THE EFFECT OF A COMBINED INTRAMAMMARY AND INTRAMUSCULAR TREATMENT OF MASTITIS ON EXCRETION OF ANTIBIOTICS IN MILK

Judith J. Poelarends[1], Henk Hogeveen[2] and Meike te Giffel[3]
[1]Research Institute for Animal Husbandry, Lelystad, The Netherlands
[2]Current address: Farm Management Group, Wageningen University, The Netherlands
[3]NIZO Food Research, Ede, The Netherlands

Most farmers use intramammary antibiotics to treat clinical mastitis in dairy cows. During the acute phase of mastitis, the swelling of the intramammary tissue causes occluded milk ducts. This may prevent distribution of the infused antibiotic and consequently result in failure of the intramammary therapy. A combination of intramuscular and intramammary therapy may overcome these problems. Therefore this combination therapy is a recognised and accepted practice among many veterinarians. Combination therapy has resulted in better bacteriological cure rates of mastitis (Ziv, 1980; Owens *et al.*, 1988; Owens *et al.*, 1990). But combination therapy is considered off label use and problems with residues in milk may occur, as the prescribed withdrawal period is not reliable anymore. The prescribed withdrawal period of an antibiotic preparation is based on the excretion time of antibiotics in milk after treatment of healthy cows according to the label. Oliver *et al.* (1990) and Pedersoli *et al.* (1995) showed that the combination of intramammary and intramuscular therapy was associated with longer excretion periods of antibiotics in milk compared to single intramammary therapy. However, these studies used healthy cows instead of cows with mastitis. To gain better insight in the possible risks of exceeding the prescribed withdrawal period when using combination therapy under practical circumstances, it is necessary to use cows with mastitis instead of healthy cows. The objective of our study was to investigate the effect of combining intramammary and intramuscular mastitis therapy on the excretion time of antibiotics in milk.

Materials and Methods

The study was carried out on five experimental farms. Three comparisons of treatments were used in the study namely, A vs. A&C or B vs. B&D or A vs. A&E. Within a farm, cows that had naturally occurring mastitis were treated alternately with either a single intramammary therapy (A or B) or a combination of intramammary (A or B) and intramuscular (C, D or E) therapy. Two consecutive treated cows were called pairs. Cows that had received an antibiotic treatment over the last 14 days or cows that needed veterinary assistance were not included in the study. In total, every treatment was applied to eight cows, with a minimum of two cows per farm. If eight cows per treatment were reached, farms were appointed to treat cows with another treatment. The antibiotic preparations that were used are presented in Table 1.

The farmers recorded appearance of the milk, udder and cow during 10 days after the start of the treatment. Sampling of composite milk samples started 4 milkings before the end of the withdrawal period (of the intramammary product) till 6 milkings after the end of the withdrawal period. It was not an objective of the present study to investigate treatment effects on milk yield and clinical cure. However, these parameters may affect the excretion time of antibiotics and for that reason these parameters were also analysed.

Table 1. Preparation code, route of administration, antibiotic preparation, administered amount and the withdrawal periods of the five preparations (WP in milkings).

Code	Route	Antibiotic preparation	Administered amount at each farm	WP
A	i.m.m.[1]	amoxicillin (200 mg) clavulanic acid (50 mg)	1½ days 2 dd[3] at farm 2 2½ days 2 dd at farm 3, 4 & 5	4
B	i.m.m.	dihydrostreptomycin (100 mg) nafcillin (100 mg) penicillin G (300.000 I.E.)	3 days 1dd at farm 1 & 4 3 days 2 dd at farm 5	10
C	i.m.[2]	amoxicillin (140 mg/ml) clavulanic acid (35 mg/ml) 1 ml/ 20kg bw/day	1 day 1dd at farm 2 2 days 1 dd at farm 3 and 5	2
D	i.m.	penicillin G (15 mg/kg bw/day)	1½ days 2 dd at farm 1 & 4 2 days 2 dd at farm 5	6
E	i.m.	sulfadoxin (200 mg/ml) trimethoprim (40 mg/ml) 1 ml/16 kg bw/day	1½ days 2 dd at farm 2 2½ days 2 dd at farm 4	6

[1] i.m.m. = intramammary; [2] i.m. = intramuscular; [3] = daily dose

Milk samples were analysed for presence of dihydrostreptomycin (Berendsen, 2000), amoxicillin, nafcillin and penicillin G (Zuidema, 2000) by means of high performance liquid chromatography using tandem mass spectrometry for identification and quantification.

