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RECOGNISING AND CONTROLLING PAIN AND INFLAMMATION IN MASTITIS

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SUMMARY

The aim of this project was to investigate if cows with clinical mastitis suffer pain. This was assessed by comparing the response of cows with mastitis to normal cows, on application of a pressure stimulus. The results strongly suggest that cows with mastitis do have increased sensitivity to pain, even when the mastitis is only mild, or moderately, severe. Treatment of cows with mild mastitis using a single, intravenous injection of a non-steroidal anti-inflammatory drug, flunixin meglumine (Finadyne, Schering-Plough Animal Health; 2.2mg/kg) combined with intramammary antibiotic therapy, reduced sensitivity to pain, but was not effective in moderately severe cases. This beneficial effect on sensitivity to pain was seen one day after the start of treatment which coincides with the duration of activity of flunixin. These results indicate that intravenous flunixin reduced sensitivity to pain in mild mastitis cases, but that repeated doses of the drug may be required to extend this beneficial effect. As cows with mastitis are in pain, improved methods are required to reduce pain and to return the udder to normal function as quickly as possible.

INTRODUCTION

Most farmers and veterinary surgeons agree that cows with severe toxic mastitis often appear to be distressed and in pain. However, it is much harder to decide whether cows affected by less severe forms of mastitis are also in pain. This may be due to our lack of ability to perceive signs of pain in stoical species such as cattle. It may be to ruminants' advantage, in an evolutionary sense, as a herd animal, and as prey rather than predator, to avoid displaying signs of weakness and pain. Development of objective, rather than subjective, methods of measuring pain are urgently required and have the potential to be used in future farm assurance schemes where adverse welfare associated with disease is assessed. While pain suffered by cows affected by diseases such as mastitis is an obvious welfare issue; cows in pain often have a reduced appetite, are less willing to move to sites of available food and have reduced milk yields, with concomitant deleterious effects on farm economics. Recognising pain and developing methods to reduce pain in mastitis, therefore, should benefit both cows and farmers.

Antibiotics are routinely used for mastitis therapy, but non-steroidal anti-inflammatory drugs (NSAID) can also be employed, although they are often reserved only for severe cases, primarily to treat systemic signs. They act to reduce inflammation (anti-inflammatory), to reduce pain (analgesic) and to reduce body temperature (anti-pyretic). There are currently two NSAID available commercially which are licensed for use in mastitis, flunixin meglumine (Finadyne Injection, Schering-Plough Animal Health) and ketoprofen (Ketofen 10%, Meriel). Both of these products must be administered by parenteral injection, and their use is restricted to 5 consecutive days for Finadyne

Injection, and 3 consecutive days for Ketofen 10%, due to potentially serious side effects, which can be fatal, resulting from prolonged use of these products. Since the withdrawal of phenylbutazone, which was commonly used by vets and farmers to treat painful conditions in cattle, from use in food producing animals, the NSAID remain the sole pain-killing drugs available. The other groups of analgesic drugs are not ideal for pain relief in cows with mastitis: the opioids are not licensed for any food producing species; corticosteroids may have immunosuppressive effects and should not be administered to pregnant animals, although they are incorporated into some intramammary antibiotic preparations; and the alpha2-adrenoceptor stimulant, xylazine, has a prolonged sedative effect. Thus, no drug is now available for controlling prolonged pain in cattle. To date, there have been few studies investigating pain in mastitis, although proteins such as bradykinin, known to mediate severe pain in humans, have been detected in milk from clinical and subclinical mastitis in cows (1).

Mastitis is defined as inflammation of the mammary glands. Inflammation is induced, in response to udder infection, by mediators that are produced to help clear infection, but that also result in damage to the milk secreting tissues of the udder. Unfortunately, damage to the udder may occur before signs of disease become obvious to the dairyman, or before somatic cells begin to rise in milk. Certain inflammatory proteins, termed acute phase proteins are present in altered milk during mastitis and may reduce milk quality (2). By measuring these proteins in milk it will be possible in future to monitor their concentration as a rapid diagnostic test for mastitis with a reduction in concentration indicating effective therapy.

