REVIEW ARTICLE

Antiviral activity of lactoferrin: a focus on hepatitis C chronic

Atividade antiviral da lactoferrina: um foco na hepatite C crônica

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ABSTRACT

Introduction: The Hepatitis C virus (HCV) is one of the main causes of chronic liver disease in the world, and despite having a treatment with sustained virologic response rate, this therapy is not resolutive for all patients. **Objectives:** To carry out a literature review on the action of bovine lactoferrin (bLf) on chronic hepatitis C to find evidence of its antiviral effect for a possible treatment associated with conventional therapy. **Methodology:** 49 articles were found according to the descriptors "lactoferrin" "immunotherapy" "chronic hepatitis C" in the pre-selected databases and seven were chosen according to the inclusion and exclusion criteria for writing the review in question. **Results:** The benefit of bLF use was related to the reduction of serum HCV RNA levels (1106 kIU/ml to 612 kIU/ml), plasma 8-isoprostane (8.6 ± 3.7 to 6.9 ± 2.1 pg/ml) and alanine aminotransferase (ALT) values (85 ± 80 to 63 ± 55 IU/l). **Conclusion:** The studies showed a possible antiviral effect of bovine lactoferrin that should be explored, through further studies, for adjuvant use in patients with chronic hepatitis C who do not show complete efficacy with the pre-established treatment for the disease.

Keywords: Lactoferrin. Chronic hepatitis C. Antivirals. Immunotherapy.

RESUMO

Introdução: O vírus da Hepatite C (HCV) é uma das principais causas de doença hepática crônica no mundo, e apesar de possuir um tratamento com taxa de resposta virológica sustentada, esta terapia não é resolutiva para todos os doentes. **Objetivos:** Realizar uma revisão da literatura sobre a ação da lactoferrina bovina (bLf) sobre a hepatite C crônica a fim de encontrar evidências sobre seu efeito antiviral para um possível tratamento associado à terapia convencional. **Metodologia:** Foram encontrados 49 artigos de acordo com os descritores "lactoferrina" "imunoterapia" "hepatite C crônica" nas bases de dados pré-selecionadas e sete foram escolhidos de acordo com os critérios de inclusão e exclusão para a escrita da revisão em questão. **Resultados:** Foi relacionado o benefício do uso da bLF na redução dos níveis séricos de RNA do HCV (1106 kIU/ml para 612 kIU/ml), plasma 8-isoprostano (8,6 ± 3,7 para 6,9 ± 2,1 pg/ml) e nos valores de alanine aminotransferase (ALT) (85 ± 80 para 63 ± 55 UI/l). **Conclusão:** Os estudos mostraram um possível efeito antiviral da lactoferrina bovina que deve ser explorado, através de mais estudos, para uso adjuvante em pacientes com hepatite C crônica que não apresentam eficácia completa com o tratamento pré-estabelecido para doença.

Palavras-chave: Lactoferrina. Hepatite C crônica. Antivirais. Imunoterapia.

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INTRODUCTION

The hepatitis C virus (HCV) is a pathogen belonging to the hepacivirus genus capable of causing a liver disease with a worldwide prevalence of around 3%, in which 85% of cases become chronic.[1] Competing with alcoholic liver disease as the leading cause of chronic liver disease, hepatitis C has a great potential for developing into liver cirrhosis and hepatocellular carcinoma (HCC) and is the most frequently diagnosed disease in cases of liver transplantation, thus constituting a serious public health problem.[2] Because of this, appropriate treatment should be recommended as soon as the diagnosis is established.

The treatment for hepatitis C was recommended in 2015 by the Ministry of Health through the Clinical Protocol and Therapeutic Guidelines (PCDT) with the recommendation of the association of new direct-acting viral agents (DAA) that increased the sustained virologic response (SVR). The scheme encompasses antiviral drugs such as Sofosbuvir, Daclatasvir, Ledipasvir, Elbasvir, Glecaprevir, and Velpatasvir with an efficacy of 90% in SVR rates without the use of interferon, thus reducing the adverse events of medication.[3] Thus, the treatment for this disease has been facilitated and its duration decreased, but even though this disease has an established treatment, the emergence of resistant viral isolates may compromise the efficacy and therapeutic success of the new direct-acting drugs (DAAs). The quasispecies nature of HCV, the high rate of viral replication, and the prolonged period of infection mean that new variants are constantly being generated in infected individuals, which may even cause resistance in the more susceptible variants.[4]

With this, the search for new compounds with antiviral properties that can be used in conjunction with already available treatments becomes crucial to improve the effectiveness of treatment, especially for patients who do not respond adequately to existing drugs or who have resistant viral strains.

