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Concentration of penicillin G in mammary tissue and secretion of end-term dairy heifers following systemic prepartum administration of penethamate hydriodide

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The aim of this study was to assess the concentration of penicillin G in mammary tissue and secretion of dry heifers following systemic administration of penethamate hydriodide. Six dairy heifers in late gestation received a single intramuscular injection of 10 g penethamate hydriodide and were sacrificed 24, 48 or 144 h after treatment. Penicillin G concentrations were measured in mammary tissue and secretion samples using HPLC. Penicillin G was detected in the udder of two animals euthanized at 24 h (mammary tissue and secretion) and at 48 h post treatment (mammary secretion only) after administration at concentrations still close to or above MIC_{90} values reported for the pathogens associated with heifer mastitis. Antibiotic concentration shortly after administration will have been substantially higher indicating a potential for systemic treatment with penethamate hydriodide to control prepartum intramammary infections in heifers without the disadvantages of local therapy such as teat contamination or risk of trauma for the administrator.

Keywords: Dairy heifer, penethamate hydriodide, prepartum treatment, systemic.

For many years there have been reports that a large proportion of dairy heifers calve with infected quarters (Fox, 2009). This condition has been referred to as heifer mastitis and studies have shown a wide variation in prevalence, from 74·6 to 29·0% (Oliver & Mitchell, 1983; Trinidad et al. 1990) and from 55·0 to 12·3% (Roberson et al. 1994; Parker et al. 2007) of quarters being reported as culture positive before and at calving, respectively. A number of pathogens have been isolated but studies have shown that infections are predominantly due to Gram-positive pathogens, specifically coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, and environmental streptococci (Fox, 2009).

Heifer mastitis can have a negative impact on future productive life (De Vliegher et al. 2004, 2005a, b), the effect depending on factors such as virulence of the pathogens involved and time of onset of the intramammary infection (IMI) (Piepers et al. 2009). The cost of heifer mastitis in early lactation on an average Dutch/Flemish dairy farm has recently been estimated to vary from €4 to €82 per heifer with an average of €31 (Huijps et al. 2009).

To address this issue, antibiotic treatments have been used empirically before calving. Various studies have shown that the use of dry or lactating cow intramammary products prior to calving in heifers can be beneficial in reducing levels of mastitis pathogens isolated post partum and in increasing milk yield during lactation (Nickerson, 2009). Systemic rather than local antibiotic treatment presents the advantages of decreased risk of teat contamination, more convenient and safer to administer, and four quarters being treated with a single administration.

Penethamate hydriodide is a prodrug which releases penicillin G on hydrolysis. It easily crosses the blood-milk barrier and concentrates in udder tissues and milk after intramuscular (i.m.) administration to lactating cows (Ziv, 1980). The spectrum of activity in vitro is mainly within the Gram-positive class of bacteria e.g. *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus* spp. A recent study on a herd struggling with *Staph. aureus* infections

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showed that i.m. treatment of heifers at parturition with penethamate hydriodide prevented IMI during the first week post partum and resulted in a significant increase in milk yield (Kreiger et al. 2007). Another study on a 350-cow dairy herd having problems with *Staph. aureus* infections showed a significant reduction in the number of IMI after introduction of a regimen to inject heifers 2 months prior to calving with penethamate hydriodide intramuscularly (Moroni et al. 2002). A prerequisite for the successful use of this treatment in heifers and cows before calving is widespread distribution of the drug in the dry udder.

The aim of this study was to assess the concentration of penicillin G in mammary tissue and secretion of heifers in the third trimester of pregnancy following a single i.m. injection of penethamate hydriodide.

Materials and Methods

Animals

The study group consisted of 6 clinically healthy primiparous dairy heifers (4 Montbeliarde and 2 cross-bred Montbeliarde × Charolais) in gestation month 8–9, weighing 540–630 kg (average 600 kg) and aged 2·5–3·5 years (average 2·6 years). Animals had received no treatment with veterinary drugs for at least 2 weeks prior to the beginning of a 7-d acclimatization period.