The primary aim of this study was to investigate whether cows with naturally occurring clinical mastitis exhibited allodynia, a decreased pain threshold, by measuring their sensitivity to pain induced by a mechanical stimulus using methods described previously (3,4). Another aim was to compare the effect of pain-killing drugs given to mastitic cows by the intravenous or intramammary route on sensitivity to pain. The levels of haptoglobin, a sensitive bovine acute phase protein in blood and the somatic cell count (SCC) of milk from mastitic cows were measured and the response to the NSAID therapy assessed.

METHODS

Animals and sampling

Holstein-Friesian cows from three institute farms and one commercial dairy farm, milked twice daily, were used. Natural cases of clinical mastitis were recognised by the dairy staff on each farm in the usual way, by observation and palpation of the udder. Each cow was visited by the same researcher within two hours of the mastitis being detected. Blood samples were taken, and after clotting serum was removed for measurement of haptoglobin. Milk samples were taken from the mastitic quarter for bacteriology, somatic cell counting and mediator analysis. Samples were also taken from an unaffected, non-mastitic, quarter on the opposite half of the udder, as controls.

Responsiveness to pain

The pain threshold of cows was assessed using a mechanical device attached to each hind limb, just below the hock, and pressure was applied via a gas-driven device that pushed a blunt-ended pin against the cow's leg. When the cow responded to the pressure by shifting her weight or moving her leg, the pressure was immediately turned off. Two or three measurements were taken from each leg

at each time point. The cows were re-assessed on six occasions during the study: once on the day of diagnosis and before treatment was given, and on five occasions one, two, four, 20, and 40 days following initial treatment.

Mastitis grades and therapy

The mastitis was classified as "mild" when clots were detected in the milk and the udder appeared normal, or "moderate" when clots were detected in the milk and the udder appeared red, hot or swollen. Any cows with more severe mastitis, which required systemic treatment or veterinary attention, were excluded from the study. All cases of mastitis received treatment. Control cows received a course of amoxycillin/clavulanic acid (3 syringes of Synulox Lactating Cow; Pfizer Ltd.), the second group received the same antibiotic course and an intramammary infusion of flunixin in a specially prepared vehicle created by Schering Plough Animal Health for this study, and the third group received the same antibiotic course and an intravenous injection with flunixin meglumine (Finadyne, Schering Plough Animal Health; 2.2 mg/kg). The first two groups of cows also received an intravenous injection of saline to act as a control for the intravenous flunixin.

Bacteriology and somatic cell counting

Bacteriological examination and somatic cell counting were performed at SAC Auchincruive using standard bacteriological identification and automated cell counting using a Fossomatic Counter. Milk samples bacteriology were taken prior to therapy being administered. Somatic cell counts were re-assessed at the same time points detailed above.

Haptoglobin

Haptoglobin levels in the blood were measured with an automated assay in the Department of Veterinary Clinical Studies University of Glasgow Veterinary School. Haptoglobin was re-assessed at the same time points detailed above

Statistical analysis

Somatic cell count data were log transformed due to being non-normally distributed. Responsiveness to pain was calculated by subtracting the threshold response in the leg on the same side of the cow as the mastitic quarter from the threshold response in the leg on the opposite side of the cow. Data were analysed using multivariate and mixed model analysis of variance (SAS, SAS Institute Inc., North Carolina).

RESULTS

Responsiveness to pain

The technique of measuring responsiveness of cows to pain using a mechanical stimulus presented no problems. All cows tolerated the attachment of the machine to their hind limbs, and their response of shifting weight or moving the limb as a result of the stimuli was easily recorded.

The responsiveness to pain in normal, non-mastitic, cows was similar on both hind limbs. In mastitic cows, increased sensitivity to pain in the leg on the **same** side as the mastitic quarter compared to the leg on the other side to the mastitic quarter, was observed for both mild and moderate mastitis. The increased sensitivity in cows with mastitis was significantly greater than in normal, non-mastitic cows at all sample days up to, and including, that taken at 4 days after start of treatment for mild cases, and up to, and including, that taken at 20 days after the start of treatment for moderate cases.

