DETERMINATION OF THE EFFECTS OF DIFFERENT DRY COW THERAPY ON UDDER HEALTH IN DAIRY COWS

SAKINE ULKUM CIZMECI[1]*, AYSE MERVE KOSE[2], IBRAHIM AYDIN[1], ZAFER SAYIN[3] AND DURSUN ALI DINC[1]

1 Selcuk University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Konya, Turkey
2 Mustafa Kemal University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Hatay, Turkey
3 Selcuk University Faculty of Veterinary Medicine Department of Microbiology, Konya, Turkey
* *Corresponding author:* ulkum@selcuk.edu.tr

ABSTRACT. The aim of this study is to determine the effects of application of antibiotic and non-antibiotic products and their combinations in preventing mastitis during drying off in dairy cows. Somatic cell count (SCC) and bacteriologic culture were performed from the milk sample which was collected during drying off on Day 7 and Day 14 of lactation. There are four groups involved in this study. The first, second and third groups were given cloxacilin-ampicilin (CA), CA with spiramycin and CA with 65% bismuth subnitrate (BS) respectively while the fourth group was given only BS, all administered intramuscularly in all groups. Observation of the SCC showed a decrease in the second group. Mastitis incidence between this period showed a decrease in groups 1 and 2 (57.25%, 48%, 34.75%; 53%, 52.75%, 36.75%), no change in the third group (41.25%, 39.5%, 41.25%) and an increase in the 4th group (43.75%, 42.25%, 51.75%). When comparing the mastitis rate and SCC counts of the four groups, it was found that the second group produced the most successful treatment option. It is concluded that parenteral antibiotic application, in addition to intramammary antibiotics, is the most successful among the dry period treatment protocols in this study.

Keywords: dry period, mastitis, cow, bismuth subnitrate

INTRODUCTION

Mastitis is the leading cause of low yield and serious economic loss in dairy cattle. Mastitis constitutes 38% of all morbidities, 30% of cows have clinical mastitis once a year, 7% of cows are culled in dairy herds and 1% die due to mastitis (Seegers *et al.,* 2003). Economic losses caused by mastitis can be summarised as milk production losses, drugs, discharged milk, veterinary services, extra labour, culling, dying, diminished genetic healing, and loss of insurance premium (Seegers *et al*., 2003; Heikkila *et al*., 2012).

The dry period begins with the removal of the lactating animal from milking and it lasts until the start of the new lactation with the birth. It is the period when the udder and the digestive system are rested (Dingwell *et al*., 2003; Dinç, 2009). This is a critical stage of the lactation cycle in dairy cows, and it is necessary for the following lactation to achieve proper milk yield and involution of the mammary glands (Bachman and Schairer, 2003; Pezeshki *et al*., 2007).

The dry period consists of active involution, involution and colostrogenesis

(Smith and Todhunter, 1982). The cause of mastitis in the dry period is presenting or newly developing intramammary infections during lactation. During the dry period, about 10%-17% of new intramammary infections develop and a large proportion of them are formed by environmental microorganisms (Pantoja *et al*., 2009). One of the most important reasons for this is the partial enlargement of the udder and increase in intramammary pressure resulting from milk remaining in the udder at the end of lactation (Church *et al*., 2008). Subclinical infections in the dry period become acute in the first few weeks of lactation (Dingwell *et al*., 2004).

In dairy cows, mastitis usually occurs through three different routes: the spread of infectious mastitis agents in the herd, the transmission of environmental microorganisms to cows in the lactation stage or in the dry period (Godden *et al*., 2003). The risk factors for mastitis formation are the beginning of dry period, first 50 days of lactation, age-birth or lactation number, lactation period, milk yield, race, milking rate and teat morphology, herd size, herd hygiene, SCC and feeding status (Richert *et al*., 2013; Nakov *et al*., 2014; Oliveira *et al*., 2015).

In order to protect against new intramammary infections that may occur during the dry and transitional periods, in addition to dry period intramammary antibiotic therapy, the cow's immune system should be supported, strengthened and the surrounding bacterial population should be minimised (O'Rourke, 2009).

Intramammary antibiotic treatments in dry cows are the most important part of classical 5-stage mastitis control programmes. The purpose of dry period treatment is to treat subclinical infections that occur during lactation and to prevent new infections that will be formed during the dry period (Janosi and Huszenicza, 2001).

