Expert Opinion

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Lactoferrin delivery systems: approaches for its more effective use

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Introduction: Recently, pharmacotherapy has advanced extensively, but there are still many refractory diseases which cannot be solved fully by existing therapeutic agents. Therefore, alternative medicine and health foods are now attracting much attention, for example, lactoferrin (LF): a multifunctional glycoprotein. As LF is non-toxic and low-cost, its application in healthcare and therapeutics is expected to be widespread.
Areas covered: In this review, LF's general basic features are described. The interaction of LF with its receptors activates the immune system, including cytokine production and balance. In particular, the immune activation of orally administered LF is considered as a new strategy for the treatment of refractory diseases, such as inflammatory bowel disease, virus infection and tumor metastasis. Also mentioned are the problems associated with the use of LF. As LF is degraded rapidly in the body due to enzymatic hydrolysis, high amounts or frequent dosing is required; an appropriate delivery system may improve these problems and increase its efficiency.
Expert opinion: Chemical modifications, such as PEGylation, can enhance the stability of LF in the body, resulting in increased efficacy. Also, liposomes and enteric or microparticulate formulations can promote the function of LF in oral administration due to target site delivery and protection of LF from enzymatic hydrolysis. These delivery systems are expected to improve the utility of LF.

Keywords: biological feature, delivery system, effective use, lactoferrin, liposome, microparticle

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1. Introduction

Lactoferrin (LF) is an 80 kD iron-binding glycoprotein found in biological fluids such as milk, blood, cervical mucus, seminal fluids, saliva and tears, and within specific granules of polymorphonuclear leukocytes (**Figure 1A**) [1-3]. LF is a multifunctional protein which exhibits antimicrobial, antiviral, antitumor, anti-inflammatory, immunomodulatory, analgesic and anti-oxidative stress effects as well as enhancement of lipid metabolism [4]. The utilization of LF in the field of healthcare or disease treatment has been proposed. Recently, its safety and tolerability were revealed in animal and human tests [5]. Namely, no apparent adverse effect was observed at 2 g/kg/day (oral dosing, 13 weeks) in rats for bovine LF [6]. In clinical tests, recombinant human LF was very well tolerated at 1.5 – 9 g/day (oral dosing, 2 weeks on/2weeks off, several months) [7]. Nowadays, LF receives much interest as a commercially available health food [8,9]. This review describes general features of LF functions first, mentions its current problems next, and provides drug delivery systems or pharmaceutical technologies to improve the use of LF in the last part.

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Article highlights.

- As lactoferrin (LF) is non-toxic and low-cost, its application in healthcare or therapeutic use is expected to a great extent.
- As LF is degraded rapidly in the body due to enzymatic hydrolysis, a high amount or frequent dosing is required. An appropriate delivery system may improve the problems for efficient use.
- Chemical modification such as PEGylation enhances the stability of LF in the body, resulting in higher efficacy. Also, liposomes and enteric or microparticulate formulations can promote the function of LF in oral administration due to their capacities of target site delivery and protection of LF from enzymatic hydrolysis. These delivery systems are expected to improve utility of LF.

This box summarizes key points contained in the article.

2. General features for LF functions

LF has many useful biological functions as stated above. The biological functions and its mechanisms are overviewed in this section.

2.1 Direct actions for antimicrobial and antiviral functions

LF exerts broad spectrum primary defense activity against bacteria, fungi and protozoa [10-12]. The activity of LF includes two major mechanisms and other additional antibacterial features (**Figure 2A**). Iron necessary for bacterial nutrition is sequestered by the chelating function of LF, which leads to the biostatic action of LF against a wide variety of bacteria and is a key host defense mechanism [13-15]. Another important point is direct binding of LF to the outer membrane of bacteria. This binding produces an increase in the permeability of the bacterial membrane, resulting in its disruption [12,16]. This bactericidal action is also observed for lactoferricin (LFcin), an N-terminal region of LF and a cationic peptide produced by cleavage of LF by a gastric enzyme pepsin (**Figure 1B**). LFcin has a more potent bactericidal effect than intact LF [12,17-19]. LFcin binds to the lipopolysaccharide of Gram-negative bacteria and teichoic acid of Gram-positive bacteria, and these bindings are considered to be an initial step of the bactericidal action of LFcin [9,20-22]. Bovine LFcin has higher bactericidal potency than human lactoferricin. LFcin is also effective against fungi and protozoa. In addition, LF exhibits antimicrobial effects through additional complementary activities in relation to virulence and pathogenicity [12,23]. For instance, LF inhibits the colonization of *Helicobacter pylori* in the human stomach and can amplify apoptotic signals in infected cells. These additional functions are also associated with antimicrobial activities.

