REVIEW ARTICLE

Milk-Derived Exosomes as a Potential Therapy for Necrotizing Enterocolitis: A Scoping Review

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ABSTRACT

Background: Necrotizing enterocolitis (NEC), a devastating gut disease primarily affecting premature infants, currently lacks effective treatment options. Human milk, known for its protective benefits against NEC, contains compositions that hold promises for addressing this urgent medical need. Thus, this study aimed to provide an overview of the therapeutic potential of milk-derived exosomes, offering valuable insights that could pave the way for future clinical interventions and advancements in NEC treatment strategies. **Methods:** Keywords such as "human milk", "exosomes", "human milk exosomes", "necrotizing enterocolitis", "therapy", and "treatment" were employed during the search strategy in Scopus, PubMed, and ScienceDirect.The articles were chosen in accordance with PRISMA-ScR, where a total of 125 articles were further screened based on the inclusion and exclusion criteria, resulting in five selected articles reviewed in this study.**Results:** Milk-derived exosomes have the ability to enhance epithelial integrity by increasing the expression of tight-junctions namely ZO-1, claudin-1, and occludin. These vesicles give protection to intestinal epithelial cell by improving the expression of goblet cells and increase mucin production and also able to reduce inflammation and stimulate cell growth and regeneration by increasing the number of ileum crypts and Lgr5 expression. In addition, milk-derived exosomes protect against cell damage by enhancing the expression of genes in the Wnt/β-catenin signalling pathway which are Axin2, c-Myc, and Cyclin D1. **Conclusion**: These findings conclude that milk-derived exosomes are beneficial to protect the intestinal epithelial cells. Various pathways can be explored and targeted in relations to creating new drugs that can effectively control the occurrence and development of NEC, including improving the prognosis of infants with NEC.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a major cause of infant mortality due to a severe gastrointestinal disorder (Martin et al., 2018). According to Boo (2016), the incidence of NEC has primarily risen in neonates with low birth weight and gestational age, despite improvements in neonatal intensive care units (NICU). For example, in Sweden, the incidence of NEC increased by about 3.1 per 10,000 live births over an eight-year period. Alsaied et al. (2020) found that seven out of every 100 very low birth weight infants admitted to NICUs are likely to develop NEC. This disease involves inflammation and tissue necrosis in the intestinal lining, which can lead to perforations or breaches in the intestinal wall. As a result, intestinal contents, including bacteria and toxins, can leak into the abdominal cavity, leading to systemic infection and further complications. For infants in critical condition who do not respond to medical therapy, surgery such as laparotomy is the standard treatment for NEC (Ginglen & Butki, 2023). Rellinger, as cited by Robinson et al. (2017), emphasized that the main goals of surgery are to control the leakage from the intestine and to remove necrotic tissues while preserving as much healthy tissue as possible, though it does not directly treat NEC itself. However, surgery carries a high risk of mortality and can lead to neurodevelopmental impairments in infants. As a result, researchers are exploring alternative therapies for this condition that do not require surgery.

Interestingly, human milk has been found to have significant benefits for infants due to its multifunctional components, such as human milk oligosaccharides (HMOs), human milk stem cells, lactoferrin, exosomes, and others. Carr et al. (2021) highlighted that human milk is a unique fluid containing various bioactive compounds essential for infant growth, development, and protection against infections. Numerous studies have shown that infants who are breastfed have higher survival rates than those who are not. Nolan et al. (2019) noted that many components of human milk provide protective effects against NEC by enhancing the intestinal immune response and offering antimicrobial properties. While research into other components like HMOs and human milk stem cells is extensive, studies on human milk exosomes are emerging, though their role and potential in NEC treatment remain less understood. As a result, there is much yet to be explored regarding the therapeutic potential of human milk exosomes. Researchers are currently focusing on these milk-derived exosomes, which are nanosized vesicles produced by cells in the body.

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Studies have demonstrated that human milk exosomes offer various benefits to intestinal epithelial cells (IEC), including promoting growth, reducing inflammation, and defending against harmful pathogens. Experiments on animal models of NEC have shown that exosomes can decrease both the incidence and severity of the disease. Based on these findings, it can be suggested that milk-derived exosomes play a significant role in treating NEC due to their protective effects on the intestinal epithelium (Dong et al., 2020). Therefore, this article aims to evaluate the existing research on milk-derived exosomes, focusing on their role in promoting the growth and regeneration of the intestinal barrier and their mechanisms in protecting the infant's intestinal health.

