Mastitis has well-recognized harmful effects on dairy farm profitability. Furthermore, mastitis impairs the milk component synthesizing ability of secretory tissues. Various therapies are available for the treatment of clinical mastitis. Meloxicam exhibits preferential binding to Cyclooxygenase-2 (COX-2) receptor and consequently generates fewer negative gastrointestinal side effects than nonspecific COX inhibitors such as flunixin meglumine and ketoprofen. Toward this end, research efforts directed at understanding the use of meloxicam alone and in combination with other antibiotics to improve milk quality and production. Therefore, in this review, we have highlighted the mechanism, biopharmaceutical challenges, and merits of meloxicam usage in dairy cattle mastitis. In addition, we also presented the integration of artificial neural network, in silico docking, and nanotechnology-driven topical drug delivery cargo as future opportunity for efficient delivery of meloxicam in the management of clinical mastitis.

Keywords: Artificial neural network, clinical evidence, mastitis, meloxicam, preclinical evidence, topical drug delivery systems

INTRODUCTION

Mastitis is a kind of inflammation of the mammary glands or udder in dairy animals such as cows, buffaloes, goats, sheep, and camels.[1-5] This happens due to invasion of milk producing tissues by pathogenic microorganisms.[6-7] It also impairs the ability of secretory tissues synthesizing milk components and in this way lowers milk yield.

Mastitis can be classified into different types: clinical, subclinical, peracute, acute, subacute, chronic, contagious, and environmental mastitis. However, clinical mastitis is primarily responsible for the extensive economic losses to the dairy industry.[8] Various therapies are available to treat clinical mastitis such as antibiotics,[9] bacteriophage therapy,[10] and nonsteroidal anti-inflammatory drugs (NSAIDs).[11] In mastitis therapy, the primary function of NSAIDs is to reduce inflammation and neutralize the endotoxin-induced effects. Diverse groups of NSAIDs were licensed for treating the pain and inflammation including diclofenac, meloxicam, etc.[11] Of all the drugs, meloxicam is widely used in clinical mastitis for selective binding to the COX-2 enzyme.

Meloxicam, an NSAID, is a member of oxicam class of drugs, showed promising effects in reducing udder pain sensitivity, edema, and body temperature without affecting the rumination time,[12,13] and has proved to be effective against mastitis in clinical trials and hence is a drug of choice in treating mastitis infections.

This review serves as one stop information on this aspect and also rephrases the preclinical and clinical evidence to unbox the relevance of meloxicam in mastitis. Moreover, the future prospect of in silico docking in the delivery of meloxicam for the management of clinical mastitis is also highlighted.

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How to cite this article: Jyothi VG, Babu CK, Kumar R, Singh PK, Khatri DK, Singh SB, et al. Meloxicam in combating clinical mastitis: Nanotechnology-driven hope and opportunities. J Pharm Bioall Sci 2022;14:121-5.
PHARMACOLOGICAL BENEFITS IN COMBATING MASTITIS

Mastitis caused by the Gram-positive and Gram-negative bacterial infections releases lipopolysaccharides which induce pyrexia. It also resulted in elevation of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). These pro-inflammatory mediators promote upregulation of cyclooxygenase (COX) expression [Figure 1],[14] and meloxicam mitigates mastitis symptoms by blocking COX-2 expression.

Meloxicam causes mitigation of pain and distress associated with mastitis[15] in lactating dairy cows.

REDUCES SOMATIC CELL COUNT

The normal level of somatic cells called somatic cell count (SCC) is approximately 1 × 10⁵ cells/ml from the milk of healthy mammary gland.[16] In general, higher SCC indicates lower milk quality [Figure 1]. Meloxicam injection in combination with antibiotics leads to lower SCC in dairy animals.[17]

DIMINISHES THE RISK OF CULLING

Culling is the process of separating cows from the main herd according to desired or undesired characteristics for slaughter.[18] One of the research findings suggested that administration of meloxicam with antimicrobials helps in reducing the risk of culling as compared to antimicrobial treatment alone.[17]

TRIM Downs Udder Edema

In mastitis, the gland often becomes blotted and painful.[19] European Medicines Agency investigated that the oral supportive therapy of meloxicam together with antibiotics diminishes the bacterial infection of udder edema.[20]

POSITIVE EFFECT ON FERTILITY

Experimental investigation by McDougall et al.[21] explained that in the cases of mastitis, PGF2α, which was released by the mammary glands, has the ability to shorten the lifespan of corpus luteum. This reduced corpus luteum level increases the likelihood of failure in recognition of pregnancy and conception which finally leads to reduced fertility. Meloxicam shows protective action against the infertility by combating the PGF2α levels.

