Pharmacokinetics and pharmacodynamics of a novel amoxicillin/sulbactam/prednisolone intramammary infusion in lactating cows after repeated administrations

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ABSTRACT: The aim of this study was to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of a novel anti-mastitis preparation, amoxicillin/sulbactam/prednisolone intramammary infusion (CAIMM), containing 200 mg amoxicillin, 50 mg sulbactam and 10 mg prednisolone per 3 g formulation, in healthy lactating cows after repeated administrations. A parallel study was performed using the available combination product (Synulox® LC) from Pfizer, with the aim of comparing the two formulations. The concentrations of drugs in quarter milk were determined using the ultra-high performance liquid chromatography tandem mass spectrometric (UPLC-MS/MS) method. No significant difference in the major PK parameters (C_{max}, T_{max}, MRT, $t_{1/2\lambda}$, and AUC_{last}) was observed. The MIC_{90} determined in 106 isolated *Staphylococcus* spp., 64 *Streptococcus* spp. and 18 *Escherichia coli* strains was 0.5, 0.25 and 2 μg/ml, respectively. The PK/PD evaluation showed that the effective duration of action ($t > MIC_{90}$) for CAIMM (42 ± 2.46 h) was increased by 0.86 times compared with Synulox® LC (34 ± 3.17 h), but the difference was not significant ($P > 0.05$). This pharmacokinetic and pharmacodynamic study revealed that CAIMM maintained high concentrations in quarter milk for the three ingredients after repeated intramammary administrations and a similar efficacy was achieved with Synulox® LC.

Keywords: amoxicillin/sulbactam/prednisolone intramammary infusion; intramammary administration; lactating cows; pharmacokinetics; pharmacodynamics

Bovine mastitis jeopardises milk production and entails expensive treatment costs so that it brings great economic loss to the dairy industry (Berry et al. 2004; Awale et al. 2012). It is reported that *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CNS), *Streptococcus uberis, Streptococcus agalactiae, Streptococcus dysgalactiae* and *Escherichia coli* are common bovine mastitis pathogens (Watts 1988). Hence, antimicrobial drugs with good efficacy against these organisms are preferred for mastitis therapy during lactation (Cattell et al. 2001). The intramammary infusion of drugs offers a convenient option for the treatment of mastitis in dairy animals (Gehring and Smith 2006). A potential advantage of this route is the achievement of high drug concentrations at the site of infection without systemic absorption, thus preventing unwanted side-effects or tissue residues (Gruet et al. 2001).

Amoxicillin (AMX) is a member of a semi-synthetic extended spectrum penicillin group of antibiotics and interferes with bacterial cell wall synthesis. Sulbactam (SUL) is a semi-synthetic compound which inhibits β-lactamases irreversibly and can extend the *in vitro* spectrum of β-lactam antibiotics. Prednisolone (PSL) belongs to the steroidal anti-inflammatory drugs and mainly aids the reduction in swelling and related pain in in-

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tramammary treatment (Lees 1991). It has been observed that AMX alone or in combination with β-lactamase inhibitors is potentially useful for the treatment of mastitis caused by pathogenic organisms (Roberson et al. 2004; Sharma et al. 2010). A very effective clinical recovery of bovine mastitis was reported in lactating cows treated with intramammary infusion of AMX plus SUL (De-Oliveira et al. 2000; Sharma et al. 2010; De and Mukherjee 2012). Synulox® LC, a combination drug comprising AMX (200 mg), clavulanic acid (CLAV, 50 mg), and PSL (10 mg) in a 3 g syringe, was developed by Pfizer Pharmaceuticals Inc. (New York, NY, USA.) and used for the treatment of bovine mastitis. However, its application in dairy farms was subjected to great restrictions because of its expensive price in China. Furthermore, the CLAV ingredient in Synulox® LC is expensive and unstable. Thus, we have developed a novel anti-mastitis preparation, amoxicillin/sulbactam/prednisolone intramammary infusion (CAIMM), in which the ingredient SUL replaces CLAV in Synulox® LC in order to reduce the product cost and optimise its preparative technology (3 g: AMX 200 mg, SUL 50 mg, and PSL 10 mg). Herein, we investigated the pharmacokinetics (PK) and pharmacodynamics (PD) of CAIMM in lactating dairy cows after repeated administrations, with the aim of providing a new therapeutic agent for the Chinese dairy industry to treat bovine clinical mastitis.

