang, L. Ding, and J. Li. 2020. The effect of *Mycoplasma gallisepticum* infection on energy metabolism in chicken lungs: through oxidative stress and inflammation. *Microb. Pathog.* 138:103848.
- Ishfaq, M., W. Zhang, Y. Liu, J. Wang, Z. Wu, S. W. Shah, R. Li, Y. Miao, C. Chen, and J. Li. 2021. Baicalin attenuated *Mycoplasma gallisepticum*-induced immune impairment in chicken bursa of fabricius through modulation of autophagy and inhibited inflammation and apoptosis. *J. Sci. Food Agric.* 101:880–890.
- Kalapos-Kovács, B., B. Magda, M. Jani, Z. Fekete, P. T. Szabó, I. Antal, P. Krajcsi, and I. Klebovich. 2015. Multiple ABC transporters efflux baicalin. *Phytother. Res.* 29:1987–1990.
- Kanci Condello, A., S. N. Kulappu Arachchige, P. K. Shil, G. J. Underwood, A. H. Noormohammadi, P. F. Markham, N. K. Wawegama, and G. F. Browning. 2020. Duration of protective immunity induced by *Mycoplasma gallisepticum* strain ts-304 vaccine in chickens. *Vet. Microbiol.* 251:108883.
- Li, N., L. Feng, Y. Tan, Y. Xiang, R. Zhang, and M. Yang. 2018. Preparation, characterization, pharmacokinetics and biodistribution of baicalin-loaded liposome on cerebral ischemia-reperfusion after *i.v.* administration in rats. *Molecules* 23:1747.
- Lu, Z., D. Xie, Y. Chen, E. Tian, I. Muhammad, X. Chen, Y. Miao, W. Hu, Z. Wu, H. Ni, J. Xin, Y. Li, and J. J. M. i. Li. 2017. TLR2 mediates autophagy through ERK signaling pathway in *Mycoplasma gallisepticum*-infected RAW264.7 cells. 87:161-170
- Morrow, C. J., Z. Kreizinger, R. R. Achari, K. Bekő, C. Yvon, and M. Gyuranecz. 2020. Antimicrobial susceptibility of pathogenic mycoplasmas in chickens in Asia. *Vet. Microbiol.* 250:108840.
- Nielsen, E., and L. E Friberg. 2013. Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacol. Rev.* 65:1053–1090.
- Noh, K., Y. Kang, M. R. Nepal, K. S. Jeong, D. G. Oh, M. J. Kang, S. Lee, W. Kang, H. G. Jeong, and T. C. Jeong. 2016. Role of intestinal microbiota in baicalin-induced drug interaction and its pharmacokinetics. *Molecules.* 21:337.
- Raviv, Z., and S. H. Kleven. 2009. The development of diagnostic real-time TaqMan PCRs for the four pathogenic avian mycoplasmas. *Avian Dis.* 53:103–107.
- Stanley, W. A., C. L. Hofacre, G. Speksnijder, S. H. Kleven, and S. E. Aggrey. 2001. Monitoring *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in breeder chickens after treatment with enrofloxacin. *Avian Dis.* 45:534–539.
- Wang, J., M. Ishfaq, Q. Fan, C. Chen, and J. Li. 2020. A respiratory commensal bacterium acts as a risk factor for *Mycoplasma gallisepticum* infection in chickens. *Vet. Immunol. Immunopathol.* 230:110127.
- Wang, J., M. Ishfaq, and J. Li. 2021a. Baicalin ameliorates *Mycoplasma gallisepticum*-induced inflammatory injury in the chicken lung through regulating the intestinal microbiota and phenylalanine metabolism. *Food Funct.* 12:4092–4104.
- Wang, J., M. Ishfaq, and J. Li. 2021b. Lactobacillus salivarius ameliorated *Mycoplasma gallisepticum*-induced inflammatory injury and secondary Escherichia coli infection in chickens: involvement of intestinal microbiota. *Vet. Immunol. Immunopathol.* 233:110192.
