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Combined effect of ampicillin, colistin and dexamethasone administered intramuscularly to dairy cows on the clinico-pathological course of E. coli-endotoxin mastitis

Gideon Ziva, Moti Shem-Tov, Frédéric Ascher

VETGENERICS LTD, PO Box 2473, Rehovot 76124, Israel
bFaculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel
cVirbac, B.P. 27., 06511 Carros cedex, France

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Abstract – The effects of a single intramuscular injection of a drug product containing ampicillin, colistin and dexamethasone, as a suspension in a diester of propylene glycol of medium-chain fatty acids, on the clinico-pathological course of experimental Escherichia coli-endotoxin mastitis was examined in 30 dairy cows. Cows were divided into five groups, six cows per group, and 24 of them were infused with E. coli endotoxin into two quarters of their udders. The drug product was injected at 25,000 IU colistin sulphate, 10.0 mg ampicillin anhydrate and 0.025 mg dexamethasone acetate kg⁻¹ body weight as follows: Group 2 cows, immediately post-endotoxin infusion (PEI); Group 3 cows, 2 h PEI and, Group 4 cows, 4 h PEI. Group 1 cows were not treated with the product and served as a positive (endotoxin only) control while Group 5 cows were not challenged with endotoxin and were not treated with the product. A clinical mastitis score (CMS) was developed to quantitatively assess the degree of inflammation. Blood biochemistry and hematological parameters were used to monitor the immediate effects of treatment on several conventional inflammatory markers. Milk somatic cell counts (MSCC), milk electrical conductivity and daily milk production were among the parameters used to monitor systemic and local inflammatory reactions. Administration of the drug product immediately PEI and 2 h PEI clearly nullified some of the most severe early systemic reactions to inflammation but the effect of therapy on the local inflammatory markers was not as obvious. Noteworthy, however, were the effects of the treatment on reducing the duration of elevated quarter MSCC and the increase in the speed of return to pre-endotoxin challenge daily milk production levels.

Keywords

bovine mastitis / E. coli-endotoxin / colistin + dexamethasone / anti-inflammatory effect / ampicillin

† Deceased
* Correspondence and reprints
Tel: (33) 4 92 08 73 03; fax: (33) 4 92 08 71 65
Résumé – Effet combiné de l’ampicilline, de la colistine, et de la déxaméthasone administrées par voie intramusculaire sur l’évolution clinique d’une mammite induite par l’endotoxine colibacillaire chez la vache laitière. L’effet d’une injection intramusculaire unique d’un médicament à base de colistine, ampicilline et dexaméthasone en suspension dans un diester de propylène glycol d’acides gras à chaîne moyenne, sur l’évolution clinique d’une mammite induite par l’endotoxine colibacillaire a été évalué sur 30 vaches. Ces vaches ont été réparties en cinq groupes à raison de six par groupe. L’endotoxine colibacillaire a été infusée dans deux des quatre quartiers de 24 d’entre elles. Le médicament a été injecté à raison de 25 000 UI de sulfate de colistine, 10 mg d’ampicilline anhydre et 0,025 mg d’acétate de dexaméthasone par kilo de poids vif selon la répartition suivante : groupe 2, immédiatement après infusion de l’endotoxine (PEI) ; groupe 3, 2 h PEI ; groupe 4, 4 h PEI. Les vaches du groupe 1, éprouvées à l’endotoxine, n’ont pas été traitées (contrôle positif, endotoxine seule), alors que celles du groupe 5 n’ont pas été éprouvées ni traitées (contrôle négatif). Un score clinique (CMS) a été établi pour évaluer quantitativement le degré d’inflammation. Biochimie sanguine et hématologie ont servi à évaluer l’effet immédiat des traitements sur plusieurs marqueurs conventionnels de l’inflammation. Comp tages cellulaires (MSCC) et conductivité électrique du lait, ainsi que production quotidienne de lait sont parmi les paramètres retenus pour l’évaluation des réactions inflammatoires systémiques et locales. L’administration du médicament immédiatement et 2 h après infusion d’endotoxine (PEI) a clairement contrecarré certaines des plus sévères réactions systémiques précoces. L’effet du traitement sur les paramètres de l’inflammation locale ne fut pas aussi évident tout en étant cependant remarquable en réduisant la durée des comptages cellulaires élevés et en accélérant la reprise d’une production laitière identique à celle ayant précédé l’infusion d’endotoxine.