MELOXICAM HELPS REDUCE PAIN AND INFLAMMATION: PRECLINICAL EVIDENCE

Preclinical investigations should follow Good Laboratory Practices in International Conference on Harmonization guidelines. Veterinary drugs intended for oral and parenteral administration are usually given on weight basis as per the following formula:

\[
\text{mg/kg} = \frac{\text{Animal weight (kg)} \times \text{dosage concentration of drug (mg/ml)}}{100}
\]

Meloxicam alone and in combination with other drugs has undergone preclinical testing. The data demonstrate its potential in the management of pain and inflammation as summarized in Table 1.

MASTITIS MAY BE SUCCESSFULLY TREATED WITH MELOXICAM: CLINICAL EVIDENCE

Clinical trials have been conducted to determine the impact of veterinary drugs on the animal subjects. The main
goal of clinical trials of a veterinary medicinal product is to determine a safe dose with effective-dosing schedule. Depending on the goal of the clinical trial, it may be categorized as either exploratory (pilot) or confirmatory (pivotal) trial [Figure 2] as per the European agency for the evaluation of medicinal products. In addition, the Committee for Medicinal Products for Veterinary Use (CVMP) or Veterinary International Conference on Harmonization guidelines provided the directions in selection of primary variables (used to estimate the sample size). Basically, two methods are used to calculate the sample size in animal studies. The most favored and reliable scientific method is power analysis. Another method employed for the calculation of sample size is G power software (performs power calculations for a wide range of experimental setups). Furthermore, resource equation is an alternative approach against power analysis to calculate the sample size in animal studies.

\[ DF = N - k = kn - k = k(n - 1) \]

Where, DF depicts degree of freedom; N refers to total number of subjects, k is number of groups, and n designates number of subjects per group.

In continuation, both randomization and blinding are the most imperative design methods employed to decrease prejudice in clinical trials. In randomized clinical trial (RCT), groups are allocated with subjects aimlessly so that they obtain dissimilar treatment(s) or no treatment. In addition to this, two major classes of RCT designs are parallel (between-group) and crossover (within-group) studies. Animals are haphazardly assigned to two groups, i.e., A and B in the parallel group design. One of the groups stands as control receiving the standard treatment or placebo, while the other group takes delivery of the experimental treatment. In crossover studies, veterinary patients are set as their own controls. Next, stratification could be carried out as a part of the randomization to stop possible inequity. In this, randomization of animals in treatment groups within strata is based on vital prognostic factors, e.g., breed, sex, age, and stage of disease. Furthermore, blinding is the second most imperative design technique, refers to the study design which averts the knowledge of few or all the individuals concerned with the test regarding the treatment assigned to the particular group of the animals. Several veterinary clinical trials in dairy cows were conducted to demonstrate the potential of meloxicam in the treatment of mastitis [Table 2].

**Table 1: Preclinical analysis of meloxicam in mice/rat models of pain and inflammation**

| Drug                        | Drug delivery systems | Remarks                          | Reference |
|-----------------------------|-----------------------|----------------------------------|-----------|
| Meloxicam                   | Solution              | Induce anti-nociceptive activity  | [22]      |
| Rutin and meloxicam combination | Dispersion            | Improve tissue inflammation       | [23]      |
| Meloxicam                   | Polymeric nanocapsule | Reduce inflammation and pain      | [24]      |
| Paracetamol and meloxicam   | Solution              | Effective in pain management      | [25]      |
| Meloxicam                   | Dermal gel            | Reduce inflammation and pain      | [26]      |
| Meloxicam                   | Self-emulsifying granules | Rapid onset of action              | [27]      |