In this context, studies suggest that the administration of lactoferrin can decrease the serum level of RNA of the HCV virus in patients with chronic hepatitis C.[3] Lactoferrin (Lf), belonging to the family of iron-binding glycoproteins, is found in breast milk and other exocrine secretions, playing a significant role in the human innate defense mechanism against pathogenic microorganisms, including viruses.[5] The high concentrations of serum proteins in colostrum, such as immunoglobulins IgA and IgG, transfer passive immunity to the organism, having as one of its functions the modulation of immunological reactions.[6] Furthermore, lactoferrin also has immunological properties both in the innate and the acquired system with anti-inflammatory activities, inhibiting several pro-inflammatory cytokines such as TNF-α and IL-1β.[7]

Several experimental studies demonstrate that bovine lactoferrin (bLf) has antiviral effects against herpes simplex virus, cytomegalovirus, human immunodeficiency virus, and rotavirus, and even induces apoptosis by H3N2 virus in cultured cells and can be considered a therapeutic option for the disease caused by HCV, given the similarity of their structures.[8,9] This antiviral functionality of bLf is due to the interaction with the virus at specific receptors such as glycosaminoglycans, generating a decrease in oxidative stress and virus RNA levels.[10]

Epidemiological and experimental studies suggest that milk proteins such as Lf also have anticancer activities by promoting apoptosis of tumor cells and activating the p53 gene, which is a tumor suppressor with an important role in cell cycle control.[10,11] Furthermore, other functionalities of lactoferrin such as antioxidant capacity and lipid peroxidation can also influence the antiviral property, since Lf, when binding to iron, creates both stable forms and forms for eliminating free iron that could stimulate oxidative reactions and consequently cell damage, thus preventing lipid peroxidation.[12]

However, even though this review had a focus on hepatitis C and direct-acting drugs (DAA) have proven high efficacy, the intent of this article became a means of adding information that can serve as a basis for studies involving the treatment of other viral diseases from the use of lactoferrin, producing beneficial outcomes for individuals, through a systematic review that compares studies with different doses and their anti-HCV outcomes which is a viral disease.

The objective of this work was to perform a systematic search for articles evidencing the antiviral action of bovine lactoferrin (bLf) in chronic hepatitis C. Although direct-acting drugs (DAA) have proven high efficacy, the intention of this article became a means to find evidence of a possible adjuvant treatment to conventional therapy, especially in patients with the disease who do not respond effectively to pre-established drugs. In addition, it may serve as a basis for studies involving other viral infections.

METHODOLOGY

Research strategy

This systematic literature review aims to gather similar studies and analyze them to answer whether the use of bovine lactoferrin is beneficial in the treatment of patients with hepatitis C. The article was produced between August and October 2021, using the databases Pubmed and Google Scholar for the study of scientific articles, by consulting the following keywords and their combinations in Portuguese and English: ‘’lactoferrin”, “Chronic hepatitis C”, “Antivirals”, “immunotherapy”.

Inclusion and exclusion criteria

The inclusion criteria for the selection of articles were studies that performed an intervention with the use of bovine lactoferrin in patients with hepatitis C, without concomitant

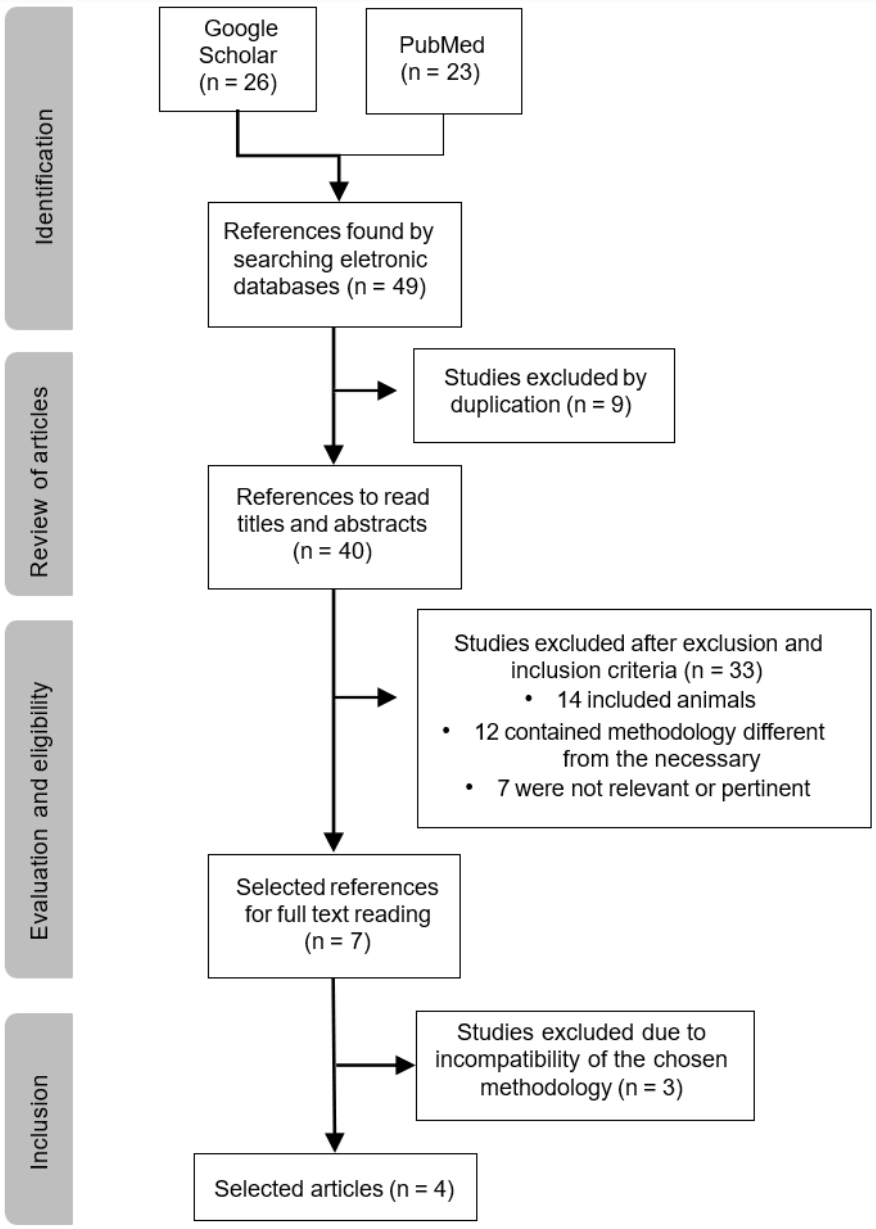
treatment with interferons, through a randomized, double-blind study, in English or Portuguese. Since studies on this subject are scarce and difficult to access, we chose to cover the time of publication in the selection of articles for the last 20 years as a selection criterion. Furthermore, articles that had doses between 0.4 and 7.2 g/day were selected, as well as those that compared the clinical outcome based on viral load and HCV genotype.