MATERIAL AND METHODS

Materials. AMX (87.2%) and SUL (89.2%) standards were purchased from the Chinese Institute of Veterinary Drug Control (Beijing, China). PSL (99%) pure standard was obtained from Sigma-Aldrich (St. Louis, MO, USA). AMX trihydrate micronization (86.5%), SUL sodium (89.2%) and PSL acetate power (99%) were supplied by Hebei Yuanzheng Pharmaceutical Co., Ltd (Shijiazhuang, China), Jingdezhen Fuxiang Pharmaceutical Co., Ltd (Jingdezhen, China), and Henan Lihua Pharmaceutical Co., Ltd (Anyang, China), respectively. Synulox® LC intramammary infusion was provided by Pfizer Pharmaceuticals Ltd (New York, USA). Soybean oil for injection was purchased from Tieling Dongbeiya Medicated Oil Co., Ltd (Tieling, China). MacConkey agar and Mannitol salt agar were bought from Beijing Land Bridge Technology Co., Ltd (Beijing, China). Reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 29213), and *S. agalactiae* (ATCC 27956) were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd (Hangzhou, China). All other reagents were obtained from the Beijing Chemical Reagents Company (Beijing, China).

Preparation of CAIMM oil suspension. The CAIMM oil suspension was formulated by suspending the active ingredients in the dispersing mixture. Briefly, the dispersing system was first prepared by dissolving suspending agents in dispersing medium, and then AMX, SUL, PSL and wetting agent were dispersed in it using a colloid mill (JM-50c, Shanghai Wangquan pump Co., Ltd, Shanghai, China). The CAIMM formulation was optimised by determining the total score of settling volume ratio after standing for 24 h and redispersibility as the indices. An orthogonal design experiment arranged in an L_9 (3^4) orthogonal table was applied to investigate three factors (the amount of suspending agent, wetting agent and antioxidant), in which each factor has three levels. The optimised formulation was hydrogenated castor oil 0.8% (v/v), Tween-20 1% (v/v) and vitamin E 0.05% (v/v).

Optimisation of the preparation procedure. A total of 0.24 kg hydrogenated castor seed oil was dissolved in 7.5 l of soybean oil for injection with an electric heater at 85 °C so as to obtain an oily gel, which was transferred into the colloid mill. Another 15 l of soybean oil was added. After that, 2 kg AMX trihydrate, 0.5 kg SUL sodium, and 0.1 kg PSL acetate, 0.3 kg Tween-20, and 0.015 kg vitamin E were added one by one and homogenised for 30 min. Finally, more soybean oil was added into the colloid mill to reach a final volume of 30 l, which was continuously homogenised for an additional 20 min. Finally, the CAIMM oil suspension was enclosed within a glass bottle and disinfected with gamma rays of cobalt 60.

Experimental design. Twelve healthy lactating cows with an average body weight of 510 ± 48.2 kg were provided by a dairy commercial farm around Beijing and randomly divided into two treatment groups (six cows in each group) named as Group Test and Group Cntl. These cows were milked twice daily with milking intervals of 12 h. Milk production was 27.32 ± 5.56 l/day (range 20.2 to 31.3 l/day) and the mean values of days in milking was 180.4 ± 68.6 day. They had not suffered from clinical mastitis for the previous two months. The somatic cell counts (SCC) and bacteriological tests were conducted in four quarters of each cow. The SCC

was required to bebelow 200 000 per ml milk and the results of bacteriological tests were negative. Animals were in optimal nutritional condition and had free access to food and water during the entire experimental period. All procedures performed on the experimental animals were approved by the China Agricultural University Animal Care and Use Committee.

After accurate milking and teat disinfection, animals in Group Test were intramammarily administered in a single quarter at a dose of 3 g CAIMM three times at consecutive milking with intervals of 12 h. Animals in Group Cntl were given, in a single quarter, at a dose of 3 g, Synulox® LC with the same dosing regimen. Milk samples were obtained by manual pressure of the teats and the first three portions of milk was discarded. Quarter milk samples were collected at 0 (pre-administration), 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 40, 46, 52, 60, 72, 84, and 96 h after the first administration. All samples were stored at –80 °C pending the running of the assay.