**Table 2: Clinical analysis of meloxicam in mastitis of dairy cattle**

| Drug                        | Route of administration | Animal model | Remarks                                                | Reference |
|-----------------------------|-------------------------|--------------|--------------------------------------------------------|-----------|
| Antibiotic and meloxicam    | Systemic                | Cows         | Improved reproductive performance                      | [15]      |
| Antibiotic and meloxicam    | Oral                    | Cows         | Reduced pain in parturition                             | [30]      |
| Antibiotic and meloxicam    | Systemic                | Cows         | Improved fertility and reduce culling rate              | [12]      |
| Antibiotic and meloxicam    | Systemic                | Cows         | Improved milk quality and reduce bacterial count        | [31]      |
| Meloxicam                   | Subcutaneous            | Cows         | Reduced pain and udder edema                            | [21]      |
| Meloxicam and penethamate hydroiodide | Parenteral            | Cows         | Reduced somatic cell count and culling rate             | [18]      |

**Future Prospects for Meloxicam in the Treatment of Mastitis: Integrated Nanotechnology-Driven Opportunities**

In India, for veterinary purposes, meloxicam injection (5 mg) is given by intramuscular, intravenous
or subcutaneous route to dairy cattle.\textsuperscript{[13]} Furthermore, pharmacokinetic analysis in a parallel study design comprising healthy and lactating cows indicated that intravenous (0.2 mg/kg) or oral (1.0 mg/kg) administration of meloxicam exhibited a decreased systemic clearance in postpartum relative to mid-lactation cows, which resulted in a longer half-life and increased total exposure independent of mode of administration.

In order to surmount confronts associated with oral and parental preparations, colloidal and particulate nanocarriers may be implicated to deliver meloxicam at the target site.

Our group also formulated meloxicam dermal spray for topical administration.\textsuperscript{[32]} A film forming polymer was incorporated in the dermal spray which formed a polymeric film on the skin surface and ensured its prolonged course of action. Nanoencapsulation of drugs requires adequate expertise for bench to bedside delivery.\textsuperscript{[33]} Customization of nanoparticulate system requires optimization which is a tedious process. Various statistical tools are being used in the optimization and preparation of nanoparticles, now alternatively ANN is being explored in the field of nanotechnology.

ANN is a computer program which simulates the neural network of human brain by learning from different experiences by using distinct learning algorithms.\textsuperscript{[34]} A set of critical attributes can be optimized by the use of ANN\textsuperscript{[35]} where a set of experimental data is given in the learning phase and the validation is to be performed to analyze the effectiveness of the model.

Amasya et al. implemented ANN for obtaining the design space for hydrogel of lipid nanoparticles. Parameters in order to achieve the desired characteristics.\textsuperscript{[36]}

Modeling of biological membrane is achieved by various software packages where the interaction of excipients with the appropriate biological membrane can be simulated to select the suitable excipient. Kaushik \textit{et al.}, studied the effect of different penetration enhancers on the permeation in the presence of different vehicles.\textsuperscript{[37]} Thus, with the molecular modeling tool, simulation of biological membrane and meloxicam in presence of different excipients can be carried out to screen the excipients in \textit{in silico}.

Hence, ANN and \textit{in silico} docking advocate integration with nanotechnology techniques in development of topical drug delivery cargo of meloxicam in the treatment of mastitis for gaining superior clinical benefits.

\textbf{Conclusion}

Mastitis is a major contributor in economic losses in dairy industry in India. Mastitis in cattle remarkably influences milk quality and quantity. Meloxicam holds great potential in the treatment of mastitis. However, physicochemical and biopharmaceutical limitations pose major hurdle in delivery of therapeutic concentration at the site of application. Novel drug delivery approaches are a good option and offer several advantages as compared to traditional topical dermal dosage forms. Therefore, substantial opportunities are available to successively deliver meloxicam at the site of action in mastitis that will ultimately help to achieve set goals of milk production as well as surely offer incredible merits to farmers and dairy industry.

\textbf{Financial support and sponsorship}

Nil.

\textbf{Conflicts of interest}

There are no conflicts of interest.