METHODOLOGY

A scoping review is an ideal research approach for examining the therapeutic potential of breast milk exosomes in treating Necrotizing Enterocolitis (NEC), as it allows for a thorough exploration of the issue and highlights gaps in current research. According to Arksey & O'Malley (2005) and Levac et al. (2010), scoping reviews are highly valuable for capturing a wide range of literature, integrating various study designs, methodologies, and sources, which enables researchers to systematically map existing evidence. This method will provide important insights into the mechanisms, limitations, and potential uses of breast milk exosomes for NEC treatment, offering a solid foundation for guiding future research and clinical practices in neonatal health. The review follows the five-stage framework outlined by Arksey & O'Malley (2005) and incorporates the checklist from the PRISMA extension for scoping reviews (Tricco et al., 2018).

Stage 1: Identify the Research Questions

The research questions were used to guide the search strategy include: 1) Can milk-derived exosomes promote growth and regeneration of the intestinal barrier of infants diagnosed with NEC? 2) How does exosome affect the integrity of tight junctions in the intestinal epithelium? The studies were then further evaluated by referring to the guided research questions and eligibility criteria.

Stage 2: Identifying Relevant Studies

Comprehensive literature research was conducted using reliable electronic databases including PubMed, Scopus, and ScienceDirect. To collect the most up-to-date information, the search was limited to articles published within the past 10 years. The search strategy for literature research focused on certain keywords, including "exosomes", "human milk exosomes", "necrotizing enterocolitis", "therapy", "treatment", and any other relevant keywords to assist the research process. The keywords were combined with the terms "AND", "OR", and "NOT" for a better research strategy. The search string was "milk-derived exosome" AND "necrotizing enterocolitis". Then, the search results were screened based on the inclusion and exclusion criteria that have been chosen as shown in Figure 1.

Three databases, namely Scopus, ScienceDirect, and PubMed, were accessed, resulting in a total of 125 articles. Among them, 92 papers were retrieved from Scopus, 17 from ScienceDirect, and 16 from PubMed (Figure 1). Then, the articles were screened, resulting in a total of 12 articles remaining after excluding 113 articles. The excluded articles were chosen based on the inclusion and exclusion criteria where the review articles, book chapters, editorial, and conference paper were excluded. There were also six articles written in other languages and 56 articles were unrelated to research questions. The 12 included articles go through a thorough review for eligibility, ensuring their reliability and validity. Among them, five articles were selected for this scoping review, while the others were excluded. Finally, data extraction was performed on the five included articles, as they met the specified inclusion and exclusion criteria as outlined in Stage 3.

Stage 3: Study Selection

The following studies were included if they meet the following criteria: (1) all papers published within the last 10 years (2) all the papers published were written in English (3) full-text article. Studies were excluded if they were unpublished studies, hand-searched articles, grey articles, review articles, book chapters, conference abstracts and letters.

Stage 4: Charting the Data

The author conducted a thorough search, screening the articles for both quality and relevance based on the established criteria and research question. Once screened, each paper was analysed and details such as author, year of publication, method of study, model involved in the studies, and outcomes were recorded.

Stage 5: Collating, Summarising and Reporting Results

Data from the finalized full articles were extensively evaluated and organized into tables, which include author, year of publication, method of study, model involved in the studies, and outcomes. The data extraction step is an important step in conducting this study. It aims to achieve the objectives and research questions of this scoping

review. The extracted table provided a descriptive summary of the results, aiding in the overall understanding of the study. Both the author and supervisor carefully examined the paper and reached a consensus on the accuracy and reliability of the findings.

