Original Article

Clinical Study on the Treatment of Piroline against Bovine Mastitis

Jian-Ping Liang[1,2] Bao-Cheng Hao[1*] Xue-Hong Wang[1] Zhi-Ting Guo[1] Wen-Zhu Guo[1] Ruo-Feng Shang[1] Lei Tao[1] Yu Liu[1] Zhao-Zhou Li[1] Lan-Ying Hua[1] Shu-Yang Wang[3]

Abstract

The study aims to investigate the efficacy of piroline and antibiotics in the treatment of bovine mastitis caused by *Streptococcus uberis* (*S. uberis*) and *Escherichia coli* (*E. coli*) during dry-milk period. 1880 cows in dry-milk period were divided into 4 groups and treated with penicillin G, ammonia benzyl penicillin, ceftiofur and piroline, respectively. The efficacy of each medicine in treating mastitis caused by *E. coli* intramammary infection (*E. coli* IMI) was followed: 31.2% for penicillin G, 36.9% for ammonia benzyl penicillin, 61.3% for ceftiofur, and 64.4% for Piroline. For those caused by *S. uberis* intramammary infection (*S. uberis* IMI), the efficacy of ceftiofur was 90% and piroline was 94.4%. The results indicated that piroline was more effective than the other three in treating the disease. The following analysis on milk samples demonstrated that there was no piroline residue in those treated cows' milk. Based on these data, it can be predicted that piroline will have a bright future in treating cow intramammary mastitis.

Keywords: bovine mastitis, efficacy, piroline

[1]*Key Laboratory of New Animal Drug Project of Gansu Province/Key Laboratory of New Animal Drug Project of CAAS, Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences, Lanzhou 730050, China*
[2]*Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730050, China.*
[3]*Lanzhou University, Lanzhou 730000, China*
***Corresponding author E-mail:** haobaocheng@sina.cn*

บทคัดย่อ

การศึกษาทางคลินิกของ Piroline ในการรักษาโรคเต้านมอักเสบในโคนม

Jian-Ping Liang[1,2] Bao-Choog Hao[1] Xue-Hong Wang[1] Zhi-Ting Guo[1] Wen-Zhu Guo[1] Ruo-Fong Shang[1] Lei Tao[1] Yu Liu[1] Zhao-Zhao Li[1] Lan-Ying Hua[1] Shu-Yang Wang[3]*

จุดประสงค์ในการศึกษาครั้งนี้ เพื่อศึกษาประสิทธิภาพของ Piroline และยาปฏิชีวนะในการรักษาโรคเต้านมอักเสบในโคนมที่เกิดจากการติดเชื้อ *Streptococcus uberis* (*S. uberis*) และ *Escherichia coli* (*E. coli*) ในช่วงหยุดให้นม โคนมจำนวน 1880 ตัวถูกแบ่งเป็น 4 กลุ่มทดลอง และทำการรักษาด้วยยา Penicillin G, ammonium benzyl penicillin, ceftiofur และ Piroline ประสิทธิภาพของยาทั้ง 4 ชนิด ในการรักษาโรคเต้านมอักเสบที่ติดเชื้อ *E. coli* (*E. coli* IM) มีค่าร้อยละ 31.2 ของ Penicillin G, ร้อยละ 36.9 ของ ammonium benzyl penicillin, ร้อยละ 61.3 ของ ceftiofur และร้อยละ 64.4 ของ Piroline ประสิทธิภาพของยาทั้ง 4 ชนิด ในการรักษาโรคเต้านมอักเสบที่ติดเชื้อ *S. uberlis* (*S. uberlis* IM) มีค่าร้อยละ 90 ของ ceftiofur และร้อยละ 94.4 ของ Piroline จากผลการทดลองพบว่า Piroline มีประสิทธิภาพที่ดีกว่ายาทั้งสามชนิดในการรักษา ผลการตรวจวิเคราะห์น้ำนม ไม่พบสารตกค้างของ Piroline ในน้ำนมของโคที่รักษา ซึ่งแสดงถึงผลของ Piroline ที่ดีในการใช้เป็นยารักษาโรคเต้านมอักเสบในโคนม

คำสำคัญ: โรคเต้านมอักเสบในโคนม ประสิทธิภาพ Piroline

[1] *Key Laboratory of New Animal Drug Project of Gansu Province/Key Laboratory of New Animal Drug Project of CAAS, Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, Chinese Academy of Agricultural Sciences, Lanzhou 730050, China*

[2] *Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730050, China.*

[3] *Lanzhou University, Lanzhou 730000, China*

***ผู้รับผิดชอบบทความ E-mail:** haobaocheng@sina.cn*

Introduction

Bovine mastitis is not only a major disease affecting the dairy industry, but also one of the major influencing factors in milk production (Yuan et al., 1992). It causes great economic losses and decreases animal health. Although much progress has been made in control of cow mastitis, producers still cannot prevent and cure it effectively. As reported in a literature (Yuan et al, 1992), 3650 strains of 24 species of bacteria and fungi were isolated and identified from 3006 milk samples, in which 2060 strains of 12 species were closely related to cow mastitis. The isolation rate of pathogenic bacteria was 62.5%. Bacteria related to mastitis were mainly *S. uberis* (38.11%), *E. coli* (7.14%), etc. Buddle and Cooper reported (Buddle and Cooper, 1980) that about 32% of found cure spontaneously over the dry period.

