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Intramuscular treatment of subclinical staphylococcal mastitis in lactating cows with penicillin G, methicillin and their esters

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The relationship between antibiotic milk concentrations and bacteriological efficacy was investigated in groups of lactating cows with subclinical mastitis due to either penicillin G-sensitive or penicillin G-resistant *Staphylococcus aureus*. Treatments consisted of the intramuscular injection of procaine penicillin G, or its weak base ester penethamate hydriodide, and sodium methicillin, or its weak base ester tamethicillin. Antibiotics were administered once daily for 2 or 4 days at accepted dosages.

After four daily treatments with procaine penicillin G and penethamate hydriodide, infections were eliminated from 56.5% and 68.8%, respectively, of quarters infected with penicillin G-sensitive staphylococci, and from 14.3% and 7.7%, respectively, of quarters infected with penicillin G-resistant staphylococci. After four daily treatments with sodium methicillin and tamethicillin, infections were eliminated from 32.4% and 48.6%, respectively, of quarters infected with penicillin G-resistant staphylococci. The better efficacy of penethamate hydriodide and tamethicillin was considered to be linked to the higher milk drug concentrations obtained with these drugs as opposed to the lower concentrations measured in the milk after treatment with the parent drugs. Cure rates were generally higher after treatment for 4 days than after the 2-day course of therapy. Treatment efficacy decreased progressively with increasing age of the cows.

Intramuscular treatment of subclinical staphylococcal mastitis in lactating cows can serve as a useful model for screening existing and new antibacterial agents and drug products intended for the parenteral treatment of clinical staphylococcal mastitis.

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INTRODUCTION

Intramammary treatment is the most common method of treating bovine mastitis with antibiotics. In streptococcal mastitis, and in some staphylococcal mastitis, such therapy usually results in a clinical cure, but the bacteriological cure rate is low (Mercer, 1971; Plommet & LeLouedec, 1975; Funke, 1982). Failures in intramammary therapy are due to poor or uneven distribution of the drug throughout the udder parenchyma, and to the compression or blockage of the milk duct system by inflammatory products (Pattison, 1958; Ullberg *et al.*, 1958). Furthermore, staphylococci are tissue invaders, and

intracisternally administered drugs may be unable to gain access to these organisms. Therefore, bacteriological failures may occur, even when the organisms are sensitive to the antibiotics used (Platonow & Blobel, 1963). In acute mastitis, systemic treatment as an adjunct to intramammary treatment has been advocated as a means of overcoming these problems (Swabrick, 1966; Uvarov, 1970; Ziv, 1975; Giesecke, 1977) and, occasionally, as the only route of administration (Sadek, 1954; Funke, 1961, 1982; Rasmussen, 1964; Schipper, 1967; Backstrom & Funke, 1978).

The optimal antibacterial and pharmacological properties of drugs intended for the systemic treatment of clinical mastitis have been elucidated (Ziv, 1975, 1980a, b). At least four requirements must be fulfilled in order to eliminate the infecting agent: (i) the infective agent should be sensitive to the drug; (ii) drug distribution throughout the udder should be good; (iii) drug concentration in the udder or milk should exceed the minimal inhibitory concentration (MIC) by a factor of one to ten depending on the minimal bactericidal concentration (MBC) value, and (iv) the duration of therapy should be sufficient to produce clinical improvement and the elimination of pathogens from the majority of cases (Funke, 1982).

The limited number of reports on the bacteriological efficacy of antibiotics administered by the intramuscular (i.m.) route only in the treatment of clinical mastitis were critically reviewed (Ziv, 1980a; Funke, 1982). It was concluded that, although the results did not seem to lend support to the proposed optimal criteria for the rational selection of antibiotics for parenteral mastitis therapy, more data were needed for an evaluation of the efficacy of the parenteral route before it could be said that the accepted principles of antibacterial chemotherapy are not applicable in the case of mastitis (Ziv, 1980a).

Although subclinical staphylococcal mastitis in lactating cows is treated almost exclusively by the intramammary route, the parenteral route can serve as a useful model for testing the validity of the principles for the rational selection of antibiotics intended for the treatment of clinical mastitis, particularly if the final objective is elimination of the causative agents and not merely clinical improvement. This model has several advantages over the clinical mastitis model: (i) it is safe and readily available; (ii) sensitivity of the isolates to the antibiotics to be tested can be determined quantitatively, in terms of MIC values, ahead of treatment; (iii) it permits the design of prospective treatment regimens using selected drugs and/or their analogues of known pharmacokinetic properties in the udder; (iv) it uses well-defined and accepted methodology for the definition of infection and cure, and (v) as a research tool, total expenditure is relatively low, and is mainly confined to the cost of drugs and discarded milk.