When mild mastitis cases were treated with antibiotics alone, the responsiveness to pain was greatest two days after the start of treatment and was still present 20 days later. Similarly, mild mastitis cases treated with antibiotics plus intramammary flunixin showed a similar effect, although the responsiveness to pain appeared to be less marked in these cows. When mild mastitis cases were treated with antibiotics and intravenous flunixin, a sharp decrease in responsiveness to pain was seen one day after the start of treatment. This coincides with the expected clinical activity of flunixin administered by the intravenous route. However, increased responsiveness to pain was again observed from the next sample day, two days after the start of treatment, and this effect persisted again until the sample on day 20 (Figure 1).

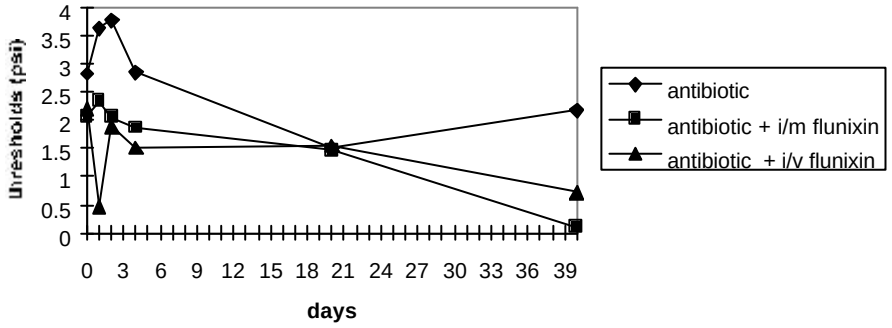

Figure 1. Threshold responses of cows with mild mastitis

With moderate mastitis treated with antibiotic alone, increased responsiveness to pain was seen throughout the study. The moderate cases also showed increased responsiveness to pain when treated with antibiotics plus intramammary flunixin at all time points sampled, and the response was greater than with antibiotic treatment alone, indicating that the intramammary flunixin may have induced an adverse effect. The responsiveness to pain in moderate cases treated with antibiotics plus intravenous flunixin was similar to those given antibiotic alone, and no reduced responsiveness to pain was seen on day one after treatment, as was the case in mild mastitis cases (Figure 2).

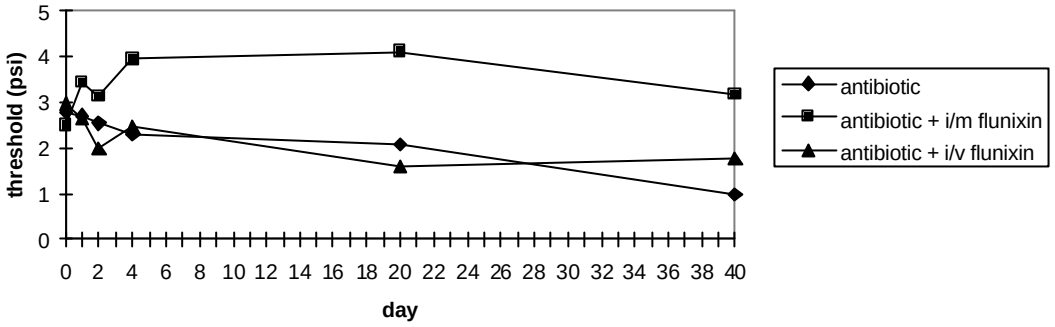

Figure 2. Threshold responses of cows with moderate mastitis

When normal, non-mastitic, cows were given the same treatments as the mastitic cows to act as controls for possible effects of the drugs themselves on responsiveness to pain, the groups given antibiotics alone or antibiotics plus intravenous flunixin, showed no increased responsiveness to pain. However, with groups given antibiotics plus intramammary flunixin, a significant increase in responsiveness to pain did occur, although not until the samples taken on day 20 and 40 following the start of treatment (Figure 3).