Analytical method. Concentrations of AMX and PSL in milk were simultaneously determined by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) according to our previously described method (Li et al. 2012). The concentrations of SUL and CLAV were determined using a different analytical method. Samples with concentrations beyond the linear range of the standard curve were quantified after dilution. Details of the validation of the method are summarised in Table 1.

Isolation of bacteria from milk samples. All bacterial isolates were collected from approximately 300 lactating cows suffering from clinical mastitis in 20 dairy farms surrounding Beijing between January, 2010 and December, 2011. Primary cultures of milk samples were performed by plating 100 µl milk on MacConkey agar for *E. coli* strain isolation, on Mannitol salt agar for *Staphylococcus* spp. strain isolation, and on K-F Streptococcus agar (Difco, NJ, USA) for *Streptococcus* spp. strain isolation. The plates were incubated at 37 °C for 24–48 h. All bacteria isolates were identified by colony morphology, Gram-staining characteristics and API microorganism identification test kits (Biomerieux, Marcy l' Etoile, France). The isolates were then stored at –80 °C in 20% sterile glycerol until further testing.

MIC determination. MIC tests were performed according to the microdilution broth method, as recommended by the Clinical Laboratory Standards Institute (CLSI 2008). Reference standards of AMX and SUL were dissolved in 0.01M phosphate buffer (pH 7.4) and then diluted in sterile Mueller-Hinton broth. Bacterial strains were prepared by diluting an overnight MH broth culture in buffered saline solution to a density of 0.5 on the McFarland turbidity scale. *E. coli* (ATCC 25922), *S. aureus* (ATCC 29213), and *S. agalactiae* (ATCC 27956) were inoculated as control strains.

Pharmacokinetic analysis. The corresponding milk concentration-time profiles of the drugs AMX, PSL, SUL and CLAV were created using Origin 8.0 (Originlab Corporation, Northampton, USA). A noncompartmental analysis (NCA) was carried out on milk drug concentrations after the last treatment using the WinNonLin Professional 6.2 software (Pharsight Corporation, Mountain View, CA, USA). All results were expressed as mean ± SD. Differences in PK parameters of $t_{1/2\lambda}$ (elimination half-life), C_{max} (maximum milk concentration), T_{max} (time to reach C_{max}), AUC_{last} (area under the milk concentration-time curve for time zero to *t*), and MRT (mean residence time) between different treatment groups were performed by two-way analysis of variance (ANOVA) using the statistical program SPSS version 17.0 (SPSS Inc., Chicago, USA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Preparation of CAIMM oil suspension

After testing and optimising the appropriate ratio of the excipients and preparation process, a novel anti-mastitis preparation-CAIMM was successfully developed. CAIMM is an almost white- to cream-coloured oil suspension that is easy to redisperse with a three-hour setting volume ratio of 100.0%. Syringeability and flowability both conformed with the Technical Standards set by the Ministry of Agriculture of the People's Republic of China (Commission of Chinese Veterinary Pharmacopoeia – CCVP 2005).

Milk pharmacokinetics

The corresponding milk concentration–time profiles of the drugs AMX, PSL, SUL and CLAV

Table 1. Analytical method and validation for AMX, PSL, SUL and CLAV determination in milk samples

