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Studies on Cefquinome Sulfate Intramammary Infusion Composited with Varied Excipients for the Prevention of Dairy Cows Mastitis in the Dry Cow Period

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ABSTRACT

Background: The prevention and treatment of dairy cow mastitis during the dry period are crucial for the dairy industry. In this work, 200 dairy cows without mastitis and normal entering dry period were selected and randomly divided into 4 groups, for the first time, to explore the prevention effects using the different formulations of Cefquinome Sulfate Breast Injection with varied excipients.

Methods: Three formulations with various excipients were further evaluated based on the drug residue analysis, somatic cell reduction in the milk and clinical symptoms of mastitis. In the control group, cloxacillin benzathine was utilized for comparisons.

Result: All three Cefquinome test groups showed a better effect on prevention than the benzathine cloxacillin group in terms of drug release time, post-partum drug residue, reducing somatic cell count and preventing mastitis in the dry period. Various excipients showed slightly different impacts on the drug residues, the changes in somatic cell counts of dairy cows before and after medication. The prevention effects on mastitis from different excipients were similar after 30 d.

Key words: Cefquinome sulfate breast injection, Cow mastitis, Dry period, Prevention, Various excipients.

INTRODUCTION

Mastitis in cows has been considered as a most common and frequently-occurring disease that greatly affects the development of the global dairy industry and seriously causes huge losses in dairy production (Ruegg, 2017; Heikkilä *et al*., 2018). The annual loss caused by bovine mastitis reaches up to 15 ~ 45 billion yuan (Wang *et al*., 2019). According to reports, the average incidence of dairy cow mastitis was from 20% to 80% (Zhang *et al*., 2016). Currently, With the rapid development of intensive dairy farming and the popularization of mechanized milking, the infection rate and incidence of mastitis have been greatly increased. Due to the diversity and complexity of the pathogenic microorganisms of mastitis (Chouhan *et al*., 2021), the prevention and treatment of mastitis seem to be a great challenge for the sustainable and safe development of the global dairy industry (Dohare *et al*., 2021; Ramasamy *et al*., 2021). Therefore, the prevention of dairy cow mastitis is of significance for both developed and developing countries.

To date, the basic prevention and treatment for the control of mastitis are based on antibiotic treatment (Krömker and Leimbach, 2017). To improve the prevention of mastitis cow during the dry period, Cefquinome injection has successfully developed. Cefquinome is a veterinary fourth-generation cephalosporin used in the treatment of coliform mastitis and other infections with great pharmacological properties (Bradley and Green, 2009), which has been widely employed as an effective medicine for the prevention and treatment of bovine mastitis.

To the best of our knowledge, in addition to the active component present in the drugs, varied excipients of composed drugs have also been considered to play a significant role in the delivery, effectiveness and even the metabolism of the active drugs. To develop a suitable drug formulation, therefore, it would be significantly crucial to explore the possible impact of excipients on the prevention of mastitis. In this work, we would like to explore the influence of various excipients on the clinical indicators, such as the drug residue, the reduction of somatic cells in milk samples and the clinical symptoms of dairy cows mastiti.

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MATERIALS AND METHODS

Drug formulations

In this work, three Cefquinome sulfate infusion solutions (3.0 g, batch number: 20180701) were formulated with various excipients. Cefquinome sulfate was provided by Aimekian (Shandong, China) biomedical Co., Ltd. The formulation I included Cefquinome (150.0 mg), excipient 1

(Sigma-Aldrich, Shanghai), excipient 2 (Hansheng H and R group) and soybean oil for injection (Zhejiang Tianyu yam Oil Co., Ltd.). The formulation II contained Cefquinome (150.0 mg), excipient 1 (Shanxi Jinyang pharmaceutical excipients Co., Ltd.), excipient 2 (Xi'an Tianzheng pharmaceutical excipient Co., Ltd.) and soybean oil for injection (Zhejiang Tianyu yam Oil Co., Ltd.). The formulation III was composed of Cefquinome (150.0 mg), excipient 1 (Sigma-Aldrich, Shanghai), excipient 2 (Xi'an Tianzheng pharmaceutical excipient Co., Ltd.) and soybean oil for injection (Zhejiang Tianyu yam Oil Co., Ltd.). Furthermore, cloxacillin benzathine infusion (batch number: 20180602, 10 ml, 0.5 g of the active drug) from the Eastern Along Pharmaceutical (Foshan, China) was utilized as the control group. Specifically, all the experimental works were carried out at the Animal Clinical lab at Eastern Along Pharmaceutical in year of 2020.