Articles based on clinical trials with animals, individuals without the disease in question, intervention time fewer than 8 weeks, and doses that differ from the others selected were excluded. Any characteristic that does not meet the inclusion criteria, such as time, language, and adequate score in the chosen criterion system, was also excluded from the composition of this review.

Selection and quality assessment

The selection and evaluation of the articles found were conducted in three stages (Figure 1). First, the titles of studies potentially relevant to this review were read. Those with characteristics that fit any of the previously decided exclusion criteria or those that were repeated were excluded. In the second stage, the abstract and introduction of each article were read and those that met the inclusion criteria were selected. In the third and last step, all studies considered relevant were read, evaluated, and verified about the intervention used, eligibility, and relevance of the research (through the JADAD scale). This analysis and integration of articles were conducted analytically, resulting in the studies that make up this systematic review, so that it could be possible to gather the knowledge produced and develop the writing of this work.

Figure 1. Flow of information through the different phases of a systematic review.



PICO Strategy

The PICO strategy, an acronym for Patient, Intervention, Comparison, and Outcome, was adopted as so that the guiding question was prepared, as well as the outline of hypotheses and methodologies that were summarized in Table 1.

We defined the target population, as patients with hepatitis C aged 20 years or more, who underwent intervention with bLf, and who were not undergoing treatment with concomitant interferon. In addition, patients should also be positive for anti-HCV antibodies, HCV level of ls 0.5-1000 KIU/mL, elevated serum ALT levels for 6 months, as well as renal function with serum creatinine and normal blood urea nitrogen levels. The intervention recommended the use of oral bovine lactoferrin managed for a period of 8 to 12 weeks in doses ranging from 1.8g/day to 7.2g/day.

In this regard, the comparison was based on patients who did not use oral bovine lactoferrin for a period shorter than 8 weeks and/or longer than 12 weeks, patients who were not positive for anti-HCV antibodies, and, finally, those who were treated with interferon for more than 12 months before the start of the study and did not respond to therapy. However, the outcome analyzed whether there was a decrease in the serum level of HCV RNA virus, whether there was a good virological response corresponding to anti-HCV activity, and the probability of a decrease in serum ALT levels, which were synthesized in Table 2.

Table 1. PICO flow diagram.