Analyte	Analytical technique	Separative conditions	Extraction procedure	Method performance									
				Fortifical levels (ng/ml)	Recovery[a] (%)	Intra-day recision[b] (%)	Inter-day precision[c] (%)	LOD[d] (ng/ml)	LOQ[e] (ng/ml)	Regression and correlation			
										slope	inter-cept	linear range (ng/ml)	R^f
AMX	UPLC-ESI(+)-QqQ; 2 MRM transitions: *m/z* 366.10/349.10 *m/z* 366.10/08.18 (AMX); *m/z* 361.08/343.10 *m/z* 361.08/147.06 (PSL). Penicillin G-d_7 and prednisilone-d_6 as internal standard (IS)	BEH C_{18} column (50 × 2.1 mm, 1.7 µm); gradient elution with a mobile phase of 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B) at a flow rate of 0.3 ml/min; i.e. 0–1 min, 98% A–2% B; 1.5–2.5 min, 15% A–85% B; 3.0–3.5 min, 2% A–98% B; 5.5 min, 98% A	AMX and PSL were extracted from 2 ml milk. Then, 6 ml acetonitrile were added to all samples, followed by centrifugation (8603 × g for 10 min), extraction again in duplicate and purified by solid-phase extraction using C_{18} cartridges (Varian Bond Elut 6cc, 500 mg). The dry residue was reconstituted with 1 ml 0.1% formic acid : 0.1% formic acid-acetonitrile (98 : 2, v/v)	2	93.4	3.8	10.8	1	2	0.1547	0.9545	2-1000	0.9993
				4	90.1	2.6	6.5						
				6	88.3	6.4	7.3						
PSL				3	101.2	3.4	12.1	1	2	0.4309	2.4961	2-1000	0.9997
				6	98.3	4.1	9.6						
				9	96.4	4.3	8.2						
SUL	UPLC-ESI(–)-QqQ; 2 MRM transitions: *m/z* 232.0/188.0, *m/z* 232.0/140.1: Penicillin G-d_7 as IS	BEH C_{18} column (50 × 2.1 mm, 1.7 µm); gradient elution with a mobile phase of 0.05% formic acid in water (eluent A) and 0.05% formic acid in acetonitrile (eluent B) at a flow rate of 0.3 ml/min; i.e. 0–0.5 min, 95% A–5% B; 1.5–2.0 min, 5% A–5% B; 3.0 min, 95% A	SUL were extracted from 2 ml milk. Then, 1 ml 0.5M hydrochloric acid and 10 ml ethyl acetate were added, followed by centrifugation at 8603 × g for 10 min, and evaporation to dryness under a stream of nitrogen at 40 °C. The residue was dissolved with 500 µl water.	10	96.3	5.2	9.1	5	10	0.0066	0.0934	10-1000	0.9961
				20	99.6	3.7	8.6						
				50	97.5	4.5	8.5						
CLAV	UPLC-ESI(–)-QqQ; 2 MRM transitions: *m/z* 197.8/135.8, *m/z* 197.8/108.2; SUL as IS	BEH Shield RP C_{18} column (50 × 2.1 mm, 1.7 µm). The same mobile phase and flow as SUL, i.e. 0–1 min, 95% A–5% B; 1.5–2.0 min, 5% A–95% B; 3.0 min, 95% A	Milk samples (2 ml) were extracted with acetonitrile. After centrifugation at 7463 × g for 10 min, *n*-hexane was added to defat. Then, the acetonitrile extracts were evaporated at 30 °C under a nitrogen stream. Dry extracts were reconstituted with 400 µl water	20	78.2	5.4	10.2	6	20	0.0007	0.0009	20-1000	0.999
				50	85.4	4.2	9.4						
				100	90.1	3.1	8.5						
				200	88.9	2.9	6.3						

[a]recovery values of the analytical method are calculated at each fortifical level (n = 6); [b]intra-day precision was calculated at each concentration level (n = 6); [c]inter-day precision was calculated at each concentration level on three different days (n=18); [d]limit of detection; [e]limit of quantitation; [f]correlation coefficient

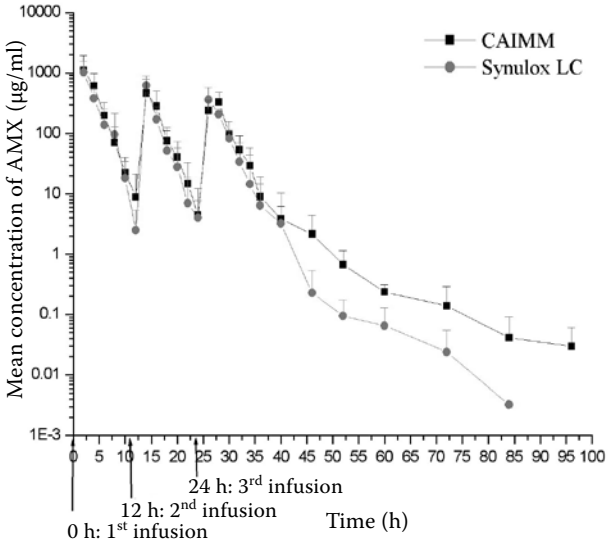

Figure 1. Milk AMX concentration-time profile after dosing with 3 g CAIMM or Synulox® LC three times at consecutive milkings at intervals of 12 h

are depicted in Figures 1 to 3, respectively. The ingredient AMX in CAIMM demonstrated rapid distribution with a high concentration (1157.37 ± 796.54 μg/ml) at a dose of 200 mg after the first administration, and decreased significantly (352.75 ± 116.81 μg/ml) after the third administration. The PK parameters of AMX, PSL, SUL and CLAV after the last treatment in different treatment groups are summarised in Table 2.