Statistical Analysis

The excretion time (EXCR) was defined as the number of milkings needed for the antibiotic residue concentration to get below the MRL after start of milk sampling. It must be noticed that the definition of excretion time used in the present study is not the same as the excretion time of antibiotics on which the withdrawal period is based. Milk yield (MILK) was defined as the milk yield at the 10th day after start of treatment. Clinical cure (NMCure) was defined as the milking after onset of treatment at which the milk, udder and cow appeared normal again. The effect of treatment on EXCR and NMCure was examined using the generalised linear mixed model analysis (GLMM) with a logarithmic link function and Poisson distribution. MILK was analysed with the residual maximum likelihood method (REML) (Genstat 5 Release 4.1, 1998). Although the study was set up to treat cows randomly, it appeared that cows were not exactly equally distributed over parities (PAR) and stage of lactation (DIM). Therefore these potential confounders were included in the statistical model. The model used for all response variables was:

$$EXCR_{ijklmno}, NMCure_{ijklmno} \text{ or } MILK_{ijklmno} = \mu + herd_i + herd(pair\ of\ cows)_{ij} + DIM_k + PAR_l + TREAT_m + COMB_n + (TREAT*COMB)_{mn} + e_{ijklmno}$$

Where

Response variable: EXCR = excretion time of antibiotics after start of milk sampling; NMCure = milking at which udder, milk and cow appeared normal again; MILK = milk yield at 10th day after onset of treatment; μ = overall mean. Random effects: $herd_i$ = herd i representing differences between herds; $herd(pair\ of\ cows)_{ij}$ = pair of cows j within herd i representing differences between pairs within herd. Fixed effects: DIM_k = k days in milk; PAR_l = parity;

$TREAT_m$ = treatment comparison group (m = A&C, A&E, B&D); $COMB_n$ = additional intramuscular administration of C, D or E (n = yes, no); $e_{ijklmno}$ = residual random error.

Results

A total of 64 cows were treated with either an intramammary therapy or with a combination of intramammary and intramuscular therapy. Of the 64 cows, 16 were withdrawn from the study because the prescribed treatment appeared to be unsuccessful. There was no difference in withdrawal between single and combined treatments. The other 48 cows were treated as described in Table 2. Because of differences in mastitis incidence between farms, the total number of treated cows varied. Mean parity was 3 and the cows were on average 132 days in milk. Mean daily milk yield during the first ten days after onset of treatment was 25.8 kg/day.

Table 2. The number of cows per treatment per farm.

Therapy	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Number of cows
A		3	2		3	8
A+C		3	2		3	8
A		6		2		8
A+E		6		2		8
B	2			4	2	8
B+D	2			4	2	8
Total	4	18	4	12	10	48

The prescribed withdrawal periods were exceeded 4 times after single intramammary therapy and 4 times after combination therapy.

The three treatment comparison groups appeared to have a significant effect on EXCR ($P<0.05$). However, the additional intramuscular administration in the combination therapy did not significantly increase EXCR ($P=0.2$), although the estimated mean EXCR was higher for combination A&C and B&D than for single A and B respectively. DIM and parity did not significantly affect EXCR. The back transformed estimated means for EXCR are presented in Table 3.

Table 3. Estimated mean EXCR and mean NMCure for every treatment (in milkings).

Treatment	Estimated EXCR	Mean NMCure
A	2.35	5.3
A&C	3.42	5.6
B	0.30	6.0
B&D	0.83	6.0
A	2.96	4.5
A&E	2.65	3.8

NMCure was not significantly influenced by any of the parameters in the model. Therefore only the mean NMCure is presented in Table 3.