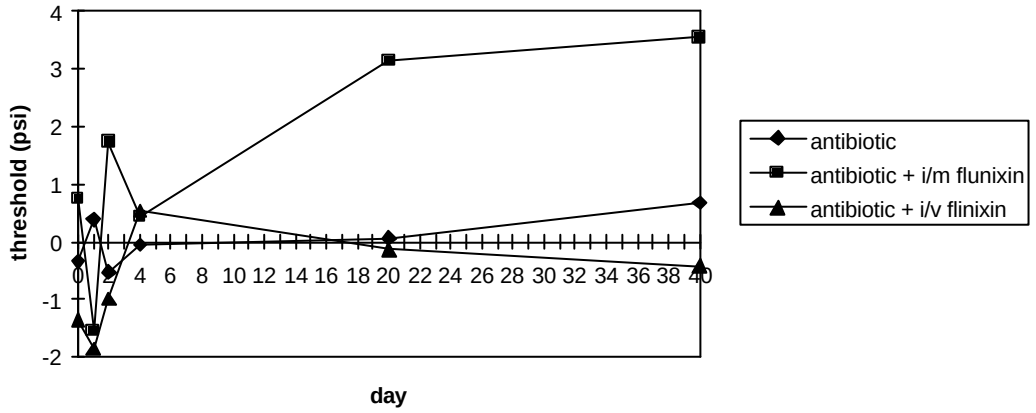

Figure 3. Threshold responses of normal, non-mastitic cows

Statistical analysis showed significant differences in sensitivity to pain among groups (mild mastitis, moderate mastitis, normal non-mastitic cows; $p<0.0001$), among treatments (antibiotics alone, antibiotics plus intramammary flunixin, antibiotics and intravenous flunixin; $p<0.05$) and there was a group-treatment interaction; $p<0.05$) on responsiveness to pain.

Haptoglobin

The moderate mastitis cases had greater levels of haptoglobin than mild mastitis cases at all time points, and both mastitic groups had higher levels than the normal, non-mastitic cows throughout the study. The haptoglobin was found to be increased in both the mild and moderate mastitis groups at

the sample taken before treatment, and the shape of the curve suggests that, particularly in the mild mastitis cases, the haptoglobin level may have been raised prior to the mastitis being noticed by the dairyman. The haptoglobin level was still significantly raised on day 4 following start of treatment in moderate cases but had returned to near the level seen in normal cows in the mild mastitis cases by the same time. The haptoglobin levels in moderate mastitis cases returned to normal levels (0.1g/l) by day 20 (Figure 4).

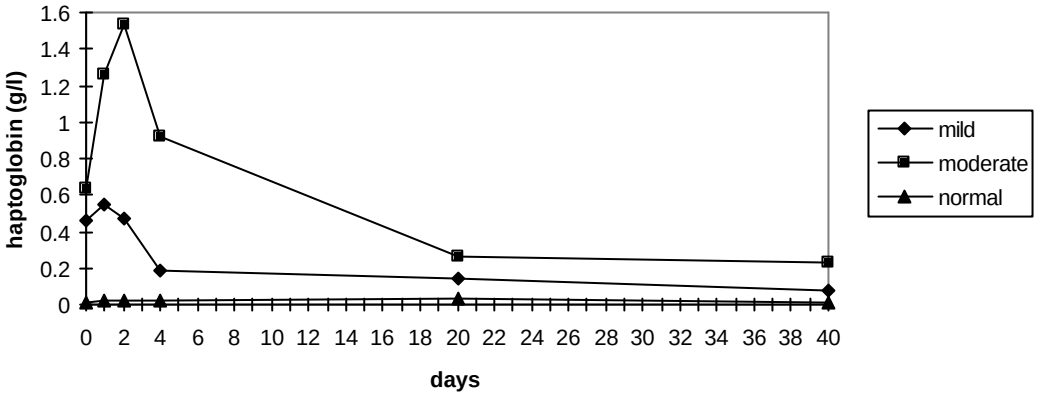

Figure 4. Serum haptoglobin levels in cows with mild mastitis, moderate mastitis and in normal, non-mastitic cows.