P – Population	I – Intervention	C – Comparation	O – Outcomes	Year	References
Ninety patients were diagnosed with hepatitis C with high serum levels of HCV RNA, type 1b HCV genotype, consumption of less than 20g of alcohol per day, and no other causes of liver dysfunction.	Participants were randomized into two groups to receive oral bLf for eight weeks at a daily dose of 3.6 g (n = 47) or a placebo (n = 43).	8-Isoprostane plasma levels and clinical laboratory data including iron metabolism parameters were measured from both groups for comparison.	The use of bovine lactoferrin for eight weeks at this respective dose brought benefits for plasma 8-isoprostane levels, ALT levels, and lipid peroxidation.	2006	[13]
Forty-five patients were diagnosed with hepatitis C, between 20-74 years of age, with positivity for anti-HCV antibodies and increased levels of ALT, as well as of HVC RNA, without other systemic alterations and with preserved liver functions.	Patients were randomized into three groups according to the following doses of oral bLf: 1.8g/day (n=15), 3.6g/day (n=15), and 7.2g/day (n=15) for eight weeks.	Comparison between groups to assess the effectiveness of the intervention was made through measurements of serum HCV, RNA, and ALT levels.	This eight-week intervention brought benefits for intolerance and potential anti-HCV activity, virological response, and HCV RNA level.	2002	[14]
One hundred and ninety-nine patients were positive for anti-HCV antibodies, with an elevated serum ALT level for at least 6 months and twice the level of ALT 200 IU/L, with normal serum creatinine and urea nitrogen levels.	Patients were randomized into two groups, one to receive 1.8 g/day of bLF (n = 97) and the other group to receive a placebo (n = 101) for 12 weeks.	The comparison was made through the virological or biochemical response rates between the groups, in addition to comparing the changes in the RNA level of HCV and ALT between the analyzed groups.	Treatment with bLf was not efficient for a virological response, as well as for the serum ALT level.	2006	[3]
Twenty-seven patients were diagnosed with hepatitis C with elevated levels of ALT as well as HCV RNA, positive for serum anti-HCV antibodies, and no other causes of liver dysfunction.	Patients received a dose of 0.4 g/day of oral bovine lactoferrin (n = 10) and another group received 3.6 g/day (n = 15).	The comparison of data obtained before and after treatment with lactoferrin was performed by analysis of variance, taking into account the serum concentration of HCV RNA and iron metabolism parameters.	Treatment with bLF proved to be efficient with serum levels of HCV RNA and ALT levels.	2002	[15]

Table 2. Articles selected for this study.

Intervention	Results	References
Ninety participants diagnosed with hepatitis C were randomized into two groups to receive oral bLF for eight weeks at a daily dose of 3.6 g (n = 47) or placebo (n = 43).	• Significantly reduced 8-isoprostane level in the intervention group, with no change in the control group; • No significant change was found in serum HCV RNA levels or iron metabolism markers after bLF treatment; • Improved lipid peroxidation and ALT levels post bLf therapy;	[13]
Forty-five patients with hepatitis C were randomized into three groups according to the following doses of oral bLF: 1.8 g/day (n = 15), 3.6 g/day (n = 15), and 7.2 g/day (n = 15) for eight weeks.	• There was no significant relationship between the dose of bLF and its effect on serum ALT or HCV RNA levels; • Association between the decrease in ALT and HCV RNA; • Treatment with bLf well tolerated and without toxic effects at the three intervention doses;	[14]
One hundred and ninety-eight patients positive for anti-HCV antibodies were randomized into two groups to receive 1.8 g/day of bLF (n = 97) or placebo (n = 101) for twelve weeks.	• There was no significant difference in IL-18, CD4+, CD8+, CD16+ and CD56+ concentration; • There was no significant difference in virological response rates between the two groups; • No favorable effect on serum ALT level in patients in the intervention group; • Treatment with bLf was well tolerated;	[3]
Twenty-seven patients diagnosed with hepatitis C were randomized to receive either a 0.4g/day dose of oral bovine lactoferrin (n = 10) or a 3.6g/day dose of the same compound (n = 15) for six months.	• Reduction in HCV RNA levels smaller in the low-dose intervention than in the higher-dose intervention; • Serum aminotransferase, iron, and ferritin levels remained unchanged throughout the study in both groups; • Elevated RNA levels after cessation of the intervention; • No serious complications occurred during treatment;	[15]

RESULTS

One of the studies on the inhibition of lipid peroxidation in patients with chronic hepatitis C was carried out by Masayoshi *et al.* (2006), in which ninety patients diagnosed with hepatitis C for eight weeks were selected. Forty-seven people received a dose of 3.6 g/day of oral bLf and the remainder received a placebo. In addition, for comparison purposes, thirty-eight healthy and HCV-negative volunteers were used as a benchmark for the other two groups. Thereafter, patients in the bLf group were classified into those who had decreased plasma 8-isoprostane levels (responders, n = 33) and those with an increase in this parameter (non-responders, (n = 14) after bLf therapy.

The results showed that platelet count and total cholesterol levels were higher in healthy individuals than in the bLf group, unlike ALT, triglycerides, and ferritin levels. Plasma 8-isoprostane levels (pg/ml) were significantly elevated in bLf-treated patients (8.6 ± 3.7) and the control group (9.1 ± 3.5) compared to healthy subjects (6.3 ± 1.6) ($P < 0.01$) and were not correlated with other laboratory parameters, such as platelet count and levels of ALT, T-CHO, TG, serum HCV RNA, serum iron, ferritin, and TS. Nonetheless, supplementation with oral bLf was found to significantly reduce plasma 8-isoprostane levels from 8.6 ± 3.7 to 6.9 ± 2.1 pg/ml after 8 weeks of bLf treatment ($P < 0, 05$) and did not change significantly in the control group. Serum ALT levels also decreased from 85 ± 80 to 63 ± 55 IU/l after bLf therapy and remained normal in the

control group, being lower in responder groups (86 ± 85 to 53 ± 38 IU/l) and remaining unchanged in non-responders (P < 0.01) after bLf treatment. Furthermore, no significant difference was found in serum HCV RNA levels, as well as markers of iron metabolism (serum iron, ferritin, TS) between both groups.