DIM and parity had a significant positive effect on MILK ($P<0.05$). The combination therapy showed a tendency ($P=0.08$) towards a higher estimated milk yield (29.0 kg/day) compared to the single therapy (26.5 kg/milking).

Discussion and Conclusion

This study showed no significant differences in excretion time of residues in milk between single intramammary therapy and combination therapy. This result is not in accordance with studies of Oliver *et al.* (1990) and Pedersoli *et al.* (1995). However, Pedersoli *et al.* (1995) used gentamycine in combination therapy for five days. The mean excretion time of combination A&C (both amoxycillin) was one milking longer than the intramammary treatment A (not significant). If A and A&C had been administered for a longer period, the difference in excretion time might have been more clear.

The prescribed withdrawal period was exceeded 8 times. In 4 cases it was after combination therapy and the other 4 cases were after intramammary therapy. In three of these 4 cases, the intramammary antibiotic was applied exactly according to the label. The fact that exceeding of the withdrawal period is associated with off label use of antibiotics seems logically, since the withdrawal period is not reliable anymore. However, the withdrawal period was also exceeded in cows that were treated exactly according to the label. Consequently, antibiotic excretion in milk from cows with mastitis may differ from the excretion of antibiotics in milk from healthy cows, on which the withdrawal period is based.

Studies of Ziv (1980) and Owens *et al.* (1988) reported a positive effect of combination therapy on bacteriological cure rate. However, they did not investigate milk production. Milk production data also give important information about the recovery of the mammary gland function. In the present study it was found that cows treated with the combination therapy tended to produce more milk after treatment than cows treated with the single therapy. There was no information about the milk production of cows before the onset of treatment, but corrections were made for DIM and parity. The slightly higher milk production in cows treated with the combination therapy might indicate that the secretory activity is increased during combination therapy, as suggested by Owens *et al.* (1990). Although it was found that milk production was higher, there were no differences in clinical cure between the single and combination therapy. In order to define differences in clinical cure between treatments, a clinical trial should be conducted. The present study was not meant as a clinical trial and did not meet the requirements for a clinical trial (Schukken and Deluyker, 1995).

Although the differences in excretion time between the single and combined treatments were not significant, there seemed to be a difference between A and A&C. Differences in excretion time might have been more pronounced if milk production was not influenced by treatment. The results give some interesting information but further study is necessary to confirm these initial findings.

Acknowledgements

The authors wish to thank NIZO food research for financing this research and Rikilt for analysing all milk samples for presence of antibiotic residues.

References

B.J.A. Berendsen. 2000. RIKILT Standard Operating Procedure A0915, the analysis of dihydristreptomycine in milk using LC-MS/MS.

Genstat 5, Release 4.1, 1998. Lawes Agricultural Trust. Rothamsted Experimental Station.

Oliver S.P., J.L. Maki and H.H. Dowlen. 1990. Antibiotic residues in milk following antimicrobial therapy during lactation. J. Food Prot. 53:693.

Owens, W.E., J.L. Watts, R.L. Boddie and S.C. Nickerson. 1988. Antibiotic treatment of mastitis: Comparison of intramammary and intramammary plus intramuscular therapies. J. Dairy Sci. 71:3143.

Owens, W.E., Z.Y. Xiang, C.H. Ray and S.C. Nickerson. 1990. Determination of milk and mammary tissue concentrations of ceftiofur after intramammary and intramuscular therapy. J. Dairy Sci. 73:3449.

Pedersoli W.M., J. Jackson and R.A. Frobish. 1995. Depletion of gentamycin in the milk of Holstein cows after single and repeated intramammary and parenteral treatments. J. Vet. Pharmacol. Therap. 18:457.

Schukken, Y.H. and H.A. Deluyker. 1995. Design of field trials for the evaluation of antibacterial products for therapy of bovine mastitis. J. Vet. Pharmacol. Therap. 18:274.

Ziv, G. Drug selection and use in mastitis: Systemic vs local therapy. 1980. J. Am. Vet. Med. Assoc. 176:1109.

Zuidema, T. 2000. RIKILT Standard Operating Procedure A0895, the analysis of amoxicillin, nafcillin and penicillin G in raw milk using LC-MS/MS.