Drug administration

After the acclimatization period each of the 6 animals was treated by deep i.m. injection with one vial of the reconstituted test item containing 7·72 g penethamate hydriodide (Mammyzine®, Mamyzin®, STOP M®, Boehringer Ingelheim Vetmedica). This equated to doses ranging from 12·3 to 14·3 mg/kg. All animals were treated, once only, on the same day. No adverse reactions were noted.

Tissue and secretion samples

Animals were slaughtered randomly in groups of two at approximately 24, 48 and 144 h post treatment. On the day of slaughter 100–120-g pieces of mammary tissue were removed from each quarter and individually minced before being placed in plastic bottles. Mammary secretions were obtained manually by pressing the mammary tissue of each quarter and collecting the liquid in plastic bottles. All the prepared samples were frozen within 2–3 h and stored at a temperature below −75 °C until analysis.

Penicillin G assay

Determination of penicillin G in mammary tissue and secretion was carried out using reversed-phase HPLC based on Tarbin et al. (1995). Samples weighing 5 g of either mammary tissue (homogenized with 10 ml water) or secretion (diluted with 10 ml saturated dibasic sodium phosphate) were acidified with 0·17 M-sulphuric acid and protein precipitated by adding 5% sodium tungstate solution. The resulting mixture was sonicated, shaken for 30 min (horizontal shaker) and the aqueous layer recovered following centrifugation at 12 °C for 10 min at 2800 ***g***. Following a second extraction step, the second aqueous layer was combined with the first, and a 20% solution of sodium chloride added. Mammary secretion extracts were adjusted to pH 8 using concentrated sodium hydroxide. Extracts obtained were then deposited on an SPE C18 cartridge (Waters, Milford MA, USA) previously washed with methanol, water and 2% sodium chloride solution. The cartridge was then washed with 2% sodium chloride solution followed by water, and dried. The cartridge was eluted with a mixture of acetonitrile, water and phosphate buffer. A derivatization mixture (1,2,4-triazol dissolved in water with 0·02 M-mercuric chloride, pH adjusted to 9·0) was added to the eluted sample and the mixture was placed in a water bath at 65 °C for 2 h. After cooling at room temperature and centrifugation for 5 min at 2800 ***g***, 150 µl of the supernatant was injected into the HPLC system (Waters, Milford MA, USA). The analyses were performed at 325 nm with a Spherisorb ODS2 column 250 mm × 4·6 mm (Waters, Milford MA, USA) attached to an appropriate guard column filled with the same material. The analyses were carried out at room temperature. The mobile phase consisted of acetonitrile/0·1 M-phosphate buffer containing 0·0157 M-sodium thiosulphate (25/75, v/v). Retention time for penicillin G was about 12 min using an isocratic elution at 1 ml/min.

Calibration curves for penicillin G concentration in mammary tissue/secretion were linear between 50 and 1000 µg/kg ($r > 0{\cdot}99$). The limit of quantification (LOQ) was set to 50 µg/kg for the two matrices and the limit of detection (LOD) was 18 µg/kg and 20 µg/kg for mammary tissue and mammary secretion, respectively. Penicillin G remained stable in extract for 24 h (mammary secretion) or 48 h (mammary tissue) at ambient temperature, and for 29 d (mammary tissue) or 90 d (mammary secretion) below −75 °C, i.e. for storage periods longer than those used in the study.

Results

Mammary tissue

Penicillin G was detected at quantifiable levels in mammary tissue from the 8 quarters of the two animals sacrificed at 24 h after treatment (ranging from 90·69 to 151·16 µg/kg) (Table 1). No major differences were seen between front and rear quarters. Penicillin G was not detected in mammary tissue from any of the quarters of the four animals euthanized at 2 and 6 d post treatment.