Dry period antibiotic treatment is to provide intramammary antibiotic preparations suitable for dry period use to all udder lobes during dry-out to heal existing mastitis cases that do not heal during lactation and to prevent or reduce new infections that may occur during the dry period (Bradley and Green, 2006).

Treatment is usually via the intramammary route because dry period antibiotic provides high local concentration. When the dry period preparation is given in the udder, the concentration that can kill the existing pathogen in the udder should be reached immediately. It must be absorbed in a limited level and passed to the systemic circulation, be able to remain in the udder for a long time and be able to be rapidly thrown away with the milk after birth (Bradley and Green, 2001).

Additional protective measures need to be taken to increase the rate of recovery from infections present during the dry period, and to reduce the rate of new intramammary infections (Nickerson, 2001a; Godden *et al*., 2006). In recent years, external and internal teat barriers have been developed (RUMA, 2015). External physical barriers applied after milking kill the microorganisms (*Staphylococcus aureus* and *Streptococcus agalactiae*) infecting the udder, and prevent the entry of environmental microorganisms (*Escherichia coli* and *Streptococcus uberis*) into the udder in the

process between two milkings (Nickerson, 2001b).

External barriers have been used for many years with dry period therapy. It forms a thin membrane on the teat, which lasts 7 to 10 days, to protect the udder from the new infection during the sensitive period (Timms, 2000; Fernandez, 2007; Lim *et al*., 2007). Internal barriers may be used alone or together with dry-period antibiotic therapy. In addition to increased cost with the dual administration, the risk of destruction in the teat channel and inoculation of pathogens may also increase (Lay *et al*., 2007; Bradley *et al*., 2011).

Paraffin-based BS is insoluble in milk when it is given to the teat, and it hardens in a short time to cover the entire teat hole and teat sinus like the keratin plug for protecting the udder during the dry period (Crispie *et al*., 2004a). Since the teat is completely closed, aseptic procedures need to be taken during application. When lactation starts, it can be easily removed by hand milking. It was reported that paraffin-based BS reduced new intramammary infections by 10 times in the fieldworks (Godden *et al*., 2003; Newton *et al*., 2008; Bhutto *et al*., 2011).

In this study, dry period antibiotic, internal mammary barrier, the combination of both, and parenteral antibiotic administration were tested to determine the effectiveness of treatment against presenting infections and the reduction of new intramammary infections during the dry period.

MATERIALS AND METHOD

The study was carried out in an intensive dairy farm which had an automatic milking system (GEA, Westfalia, DE) and herd tank milk. Daily milk yield records for every milking were kept and BSCC (bulk somatic cell count) was made on the farm. There was a controlled feeding programme according to milk yield and lactation period applied, and dry-off was gradual for 60 days prior to the expected date of birth. The farm kept animal health records and an internationally certified herd management programme called Dairy Plan. The animal population in the farm consisted of 100 heads of Holstein cows between the ages of 3 and 4 years and 400 udder quarters. For this study, cows that needed to be dried off were randomly selected without evaluating whether mastitis occurred during the other period of lactation, general health problems, and other findings. Animals treated in the last 30 days due to clinical mastitis were excluded.

Animals were randomly assigned to four groups (n=25 per group) according to the treatment methods applied (Table 1). Cloxacillin (500 mg) and ampicillin (250 mg) was administered intramammary to each mammary lobe in the first group (CA). CA was administered to each mammary lobe and at the same time, spiramycin (600,000 IU) was given parenterally to the second group (CAS). CAS + 65% bismuth subnitrate (BS) were administered to each mammary lobe in animals in the third group (CABS). In the fourth group, only BS was administered to each mammary lobe (BS). Sterile tubes of milk samples were taken on 7th and 14th day postpartum (PP) immediately after birth and

Table 1. Dry-period treatment protocol.

Group (n=25)	Dry-period therapy method
CA	Bovoclax (cloxacillin 500 mg + ampicillin 250 mg)
CAS	Bovoclax (cloxacillin 500 mg + ampicillin 250 mg) + spiramicin 600,000 IU, İM
BS	Bizmuth subnitrate at 65% concentration
CABS	Bovoclax (cloxacillin 500 mg + ampicillin 250 mg)+ bismuth subnitrate at 65% concentration

Table 2. Rate of infected mammary lobes and average SCC at day 0 postpartum.