Cows management

In the experiment, 200 healthy dairy cows were randomly divided into 4 groups. During daily feeding, no antibiotics or other medical drugs were added. The drug administration was carried out on the first day of the dry period after milking in each milk area. On the day of the dry period, the milk samples were collected for somatic cell counting. Meanwhile, the body temperature, any redness, swelling, hotness and pain were all recorded. The corresponding details were summarized in Table 1.

Sampling and measurements

The experimental cows were regularly observed by professional clinical veterinarians and breeders. Any corresponding mastitis symptoms were recorded. On the 50th day of drug administration, milk samples were taken on the first day and the fourth day in the postpartum period, respectively. Before the sampling, the cow udder was initially rinsed with clean pure water and rapidly cleaned with a clean towel. Subsequently, the nipples test area was sterilized using 75% alcohol. After the alcohol was evaporated, the fourth squeezed milk sample was collected into a sterilized test tube. During the milk sampling, 10 mL of milk was collected after the first three squeezed milk was dumped. Immediately, the milk sample was sent to the laboratory for further index determination. The E50 drug residue testing kit (Delvotest ® SP NT) was used for the drug residue detection. The milk samples were subsequently analyzed by the Milk Somatic Cell Counter (SCC600 Beijing Tongde Venture Technology Co., Ltd.).

The clinical symptoms observation was also performed during the dry period and within postpartum 30 days, in which the overall condition of sick cows and the symptoms of the infected milk area were recorded. Initially, the milk juice was an aqueous sample with fine particles or a small clump of floccules. After several days, the milk gradually turned into brownish yellow liquid. Any swelling, fever and hardness were fully documented. All data statistics and analyses above were performed using the SPSS18.0 software. Specifically, values ≤ 0.05 were regarded as statistically significant.

Results and Discussion

Drug residue in milk production

On the 50th day after drug administration, the first day and the fourth day during the post-partum period, the concentration of antibiotic drugs in the milk samples was analyzed using the E50 drug residue testing kit. The corresponding results were summarized in Table 2. After the 50th day of the drug administration, 88% of the cows were positive for drug residues and 12% with testing results were close to the detection limit. Group 3 had similar results as compared to group 1. Group 2 showed the highest negative percentage. Particularly, no cow showed the Cefquinome residue in the milk.

Furthermore, control group 4 also showed no significant difference as compared to group 3. The drug residue on day 1 in the postpartum period was also tested. Group 1 demonstrated the highest negative percentage of 40% and the lowest positive percentage (38%), while groups 2 and 3 showed slightly lower percentages (34% and 38%). But group 2 (46%) had a slightly higher positive percentage than group 3 (44%). As compared to the administration of Cefquinome sulfate, control group 4 had a much higher positive percentage (62%). Specifically, only 20% of cows were negative for the drug residues. On the 4th day of the postpartum period, all the groups injected with Cefquinome were 100% negative. However, 10% of cows in group 4 had the presence of cloxacillin benzathine.

The drug residues analyzed from the milk indicate the metabolism of these drugs (Gan *et al*., 2013). As the data on the 50th day after injection, the majority of cows ($\geq$ 88%) in all the groups showed a positive with antibiotics in the milk. These experimental groups 1-3 were slightly different as compared to the control group 4. Therefore, Cefquinome sulfate and cloxacillin benzathine showed differences in metabolism. In addition, various excipients utilized in these formulations 1-3 induced a slight difference in the metabolism of Cefquinome. The utilization of excipient 2 slightly increased the metabolism rate of the drug since 12% of cows were negative in the drug residues analysis. In contrast, excipient 1 seems to slow down the metabolized profile of Cefquinome sulfate as compared to excipients composited in groups 2 and 3.