The present report deals with the relationship between antibiotic milk levels and the efficacy of penicillin G, methicillin and their respective lipophilic esters in eliminating penicillin G-sensitive and penicillin G-resistant staphylococci from the udders of lactating cows after i.m. administration for 2 and 4 consecutive days.

MATERIALS AND METHODS

Cows and herds

Studies were conducted during 1982–1983 on 213 clinically normal Israeli Holstein cows. Cows were located in eight dairy herds and were housed, fed and milked under the usual local conditions. In several herds cows were milked twice daily, and in others three times daily. Post-milking teat disinfection, using an approved germicidal teat dip, was used in each herd. The status of udder health in each herd was known from results of bacteriological tests conducted periodically on aseptically collected milk samples. Cows selected for the trial were in their third to sixth month of lactation, and were all infected subclinically with *Staphylococcus aureus* in at least one quarter of the udder. Attempts were made to select similar proportions of infected cows which were in their first, second, third, and fourth or subsequent lactations.

Antibiotic sensitivity tests

All *S. aureus* isolates recovered from the udders of the cows selected were tested for

their sensitivity to penicillin G and methicillin using the paper disc method (Barry *et al.*, 1970). Approximately 50% of the isolates were resistant to penicillin G and all were sensitive to methicillin. The MIC of penicillin G for twenty-five penicillin G-sensitive and thirty-eight penicillin G-resistant isolates was determined by the method of Barry & Sabath (1974). The MIC of all the penicillin G-sensitive isolates was ≤ 0.1 IU/ml, whereas the MIC for all the penicillin G-resistant isolates tested was ≥ 1.0 IU/ml. The MIC of methicillin for thirty-five of the thirty-eight (92%) penicillin G-resistant isolates ranged from $<$ 0.1 to 0.5 μg/ml, and the MIC values for the remaining three isolates were either 1.0 or 2.0 μg/ml. On the basis of these results, the cows were classified as infected with either penicillin G-sensitive or with penicillin G-resistant *S. aureus*. Cows which were infected in one quarter of the udder with a penicillin G-sensitive isolate and in the other quarter with a penicillin G-resistant isolate were excluded from the trial.

Drugs and treatments

The following drugs, each obtained from commercial sources as a dry powder, were used: (i) procaine penicillin G (Assia Maabarot Ltd, Israel); (ii) penethamate hydriodide (Mamyzin-Forte Krist, Boehringer Ingelheim Vetmedica GmbH, FRG); (iii) sodium methicillin (Sigma Chemical Co., St Louis, MO), and (iv) tamethicillin (Andreu Laboratories, Barcelona, Spain).

All drugs were administered by a deep i.m. injection into the upper one-third region of the neck, either as an aqueous solution (sodium methicillin) or as aqueous suspensions (the other three drugs). Multiple injections were given at 24-h intervals. Cows in herds milking three times daily were treated for 2 days, and cows in herds milking twice daily were treated for 4 days. The four drugs were used in each of the eight herds, and in each herd approximately the same number of cows were injected with a given drug. The following standard doses/cow/day were used: procaine penicillin G — 6×10^6 IU; penethamate hydriodide — 5×10^6 IU penicillin G equivalent; sodium methicillin — 5.0 g; tamethicillin — 5.0 g (equivalent to 4.0 g sodium methicillin).

Milk sampling and testing

Duplicate quarter milk samples for bacteriological analyses were collected immediately before the first treatment, and again 3–4 weeks later, using accepted procedures (Brown *et al.*, 1981). Recommended criteria (Anon., 1982) for defining infection and cure were employed. Pool (jar) milk samples were collected from ten, twelve, eight and fourteen cows treated four times with procaine penicillin G, penethamate hydriodide, sodium methicillin and tamethicillin, respectively, at 3, 6, 12 and 24 h after each treatment. Concentrations of penicillin G and methicillin in the samples were determined by microbiological assay using a paper disc, agar diffusion procedure (Grove & Randall, 1955).