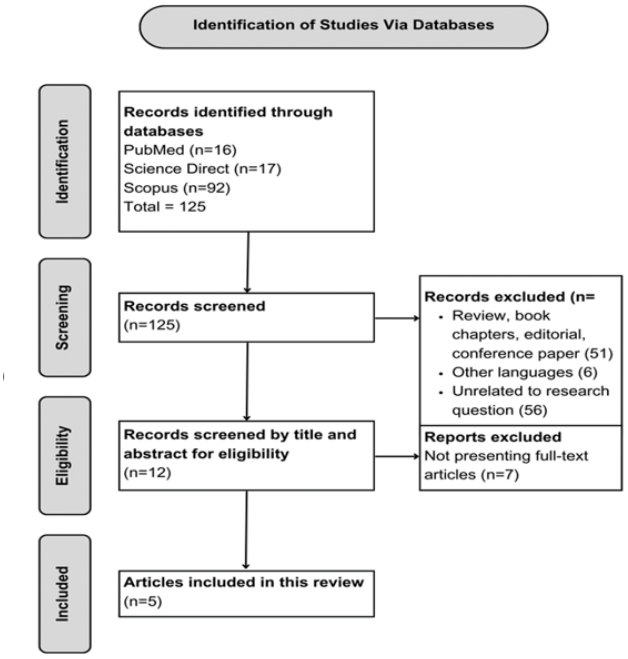

Figure 1: PRISMA ScR flow diagram

RESULTS

Study Characteristics

The selected studies focused on experimental research This review includes various research from different journals; where He et al. (2021) from Springer Nature, Li et al. (2019) from PLOS ONE, Hu et al. (2022) from Springer, and both Dong et al. (2020) and Martin et al. (2018) from SAGE Publication. Four studies using human breast milk and only Li et al. (2019) used bovine breast milk. Despite the different sources, all the studies approved that milk-derived exosomes are able in treating NEC.

Ability of Exosomes in Promoting Growth and Regeneration of the Intestinal Barrier

All five research articles confirmed the ability of exosomes in promoting growth and regeneration of the intestinal barrier (Table 1). He et al. (2021) stated that the strong integrity of the intestinal epithelium was because HBM exosome were able to increase the epithelial tight-junction proteins both in mRNA expression and protein content. According to Li et al. (2019), exosomes could promote intestinal epithelial cell viability, enhance proliferation, and stimulate intestinal stem cell activity under healthy condition. The therapeutic effect of HBM exosome on experimental NEC have been verified by Hu et al. (2022) due to its ability in rescuing intestinal injury, restoring epithelial regeneration, and inhibiting intestinal inflammation. Dong et al. (2020) stated that HBM exosome was a potential therapy to decrease cell toxicity directly in intestinal stem cells (ISCs). Lastly, Martin et al. (2018) able to demonstrate that human breast milk-derived exosomes reduce oxidative stress-related injury on intestinal epithelial cells (IECs).

Table 1: Data extraction of the ability of exosomes in promoting growth and regeneration of the intestinal barrier

Author's Name and country	Year of Publication	The ability of exosomes in promoting growth and regeneration of the intestinal barrier
He et al. China	2021	This study showed that the presence of milk-derived exosomes is found to be beneficial maintaining an intact and healthy intestinal lining since it can strengthen the intestinal epithelium's integrity by increasing the levels of epithelial tight junction proteins ($p<0.05$).
Li et al. Canada	2019	According to this study, milk-derived exosomes give protection to the intestinal epithelial cells (IECs) by promoting the cell viability ($p<0.01$), enhancing cell proliferation ($p<0.05$), and stimulating intestinal stem cells activity ($p<001$).
Hu et al. China	2022	This study confirmed that milk-derived exosomes have the therapeutic effect due to its ability in promoting cell growth and regeneration while minimizing intestinal inflammation ($p<0.05$).
Dong et al. China	2020	This study demonstrated that milk-derived exosomes can protect intestinal stem cells (ISCs) against cellular harm and cell toxicity.
Martin et al. USA	2018	According to this study, milk-derived exosomes could minimize oxidative stress and reduce injury in intestinal epithelial cells caused by oxidative stress.

Mechanism of Action of Milk-Derived Exosomes in Protecting the Infant's Intestinal Barrier

It has been disclosed that all the research articles concentrated on various components, indicating that multiple factors are involved in the regeneration of the intestinal barrier. He et al. (2021) investigated the impact of exosomes on intestinal epithelial tight-junction proteins, such as ZO-1, claudin-1, and occludin, while Li et al. (2019) focused on how exosome administration affects mucin production, goblet cell expression, MPO expression, and GRP94 expression. Hu et al. (2022) provided evidence by examining the number of ileum crypts and Lgr5 expression, and they identified several biological processes that are regulated during exosome administration. Dong et al. (2020) found that HBM exosome administration significantly improved the viability of ISC exposed to H2O2. Martin et al. (2018), who concentrated on the damage caused by oxidative stress, discovered that milk exosomes deliver microRNA-125b, which targets and inactivates the apoptosis-inducer p53.

DISCUSSION

The key findings of this scoping review pertain to the ability of exosomes in promoting growth and regeneration of the intestinal barrier and the effect of exosomes administration to the components in the intestinal barrier. Necrotizing enterocolitis has been controlled for a long time despite the fact that numerous therapies, including prebiotics and surgical procedures, have been employed. However, the surgery itself has a high risk of death and neurodevelopmental impairment in infants with very low birth weight (Robinson et al., (2017). According to Carr et al. (2021), breast milk is a unique fluid consists of many bioactive compounds that are necessary for an infant's growth development and protection against infections. It is amazing that many studies found infants with breastfeeding have a higher survival rate than infants without breastfeeding. Exosomes are known as an integral component of the complex composition of breast milk, as this fluid naturally contains a high concentration of exosomes. These substances have many functions, including intercellular communication, immune system development, gastrointestinal health, and therapeutic potential (Carr et al., 2021).