Statistical analysis showed a significant effect of group (mild mastitis, moderate mastitis, normal non-mastitic cows; $p < 0.0001$), and time ($p < 0.0001$) but not treatment, on levels of haptoglobin in blood.

Bacteriology and somatic cell counting

Bacteria isolated from the clinical cases included the usual range of pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus uberis* and *Arcanobacterium pyogenes*. A number of minor pathogens and contaminants, including *Staphylococcus epidermidis*, micrococci. and Bacillus sp. were also recognised. A small number of cases showed no growth on bacteriological examination.

The SCC increase was greatest with the moderate mastitis cases and peaked at approximately 13,000,000 cells/ml milk on day 1 after initiation of treatment. The mild mastitis cases also showed an increase in SCC compared to normal non-mastitic cows and again the peak levels were recorded at the same time point (Figure 5). The SCC in mild mastitis cases declined to normal levels by day 4 after the start of treatment but were still elevated in the moderate mastitis cases at day 20. Cell counts were also increased in normal non-mastitic cows following all treatments, but the SCC levels did not rise above 4,000,000 cells/ml milk in these cows. The SCC was high in a number of the normal cows, some of which had bacteria isolated from them and were, therefore, subclinically infected.

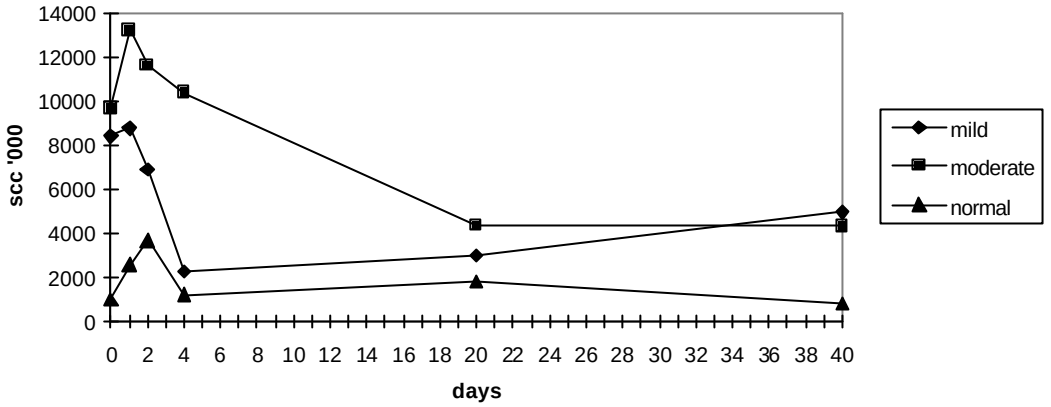

Figure 5. Somatic cell counts from quarters with mild mastitis, moderate mastitis and normal, non-mastitic quarters.

Statistical analysis showed a significant effect of group (mild mastitis, moderate mastitis, normal non-mastitic cows; $p < 0.0001$), and time ($p < 0.0001$) but not treatment, on SCC in milk.

DISCUSSION

The bacteriology results from the clinical cases of mastitis were typical of the bacteria associated with mastitis in the UK.

The increased responsiveness to pain was very marked and of a similar level in both mild and moderate cases of mastitis. The duration of this increased sensitivity was long, and extended to day four following detection of mastitis for mild cases, and day 20 for moderate cases. This implies that cows with mastitis may find painful stimuli that would normally be innocuous, with the potential that everyday activities may cause the cow more pain than usual.