RESULTS

Two and four i.m. injections of procaine penicillin G at 24-h intervals eliminated infection from 56.5% and 48.9% of quarters subclinically infected with penicillin G-sensitive *S. aureus*, but the cure rate among quarters infected with penicillin G-resistant *S. aureus* was $<$ 15% (Table I). The administration of penethamate hydriodide to cows with subclinical mastitis due to penicillin G-sensitive *S. aureus* eliminated infection from 62.7% and 68.8% of quarters, but was rather ineffective in eliminating infections from quarters infected with penicillin G-resistant *S. aureus* (Table II). Methicillin therapy eliminated 24.4% and 32.4% quarter infections, and treatment with tamethicillin eliminated 20.0% and 48.6% quarter infections due to penicillin G-resistant *S. aureus* (Table III).

Concentrations of penicillin G and methicillin in milk are depicted in Fig. 1, and ranges of drug concentrations are given in Table IV. Mean peak penicillin G concentrations after treatment with penethamate hydriodide were eight to ten times higher than after procaine penicillin G therapy. Lowest concentrations of penicillin G were observed 24 h after treatment, but mean nadir levels after treatment

TABLE I. Efficacy of intramuscular injection of procaine penicillin G in the treatment of subclinical mastitis in lactating cows associated with penicillin G-sensitive and penicillin G-resistant *Staphylococcus aureus* (dose = 6×10^6 IU/cow/day)

Sensitivity of isolates to penicillin G	Duration of treatment (days)	Lactation	No. of infections before treatment		% infections eliminated	
			Cows	Quarters	Cows	Quarters
(a) Sensitive	2	1st	3	3	33.3	33.3
		2nd	9	14	33.3	57.1
		4th & more	3	6	33.3	66.7
		All	15	23	33.3	56.5
	4	1st	6	5	66.7	80.0
		2nd	7	10	42.9	40.0
		3rd	8	12	37.5	50.0
		4th & more	11	18	36.4	44.4
		All	32	45	43.6	48.9
(b) Resistant	2	1st	3	5	0.0	20.0
		2nd	5	7	0.0	0.0
		3rd	2	4	50.0	25.0
		4th & more	3	7	0.0	0.0
		All	13	23	7.7	8.7
	4	1st	2	2	50.0	50.0
		2nd	2	3	0.0	0.0
		3rd	3	5	0.0	0.0
		4th & more	2	4	0.0	25.0
		All	9	14	11.1	14.3

with penethamate hydriodide were significantly ($P < 0.05$) higher than the corresponding values measured after treatment with procaine penicillin G. Mean peak milk methicillin concentrations after treatment with tamethicillin were 25–50% higher than the corresponding concentrations observed following sodium methicillin therapy. Whereas methicillin concentrations in milk decreased very rapidly after treatment with sodium methicillin to less than 0.1 μg/ml, the lowest concentrations were ⩾ 0.5 μg/ml after treatment with tamethicillin (Table IV).

DISCUSSION

The overall bacteriological efficacy of i.m. administration of procaine penicillin G in the treatment of forty-seven cows having sixty-eight quarters subclinically infected with penicillin G-sensitive *S. aureus* (Table I) was very similar to the cure rate reported by Funke (1982) in ninety-seven similarly infected and treated cows. Sadek (1954) administered sodium penicillin G and procaine penicillin G (1/3 ratio) i.m. twice at 24-h intervals at 10,000 IU/kg of body weight to six cows with chronic staphylococcal mastitis and reported that infection was eliminated from five (83.3%) of the treated cows. Additional reports are not available on the bacteriological efficacy of penicillin G, and data could not be found on the efficacy of penethamate hydriodide, methicillin or tamethicillin in eliminating subclinical *S. aureus* infections in lactating cows.

Data obtained in the course of the present studies revealed a direct positive relationship between the sensitivity of *S. aureus* to penicillin G isolated before treatment, and the percentage of infections eliminated by i.m. penicillin G and penethamate hydriodide therapy. Furthermore, treatment with the analogues of penicillin G and methicillin was clearly more effective in eliminating penicillin G-sensitive

TABLE II. Efficacy of intramuscular injection of penethamate hydriodide in the treatment of subclinical mastitis in lactating cows associated with penicillin G-sensitive and penicillin G-resistant *Staphylococcus aureus* (dose = 5×10^6 IU/cow/day)