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mammite bovine / endotoxine colibacillaire / colistine + dexaméthasone / ampicilline / effet anti-inflammatoire

1. INTRODUCTION

Coliform bacteria are the major cause of clinical mastitis in well-managed dairy herds with low bulk (tank) milk somatic cell counts (MSCC). Endotoxins play an important role in the pathogenesis of coliform mastitis. The pathophysiological changes following intramammary infusion of endotoxin are generally the same as those found in field cases of coliform mastitis and consist of a wide range of clinical, hematological, metabolic and hormonal changes. The time span following infusion, however, is shorter and recovery is usually quick and uneventful [6, 7, 12, 15, 16, 20, 23, 25, 27].

The main therapeutic objectives against acute and peracute coliform mastitis are to shorten the period where systemic signs of disease are observed, to minimize losses in milk production and to prevent the establishment of subclinical mastitis [1, 5]. Antibacterial treatment is often practiced and may be useful in acute coliform mastitis [13], but its importance should not be over estimated [9-11, 22, 28]. Among the antimicrobial drugs used, the polymyxins (polymyxin B and colistin) occupy an important place by virtue of their bactericidal and endotoxin-neutralizing effects [21, 29]. Symptomatic and anti-inflammatory treatment may be indicated in severe cases of coliform mastitis. Two groups of drugs are available for this purpose: glucocorticosteroids (GCS) and non-steroidal anti-inflammatory drugs (NSAIDs). Dexamethasone is among the GCS found to be useful and effective in the treatment of acute endotoxin-induced mastitis [2] and experimental infectious E. coli mastitis [3, 17].
This subject area was reviewed by DeGraves and Anderson [8] and Lohuis [14]. Dexamethasone administered intramuscularly at 0.44 mg·kg⁻¹ at the time of endotoxin infusion, or E. coli bacterial inoculation, ameliorated the systemic clinico-pathological response and also significantly reduced the local (mammary gland) inflammatory response. A common clinical observation is that when administered separately, the polymyxin antibiotics (at 2.5–5.0 mg·kg⁻¹) and the GCS may be effective in the treatment of coliform mastitis [28]. However, data are not available on combined polymyxin/GCS therapy in the management of acute coliform mastitis. Ideally, the usefulness of such combined treatment should be evaluated under field conditions in naturally occurring coliform mastitis. This approach, however, is riddled with practical problems, due mainly to the epidemiology of the disease. Another approach involves the experimental infectious coliform mastitis model but, although many infectious and inflammatory variables can be controlled, these studies are rather expensive [24]. A third approach may involve assessment of the combined polymyxin/GCS therapy as anti-inflammatory drugs potentially capable of modifying the clinico-pathological course of experimentally induced E. coli-endotoxin mastitis.

The objective of the present study was to assess in lactating dairy cows the effect of a single intramuscular (i.m.) administration of a product containing colistin/ampicillin and dexamethasone as suspension in a diester of propylene glycol of medium-chain fatty acids on the clinical and pathological features of a well-studied and established E. coli-endotoxin mastitis model. This formulation was selected because it was available commercially in a ready-to-use injectable form, although it was realized that the antibiotic ampicillin may have very little specific anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1. Herd and cows

The studies were conducted on 30 clinically normal Holstein cows located in an experimental dairy herd. The cows were within 150 days of their second to sixth calving. They produced 22.5–34.5 L milk per day, had at least two quarters of the udder free from major udder pathogens. Their milk somatic cell count (MSCC) was < 300 000·mL⁻¹. The cows were kept in shaded free stalls and were fed antibiotic-free total mixed ration consisting of corn or grain silage, high quality lucerne hay, cottonseed or sunflower-seed meal and by-products of the fruit and vegetable canning industry. The cows were milked twice daily in a milking parlor equipped with an automatic removal of the milking units. The milking-time hygienic practices aimed at minimizing new udder infections included a premilking udder wash with warm tap water, use of disposable paper towels to wipe off excess moisture from the teats, foremilk stripping, use of an approved post-milking iodine teat dip and antibiotic drying-off therapy.