\section*{References}

\begin{enumerate}
\item Donadue FX, Howes NL, Esteves CL, Howes MP, Byrne TJ, Macrae AI. Farmer and veterinary practices and opinions related to the diagnosis of mastitis and metabolic disease in UK dairy cows. Front Vet Sci 2020;7:127.
\item Tanamati F, Taylor JF, Behura SK, Santos DJ, Stafuzzza NB, Andrade WB, \textit{et al}. Short communication: Characterization of the milk protein expression profiles in dairy buffaloes with and without subclinical mastitis. J Dairy Sci 2020;103:2677-84.
\item Polveiro RC, Vidigal PM, Mendes TA, Yamatogi RS, Lima MC, Moreira MA. Effects of enrofloxacin treatment on the bacterial microbiota of milk from goats with persistent mastitis. Sci Rep 2020;10:4421.
\item Vasileiou NG, Cripps PJ, Ioannidi KS, Katsafadou AI, Chatzopoulos DC, Barbagianni MS, \textit{et al}. Experimental study for evaluation of the efficacy of a biofilm-embedded bacteria-based vaccine against \textit{Staphylococcus chromogenes}-associated mastitis in sheep. Vet Microbiol 2019;239:108480.
\item Hayajneh FM. The effect of subclinical mastitis on the concentration of immunoglobulins A, G, and M, total antioxidant capacity, zinc, iron, total proteins, and calcium in she-camel blood in relation with pathogens present in the udder. Trop Anim Health Prod 2018;50:1373-7.
\item Derakhshani H, Fehr KB, Sepahri S, Francoz D, De Buck J, Barkema HW, \textit{et al}. Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. J Dairy Sci 2018;101:10605-25.
\item Keane OM. Symposium review: Intramammary infections-major pathogens and strain-associated complexity. J Dairy Sci 2019;102:4713-26.
\item Rollin E, Dhuyvetter KC, Overton MW. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev Vet Med 2015;122:257-64.
\item El-Sayed A, Kamel M. Bovine mastitis prevention and control in the post-antibiotic era. Tropical animal health and production 2021;53:1-6.
\item Titze I, Krömker V. Antimicrobial activity of a phage mixture and a lactic acid bacterium against \textit{Staphylococcus aureus} from bovine mastitis. Vet Sci 2020;7:E31.
\item Breen J. The importance of non-steroidal anti-inflammatory drugs (NSAIDs) in mastitis therapeutics. Livestock 2017;22:182-5.
\end{enumerate}
12. van Soest FJ, Abbeloos E, McDougall S, Hogeveen H. Addition of meloxicam to the treatment of bovine clinical mastitis results in a net economic benefit to the dairy farmer. J Dairy Sci 2018;101:3387-97.
13. Coetzee JF, KuKanich B, Mosher R, Allen PS. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet Ther 2009;10:E1-8.
14. Furst DE. Meloxicam: Selective COX-2 inhibition in clinical practice. Semin Arthritis Rheum 1997;26:21-7.
15. McDougall S, Abbeloos E, Piepers S, Rao A, Astiz S, Werven TV, et al. Addition of Metacam®(Meloxicam) to the treatment of clinical mastitis improves subsequent reproductive performance. In: British Mastitis Conference 2015. Worcester, UK: The Dairy Group; 2015. p. 61-3.
16. Allhusien MN, Dang AK. Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Vet World 2018;11:562-77.
17. McDougall S, Bryan MA, Tiddy RM. Effect of treatment with the nonsteroidal anti-inflammatory meloxicam on milk production, somatic cell count, probability of retreatment, and culling of dairy cows with mild clinical mastitis. J Dairy Sci 2009;92:4421-31.
18. Edwards-Callaway LN, Walker J, Tucker CB. Culling decisions and dairy cattle welfare during transport to slaughter in the United States. Front Vet Sci 2018;5:343.
19. Jones T. Treatment of clinical mastitis in dairy cattle. Vet Rec 2016;178:614.