The study published by Ueno H *et al.* (2006) analyzed one hundred and ninety-nine patients in which one hundred and ninety-five patients rigorously completed the scheduled twelve weeks of treatment. Thereafter, treatment consisted of bLf at a dose of 1.8 g/day or placebo, administered orally twice a day for twelve weeks. Virological efficacy was the primary outcome and was observed in fourteen of ninety-seven participants (14.4%) in the bLf group and nineteen of one hundred and one (18.8% in the placebo group), in other words, the response rate virological comparison of both groups showed no significant difference (-4.4%, 95% CI - 14.8; 6.1). Regarding the change in HCV RNA level at twelve weeks from baseline, it was evaluated in one hundred and ninety participants (93 in the bLf group and 97 in placebo groups) with a change in the mean log of the RNA level of -0.09 in both groups. Biochemical efficacy also did not indicate significant differences (P=0.93) due to no change in serum aspartate aminotransferase (AST) level assessed in one hundred and ninety-two participants (93 bLf group, 99 placebo groups). The virologic rates demonstrated that participants with low levels of HCV RNA (<100 KIU/mL) had a virologic response of 29.4% in the bLf group and 15.4% in the placebo group, thus indicating that the virologic responses do not differ significantly from each other (14.0%, 95% CI -15.2, 43.2).

Regarding biochemical efficacy, no significant differences were indicated either (P=0.93) due to no change in serum AST level assessed in one hundred and ninety-two participants (93 bLf group, 99 placebo group). Likewise, the analysis of IL-18 in the bLf group was 293.9 pg/ml and 309.9 pg/ml at baseline and 280.7 pg/ml and 291.5 pg/ml at twelve weeks, respectively indicating that there were no differences in the serum concentration of IL-18 and the percentage of peripheral blood lymphocytes such as CD4+, CD8+, CD16+, and CD56+ remained unchanged throughout the study. Regarding biochemical efficacy, no significant differences were indicated either (P=0.93) due to no change in serum AST level assessed in one hundred and ninety-two participants (93 bLf group, 99 placebo group).

Another study by Iwasa *et al.* (2002) on the inhibition of hepatitis C virus viremia by lactoferrin separated twenty-five patients diagnosed with hepatitis C into two groups that differ from the intervention dose of oral bLf, namely: ten patients with 0.4g/day and fifteen with 3.6 g/day for six months. As a result, there were no significant differences between the two groups regarding age, sex, serum ALT, and HCV RNA levels. The serum concentration of HCV RNA was 1627 kIU/ml at baseline and 1049 kIU/ml after six months of treatment in the low-dose group, in contrast to the high-dose group, in which the concentration of HCV RNA decreased from 1106 kIU/ml at baseline to 612 kIU/ml after six months of treatment (p = 0.01). Serum levels of aminotransferases, iron, and ferritin remained unchanged throughout the study and two months after cessation of lactoferrin, however, HCV RNA levels were elevated.

The article by Shuichi Okada *et al.* analyzed forty-five patients who received bLf at three dose levels orally, twice or three times a day in an eight-week course on an outpatient basis and then followed for the next eight weeks. Fifteen patients were entered into each of the three bLf dose levels, namely: 1.8, 3.6, and 7.2 g/day. The biochemical response was observed in two patients who received bLF of 1.8g/day and 3.6g/day at the end of treatment, but none reached ALT normalization. The virological response was observed in four patients with a bLF of 1.8 g/day at the end of treatment, although all had persistently detectable serum levels of HCV RNA. There was no significant difference between the dose of bLf and the effect of bLF on serum ALT (P=0.30) or HCV RNA levels (P=0.20) at the end of treatment.

In virologic responders, a reduction in HCV RNA levels was associated with a reduction in serum ALT levels. The effects of bLf on serum levels of HCV RNA and LT represented 341 Kcopies/ml (35-1492) and 82 IU/l (52-270) in patients who received 1.8g/day, 224 Kcopies/ml (<1-1354), and 107 IU/l (51-375) in patients who received 3.6g/day and 138 Kcopies/ml (3-1460) and 99 UI/ l (52-169) in patients who received 7.2g/day, respectively, demonstrating that although the decrease in serum HCV RNA levels preceded the decrease in serum ALT levels, the time course of serum HCV RNA levels was parallel to serum ALT levels during and after bLf treatment.