Group	The rate of infected mammary OBE during dry-off (%)	Infected (SCC) (thousand/ml)	Uninfected (SCC) (thousand/ml)
CA	56.7	841	140
CAS	52.7	1,237	178
BS	42.2	748	129
CABS	41.1	687	65
Average	48.18	878	128

Table 3. Mean somatic cell counts (SCC) in thousand/ml.

	SCC	Micro-organism +	Micro-organism -	SCC	Micro-organism +	Micro-organism -	SCC	Micro-organism +	Micro-organism -	Average
Group	Sample 1			Sample 2			Sample 3			
CA	571	841	140	692	1,077	174	562	1,272	190	608[a]
CAS	811	1,237	178	743	930	259	713	1,401	232	755[a]
BS	499	748	129	418	1,086	168	372	869	184	430[b]
CABS	308	687	65	601	882	124	381	559	160	489[a]

Note: a and b signifies statistical difference, p>0.05

Table 4. Rate of infected mammary lobes after dry-off and after treatment, postpartum (PP).

	Sample timing of mastitis rates in general (%)		
Group	Day 0 PP	Day 7 PP	Day 14 PP
CA	56.7	48.2	35.5
CAS	52.7	52.7	36.4
BS	42.2	42.4	51.5
CABS	41.1	39.3	41.1
Average	48.18	45.65	41.12

Table 5. Persistent and new infections on days 7 and 14 postpartum (PP).

Group	Day 7 PP		Day 14 PP		
	Treatment Rate %	New Infection Rate %	Treatment Rate %	New Infection Rate %	Persistent Infection Rate %
CA	25.5	9.8	29.3	25.9	5.3
CAS	37.9	17.2	41.4	15.0	7.3
BS	40.7	53.6	39.3	50.0	7.6
CABS	43.5	34.8	47.8	50.0	10.7
AVERAGE	36.9	28.85	39.45	35.22	7.72

Table 6. Dry-off, *S. aureus* (SA) percentages on days 7 and 14 postpartum (PP).

Group	Day 0 PP	Day 7th PP	Day 14th PP
CA	32.8	56.1	50.0
CAS	62.1	51.7	45.0
CABS	57.7	54.5	43.5
BS	55.6	42.9	50.0
In general	52.3	53.4	47.6

Table 7. Dry-off, coagulase negative *Staphylococcus* (CNS) rates in % on days 7 and 14 postpartum (PP).

Group	Day 0 PP	Day 7 PP	Day 14 PP
CA	45	61	46
CAS	48	55	36
CABS	48	41	52
BS	56	46	56
In general	48	50	49

Table 8. Other bacteria species isolated in infected mammary lobes during day 0 postpartum (PP) and new intra-mammary infection rates (%)

AGENT	Day 0 PP	Day 7 PP	Day 14 PP
Aspergillus spp. (Asp)	3.85	4.26	4.55
Corynebacterium spp. (C)	13.46	14.89	11.36
E. coli (EC)	30.77	34.04	38.64
Fungus	13.46	12.77	22.73
P. multocida (PM)	9.62	2.13	2.27
Klebsiella spp. (Klb)	5.77	10.64	9.09
Trueperellapyogenes (Tp)	23.08	21.28	11.36
Mix	35.09	40.03	34.58

just before the application of dry period for culture to determine SCC. Milk samples were transferred to the microbiology laboratory in the cold chain within 24 hours. SCC rate in the milk samples was measured with DeLaval Cell counter DCC. Various mediums were used for isolation and identification of various microorganisms from milk samples such as blood agar base (Oxoid), MacConkey agar, Sabouraud dextrose agar, Baird Parker agar (Oxoid) and Edwards medium. Identification of breeding microorganisms was performed according to classical methods (Ateş *et al.,* 1991, Erganiş *et al.,* 1995). Treatment with dry or periodic therapy was considered successful if there were no postnatal specimens as a result of dry period therapy or if there were no mastitis pathogens (major and minor) in postnatal specimens.

Analysis of differences in characteristics such as lactation mastitis incidence, aetiology, efficacy of treatment methods between healthy and infected mammary lobes were determined. Chi-square tests, and *t*-tests were conducted between the ratios if differences were found significant.