2.2. Drug product

The product tested was MULTIBIO-D Injectable Suspension (VIRBAC, Carros cedex, France, Lot # 781, exp. date Nov. 96) containing, per mL: 0.25 mg dexamethasone acetate, 100 mg ampicillin, 250 000 IU colistin sulfate in a vehicle based on a diester of propylene glycol of medium-chain fatty acids. The manufacturer’s recommended procedure is a dose of 10 mL·100 kg⁻¹ body weight, which is equivalent to 0.025 mg dexamethasone acetate, 10 mg ampicillin and 25 000 IU colistin-kg⁻¹, injected intramuscularly at two sites 10–15 cm apart.

2.3. Induction of E. coli-endotoxin mastitis

Immediately after a normal morning milking, the left front (LF) and the right rear (RR) quarters of the udder of each cow were aseptically infused with 100 μg of E. coli lipopolysaccharide 026:B6 (Difco) dissolved in 5 mL of a sterile phosphate buffered saline solution.
The relatively large dose of endotoxin was used in order to clearly induce a typical clinical systemic reaction [2, 3, 12, 15].

2.4. Experimental design

Cows were divided into five groups, six cows per group, and 24 were challenged with the endotoxin. The drug product was injected into Group 2 cows immediately post-endotoxin infusion (PEI), to Group 3 cows 2 h PEI and to Group 4 cows 4 h PEI. Group 1 cows were not treated with the drug product and served as positive (endotoxin only) controls. Group 5 cows were not challenged with endotoxin and were not treated with the drug product, serving as contemporary normal cow controls. An analysis of the number of lactations, days since calving and daily milk production for each group of cows indicated that the differences in the means for each of these parameters were not significant at the 95% confidence level (Student t-test).

2.5. Clinical observations, sampling schedules and tests

Clinical examinations and observations leading to a clinical mastitis score (CMS, [3]) were performed on d 2, d 1 and immediately before endotoxin infusion, to establish baseline values, and 2, 4, 6, 8, 12, 24, 32 and 48 h PEI. Additional clinical examinations involving rectal temperature, heart and respiration rates, ruminal contraction rate, california mastitis test (CMT), quarter MSCC and electrical conductivity of pooled milk were made once per day until the 7th day PEI, and on the 14th day PEI. Jugular vein blood samples were collected each time the CMS was determined. Blood was subjected to a complete blood count (CBC) and the concentrations of albumin, globulin, aspartate amino transferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), glutamic transaminase (GTA), lactate dehydrogenase (LDH), total bilirubin, blood urea nitrogen (BUN), creatinine, uric acid, calcium, magnesium, and inorganic phosphorus were determined by accepted procedures. Milk production was recorded at the end of each milking from 1 week before endotoxin infusion to 1 week PEI. Duplicate quarter milk samples for bacteriological analyses were collected before endotoxin infusion and 24 and 48 h PEI.

2.6. Statistical analysis

Data were analysed by a repeated measure analysis of variance design using a computer statistical package (SAS/STAT® Guide for Personal Computers, Version 6 Edition, SAS Institute, Inc., Cary, NC, USA, pp. 378, 1985). Categorical data were analysed by the same computer program using χ²-square analysis. Data are presented as mean ± SD.

3. RESULTS

3.1. Clinical signs

Mild to moderate quarter inflammation was developed in all 48 endotoxin-challenged quarters. Local clinical signs included diffuse oedema, swelling, increased skin temperature and pain. Changes in the milk from the endotoxin-challenged quarters were noted at 2 h PEI in approximately 35% of the quarters and in all the quarters at 4 h PEI. These changes included the presence of clots and flakes turning into thick secretions in some quarters and watery milk in others. Systemic clinical signs of mild to moderate but transient endotoxemia were observed in each of the endotoxin-challenged cows. All Group 5 cows were clinically normal throughout the experimental period and their appetite, demeanor, milk production and results of tests conducted on samples of their blood and milk did not deviate from normal, pretreatment values. Detailed results from this group of cows, therefore, will not be further presented or discussed.