The results in Table 2 show that the PK parameters of AMX, SUL and PSL of CAIMM are similar to those of Synulox® LC. There was no significant difference ($P > 0.05$) in any of the PK parameters ($t_{1/2\lambda}$, T_{max}, C_{max}, AUC_{last} and MRT) after the third infusion, indicating that CAIMM had a similar distribution and elimination through the quarter milk compared with Synulox® LC.

Pharmacokinetic/pharmacodynamic integration

A total of 216 bacteria isolates from approximately 300 lactating cows suffering from bovine clinical mastitis were obtained in this study. The obtained MIC range, MIC_{50} and MIC_{90} values for AMX and AMX/SUL (4 : 1) toward these isolated strains are summarised in Table 3. The MICs of isolated strains decreased 2- to 8-fold for AMX/SUL (4 : 1) compared with AMX. The relationship between PK profile and the MIC_{90} of the strains is shown in Figure 4. Milk AMX concentrations were five times higher than the MIC_{90} of isolated bovine pathogens at 48 h after the third administration. To illustrate the efficacy of CAIMM, the comparisons of PK/PD parameters were calculated with the MIC and PK parameters of AMX only. The $t > MIC_{90}$ and $t > MIC_{50}$ of CAIMM against isolated bovine mastitis pathogens were 42 ± 2.46 h and 48 ± 1.12 h, respectively (Table 2). The effective duration of action ($t > MIC_{90}$) for CAIMM (42 ± 2.46 h) was increased by 0.86 times compared with Synulox® LC (34 ± 3.17 h), but the difference was not significant ($P > 0.05$).

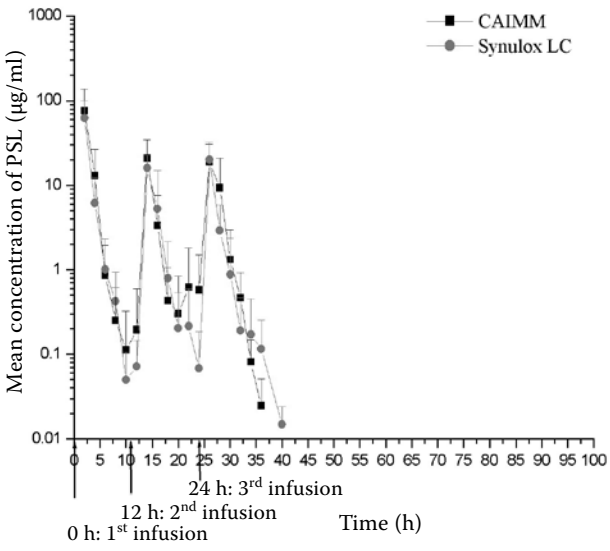

Figure 2. Milk PSL concentration-time profile after dosing with 3 g CAIMM or Synulox® LC three times at consecutive milkings at intervals of 12 h

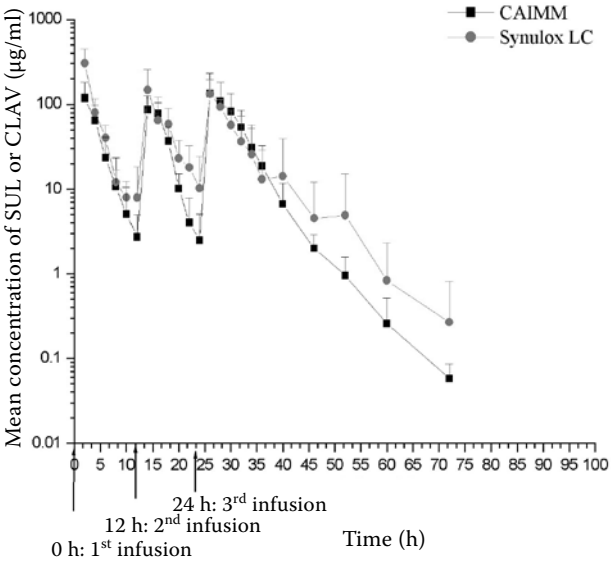

Figure 3. Milk SUL or CLAV concentration-time profile after dosing with 3 g CAIMM or Synulox® LC three times at consecutive milkings at intervals of 12 h