DISCUSSION

In the study performed by Iwasa *et al.* (2001), it was concluded that lactoferrin reduces the serum levels of HCV RNA in patients with chronic hepatitis in those patients whose doses were higher. In the study by Okada *et al.* (2003), there was a reduction in HCV RNA levels, but there was no significant difference in the result between the doses used in the intervention. The mechanism of HCV RNA reduction can be explained by the ability of bLf to bind and neutralize the virus activity, inhibiting the adsorption of the HCV-lactoferrin complex in the host's hepatocytes.[7,16] Regarding the difference in the results obtained due to the doses, it may have been caused by the very discrepant doses, since, in the first study, a dose of 0.4 g/day is compared with a dose of 3.6 g/day, and in the second study, the results of doses of 1.8 g/day, 3.6 g/day, and 7.2 g/day are compared. Therefore, this may indicate that the result above 1.8 g/day is the same as the higher doses, but a dose lower than this may not have such a significant effect. In contrast, studies by Ueno *et al.* (2006) and Masayoshi *et al.* (2006) did not obtain significant responses with a dose of 1.8 g/day and 3.6 g/day of oral bLf, respectively, which makes us think about the divergence of results between this study and the one carried out by Okada. These unchanged levels of HCV RNA can be explained by the fact that the dose had no effect on the host and did not inhibit the absorption of the viral complex in hepatocytes, thus allowing them to be internalized and that the HCV RNA remained unchanged, different from patients who did not receive previous treatment.[17]

In the studies carried out by Masayoshi *et al* (2006) and Iwasa *et al* (2002), the parameters of iron metabolism appear unchanged after supplementation, which may indicate that bLf does not help in lipid peroxidation due to its ability to modulate the transport of iron, as showed in the study by Gutteridge *et al.* (1981) or as the study by Gibbons *et al.* (2015) in which iron-saturated bLF would exert a cytotoxic effect depending on the type of cell line.[17,18,19] Some studies indicate that the antiviral activity of lactoferrin, detected in monolayers of cultured cells infected with involved or uninvolved viruses, is unrelated to the iron saturation degrees of lactoferrin, while other types of lactoferrin, such as Zn- and Mn-saturated lactoferrin, exerted potent antiviral ability against HSV, HIV infection and polioviruses.[8]

In the study by Masayoshi *et al.* (2006), an increase in 8-isoprostane was observed in intervention groups and its consequent reduction after eight weeks of treatment with bLf, thus indicating a reduction in lipid peroxidation. Unlike the thought that this mechanism occurs due to the iron-binding capacity of bLf, it can be said that the reduction in lipid peroxidation occurs through the metabolism of the N-terminal peptide, as some of the undigested peptides of bLf can play a beneficial role in the lower gastrointestinal tract.[20,21]

It was observed in the studies carried out by Masayoshi *et al.* (2006) and Okada *et al.* (2002) that the reduction in ALT levels occurred concomitantly with the elevation of 8-isoprostane and the decrease of RNA HCV, respectively. Furthermore, in this first study, ALT levels were decreased after bLf therapy, which can be explained by the hypothesis that HCV stimulates iron absorption in patients with HCC and this affects ALT activity.[21,22] In a study by Takikawa *et al.* (1994) with rodents, it was found that the serum ferritin concentration reduced ALT activity in patients with HCV, this mechanism having been understood with the alteration in the iron metabolism in the intervention group since hyperferritinemia had synergistic effects with overload HCV virus.[23] However, in the studies observed in this work, iron metabolism remained unchanged and, therefore, there is a need for more experimental work to understand the relationship between the levels of ALT, RNA, and HCV and, consequently, their properties of lipid peroxidation.

A recent clinical study by Ishii K *et al.* (2003) demonstrated that oral administration of bLf (0.6 g/day) for three months in thirty-six patients with chronic hepatitis C significantly increased the serum level of IL-18 compared to baseline due to bLf stimulating the IL-18 production from intestinal enterocytes, in addition to increased splenic cell NK activity and blood CD8+ production. This may indicate, therefore, that the activity of lactoferrin in increasing blood lymphocyte levels and consequently inhibiting lipid peroxidation is due to suppression of the inflammatory response caused in the infected host.[24,25]

However, contrary to what this first study points out to us, the study by Ueno H *et al.* (2006) did not obtain serum alteration in the production of IL-18, which may be a hypothesis of this result the fact that bLf administered orally had no relevant efficacy, including anti-HCV activity in patients with chronic hepatitis C, although the virological response rate was higher than that reported in the study by Okada S *et al.* (2002) in which only 8.9% of patients had a significant virological response.[14]

The immunomodulatory role of the bLf protein was highlighted in specific examples and illustrated in the context of SARS-CoV-2 in a prospective observational study by Serrano et al. (2020), in which they elucidated the potential doses for prevention and infection by COVID-19. The study was based on the use of liposomal bovine lactoferrin (LLF) as a food supplement Lactoferrin™ associated with vitamin C, was recommended for 10 days and obtained qualitative responses in the first 3 to 4 days with the reduction of pro-inflammatory cytokines (IL-2 and IL-6), TNF and ferritin, which also helped in the prevention of acute respiratory distress.[26]