A summary of mean rectal temperature, rumen contraction rate and the CMS is shown in figure 1A, B and C, respectively. During 4, 6, 8 and 12 h PEI, the mean rectal temperature for Groups 1 and 4 were significantly higher than for Groups 2 and 3. Normal rectal temperature was
Figure 1. Effect of intramuscular administration of dexamethasone/colistin/ampicillin on rectal temperature (A), rumen contraction rate (B), the clinical mastitis score (CMS) (C), white blood cell (WBC) count (D), serum lactate dehydrogenase (LDH) activity (E), and serum calcium concentration (F) in cows with experimental *E. coli*-endotoxin mastitis. (Group 1, not treated; Group 2, treated at time of endotoxin infusion; Group 3, treated 2 h after endotoxin infusion; Group 4, treated 4 h after endotoxin infusion.) Values are presented as mean ± SD.
recorded in all the cows at 24 h PEI. Rumen contraction rate was depressed in nearly all the endotoxin-challenged cows from 4–12 h PEI (figure 1B) but during 6 and 8 h PEI the magnitude of depression was significantly greater in Groups 1 and 4 cows than in Groups 2 and 3 cows. A significant increase in the CMS from 4 to 8 PEI h was calculated for all the endotoxin-challenged cows (figure 1C). The scores for Groups 2 and 3 cows, however, were significantly lower than those for Groups 1 and 4 cows from 6 to 24 h PEI.

3.2. Hematological and blood biochemistry findings

The most consistent significant changes from the normal hematological and blood biochemistry test results were transient leukopenia, hypocalcemia and elevated serum LDH values.

Figure 1D presents the mean change from pretreatment WBC count. This form of data presentation was selected because baseline (pre-endotoxin infusion) values for the WBC count among cows varied greatly. Although differences among treatment groups were not significant during any sampling time PEI, a trend toward a greater reduction in WBC count was noted for Groups 1 and 4 cows. An increase in the WBC count was noted 24 h PEI. Because of large variations among cows in the magnitude of increase in serum LDH activity, differences in mean values for Groups 1 and 4 cows versus Groups 2 and 3 cows were not significant at the 95% confidence level (figure 1E). The degree of hypocalcemia for Group 1 cows versus the other groups was significantly greater at 4 and 8 h PEI (figure 1F). At 12 h PEI however, the reduction in serum calcium concentration for Groups 1 and 4 cows was significantly greater than for Groups 2 and 3 cows.

3.3. MSCC

The MSCC was greatly elevated in samples collected from all the 48 challenged quarters. The highest and most persistent rise in MSCC was noted for Group 1 cows followed by Group 4 cows (figure 2). Although the mean MSCC in each of the challenged quarters were not significantly different during the first 24–32 h, thereafter the mean values for the cows in Group 2 and 3 were significantly lower than for the Group 1 and 4 cows. Thus, on day 7 PEI, 20 out of the 24 (83.3 %) challenged quarters in Group 2 and 3 cows and 9 out of the 24 (37.5 %) challenged quarters in Group 1 and 4 cows secreted milk with MSCC near the pre-challenge values. A sharp drop in resistance to electrical conductivity was seen in milk from all the challenged quarters. This drop was significantly greatest, and the reduction most persistent, in the milk from Groups 1 and 4 in comparison with the corresponding values for the milk from cows in Groups 2 and 3 (figure 3).
3.4. Milk production

Because of large variations among cows, only typical examples of daily milk production over 13 d starting 6 d before and 7 d PEI are presented (figure 4). The largest decrease was in Group I and 4 cows, whereas Group 2 and 3 cows returned significantly more quickly to near pre-challenge daily milk production.