- Wei, Y., J. Liang, X. Zheng, C. Pi, H. Liu, H. Yang, Y. Zou, Y. Ye, and L. Zhao. 2017. Lung-targeting drug delivery system of baicalin-loaded nanoliposomes: development, biodistribution in rabbits, and pharmacodynamics in nude mice bearing orthotopic human lung cancer. *Int. J. Nanomed.* 12:251–261.
- Wei, Y., C. Pi, G. Yang, and X. Xiong. 2016. LC-UV Determination of baicalin in rabbit plasma and tissues for application in pharmacokinetics and tissue distribution studies of baicalin after intravenous administration of liposomal and injectable formulations. *Molecules.* 21:444.
- Wu, Z., C. Chen, Y. Miao, Y. Liu, Q. Zhang, R. Li, L. Ding, M. Ishfaq, and J. Li. 2019. Baicalin attenuates *Mycoplasma gallisepticum*-induced inflammation via inhibition of the TLR2-NF- κ B pathway in chicken and DF-1 cells. *Infect. Drug Resist.* 12:3911–3923.
- Wu, Z., C. Chen, Q. Zhang, J. Bao, Q. Fan, R. Li, M. Ishfaq, and J. Li. 2020. Arachidonic acid metabolism is elevated in *Mycoplasma gallisepticum* and Escherichia coli co-infection and induces LTC4 in serum as the biomarker for detecting poultry respiratory disease. *J. Virul.* 11:730–738.
- Xiao, X., J. Sun, T. Yang, X. Fang, J. Cheng, Y. Q. Xiong, and Y. H. Liu. 2016. Pharmacokinetic/pharmacodynamic profiles of tiamulin in an experimental intratracheal infection model of *Mycoplasma gallisepticum*. *Front. Vet. Sci.* 3:75.
- Xiao, X., J. Sun, T. Yang, X. Fang, D. Wu, Y. Q. Xiong, J. Cheng, Y. Chen, W. Shi, and Y. H. Liu. 2015. *In vivo* pharmacokinetic/pharmacodynamic profiles of valnemulin in an experimental intratracheal *Mycoplasma gallisepticum* infection model. *Antimicrob. Agents Chemother.* 59:3754–3760.
- Xing, J., X. Chen, and D. Zhong. 2005. Absorption and enterohepatic circulation of baicalin in rats. *Life Sci.* 78:140–146.
- Xu, T., X. Li, M. Huang, Q. Wang, C. Li, G. Tian, and Y. Chen. 2020. A preferable approach for the quality control of Xiaoe Chiqiao Qingre granules based on the combination of chromatographic fingerprints and chemometrics. *J. Analyt. Methods Chem.* 2020:6836981.
- Zhang, W., Y. Liu, Q. Zhang, S. Waqas Ali Shah, Z. Wu, J. Wang, M. Ishfaq, and J. Li. 2020. *Mycoplasma gallisepticum* infection impaired the structural integrity and immune function of bursa of fabricius in chicken: implication of oxidative stress and apoptosis. *Front. Vet. Sci.* 7:225.
- Zhang, Y., G. Hill, Z. Ge, N. Park, H. Taylor, V. Andreasen, L. Tardy, A. Kavazis, C. Bonneaud, W. J. P. Hood, and b. z. PBZ. 2021. Effects of a bacterial infection on mitochondrial function and oxidative stress in a songbird. 94:71-82.
- Zhiyong, W., D. Liangjun, B. Jiixin, L. Yuhao, Z. Qiaomei, W. Jian, L. Rui, I. Muhammad, and L. Jichang. 2019. Co-infection of *Mycoplasma gallisepticum* and escherichia coli

- triggers inflammatory injury involving the IL-17 signaling pathway. *J. Front. Micro.* 10:2615.
- Zhu, H., Z. Qian, F. He, M. Liu, L. Pan, Q. Zhang, and Y. Tang. 2013. Novel pharmacokinetic studies of the Chinese formula Huang-Lian-Jie-Du-Tang in MCAO rats. *Phytomedicine.* 20:767–774.
- Zhu, H., Y. Qiu, D. Gong, J. Wang, and G. Sun.. 2021. A novel method for quality consistency evaluation of Yankening tablet by multi-wavelength fusion profiling combined with overall components dissolution method and antioxidant activity analysis. *J. Pharm. Biomed. Anal.* 196:113910.