Table 2. Comparison of pharmacokinetic parameters (mean ± SD) for the ingredients AMX, PSL, SUL, or CLAV in quarter milk of cows (n = 6) after dosing with 3 g CAIMM or Synulox® LC three times at consecutive milkings at intervals of 12 h

Drug	Parameters	Group test (CAIMM, n^a = 6)	Group Cntl (Synulox®LC, n^a = 6)	P-value (ANOVA)
		mean ± SD		
AMX (200 mg)	$t_{1/2\lambda}$ (h)	7.17 ± 3.38	5.34 ± 3.30	0.20
	T_{max} (h)	3.33 ± 1.03	2.67 ± 1.03	0.61
	C_{max} (µg/ml)	352.8 ± 116.8	387.7 ± 174.7	0.74
	AUC_{last} (h/µg/ml)	1585.5 ± 521.8	1445.4 ± 700.7	0.77
	MRT (h)	4.63 ± 0.82	3.77 ± 0.62	0.14
	$t > MIC_{90}$ (h)	42 ± 2.46	34 ± 3.17	0.42
	$t > MIC_{50}$ (h)	48 ± 1.12	40 ± 2.54	0.57
PSL (10 mg)	$t_{1/2\lambda}$ (h)	0.95 ± 0.33	1.64 ± 1.57	0.33
	T_{max} (h)	2.50 ± 1.00	2.00 ± 0.00	0.39
	C_{max} (µg/ml)	26.2 ± 7.6	19.5 ± 13.9	0.69
	AUC_{last} (h/µg/ml)	81.9 ± 40.2	48.7 ± 37.7	0.50
	MRT (h)	2.80 ± 0.48	2.46 ± 0.27	0.43
SUL/CLAV (50 mg)	$t_{1/2\lambda}$ (h)	3.59 ± 1.02	5.15 ± 1.26	0.17
	T_{max} (h)	2.33 ± 0.82	3.33 ± 1.63	0.20
	C_{max} (µg/ml)	134.7 ± 96.5	122.9 ± 61.2	0.83
	AUC_{last} (h/µg/ml)	931.8 ± 612.6	835.7 ± 425.6	0.76
	MRT (h)	6.21 ± 0.44	7.17 ± 4.22	0.56

[a]the number of lactating cows in each group

DISCUSSION

In China, recent investigations have shown that the annual incidence of clinical mastitis is about 54.3%, and the annual economic loss is estimated to be about 316 million Yuan (Liu 2004; Li et al. 2009). It has been reported that *E. coli, S. uberis, S. aureus, S. dysgalactiae* and *S. agalactiae* are prevalent pathogens of mastitis (Cheng et al. 2010). *In vivo* studies have demonstrated that a bacteriological cure rate of 67% was achieved when intramammary AMX was used against these common pathogens (Roberson et al. 2004). In human medicine, several studies have reported the efficacy of a AMX/SUL combination in the treatment of bacterial infections, including *E. coli* and *Acinetobacter baumannii* (Bantar et al. 1999, 2009). Moreover, the results of our preliminary study showed that a good bactericidal activity *in vitro* was achieved for the AMX/SUL (4 : 1) combination against these common mastitis pathogens (unpublished results). At present, Synulox® LC is widely used for the treatment of dairy cow mastitis and a good clinical efficacy is obtained in China. However, its application in dairy farms is subject to great restrictions because of its expensive price. Therefore, it is imperative to develop a novel anti-mastitis preparation in order to reduce mastitis treatment costs, as such a combination product is not available in China.