Mammary secretions

Quantifiable concentrations of penicillin G were detected in mammary secretion from all quarters of the two animals

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Table 1. Penicillin G concentrations (μg/kg) in mammary tissue and secretion of end-term dairy heifers following administration of penethamate hydriodide

Time of sacrifice after administration	Animal number (month of gestation)	Quarter position†	Mammary tissue	Mammary secretion
1 d (24 ± 0·5 h)	2 (8^{th})	RL	96·49	74·53
		RR	109·47	98·67
		FL	139·18	119·18
		FR	102·61	152·42
	4 (9^{th})	RL	151·16	291·31
		RR	90·69	290·32
		FL	133·32	256·78
		FR	102·98	226·52
2 d (48 ± 0·5 h)	3 (9^{th})	RL	<LOD‡	77·39
		RR	<LOD	63·81
		FL	<LOD	81·76
		FR	<LOD	69·25
	5 (9^{th})	RL	<LOD	66·31
		RR	<LOD	73·97
		FL	<LOD	<LOQ§
		FR	<LOD	<LOQ
6 d (144 ± 0·5 h)	1 (9^{th})	RL	<LOD	<LOQ
		RR	<LOD	<LOQ
		FL	<LOD	<LOQ
		FR	<LOD	<LOQ
	6 (9^{th})	RL	<LOD	74·62
		RR	<LOD	<LOQ
		FL	<LOD	<LOQ
		FR	<LOD	<LOQ

† RL: rear left, RR: rear right, FL: front left, FR: front right
‡ Level of detection (for tissue and secretion = 18 and 20 μg/kg, respectively)
§ Level of quantification (= 50 μg/kg)

sacrificed on the first day after treatment (ranging from 74·53 to 291·31 μg/kg) (Table 1). Penicillin G concentrations were either similar to the ones observed in mammary tissue (animal 2, average 111·2 and 111·9 μg/kg in secretion and tissue, respectively) or approximately twice the concentrations in mammary tissue (animal 4, average 266·2 and 119·5 μg/kg in secretion and tissue, respectively). In animals euthanized on the second day after treatment, quantifiable concentrations of penicillin G were present in mammary secretions from the 4 quarters of animal 3, and from the rear quarters of animal 5. In animals sacrificed on the sixth day after treatment quantifiable concentrations of penicillin G were not detected in secretion samples from 7 of the 8 quarters. Penicillin G was detected at a low level in mammary secretion from the remaining quarter at a value close to the LOQ of 50 μg/kg.

Discussion

Penicillin G reached tissues from all quarters of the udder of non-lactating heifers in high concentrations. Assuming the density of milk secretion and mammary tissues is higher than that of milk and colostrum, it appears that antibiotic concentrations present in the udder 24 h after administration of penethamate hydriodide were near or above the MIC_{90} of penicillin for *Staph. aureus* (0·125 to >100 μg/ml) and streptococci (0·07–2 μg/ml) as summarized by Erskine et al. (2004) and CNS (e.g. Gentilini et al. 2002, 4·4 μg/ml) i.e. the target pathogens commonly associated with heifer mastitis. Previous work shows that after a single i.m. injection of penethamate hydriodide in lactating cows the mean maximum penicillin G concentration is reached after 3·76 h in plasma and after 5·91 h in milk. With a mean half life of 4·27 h (plasma) and 4·00 h (milk) the maximum concentration are at least 5-times those recorded at 24 h post administration (Friton et al. 2003). This supports the view that in the current study penicillin G concentrations well above the MIC_{90} for the more common mastitis pathogens associated with heifer mastitis will have been reached.

Previous studies have shown that i.m. administration of penethamate hydriodide for 3 d can be efficacious in the treatment of clinical and subclinical mastitis in lactating cows (Serieys et al. 2005; Salat et al. 2008). Additionally, in another study where heifers were treated intramuscularly with penethamate hydriodide on one occasion 7 d prior to calving, periparturient mastitis incidence was 22% in treated heifers *v.* 46% in non-treated control heifers (Bryan & Friton, 2005).

We conclude that systemic use of penethamate hydriodide prior to calving can result in levels of penicillin

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G in mammary tissue and secretion substantially higher than the MIC_{90} of pathogens associated with heifer mastitis. These findings support the view that penethamate hydriodide administered via the i.m. route to heifers prior to calving could be an appropriate, although temporary, therapeutic choice while preventive measures are being implemented by the herd manager/farmer. Systemic treatment of end-term heifers that have never been constrained before has obvious advantages over local therapy using lactating or dry cow products. However, this therapeutic approach needs to be verified under field conditions to quantify the short- and long-term effects on udder health (somatic cell counts and clinical mastitis cases), and milk production.

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