Although there are effective therapies for hepatitis C virus (HCV) infection, some barriers may compromise the treatment of HCV infection, such as adverse effects of drug treatment and the presence of comorbidities.[27] Among the comorbidities that most negatively impact the efficacy of drug treatment for hepatitis C, we have psychiatric disorders such as depression, anxiety, and insomnia; co-infections, patients co-infected with HCV and HIV; endocrine-metabolic disorders such as obesity and insulin-resistant diabetes mellitus, in addition to conditions associated with the advanced age of some patients and pictures of advanced liver fibrosis and multicirrhosesosis.[28]

Among the drug treatment options, ribavirin, a guanosine analog, has antiviral activity against a range of DNA and RNA viruses, including hepatitis C. In addition, ribavirin modifies the immune system response and enhances the antiviral activity of interferon.[29] However, ribavirin, although associated with reduced hepatic inflammatory activity and induction of biochemical responses such as ALT reduction, is not recommended as monotherapy, since its benefits are only seen in association with interferon, thus ensuring a sustained virologic response in more than 50% of patients.[30] Conversely, remarkable resistance mechanisms developed by HCV interfere with innate and adaptive host immune responses, contributing to resistance to exogenously administered interferon used for HCV treatment.[31] Understanding the molecular basis of interferon treatment failure in HCV infection, some treatment strategies for patients not responding to interferon treatment may emerge. In this sense, studies show that bLF, an iron-binding glycoprotein found in body fluids, such as milk, tears, and saliva, and that is currently isolated from bovine milk, has shown several actions on the immune system, including induction of IFN-α/β production in Peyer's follicles and activation of NK, T CD4+, CD8+, and B cells, besides suppressing symptoms of influenza virus, rotavirus, and herpes-virus infections.[32] In this sense, from the immunological point of view and based on evidence collected in this review, we hypothesize that bLF plays an "interferon-like" role,[7,14] being associated with interferon, so that both,

along with ribavirin, may play an adjuvant role, especially for patients whose antiviral response is not sustained during conventional treatment, such as patients with previous use of DAAs and no response, disease relapse, non-responders to interferon, male gender, age > 40 years, genotype 3 or patients classified by Child-Pugh as B and C.[33] However, new studies involving the use of bLf, alone or in association with ribavirin/interferon treatment, are necessary for the role of bLf in a probable therapeutic scheme to be evaluated, revealing a possible adjuvant option in the treatment of hepatitis C, especially in the group of patients who present therapeutic failure in drug treatment, such as those with comorbidities cited in this review.

CONCLUSION

Given the studies presented, lactoferrin showed a virological response in two studies that were correlated with decreased serum ALT levels, since the progression to cirrhosis in HCV patients depends on liver severity that will alter the natural history of the disease. Therefore, because of the potential of bLf, further studies should be conducted, especially involving a large population of HCV patients, to evaluate the long-term effects of bLf, in addition to its possible adjuvant role in the treatment of hepatitis C, especially in the population of patients where the presence of comorbidities is an important factor in the pharmacological ineffectiveness of conventional treatment.

REFERÊNCIAS

1. Siciliano RF, Boulos M. Hepatite C: tratamento revisitado. Arq Gastroenterol. 2004;41(1):1-2.

2. Strauss E. Hepatite C. Rev Soc Bras Med Trop. 2001;34(1):69-82.

3. Ueno H, Sato T, Yamamoto S, Tanaka K, Ohkawa S, Takagi H, et al. Randomized, double-blind, placebo-controlled trial of bovine lactoferrin in patients with chronic hepatitis C. Cancer Sci. 2006;97(10):1105-10.

4. Rocha LS. Avaliação das mutações de resistência ao tratamento com os novos antivirais de ação direta (DAA) em pacientes com Hepatite C crônica [dissertação]. Salvador: Instituto Gonçalo Moniz, Fundação Oswaldo Cruz; 2019. 102 p.

5. Queiroz VA, Assis AM, R. Júnior HC. Efeito protetor da lactoferrina humana no trato gastrintestinal. Revista Paulista de Pediatria. 2013;31(1):90-5.

6. Sgarbieri VC. Propriedades fisiológicas-funcionais das proteínas do soro de leite. Rev Nutr. 2004;17(4):397-409.

7. Tanaka K, Ikeda M, Nozaki A, Kato N, Tsuda H, Saito S, et al. Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. Jpn J Cancer Res. 1999;90(4):367-71.

8. Berlutti F, Pantanella F, Natalizi T, Frioni A, Paesano R, Polimeni A, et al. Antiviral properties of lactoferrin - a natural immunity molecule. Molecules. 2011;16(8):6992-7018.

9. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J. Lactoferrin structure and functions. Adv Exp Med Biol. 2008;606:163-94.

10. García-Montoya IA, Cendón TS, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin a multiple bioactive protein: an overview. Biochim Biophys Acta. 2012;1820(3):226-36.

11. Haraguchi FK, Abreu WCP, Paula H. Proteínas do soro do leite: composição, propriedades nutricionais, aplicações no esporte e benefícios para a saúde humana. Rev Nutr. 2006;19(4):479-88.