During the post-partum period, the metabolized profiles of two drugs (Cefquinome sulfate and cloxacillin benzathine) were different. On the first day, group 1 showed the highest negative percentages (40%), while the control group had the lowest (20%). In addition, group 3 showed higher numbers of the cow without a drug in the milk. It seems that excipients play a significant role in the metabolism of these drugs. Excipient 1 can most effectively promote drug utilization. But the varied component of excipients also had a slightly different influence. As the data shown in Table 2, the trends of influence on the metabolism of Cefquinome sulfate on the first day was observed like excipient 1> excipient 3> excipient 2, in which the drug metabolism of Cefquinome in groups 1 and 3 was significantly more efficient than that in group 2. Interestingly, after 4 days, groups 1 to 3 injected with the Cefquinome showed 100%

Table 1: The grouping and treatment details for dry dairy cows.

Group	Cow numbers	Milk area	Treatments (one time)
1	50	196	Breast tube administration of formulation I (3.0 g, 150 mg cefquinome)
2	50	195	Breast tube administration of formulation II (3.0 g, 150 mg cefquinome)
3	50	198	Breast tube administration of formulation III (3.0 g, 150 mg cefquinome)
4	50	196	Breast tube administration of cloxacillin benzathine (10 ml, 0.5 g of the active drug)

Table 2: Results of drug residues in dairy cows at different times.

Sampling time	Group	Cow numbers (percentage)		
		Positive (+)	Negative (-)	Approaching detection limit (±)
50 days after drug administration	1	44 (88%)	0 (0%)	6 (12%)
	2	40 (80%)	6 (12%)	4 (8%)
	3	42 (84%)	2 (4%)	6 (12%)
	4	40 (80%)	3 (6%)	7 (14%)
Day 1 in postpartum period	1	19 (38%)	20 (40%)	11 (22%)
	2	23 (46%)	17 (34%)	10 (20%)
	3	22 (44%)	19 (38%)	9 (18%)
	4	31 (62%)	10 (20%)	9 (18%)
Day 4 in postpartum period	1	0 (0%)	50 (100%)	0 (0%)
	2	0 (0%)	50 (100%)	0 (0%)
	3	0 (0%)	50 (100%)	0 (0%)
	4	2 (4%)	45 (90%)	3 (6%)

negative in the analysis of drug residue, indicating the fast utilization of cefquinome sulfate drug. In group 4, 10% of cows were still observed with the presence of cloxacillin benzathine. These results evidenced that Cefquinome sulfate had a higher metabolism rate during the postpartum period as compared to cloxacillin benzathine. Based on the drug residue analysis on day 1 in the postpartum period, group 1 showed the lowest percentage. Moreover, no difference was obtained on the fourth day among these various excipients. Therefore, we presumed that excipient 1 might have a potentially best-induced role in the metabolism of Cefquinome sulfate.

Milk somatic cell count in the milk sample

Milk samples of each cow were collected before the drug administration and on the 2nd, 5th and 10th day after the parturition, in which the corresponding somatic cells were counted (Table 3). In particular, group 1 had the highest SCC before the drug administration and it also demonstrated the lowest SSC during the treatment among groups 1-3. SSC observed in experimental groups had a trend like a group 1< group 3< group 2. Before the treatment, group 4 showed a lower SSC than the other 3 experimental groups. However, on the 2nd, 5th and 10th day of the post-partum period, all SCC obtained from group 4 were much higher (>25%) than those collected from experimental groups (1-3).