Dry cow treatment (DCT) is an important step of a mastitis control program, the advantages of DCT include reducing incidence of intramammary infections (IMI) at parturition and increasing cure rate of IMI (Buddle and Cooper, 1980). The aims of DCT are to cure existing intramammary infection (IMI), and to prevent new infection during the dry period. A previous study that bacteriological cure rates for various intramammary dry cow treatments ranged from 25% to 75%. Systemic DCT was previously studied in an attempt to improve the cure rates. The systemic administration of antibiotics was evaluated in different studies. Owing to antibacterial drug resistance, only a few of antimicrobial agents demonstrated an improved cure rate over conventional intramammary DCT.

Piroline is a mainly active component isolated from the *Rubia Cordifolia*, which has been used in China to treat bovine mastitis for a long time (Liang et al, 1993; Liang et al, 2000). The pharmacological and antibacterial properties suggested that there was much less or no drug resistance to piroline, and thus it might be valuable for the treatment of bovine mastitis. The purpose of the present study was to compare the efficacy of piroline with that of other antibiotics commonly used in the systemic DCT. The results would be helpful for better prevention and cure of bovine mastitis.

Materials and Methods

Herds: The DCT field trials were conducted in three factory-supply dairy herds under seasonal-calving

conditions in Lanzhou, Gansu of China. Owners agreed to the following conditions: 1) provide calculated calving dates; 2) schedule dates for drying off, sampling, and treatment; and 3) permit some cows to serve as untreated controls. General information on several herds is presented in table 1.

Udder selection and milk sample collection: Udders and milk samples were collected through recommended procedures. Composite milk samples were collected from 1880 lactating cows from three herds within 4 weeks prior to drying off. Duplicate quarter milk samples were collected at drying off. And within 21 days, single sample was collected from all quarters of cows in treatment 1 and 3 at the prepartum period prior to infection of the lactating cow product (LCP).

Drugs: Penicillin G (batch number, h0605071) and Ammonium benzyl penicillin (batch number h06050714) was purchased from North China Pharmaceutical Group Corporation, Shijiazhuang city, China. Ceftiofur (batch number y06030914) was from Heibei Yuan Zheng Pharmaceutical Co., Ltd. Shijiazhuang, China. And Piroline was made by Lanzhou Institute of Animal & Veterinary Pharmaceutics Sciences of Chinese Academy of Agricultural Sciences, Lanzhou city, China.

Assignment of cows to treatment groups: Cows with *E. coli* IMI, based on cultural results of composite milk samples, were assigned randomly to the four treatment groups, a minimum of 80 *E. coli* IMI was included in each of the four treatment groups. More than 90 SU IMI were included initially in treatment 1 and 3 in anticipation of missed infusions due to early calving or incorrect calculated calving dates. Cow missed scheduled prepartum treatment were included in treatments 2 and 4, respectively. All four treatment groups were represented within each herd in table 2.

Treatment regimens: Each group of treatments was listed in table 2. Treatment 1 received an infusion at drying off with penicillin G 2,000,000 IU/50 ml (water), treatment 2 with ammonium benzyl penicillin 0.5 g/50 ml (water), treatment 3 with ceftiofur 0.5 g/50 ml (water), and treatment 4 with piroline 0.4 g/50 ml(water) (Brander, 1969).

Table 1 Information on cooperation dairy farms

Herd	Management Practices				
	Number of cows	Wash udders	Spray treats	Treat dry-cows	Overall management
A	620	+	+	+	Good
B	708	+	+	+	Good
C	552	+		+	Fail

Table 2 Experimental design of dry-cow therapy field trial

Treatment	Time of treatment	
	Drying off	Prepartum
1	+	+
2	+	+
3	+	+
4	+	+

+: Intramammary infections

Microbiological procedures: Method recommended by the National Mastitis Council, U.S.A., was followed. Presumptive identification of the following microorganisms was made; *Escherichia coli* (*E. coli*); *Staphylococcus epidermidis* (SE); *Streptococcus galactiae* (SAg); *Streptococcus uberis* (*S. uberis*); other streptococci (OS); *Corynebacterium bovis* (CB) and Coliforms (CO).

Definition of terms:

Infection: the number of somatic cells in the milk samples exceeds regular range and determination of pathogenic bacteria in the milk shows positive.

Cure: clinical symptoms ease off or disappear, the number of somatic cells in the milk returns to regular range, and determination of pathogenic bacteria in the milk shows negative.

Fail: clinical symptoms do not ease off, the number of somatic cells in the milk does not return to regular range, and determination of pathogenic bacteria in the milk shows positive.

Statistical analysis: An analysis of variance was conducted only on the *E. coli* and *S. uberis* data. The number of IMI with other pathogens was insufficient to conduct valid analysis. Developed (%) = (developed quarters/treated quarters) x 100%, and Cured% = (cured quarters/treated quarters) x 100%.