When examining the SCC values, differences between sampling times were tested using paired *t*-tests. Student *t*-tests were used for differences between therapy groups (İnal, 2005).

RESULTS AND DISCUSSION

It was observed that in the samples made during dry-off, nearly half of the mammary lobes (averaging 48.18%) were infected with mastitis and the mean SCC counts in infected and uninfected animals were 878 per ml and 128 per ml, respectively (Table 2). It was found that SCC increased in the second sample in the proliferative (+) and non-proliferative (-) quarters. It was found that while the mean SCC decreased after the treatment that the non-reproduction milk was within the acceptable range of SCC, SCC in the breeding milk even exceeded the 1,000,000 limit of the average (Table 3). The statistical difference was significant in the mean of SCCs between the groups ($p < 0.05$).

Mastitis and infected mammary lobe ratios were found to be similar between the groups during dry-off and the average of all groups was 48.18%. On the 7th day postpartum, mastitis rate was decreased in group 1. In addition, mastitis rates were lower in groups 1 and 2 on the 14th day compared to the other groups (Table 4).

On the 7th day postpartum, group 4 showed better improvement to treatment than the other groups. Group 1 was more successful in the second sampling in preventing new infection formation and in the third sampling group 1 fell behind group 2. Persistent infection rate was found to be lower in group 1 (Table 5).

The incidence rate of *S. aureus* was higher in the other groups except in the first group during dry-off. However, it was higher in the second sampling in the first group. Considering the general average, half of the samples (52.3%) in all groups and in all samplings were found to be infected with *S. aureus*. The rate of *S. aureus* was the lowest in the second group after treatment (Table 6). In the general average, 50% of all mammary lobes were infected with coagulase negative staphylococcus (CNS). The best response

was in the second group. The therapy in the fourth group was unsuccessful (Table 7).

The bacteria species isolated in infected mammary lobes and new intramammary infections during the dry-off process were examined. It was found that the incidence rate of *E. coli* in all three samples was higher than that of other microorganisms and that *Aspergillus* spp. and *Pasteurella multocida* were found to be the least common microorganisms naturally (possibly because there were no major mastitis agents) (Table 8).

As a result of the statistical analysis made on the mammary quarters level, where *S. aureus* was detected, there was a statistical difference between the days dry-off on the left anterior lobes at 14 days postpartum as well as between the first and second groups. When the CNS was examined at the level of the mammary quarters, it was found that the difference between the first and second groups was significant in the left back quarters. In the examination performed at the level of other microorganisms, it was found that the difference between the first and third groups in the left anterior, right anterior and right anterior quarters was significant ($p < 0.05$).

CONCLUSION

In this study, only dry period antibiotic, only internal teat barrier, the combination of both, and parenteral antibiotic administration during dry-off treatment were tested to determine the effectiveness in reducing the incidence of new intramammary infection and treatment of existing infections. It was observed that nearly half of mammary lobes (48.18%) during the dry-off process were infected with mastitis and that the mean number of SCC in infected animals was very high.

Studies have shown that when the intramammary teat barrier was administered to a cow with a SCC of less than 200,000, while it prevented the rate of new intramammary infections more than that of untreated patients, it was equally effective with those treated with antibiotic dry-period therapy. In this study, the average SCC was more than 200,000 in all groups and the ratio of mammary lobes with SCC lower than 200,000 was very small. The intramammary teat barrier was found to be not effective enough. Some researchers suggested that general dry-period antibiotic treatment in animals with lower SCC (<200,000) should be rediscussed (Huxley *et al*., 2002).

It has been reported that internal teat barriers (plugs) significantly reduce the prevalence of new intramammary infections during delivery (Huxley *et al*., 2002; Newton *et al*., 2008). There is little economic difference at the level of individual cows between dry-period antibiotic treatment and internal teat barriers to protect against new mammary infections when all mammary lobes are free from infection during dry-off. If the infected cow is not treated, approximately 110 Turkish Lira (₺) of economic loss occurs. While the internal teat barriers remain ineffective in Corynebacterium-infected cows during dry-off, dry-period antibiotic therapy alone is more profitable (Berry *et al*., 2004). In this study, the majority of the mammary lobes were infected (48.18%) during the dry period. Therefore, the combined or single internal administration was ineffective (CA. 48.2, CAS.