In this study, a novel CAIMM oil suspension was developed after testing and optimising the appropriate ratio of suspending agents, wetting agent, antioxidant and the active constituents. We have produced three batches of the combination products using colloid mill in the good manufacturing practice (GMP) workshop in the Chinese Animal Husbandry Industry Chengdu Biopharm Itd (Chengdu, China). The volume of each batch was 30 l.

The milk concentration-time relationship was modelled from lactating cows administered the

Table 3. MIC determination for AMX and the AMX/SUL (4 : 1) combination against *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli* strains isolated from milk samples

Strain	n[a]	AMX (μg/ml)			AMX/SUL (4 : 1) (μg/ml)		
		range	MIC_{50}[b]	MIC_{90}[c]	range	$MMIC_{50}$[b]	$MMIC_{90}$[c]
Staphylococcus aureus	28	0.25–8	4	8	0.125–4	0.5	1
Staphylococcus spp.	106	0.25–4	1	2	0.125–1	0.25	0.5
Streptococcus agalactiae	24	0.5–2	0.5	1	0.125–1	0.125	0.25
Streptococcus dysgalactiae	18	0.25–1	0.25	1	0.125–0.5	0.125	0.25
Streptococcus uberis	22	0.5–4	1	2	0.125–2	0.125	0.25
Escherichia coli	18	4–8	4	8	1–4	1	2

[a]number of isolated strains; [b]minimum inhibitory concentration required to inhibit the growth of 50% of organisms; [c]minimum inhibitory concentration required to inhibit the growth of 90% of organisms

new formulation. The ingredients AMX, SUL and PSL of CAIMM in quarter milk had a similar PK property (Figure 4), which is important when examining two or more drugs in a combination product because the PK characteristics of one active ingredient could be affected by the other ingredients, vehicle or excipients. AMX was generally quantifiable for 60 h after the last administration (Figure 1). The milk elimination half-time ($t_{1/2\lambda}$) of AMX (7.17 ± 3.38 h) in CAIMM was similar to that in Synulox® LC (5.34 ± 3.30 h, $P > 0.05$). The different antibiotic and formulation vehicle could contribute to this PK difference in CAIMM and Synulox® LC. Similar observations have been reported for a plethora of β-lactam antibiotics used in mastitis therapeutics (Whitten and Hanlon 1997; Cagnardi et al. 2010; Zonca et al. 2011).

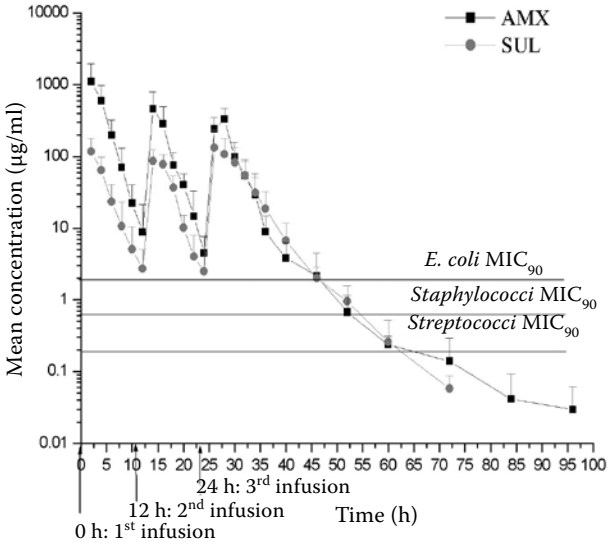

Figure 4. The relationship between mean milk concentration-time profile of AMX and SUL in CAIMM and MICs for the AMX/SUL (4 : 1) combination on strains isolated from milk samples

PSL, a short-acting glucocorticoid, was basically undetectable in milk after about 12 h post-3rd administration. Another PK study of Synulox® LC demonstrated that PSL did not have a prolonged residence time in the milk/udder (about 8–10 h post-infusion, levels of PSL were less than 1 μg/ml) (Synulox Lactating Cow Article 34 referral – European Medicines 2011). A rapid elimination half-time of PSL in CAIMM was observed ($t_{1/2\lambda}$ = 0.95 ± 0.33 h, Table 2) in this study. PSL was rapidly distributed after the first administration with a peak milk concentration of 76.91 ± 59.51 μg/ml, which decreased to a mean C_{max} value of 26.20 ± 7.56 μg/ml post-3rd administration. This indicates that the drug is rapidly released from the formulation and that a high system absorption could be foreseen when PSL is administrated intramammarily.