20. EMA/CVMP/259397/2006, “Metacam (meloxicam),” 2006. [Online]. Available: https://www.ema.europa.eu/en/documents/overview/metacam-epar-summary-public_en.pdf. [Last accessed on 2021 Dec 19].
21. McDougall S, Abbeloos E, Piepers S, Rao AS, Astiz S, van Werven TV, et al. Addition of meloxicam to the treatment of clinical mastitis improves subsequent reproductive performance. J Dairy Sci 2016;99:2026-42.
22. Miranda HF, Noriega V, Sierraalta F, Poblete P, Aranda N, Prieto JC. Nonsteroidal anti-inflammatory drugs in tonic, phasic and inflammatory mouse models. Drug Res (Stuttg) 2019;69:572-8.
23. Fikry EM, Hasan WA, Mohamed EG. Rutin and meloxicam attenuate paw inflammation in mice: Affecting sorbitol dehydrogenase activity. J Biochem Mol Toxicol 2018;32:1-7.
24. Villalba BT, Ianiski FR, Wilhelm EA, Fernandes RS, Alves MP, Luchese C. Meloxicam-loaded nanocapsules have antinoceptive and antiedematogenic effects in acute models of nociception. Life Sci 2014;115:36-43.
25. Miranda HF, Puig MM, Prieto JC, Pinardi G. Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain. Pain 2006;121:22-8.
26. Gupta SK, Bansal P, Bhardwaj RK, Jaiswal J, Velpandian T. Comparison of analgesic and anti-inflammatory activity of meloxicam gel with diclofenac and piroxicam gels in animal models: Pharmacokinetic parameters after topical application. Skin Pharmacol Appl Skin Physiol 2002;15:105-11.
27. Parekh VJ, Desai ND, Shaikh MS, Shinde UA. Self nanoemulsifying granules (SNEGs) of meloxicam: Preparation, characterization, molecular modeling and evaluation of in vivo anti-inflammatory activity. Drug Dev Ind Pharm 2017;43:600-10.
28. The European Agency for the Evaluation of Medicinal Products. Veterinary Medicines and Inspections. London, UK: European Medicines Agency; 2001.
29. Pallmann P, Bedding AW, Choodari-Oskooei B, Dimairo M, Flight L, Hampson LV, et al. Adaptive designs in clinical trials: Why use them, and how to run and report them. BMC Med 2018;16:29.
30. Shock DA, Renaud DL, Roche SM, Poliquin R, Olson ME. Evaluating the impact of meloxicam oral suspension administration at parturition on subsequent production, health, and culling in dairy cows: A randomized clinical field trial. PLoS One 2018;13:e0210326.
31. Das G, Lalnunpuia C, Sarma K, Behera SK, Dutta TK, Bandypadhyay S. Prevalence of Staphylococcus aureus associated sub-clinical mastitis in crossbred cows in Mizoram. Rumin Sci 2015;4:167-70.
32. Jyothi VG, Pawar J, Madan J, Kumar R, Khatri D, Singh SB. Film forming topical dermal spray of meloxicam for the management of pain and inflammation. Indian Patent 2020:20204104763.
33. Mandal S, Kang G, Prathipati PK, Zhou Y, Fan W, Li Q, et al. Nanoencapsulation introduces long-acting phenomenon to tenofovir alafenamide and emtricitabine drug combination: A comparative pre-exposure prophylaxis efficacy study against HIV-1 vaginal transmission. J Control Release 2019;294:216-25.
34. Algahtani MS, Ahmad MZ, Nourine IH, Ahmad J. Co-delivery of imiquimod and curcumin by nanoemugel for improved topical delivery and reduced psoriasis-like skin lesions. Biomolecules 2020;10:E968.
35. Elkomy MH, Elmenshawe SF, Eid HM, Ali AM. Topical ketoprofen nanogel: Artificial neural network optimization, clustered bootstrap validation, and in vivo activity evaluation based on longitudinal dose response modeling. Drug Deliv 2016;23:329-306.
36. Amasya G, Aksu B, Badilli U, Onay-Besikci A, Tarimci N. QbD guided early pharmaceutical development study: Production of lipid nanoparticles by high pressure homogenization for skin cancer treatment. Int J Pharm 2019;563:110-21.
37. Kaushik D, Costache A, Michniak-Kohn B. Percutaneous penetration modifiers and formulation effects. Int J Pharm 2010;386:42-51.