Ability of Exosomes in Promoting Growth and Regeneration of the Intestinal Barrier

Chen et al. (2020) stated that exosomes protect mucus barrier, improve IECs proliferation and intestinal stem cells viability, reduce oxidative stress and inflammation of IECs, and increase neutrophil recruitment hence are ideal therapeutic agents in NEC. Hence, based on the results of this scoping review, the five final articles provide a strong evidence to support that statement and confirmed that exosome are able to; enhance epithelial integrity (He et al., 2021), give protection to intestinal epithelial cells (Li et al., 2019), reduce inflammation and stimulate cell regeneration (Hu et al., 2022), protect from cell damage (Dong et al., 2020), and reduce oxidative stress (Martin et al., 2018). He et al. (2021) suggested that exosomes can improve the integrity of the intestinal epithelium due to their ability to increase the expression and content of proteins responsible for tight junctions. The RT-qPCR result of the study showed a decrease of tight-junction protein expression in group that have been stimulated with lipopolysaccharide (LPS) and group that has pre-treated with exosome-free HBM while stimulated with LPS. On the other hand, the protein increased in a group that have been pre-treated with exosomes while stimulated with lipopolysaccharide. This result showed that the presence of exosome can increase the expression of tight-junction protein, hence, improving the integrity of the intestinal epithelium.

Interestingly, Li et al. (2019) provided evidence that exosomes promote intestinal epithelial cell viability and enhance proliferation. Result of the study showed that the number of goblet cells per villus were significantly reduced during NEC compared to control, and the number increased after administration of milk-derived exosomes in the NEC group. Goblet cells secrete mucins, forming a vital mucus layer that shields the gastrointestinal tract surface from injury (Johansson et al., 2013, as stated in Li et al., 2019). Thus, exosomes increased the expression of goblet cells that are able to produce mucins that can protect intestinal epithelial cells (IECs). Hu et al. (2022) pointed out the therapeutic potential of HBM-exosomes in treating NEC by stating that exosomes can promote intestinal healing by rescuing injury, restoring epithelial regeneration, and inhibiting inflammation.

Dong et al. (2020) highlighted the potential of HBM exosomes to directly decrease cell toxicity in intestinal stem cells (ISCs) which gave protection from cell damage. This protective effect is crucial for maintaining the regenerative potential of the intestinal barrier. During in-vitro experiment, they observed a significant increase in the relative gene expression of Lgr5 after the administration of exosome. Studies have shown that the Lgr5+ is a highly active ISCs necessary for intestinal epithelium renewal by continuously supply new intestinal epithelial cells (Barker et al., 2007, as stated in Hou et al., 2020). Lastly, Martin et al. (2018) contributed to the understanding of exosomes' multifaceted role by demonstrating their ability to lessen oxidative stress-

induced damage in intestinal epithelial cells (IECs). According to Li et al. (2016) as stated in Martin et al. (2018), oxidative stress on the intestinal epithelial compartment is one potential mechanism leading to NEC in neonates. Cell treated with 1 and 10 μg of exosomes in the presence of H_2O_2 had a complete recovery, while 50% cell death was observed in cell without exosomes. Thus, it is confirmed that the presence of exosome is able to lessen the oxidative stress-induced damage in IECs.

Mechanism of Action of Milk-Derived Exosomes in Protecting the Infant's Intestinal Barrier

This scoping review able to highlight the multifaceted nature of exosome-mediated intestinal barrier by collecting research articles that focusing on different components of intestinal barrier. Firstly, He et al. (2021) demonstrated that HBM-exosomes can sustain the intestinal epithelial tight-junction proteins, specifically zonula occludens-1 (ZO-1), claudin-1, and occludin, both in-vitro and in-vivo. Volksdorf et al. (2017) stated that these proteins are present early in newly formed regenerating epidermis of normal wound healing.