This study is the first to report the PK characteristic of SUL in lactating cows after repeated intramammary administration. Metabolic studies using UPLC-TOF/MS showed that SUL was mainly eliminated in the way of the parent compound in quarter milk samples. The metabolites did not contribute significantly to its elimination (data not shown). After intramammary administration of CAIMM containing 50 mg of SUL in a single quarter of lactating cows, the mean peak concentration was 134.7 ± 96.5 μg/ml and the time to C_{max} (t_{max}) was 2.33 ± 0.82 h. The mean elimination half-time and AUC_{last} did not have a significant difference from those of CLAV at the same dose (Table 2). To better understand drug efficacy and residues within the bovine udder, a more comprehensive understanding of milk drugs concentrations in the different compartments of the mammary gland

would be helpful (Whitten et al. 2012). Therefore, the compartmental and NCA model was studied to model the milk concentration–time data of SUL. We found that milk SUL concentration-time data fit a single one compartment (data not shown). However, the single compartment that may be appropriate for consideration of drug residues does not assist in understanding efficacy because this model is simple and sampling data are limited (Whitten et al. 2012). Considering the PK/PD integration of CAIMM and time-rich data with many collection time points, the NCA model was finally selected in this study.

According to Whitten et al. and Stockler et al., milk composition and milk drug concentrations differed between fore–milk, pooled milk and milk strippings (Stockler et al. 2009; Whitten et al. 2012). In this study, fore–milk was collected from a single intramammary quarter to study the PK-PD of CAIMM in lactating cows. However, pooled milking samples are inappropriate for estimation of milk drug concentrations needed for evaluation of efficacy (Stockler et al. 2009). Pharmaceutical products which are administered to food producing animals have the potential of leading to the introduction of drug residues into the human food chain. Thus, it is very important to pay attention to residual problems of new compounds. The maximum residual limits (MRLs) of AMX and PSL in milk are 4 and 6 μg/kg, respectively. At present, there is no MRL set for SUL in milk. Considering similar chemical properties with SUL and CLAV, the MRL of CLAV in milk (200 μg/kg) was set as the permissible limit of residual analysis for SUL. Our preliminary residual depletion studies revealed that the milk withdrawal time of AMX and SUL after IMM infusion of CAIMM in healthy dairy cows was 53.7, 31 h, respectively (unpublished results).

Finally, the PK/PD evaluation was further studied according to milk AMX pharmacokinetics and the MIC_{90} and MIC_{50} of the isolated strains to predict clinical efficacy. It is generally accepted that the *in vivo* efficacy of β-lactam antibiotics is primarily correlated with the duration for which antibiotic concentration at the site of infection is greater than the MIC for the infective organism (t > MIC) (Erskine et al. 2003). Failure to maintain concentrations above the MIC for a sufficient time may result in treatment failure. In this study, the MIC_{90} calculated for the 28 *S. aureus* and *Streptococcus* spp. isolates was 0.5, 0.25 μg/ml, respectively. The MIC_{50} was also used to avoid excessive doses as it is more precisely calculated (Schentag 2000). Milk AMX concentrations were five times higher than the MIC_{50} of these isolated pathogens at 48 h after the third infusion. As shown in Table 3, the MICs of isolated strains decreased 2- to 8-fold for AMX/SUL (4:1) compared with AMX. It was concluded that the presence of SUL in CAIMM meant that AMX had a bactericidal activity against strains that would otherwise be resistant because of β-lactamase production.

In summary, in this study a new oily suspension-amoxicillin/sulbactam/prednisolone intramammary infusion (CAIMM) was successfully developed to control bovine udder infection. The quality of the new combination product is consistent with the Technical Standards set by the Ministry of Agriculture of People's Republic of China. Pharmacokinetic experiments revealed that CAIMM maintained high concentrations in quarter milk for the three ingredients after repeated intramammary administrations and the pharmacokinetic characteristics of the two combination products were similar. The PK/PD evaluation demonstrated that the effective duration of action (t > MIC_{90}) for CAIMM against bovine mastitis pathogens was 42 ± 2.46 h.

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