Before the endotoxin infusion, six non-challenged quarters of five cows were infected with major udder pathogens. The infecting microorganisms were isolated from four of these quarters 1, 2 and 7 d PEI, whereas two new quarter infections were acquired by two other cows. The bacteriological status of all other quarters did not change throughout the observation period.

4. DISCUSSION

Cows selected for the present trial were similar in their udder health status, stage of lactation and daily milk production. The inflammation model selected produced uniform clinico-pathological changes, both local and systemic, in all cows. The most consistent local features were the swelling of the affected quarters, physical changes in the secretions, an elevated MSSC, and a very sharp yet transient reduction in milk produced by the challenged quarters. The most consistent systemic features were pyrexia, reduction in rumen contraction rate, leukopenia followed 24 h later by transient leukocytosis a 'rebound' phenomenon which appears to be characteristic for the model [2, 23], elevation in serum LDH activity, hypocalcemia, and a reduction in daily milk production. These local and systemic changes coincided with a rise in the CMS (figure 1) and were typical for the model [6, 7, 15, 16].

The influence of a single i.m. administration of a product containing colistin and dexamethasone on the local and systemic symptoms, and the effects on the inflammation parameters were quite obvious. It is doubtful whether ampicillin, which was present in the product, had any effect on the inflammatory parameters examined in the present study. There appeared to be a correlation between the severity of inflammation and the time of administration. A partial suppression of the most severe clinico-pathological signs was seen in the cows treated with the drug product at the time of the intramammary endotoxin challenge. The injection of the drug product 2 h PEI still resulted in the amelioration of the expected local and systemic signs of inflammation. The inflammation was not modified, however, when the administration was delayed to 4 h PEI. Thus, the most obvious effects of i.m. dexamethasone/colistin/ampicillin therapy were confined to the immediate systemic inflammatory response and much less to the local effects. The treatment Group 2 and 3 cows resulted in a lower CMS and was most effective in depressing the rise in serum LDH activity and lessening the depression in serum calcium levels. The immediate effects on the MSCC, however,
were minimal. Very similar results were reported by Lohuis et al. [18] using dexamethasone, betamethasone, \(\alpha\)-fluoroprednisolone and flumethasone.

Temporary suppression or marked reduction in milk production is a characteristic finding for the *E. coli*-endotoxin mastitis model [26], as was observed in the present study (figure 4), and for the *E. coli*-infectious mastitis model [19, 24]. It was remarked [19] that the most severe early systemic signs were associated with the greatest and most persistent reduction in milk production. In the present study, in the face of the great reduction in milk production due to the acute inflammation, it

**Figure 4.** Representative examples of the effect of intramuscular administration of dexamethasone/colistin/ampicillin on the daily milk production in cows with experimental *E. coli*-endotoxin mastitis.
was impossible to evaluate the effect, if any, dexamethasone may have had in increasing milk loss [2]. The results of the present study suggest that treatment must have had some positive effects on the mammary gland as well because, in Group 2 and 3 cows, the duration of the elevated MSCC was shorter and the return to pre-challenge daily milk production levels was sooner than in Group 1 and 4 cows. The small number of cows involved precluded a statistical analysis of the significance of these latter effects. A confounding factor which must be considered in analysing the data was the observation that the cows did not develop clinically detectable manifestations of mastitis at the same time after endotoxin infusion. In the present study it seemed that local symptoms appeared 2 h PEI for some cows, but 4 h were necessary for all infused quarters to develop obvious inflammatory signs. The fact that Group 4 cows did not respond to treatment as well as cows of Group 2 and 3 could possibly be linked to slow mobilization of inflammatory mediators [7, 27] and subsequently poorer response to anti-inflammatory drug therapy [14].

There are still unanswered questions regarding the relative contribution of colistin and dexamethasone to the amelioration of the acute clinico-pathological inflammatory response to intramammary endotoxin challenge, and the relevance of the present findings to the treatment of acute coliform mastitis in the field. These two aspects have both academic and practical implications. Nevertheless, results of the present trial point to the possibility that the therapeutic response to acute coliform mastitis could be perhaps improved by optimizing the use of dexamethasone and colistin.

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