As shown in Table 3, somatic cells present in the milk samples demonstrated great differences between the experimental groups and the control group. Initially, SCC in each group showed no great difference (83.3~86.6 ×10000), but all of them were higher than 500,000/mL. Moreover, there was no significant difference in SCC ($P>0.05$) for both the experimental and control groups, indicating that these groups can be useful for the drug efficacy evaluation. After the drug administration, therefore, SCC for both test groups and the control group significantly decreased. Even group 4 had a lower SSC number than all 3 experimental groups before the drug administration, but all SCC in groups 1-3 were much lower (>25%) than those obtained in group 4. Thus, the results show that the test group can effectively reduce the somatic cell number, particularly, whose effect was better than the control group. In three experimental groups, SSC showed a trend like a group 1< group 3< group 2, which indicates that excipient components have a specific impact on the controlling of SSC. Particularly, excipient 1 can better control the somatic cell number as compared to groups 2 and 3.

Clinical symptoms of mastitis

The clinical symptoms of mastitis for all experimental and control groups were recorded during the dry period and within post-partum 30 days in Table 4. Specifically, a cow was observed with mastitis within 30 d during the post-partum period in group 1. In the experimental group, cow numbers observed with mastitis within post-partum 30 d were determined to be 1, 2, 1 (group1, 2 and 3), respectively. As for control group 4, four cows during the dry period were found with mild or serious clinical symptoms of mastitis.

Based on the clinical symptoms of mastitis for all experimental and control groups summarized in Table 4, groups 1 and 3 showed the lowest cow numbers with mastitis. Therefore, both formulations 1 and 3 would be suitable for the prevention of mastitis either in the dry period or 30 days post-partum period. All experimental groups 1-3 had lower numbers of cow mastitis as compared to group 4. So Cefquinome demonstrated a better effect on the

Table 3: The number of milk somatic cells before and after the treatment.

Group	Somatic cell count (SCC, 10000/ml)			
	Before drug administration	Day 2 in post-partum period	Day 5 in post-partum period	Day 10 in post-partum period
1	86.6±16.8	31.1±15.7[a]	30.6±15.5[a]	33.0±16.1[a]
2	84.9±17.7	34.9±15.9[a]	34.3±15.2[a]	35.6±16.8[a]
3	87.1±18.4	33.3±16.8[a]	32.5±16.4[a]	34.8±17.3[a]
4	83.3±19.8	59.7±21.2[b]	60.4±21.3[b]	62.8±23.7[b]

Note: a with a significant difference between groups (P<0.05); b with no significant difference between groups (P>0.05).

Table 4: Clinical mastitis observation results.

Group	Cow numbers observed with mastitis during the dry period	Cow numbers observed with mastitis during the post-partum period (within 30 d)
1	0	1
2	0	2
3	0	1
4	1	4

prevention of cow mastitis as compared to cloxacillin benzathine (Davis *et al*., 1975). Typically, all cows with mastitis showed similar behaviors, which were observed with mild depression, slightly poor appetite, mild swelling, pain, fever, flocculent milk (Kvist, 2016). Therefore, various excipients would not influence the clinical symptoms of mastitis. Specifically, only one cow was observed with mastitis in group 4. But no cow was found with any symptoms of mastitis for groups 1-3. These results concluded that the Cefquinome can effectively prevent the occurrence of postpartum clinical mastitis. Moreover, the effect derived from the Cefquinome seemed to be better than the control group. Group 1 and 3 demonstrated better prevention effects as compared to group 2. Therefore, we presumed that formulations 1 and 3 would be better Cefquinome composites in preventing postpartum clinical mastitis.

Conclusion

In this work, the effects of Cefquinome sulfate intramammary infusion composited with varied excipients were specifically investigated for the prevention and treatment of dairy cows mastitis in the dry cow period in China. Our results showed that various excipient components showed a slight influence on the clinical indicators including drug residue, reduction of somatic cells in milk and clinical symptoms of mastitis. According to the drug release time and these clinical indicators, all Cefquinome test groups demonstrated a better prevention effect on mastitis, as compared to the control group with cloxacillin benzathine. Particularly, group 1 with formulation I was proven to show the longest drug release time, lowest drug residue during the postpartum period, the best reduction of somatic cells in milk and suitable control of clinical symptoms of mastitis. So the formulation I had the optimal component of cefquinome sulfate breast injection, which might be useful to effectively prevent and treat the dairy cows mastitis in the dry cow period.

Conflict of interest

All authors declare that they have no conflict of interest.

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