Sensitivity of isolates to penicillin G	Duration of treatment (days)	Lactation	No. of infections before treatment		% infections eliminated	
			Cows	Quarters	Cows	Quarters
(a) Sensitive	2	1st	4	6	100.0	100.0
		2nd	4	5	75.0	80.0
		3rd	6	10	33.3	60.0
		4th & more	3	6	0.0	16.7
		All	17	27	64.7	62.7
	4	1st	6	8	83.3	87.5
		2nd	5	7	80.0	85.7
		4th & more	9	17	44.4	52.9
		All	20	32	65.0	68.8
(b) Resistant	2	1st	2	3	50.0	33.3
		2nd	3	3	0.0	0.0
		3rd	2	4	0.0	0.0
		4th	4	6	0.0	16.7
		All	11	16	9.1	12.5
	4	2nd	3	3	0.0	33.3
		3rd	3	4	0.0	0.0
		4th & more	3	6	0.0	0.0
		All	9	13	0.0	7.7

staphylococci and methicillin-sensitive staphylococci than treatment with the parent drugs. The better efficacy of the analogues can perhaps be linked to the higher milk drug concentrations obtained compared to the concentrations measured after the parent compounds were administered.

Milk penicillin G concentrations observed by us after i.m. injection of procaine penicillin G were very similar to those reported by others (Sadek, 1954; Hovmand & Overby, 1955; Hogh & Rasmussen, 1966; Schipper, 1967; Van Os *et al.*, 1974) using a similar dose of the drug. Our finding that milk penicillin G concentrations after treatment with penethamate hydriodide were considerably higher than after treatment with procaine penicillin G is in accord with previous studies (Hovmand & Overby, 1955; Hogh & Rasmussen, 1966; Schipper, 1967; Van Os *et al.*, 1974; Ziv, 1975, 1980a, b) and can be explained on the basis of the pH–pK passive diffusion principle which determines the movement of weak acids and weak bases across the blood–milk barrier (Ziv, 1975). The better penetration of methicillin into the milk, and the higher milk drug concentrations observed following tamethicillin administration (Fig. 1), can also be explained by the same principle as was noted in two earlier studies (Lazaro *et al.*, 1979; Ziv *et al.*, 1983).

Following treatment with procaine penicillin G, the peak and nadir milk drug concentrations ranged between 0.14 IU/ml and 0.04 IU/ml, respectively (Table IV), i.e. at concentrations equivalent to or 1.5 times greater than the MIC for twenty-five penicillin G-sensitive isolates, but much lower than the MIC for the thirty-eight penicillin G-resistant isolates tested. This course of treatment was effective in eliminating penicillin G-sensitive *S. aureus* quarter infections at rates (Table I) similar to cure rates reported following recommended courses of intramammary therapy using commercial products containing either penicillin G only or combinations with an aminoglycoside antibiotic (Mercer, 1971; Plommet & LeLouedec, 1975; Funke, 1982). Treatment

TABLE III. Efficacy of intramuscular injection of methicillin and tamethicillin in the treatment of subclinical mastitis in lactating cows associated with penicillin G-resistant *Staphylococcus aureus* (doses of methicillin and tamethicillin = 5.0 g/cow/day)

Antibiotic	Duration of treatment (days)	Lactation	No. of infections before treatment		% infections eliminated	
			Cows	Quarters	Cows	Quarters
Methicillin	2	1st	5	7	20.0	42.9
		2nd	8	11	25.0	36.4
		3rd	9	13	0.0	15.4
		4th & more	6	10	0.0	10.0
		All	28	41	10.7	24.4
	4	2nd	6	13	33.3	46.2
		3rd	6	11	33.3	36.4
		4th & more	8	13	12.5	15.4
		All	20	37	25.0	32.4
Tamethicillin	2	2nd	4	7	25.0	28.8
		3rd	2	4	0.0	0.0
		4th & more	9	14	0.0	21.0
		All	15	25	6.6	20.0
	4	1st	6	7	33.3	57.1
		2nd	4	6	50.0	50.0
		3rd	8	11	37.5	45.5
		4th & more	7	11	28.6	45.5
		All	25	35	36.0	48.6