52.7, BS. 42.4, CABS. 39.3). Hence, internal teat barriers should be used in animals which are not infected with mastitis or have low SCC during the dry period.

The use of bismuth subnitrate, an internal teat barrier, in mastitis-infected mammary lobes is not recommended during dry-off (Green, 2003). However, from the results of the culture performed after treatment in animals, it was found that 48.18% of mammary lobes were infected with mastitis. This shows that the internal teat barrier is not suitable for the purpose. There was a success in recovery (rates were 40.7% and 39.3% in the mammary lobes treated with the internal teat barrier) but new infections developed (53.6% and 50% of the samplings). Mastitis rates also showed no difference between the three samplings and were ineffective.

Previous studies have shown that the use of internal teat barriers in uninfected mammary glands during the dry period is at least as effective as long-acting dry-period treatment or could reduce the rate of new intramammary infections during the dry period (Godden *et al*., 2003; Cook *et al*., 2004; Crispie *et al*., 2004a,b). In this study, the effect of the internal teat barrier on non-infected mammary lobes during dry-off to prevent the formation of new infections was as follows; the rate of new intramammary infection in non-infected group was 53.6% in the first sampling in the BC group and 50% in the second sampling, and 34.8% and 50% in the CABS group.

Bacteriocins and non-antibiotic additives such as strong proteins produced by some bacteria which are capable of killing other microorganisms have been used to enhance the effectiveness of the internal teat barriers, and new generation of teat barriers have been made with these combinations (Crispie *et al*., 2004b). For example, non-antibiotic teat barrier containing chlorhexidine or lacticin has been shown to provide better protection than the conventional teat barrier by reducing the prevalence of new intramammary infections caused by all pathogens (Crispie *et al*., 2004b; Petrovski *et al*., 2011). In this study, it is found that the use of classical teat barriers containing only bismuth subnitrate was inadequate due to the reduction of intramammary infections rates including the risk of contamination during treatment, especially the lack of components with antimicrobial properties.

Over the years there have been changes in the aetiology of mastitis. Bacterial agents in the aetiology are shifting towards contagious environmental microorganisms. The incidence of agents such as CNS and *Corynebacterium bovis* (also known as minor pathogens), has increased (Bradley *et al*., 2007). The trend is clearer in free system operations (Olde Riekerink *et al*., 2008). Classical mastitis control systems has become ineffective. Among the agents isolated in this study were *E. coli, Klebsiella* spp. and *P. multocida*. Results show that *E. coli* had the highest occurrence on days 0 PP, 7 PP and 14 PP with 30.7%, 34.04% and 38.64%, respectively..

In this study, it was found that about 50% of all mammary quarters were infected with CNS. On average, the best response after treatment was in group 2 but all of the groups continued to have CNS infection. Therefore, it shows that the treatment failed.

Another study reported that nearly half of post-treatment mastitis in CNS-generated mastitis was seen as a persistent infection that could not be cured (Taponen *et al*., 2012).

It was reported that there were fewer new clinical mastitis in the cefquinome-treated group than in the group treated with cloxacillin (Bradley *et al*., 2011) and no difference was found between cloxacillin and cefquinome in ameliorating intramammary infections caused by major pathogens. Similar results were obtained in this study. The high rate of intramammary infections (CAS: 17.2% -15%, BS: 53.5-50%, CABS: 34.8-50%) is due to the effectiveness of the dry-period preparation containing narrow-spectrum cloxacillin.

Taking into consideration the treatment rate of infection in this study, the best result was obtained in groups 3 and 4. In the protection from new infections, CA and CAS groups were found to be more effective than the other two groups. It is concluded that the administered systemic antibiotic may have reduced the incidence of new infections.

Dry period systemic administration may be advantageous in preventing new intramammary infections by choosing the appropriate drug with better injection in the mammary tissue and reducing some risks of intramammary administration. In parenteral administration, spiramycin, which is well spread in mammary tissue, is thought to have a positive effect on dry-period treatment (Prescott and Baggot, 1988; Du Preez, 2000). In the group treated with spiramycin + cloxacillin, it was observed that the treatment rate in the second sampling was higher than that of cloxacillin alone. However, spiramycin + cloxacillin was found to be inadequate in preventing new infections, and in the third sampling, it was found to be more successful in both parameters.