12. Actor JK, Hwang SA, Kruzel ML. Lactoferrin as a natural immune modulator. Curr Pharm Des. 2009;15(17):1956-73.

13. Konishi M, Iwasa M, Yamauchi K, Sugimoto R, Fujita N, Kobayashi Y, et al. Lactoferrin inhibits lipid peroxidation in patients with chronic hepatitis C. Hepatol Res. 2006;36(1):27-32.

14. Okada S, Tanaka K, Sato T, Ueno H, Saito S, Okusaka T, et al. Dose-response trial of lactoferrin in patients with chronic hepatitis C. Jpn J Cancer Res. 2002;93(9):1063-9.

15. Iwasa M, Kaito M, Ikoma J, Takeo M, Imoto I, Adachi Y, et al. Lactoferrin inhibits hepatitis C virus viremia in chronic hepatitis C patients with high viral loads and HCV genotype 1b. Am J Gastroenterol. 2002;97(3):766-7.

16. Yi M, Kaneko S, Yu DY, Murakami S. Hepatitis C virus envelope proteins bind lactoferrin. J Virol. 1997;71(8):5997-6002.

17. Gibbons JA, Kanwar JR, Kanwar RK. Iron-free and iron-saturated bovine lactoferrin inhibit survivin expression and differentially modulate apoptosis in breast cancer. BMC Cancer. 2015;15:425.

18. Gutteridge JM, Paterson SK, Segal AW, Halliwell B. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochem J. 1981;199(1):259-61.

19. Almeida MA. Ativação da apoptose mediada por Lactoferrina Bovina (bLf) em células Vero [dissertação]. Rio de Janeiro: Universidade Federal do Estado do Rio de Janeiro; 2019. 68 f.

20. Wakabayashi H, Matsumoto H, Hashimoto K, Teraguchi S, Takase M, Hayasawa H. Inhibition of iron/ascorbate-induced lipid peroxidation by an N-terminal peptide of bovine lactoferrin and its acylated derivatives. Biosci Biotechnol Biochem. 1999;63(5):955-7.

21. Takikawa T, Hayashi H, Nishimura N, Yano M, Isomura T, Sakamoto N. Correlation between serum levels of alanine aminotransferase and ferritin in male blood donors with antibody to hepatitis C virus. J Gastroenterol. 1994;29(5):593-7.

22. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology. 1992;102(6):2108-13.

23. Ishii K, Takamura N, Shinohara M, Wakui N, Shin H, Sumino Y, et al. Long-term follow-up of chronic hepatitis C patients treated with oral lactoferrin for 12 months. Hepatol Res. 2003;25(3):226-33.

24. Shin K, Wakabayashi H, Yamauchi K, Teraguchi S, Tamura Y,

Kurokawa M, et al. Effects of orally administered bovine lactoferrin and lactoperoxidase on influenza virus infection in mice. J Med Microbiol. 2005;54(Pt 8):717-23.

25. Wolf JS, Li G, Varadhachary A, Petrak K, Schneyer M, Li D, et al. Oral lactoferrin results in T cell-dependent tumor inhibition of head and neck squamous cell carcinoma in vivo. Clin Cancer Res. 2007;13(5):1601-10.

26. Serrano G, Kochergina I, Albors A, Diaz E, Oroval M, Hueso G, et al. Liposomal Lactoferrin as Potential Preventative and Cure for COVID-19. Int J Res Health Sci. 2020;8(1):8-15.

27. McGowan CE, Fried MW. Barriers to hepatitis C treatment. Liver Int. 2012;32 (Suppl 1):151-6.

28. Hassanein T, Shiffman ML, Zein NN. The practical management of treatment failure in chronic hepatitis C: a summary of current research and management options for refractory patients. Gastroenterol Hepatol (N Y). 2007;3(6 Suppl 20):4-32.

29. Saito EY. Ribavirin® [Internet]. Blau Farmacêutica: São Paulo; 2019 [acessado em 25 out. 2023]. Bula de remédio. Disponível em: https://www.blau.com.br/storage/app/media/Bulas%20Atualizacao%2006%2012%2018/Ribavirin%20-%20Medicamento_Bula_Profissional.pdf

30. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140(5):346-55.

31. Tai AW, Chung RT. Treatment failure in hepatitis C: mechanisms of non-response. J Hepatol. 2009;50(2):412-20.

32. Kubo S, Miyakawa M, Tada A, Oda H, Motobayashi H, Iwabuchi S, et al. Lactoferrin and its digestive peptides induce interferon-α production and activate plasmacytoid dendritic cells ex vivo. Biometals. 2023;36(3):563-73.

33. Moles L, Manzano S, Fernández L, Montilla A, Corzo N, Ares S, et al. Bacteriological, biochemical, and immunological properties of colostrum and mature milk from mothers of extremely preterm infants. J Pediatr Gastroenterol Nutr. 2015;60(1):120-6

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