These proteins are a critical structure that resist invasion of pathogens in the epithelial barrier (He et al., 2021). Results showed that lipopolysaccharide (LPS) can insult the protein. However, the presence of exosomes was able to protect it against LPS. Western blot also showed the decrease of these proteins in a group of pups that have been introduced to NEC and the levels of proteins was higher when there was a presence of exosomes. Therefore, it proved that exosomes could enhance tight-junction function which can create a strong seal between epithelial cells and consequently maintain the barrier integrity. He et al. (2021) also pointed out that levels of the pro-inflammatory cytokines IL-6β and TNF-α were significantly reduced when there was an administration of exosome. According to Hui et al. (2017), these two cytokines were correlated with intestinal inflammation in NEC patients. So, when there is a high level of these cytokines, it means that there might be the occurrence of inflammation.

Li et al. (2019) explored the influence of exosomes on mucin production, goblet cell expression, MPO (myeloperoxidase) levels, and GRP94 (heat shock protein 90) expression. The study showed a significant reduction in MPO expression after the introduction of exosome, which means that exosomes are able to reduce intestinal mucosal inflammation. The number of goblet cells also increased in the NEC group after the administration of milk-derived exosomes. High number of goblet cells conveyed that a thick layer of mucus layer due to the high mucins production. According to Dharmani et al. (2011) as stated in Li et al. (2019), it has been reported that in the inflamed intestine, depletion of mucin production from goblet cells occurs prior to epithelial cell damage, inflammation and elevation of MPO. The abundant protein in endoplasmic reticulum, GRP94 is crucial for goblet cell function and gut barrier integrity (Liu et al., 2013). Therefore, it can be marker for inflammation and this study observed that GRP94 was reduced in NEC and back to normal after the administration of exosomes.

Hu et al. (2022) focused on the number of ileum crypts and Lgr5 expression (a marker for intestinal stem cells). They observed an increase in both, indicating that exosomes may stimulate stem cell activity and proliferation. The increase in the number of ileum crypts suggests that exosomes may promote the formation of new crypts, potentially expanding the niche for ISCs and enhancing the overall regenerative capacity of the gut. The increase of Lgr5 expression indicated that exosomes may be stimulating the activity of existing ISCs, prompting them to divide and differentiate into new intestinal epithelial cells at a higher rate. Dong et al. (2020) investigated the effects of human milk-derived exosomes in ISCs that have been damaged by oxidative stress by using H_2O_2 exposure. Their results suggest that exosomes can significantly increase ISC viability, protecting them from damage caused by free radicals which were possibly mediated via the Wnt/β-catenin signaling pathway. According to Dong et al. (2020), Wnt/β-catenin signaling pathway is the signature pathway, and the genes that are regulated downstream of this route may serve as possible markers of ISCs. Pai et al. (2017) stated that the regeneration of stem cells, differentiation, migration, genetic stability, apoptosis, and proliferation are all regulated by the highly conserved Wnt/β-catenin signaling mechanism. They also found that human milk-derived exosomes were able to enhanced ISCs proliferation as there was a significant increase in the expression of Lgr5 after the administration of exosomes and the small intestinal epithelium of the mouse completely renews every three to five days. Therefore, it has been proved that human milk-derived exosomes have a potential to protect ISCs from oxidative stress.

Martin et al. (2018) explored the role of breast milk-derived exosomes in mitigating oxidative stress damage by inducing H_2O_2 to the cells and confirmed that breast **milk-**derived exosomes are protective against cell toxicity. According to Simon et al. (2009) as stated in Martin et al. (2018), oxidative stress is one of the factors causing cell apoptosis due to the increase of p53 expression. Hence, they discussed about the ability of exosomes in delivering microRNA-125b, which is a molecule that can target and inactivate the apoptosis inducer p53. Regulating the

expression of p53 may help in preventing excessive cell death within the intestinal epithelium. Thus, it is true that the administration of milk-derived exosomes can prevent the occurrence of apoptosis and consequently promoting the health of intestinal barrier.

CONCLUSION

Current treatments for Necrotizing Enterocolitis (NEC), such as surgery, carry significant risks, including death and neurodevelopmental impairment. This review highlights exosomes as a safer, more targeted alternative. Exosomes support intestinal barrier health by enhancing epithelial integrity through tight-junction proteins like ZO-1, claudin-1, and occludin. Milk-derived exosomes promote cell growth and regeneration by increasing goblet cell expression, leading to higher mucin production and improved epithelial cell viability and proliferation. They also have anti-inflammatory effects, potentially reducing the inflammation characteristic of NEC. Additionally, exosomes protect against oxidative stress-induced cell damage. However, further research is needed to determine the optimal dosage, delivery method, and long-term safety of exosome therapy. Issues such as efficient delivery to target cells and the unknown long-term effects of exosome therapy require continued investigation, with nanotechnology potentially aiding in exosome delivery.

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