Results

The efficacy was 61.3% against *S. uberis* IMI in treatment 3 and 64.4% in treatment 4 (Table 3), and the difference was not significant. The efficacy of treatment 4 was significantly greater ($p<0.01$) than the 36.9% in treatment 2, and treatment 4 was also significantly different from treatment 1 (31.2%, ($p<0.01$). Treatment 4 and 3 were significantly different from treatment 1 and 2.

Approximately 10.5% of quarters developed from new IMI with *E. coli* or *S. uberis* during the dry period (Table 4). These resulting pathogens were accounted for 93% of new IMI. Other new IMI were caused by SAg and OS, about 3% for each one. Though differences were observed between the treatment groups, the incidence of the new IMI was similar to *E. coli* and *S. uberis*. In treatment 1 and 2, the incidence of IMI with S. uberis almost doubled than that in treatments 3 and 4 (Liang et al., 1993; Liang et al., 2000). Efficacy of prepartum treatment with the ceftiofur and piroline against new SAg IMI was low, but it was 61.3% and 64.4% against the new IMI with *S. uberis* respectively (Table 3).

The rate of new dry period IMI with SU ranged from 3.6% to 4.9% of quarters for treatment 1, 2 and 3. 10.5% of quarters become infected with SU in treatment 4 (Table 4), prior prepartum samples were analyzed from cows in treatment 3 and 4 , so efficacy of ceftiofur and piroline were excellent, 90% and 94.4%, respectively for treatment 3 and 4. No prepartum samples were analyzed from cows in treatment 1 and 2 and spontaneous recoveries were determined for the entire dry period using samples collected postpartum.

Table 3 Efficacy of dry-cow therapy against *S. uberis*

Treatment	Cows	Quarters	Quarter Number Intramammary infections		Efficacy (%)
			Drying off	Postpartum	
1	91	243	99	61	31.2
2	102	288	102	64	36.9
3	83	211	89	66	61.3
4	98	270	91	70	64.4
Totals	374	1012	381	261	

Table 4 New dry-period intramammary infections

Group	Number.		Intramammary infections					
			E. coli			*S. uberis*		
	Cows	Quarters	Quarter Number	Developed[a]	Cured[b]	Quarter Number	Developed[a]	Cured[b]
1	98	270	21	7.8	0	10	3.9	0
2	102	288	10	3.6	0	10	3.6	0
3	91	243	8	3.4	45	12	4.9	90
4	83	211	15	7	60	22	10.5	94.4
Totals	374	1012	54	5.3		54	5.3	

[a]Developed after prepartum treatment, [b]Expressed as percentage cure of IMI that developed prepartum sampling.

Discussion

Treatment 4, treated with piroline, did not significantly reduce the number of *E. coli* infections postpartum when compared with treatment 3. On the contrary, ceftiofur showed good effect in reducing the number of *E. coli* IMI significantly when compared with treatment 1 and 2. Treatment 4, treated with piroline, did not significantly reduce the level of *E. coli* IMI only with ammonium benzyl penicillin for prepartum treatment as compared with penicillin. The rate of spontaneous recovery for *E. coli* during dry period was consistent with the figures previously reported. Efficacy of piroline was 91.3% when compared with other reports on piroline against *E. coli*. The range in efficacy among herds was from 83% to 95%, which corresponded with earlier studies (Liang et al., 1993).

The rate of new *E. coli* dry period IMI could be reduced 50% by DCT and supported earlier work. The incidence of new IMI with *S. uberis* was similar to that observed with E. coli and was reduced about 50% by DCT (Brander, 1969; Brown et al, 1969; Christie et al, 1974 ; Buddle and Cooper, 1980). However, the incidence of new *S. uberis* IMI was similar to that in treatment 3 and 4. Further studies are required to explain these results since no prepartum samples were collected and detected from cows in treatment 1 and 2. Additionally, the rate of spontaneous recovery was high for new *S. uberis* IMI during the late dry or early postpartum period. Philpot (Philpot, 1969; Philpot, 1979) reported that spontaneous recovery over the dry period was 70% for Streptococci, compared with 27% for *E. coli*.

Prepartum therapy with piroline was effective in eliminating over 90% of new *S. uberis* IMI, but it was not effective in eliminating new *S. uberis* IMI (Liang, 2000). The average efficacy against new *E. coli* IMI was less than 60%, but the number of new *E. coli* was largely reduced. A wide variation in the efficacy was observed when compared with the earlier reports, but these data may not be conclusive. The results validated many earlier reports on piroline reducing the incidence of new dry period IMI with *E. coli* and *S. uberis*. Prepartum therapy with piroline appeared to be of marginal benefit and probably would be of practical value in dairy herds experiencing significant clinical mastitis cows. Philpot reported a 68% reduction of clinical cases during the first week of lactation when cows received penicillin G at parturition and the end of lactation. Results from this field trial provided supportive evidence of the effectiveness of piroline, eliminating many new IMI by educing levels of infected quarters before and after parturition, especially for the IMI infected with *S. uberis*.

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