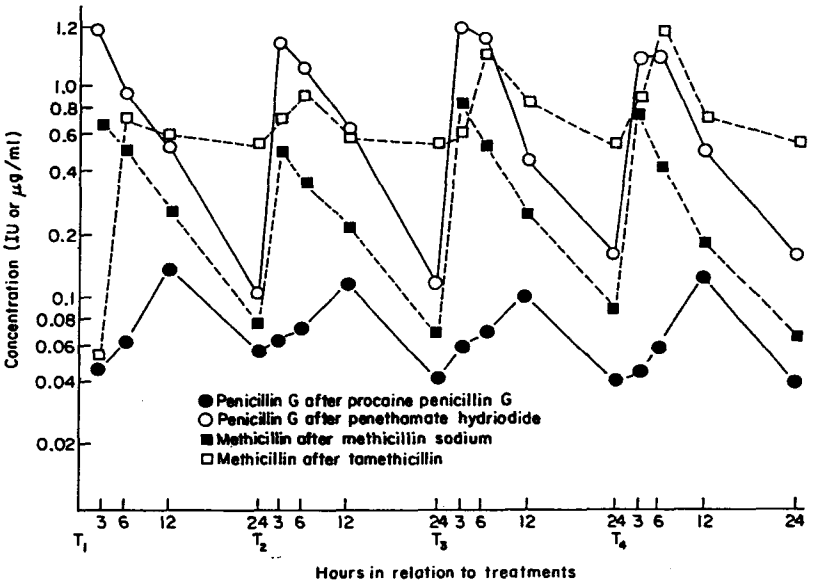

FIG. 1. Mean concentrations of penicillin G and methicillin in the milk of dairy cows after intramuscular injection of (●) procaine penicillin G at 6×10^6 IU/cow, (○) penethamate hydriodide at 5×10^6 IU penicillin G equivalent/cow, (■) sodium methicillin at 5.0 g/cow, and (□) tamethicillin at 5.0 g/cow, once daily for 4 days.

TABLE IV. Peak and nadir antibiotic concentrations in the milk after intramuscular injection of procaine penicillin G, penethamate hydriodide, sodium methicillin and tamethicillin once daily for 4 days

	Concentration (IU or μg/ml)							
	Day 1				Day 4			
	Peak		Nadir		Peak		Nadir	
Antibiotic	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Procaine penicillin G	0.142	0.103	0.055	0.010	0.125	0.05	0.042	0.010
Penthamate hydriodide	1.20	0.36	0.10	0.04	1.12	0.04	0.15	0.06
Sodium methicillin	0.66	0.08	0.07	0.02	0.70	0.10	0.065	0.02
Tamethicillin	0.72	0.12	0.50	0.08	1.18	0.17	0.55	0.07

with penethamate hydriodide resulted in fluctuating milk penicillin G levels equivalent to or twelve times greater than the MIC for the penicillin G-sensitive isolates, but only near the MIC for very few of the penicillin G-resistant isolates. Penethamate hydriodide i.m. therapy was thus substantially more effective than procaine penicillin G i.m. therapy (Table II), and more efficacious than intramammary penicillin G therapy.

After each treatment with sodium methicillin, drug concentrations equivalent to or higher than the MIC for the penicillin G-resistant isolates (0.5 μg/ml) were maintained in the milk for only 6 h (Fig. 1), whereas methicillin concentrations in excess of 0.5 μg/ml were maintained in the milk for 24 h after each administration of tamethicillin (Table IV). The bacteriological efficacy of i.m. tamethicillin therapy for 4 consecutive days was substantially higher than for a similar course of sodium methicillin administration (Table III). Intramuscular tamethicillin cure rates were similar to or better than those reported for intramammary treatment using commercial products containing the semi-synthetic isoxazolyl-type penicillins, like cloxacillin and oxacillin, in eliminating penicillin G-resistant staphylococci from the udders of subclinically infected lactating cows (Plommet & LeLouedec, 1975; Funke, 1982).

Results of numerous studies on the bacteriological efficacy of parenteral antibiotic therapy in the management of clinical bovine mastitis due to Gram-positive pathogens were reviewed (Ziv, 1980a; Funke, 1982). In the majority of these studies a progressive increase in cure rates was noted with the increase, from 2 to 6 days, in the duration of therapy. Furthermore, for a given course of therapy, the cure rate declined progressively with increasing cow's age. These relationships were generally observed in the present study which used the subclinically infected cow model. Admittedly, it is rather questionable whether, in practice, a 4–6-day course of i.m. antibiotic therapy can be economically justified for the treatment of subclinical staphylococcal mastitis in lactating cows. The model used in the present investigation can, however, be of value in screening existing, and particularly new, antibacterial agents and drug products intended for clinical staphylococcal mastitis parenteral therapy.

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