In a study using systemic enrofloxacin or tylosin (a spiramycin-related macrolide), it was observed that the efficacy of local treatment with penicillin, nafcillin, and dihydrostreptomycin did not increase (Bolourchi *et al*., 1995). In this study, it was observed that parenteral administration (spiramycin) increased the efficacy of local treatment when comparing CA (25.5%) and CAS (37.9%) groups at day 7PP.

It was observed that the administration of only IM spiramycin during dry-off is not effective against *S. aureus* mastitis. It was reported that when 30,000 IU/kg IM spiramycin was administered during the first four days of the dry period, the recovery rate in chronic subclinical *S. aureus* mastitis cows was less than 50% (Janosi *et al*., 2001). It was reported that the effectiveness of dry-period systemic applications has not been substantiated yet in practical terms (Janosi and Huszenicza, 2001). It was argued that recurrent practices should not be preferred due to high cost and low efficiency (Janosi *et al*., 2001). In this study, it was observed that the application of IM spiramycin did not show the expected effectiveness, and the rate of *S. aureus* was 62.1% during dry-off, 51.7% in the second sample and 45% in the third sample. In a study comparing four different dry-period treatment protocols with systemic tylosin alone (12 g, IM), tylosin combination with internal mammary gland barrier, tylosin combination with cephapirin

+ internal barrier and finally cephapirin + internal barrier application, similar to the study presented, it was reported that the combination of tylosin with intramammary antibiotics increased the rate of healing of gram-positive intramammary infections and also that the combination of tylosin + internal teat barriers or tylosin alone is as effective as cephapirin + teat barrier combination (Contreras *et al*., 2013). However, in this study, it was found that the intramammary antibiotic + teat barrier provided the highest treatment success rate, whereas the intramammary antibiotic applied alone had the lowest treatment rate (25.5%).

It was reported that during the dry period, the rate of occurrence of new intramammary infections in mammary quarters varies widely among herds (6-26%) (Godden *et al*., 2003; Cook *et al*., 2004; Dingwell *et al*., 2004). It was found that the incidence rates of new intramammary infections in this study were 36.9% in the first sampling and 39.45% in the second sampling. The CA group was found to be more successful in the first sampling in preventing the formation of new infections among the groups; however, in the second sampling, it appeared to fall behind the CAS group. In the BS and CABS groups, it was found that the rates of new infections were very high in both samplings. What might have caused the decrease of the treatment success and the increase of the rate of new infection was because the individual SCC during dry-off in cows included in the study were very high, the operator was a large-scale enterprise, three milkings in a day were being applied, and a different system was used in manure management.

It was reported that internal teat barriers act as a physical barrier against mastitis-forming bacteria and mechanically block mastitis-forming bacteria penetration into mammary during the dry period (Zoetis Inc, 2013). It was reported that using internal teat barrier with dry period antibiotic treatment as a combination in herds which have a high rate of new intramammary infections during the dry period, has reduced new intramammary infections caused by environmental streptococci in the first 100 days of lactation (Cook *et al*., 2005).

In this study, it was concluded that new intramammary infections in both groups (alone or in combination) were formed by *S. aureus* and CNS and that the effect of the internal barrier was limited. In the second sampling, it was found that the rate of new infection was 53.6% in the BS group, 34.8% in the CABS group, and 50% in both groups in the third sample. In addition, the rate of persistent infection was 7.6% and 10.7% in these two groups.

In a study comparing the use of cloxacillin (Contreras *et al*., 2013), bismuth subnitrate and the combination of both for dry-period therapy, the combination was reported to be more useful in preventing new intramammary infections (Sanford *et al*., 2006). The same effect was not found in this study. This could be due to the rate of infected mammary lobes, the average SCC being high, and not being the broad spectrum of cloxacillin during dry-off.

In the study of testing dry-period therapy options, the incidence of mastitis during dry-off and average somatic cell

counts influence the rate of catching mastitis in the following lactation and high mastitis rate reduces the chance of success.

In conclusion, as reflected by the results of the treatment trials, it can be concluded that internal teat sealants would be a more successful option when used in animals with somatic cell numbers below 200,000. It is more useful to use the internal teat sealants in combination with antibiotics in infected quarters during dry-off, and parenteral administration enhances the effectiveness of local treatment.

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