Sensitivity to pain was reduced when mild mastitis cases were treated with a single intravenous injection of flunixin, in combination with intramammary antibiotic. This response was seen one day after the start of treatment, which coincides with the clinical activity of flunixin, after which the cows returned to a state of increased sensitivity to pain. This suggests that a single intravenous injection of flunixin altered pain mechanisms for a short time and indicates that repeated doses of this drug may potentially maintain this beneficial effect. These data also suggest that changes in pain perception are occurring away from the site of inflammation, the udder, because flunixin given locally showed no beneficial effect in mild mastitis cases, whereas the same drug, administered intravenously, successfully reduced sensitivity to pain. Mastitis cases, classified as moderately severe, did not show a similar beneficial effect of treatment with flunixin as the mild mastitis cases, and it is possible that either an increased dose of flunixin or alternative drugs are required to relieve pain in cows with moderate, or more severe, mastitis. The intramammary flunixin appeared to increase pain sensitivity a few weeks after treatment and it is possible that either the drug or the vehicle in which the drug was suspended induced a delayed inflammatory response in the udder.

The detection of haptoglobin in both mild and moderate mastitis cases indicated that an acute phase response was occurring and that this protein has the potential to be used for an early warning marker of mastitis. This suggests that especially milder cases of mastitis may have measurable inflammatory changes prior to recognition of milk clots or udder changes by the dairyman.

The SCC showed predictable responses to mild and moderate mastitis, with the SCC being greater and increased for a longer period in moderate cases than in mild cases. The SCC in mild mastitis cases had almost peaked on the day of detection, whereas the SCC in moderate cases continued to rise for another 24 hours. The pattern of SCC in mild and moderate mastitis was similar to that seen from the haptoglobin results, again suggesting that inflammatory changes precede clinical detection particularly in mild cases. The cell counts in normal cows, even when not subclinically infected, were higher than would be expected by normal sampling regimes. This is due to the milk sample being collected after the udder had been emptied at milking. At this time, the cells are concentrated in the secretion remaining in the glands. This means that the SCC in this study can not be directly compared to SCC taken routinely from cows or milking jars.

The treatments given in this study had no effect on either haptoglobin levels or on SCC indicating that a different therapeutic approach is required to increase speed of recovery of inflammation. It is possible that different types or doses of NSAID may be more effective in reducing udder inflammation, or it may be necessary to develop novel drugs to treat mastitis in the future.

CONCLUSIONS

- It is possible and practicable to measure the responsiveness of cows with clinical mastitis to pain
- Cows with mild and moderate mastitis have increased responsiveness to pain on the leg nearest the side with the mastitic quarter
- Increased responsiveness to pain, persists for many days after mild mastitis and some weeks following moderate mastitis
- A reduction in responsiveness to pain resulted from treatment of mild mastitis cases with intramammary antibiotic and intravenous flunixin one day after treatment. This indicates a beneficial effect on pain relief.
- The reduction in responsiveness to pain which resulted from treatment of mild mastitis cases with intramammary antibiotic and intravenous flunixin was shortlived and the increased responsiveness to pain returned to former levels and persisted. This indicates that repeated doses of intravenous flunixin may be required for prolonged pain relief.
- Treatment of mastitis cases with intramammary antibiotics and intramammary flunixin caused an adverse effect with increased responsiveness to pain being recorded

- Mild and moderate mastitis induced an increase in blood haptoglobin levels and were unaffected by treatment

- The somatic cell counts were raised in mild and moderate mastitis cases and were unaffected by treatment

- The haptoglobin levels and SCC in mild mastitis cases may have been elevated significantly prior to clinical detection

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REFERENCES

1. ESHRAGHI H R, I J ZEITLIN, J L FITZPATRICK, H TERNENT & D N LOGUE (1996) British Journal of Pharmacology 119**,** 202
2. HONKANEN-BUZALSKI T, T KATILA & M SANDHOLM (1981) Acta Veterinaria Scandinavia 22, 360-368
3. NOLAN A M, A LIVINGSTON, R MORRIS & A WATERMAN (1987) Journal of Pharmacological Methods 17, 39-49
4. WELSH E M & A M NOLAN (1995) Pain, 60, 159-166