LF creates strong interest in antiviral therapy, because there are less useful treatments against viral infection as compared with the antimicrobial therapy. The antiviral activity of LF has been demonstrated against various types of viruses [24,25]. Viral infection involves attachment of a virus to the host cell surface and fusion between the viral envelope and host cell membrane [26]. Viral glycoproteins work in each process. Both attachment and fusion are required for viral entry. Heparan sulfate (HS) of the host cells functions as an attachment receptor for the virus via its glycoproteins. Viral glycoproteins interact with co-receptors on the surface of host cells, leading to fusion of the virus with the host membrane. The antiviral activities of LF include different mechanisms (**Figure 2B**), one of which is the interaction of LF with host cells. For this mechanism, it was demonstrated that preincubation of LF with the virus prior to infection gave no improvement. LF binds to HS of the host cells with high affinity, and this binding inhibits the attachment of the virus to the host cell surface. This phenomenon is observed in HBV and herpes simplex virus [26,27]. On the other hand, for infection with adenovirus, feline herpes virus 1, HCV and HIV, LF exerts its activity by direct interaction with viral particles [26,28]. In addition, LFcin has antiviral activity, but its potential is less than that of LF itself. LFcin is considered to directly inactivate virus and upregulate host defense [22].

2.2 Biological functions on antitumor and immunological effect

Although LF has a high molecular mass and is susceptible to proteolytic enzymes in the gastrointestinal pathway, it shows multifunctional activities via oral administration. Other functional mechanisms exist in LF action. Recently, it has been clarified that LF plays an important role in host defense through the immune system [29,30]. In particular, regarding oral administration of LF, gut-associated lymphatic tissue (GALT) has been elucidated to be importantly associated with various host defense functions by LF. LF activates or modulates the immune system by binding to LF receptors on the intestinal mucosal membrane and GALT-related cells [31,32]. Immunomodulatory or anti-inflammatory activities and enhancement of immune response are caused by the action of LF on the immune system. Activation of the immune system is importantly related to host defense, including antimicrobial effects [33]. LF receptors have been found in the small intestinal membrane, M cells, monocytes, macrophages, lymphocytes, liver cells and so on (**Table 1**). LF activates NK cells, polymorphonuclear leukocytes, macrophage cytotoxicity and so on, leading to antitumor and antimetastatic activities [34]. Furthermore, LF affects the cell growth, which is related to its antitumor activity [35]. LFcin also has antitumor potential as well as LF [36]. Bovine LFcin is reported to exhibit antitumor effect through direct action to tumor cells [37].

Recently, anti-inflammatory activities of LF have been reported to be exerted by immunomodulation mainly through cytokine balance (**Table 1**) [29]. As many refractory diseases are closely associated with abnormalities of the

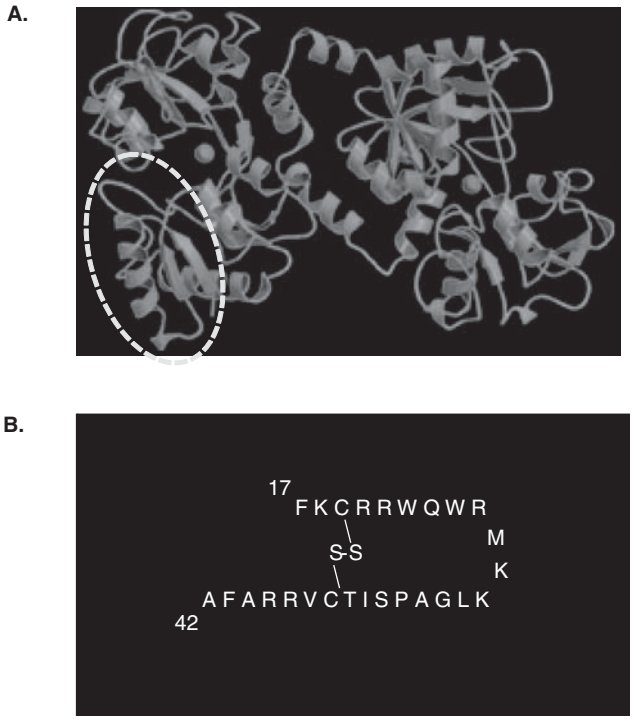

Figure 1. Structures of bovine lactoferrin (A) and bovine lactoferricin **(B)**. **A.** Lactoferricin is present in the dotted region. **B.** Lactoferricin is expressed with amino-acid codes.

topical or systemic immune system, the potential of LF as an immunomodulator is considered to be useful against those diseases [38]. LF was demonstrated to improve diseased conditions in rats with dextran sulfate sodium (DDS)-induced colitis, when orally administered [39]. LF corrected the disturbed balance of cytokines at inflammatory sites. Namely, LF, orally administered consecutively once a day from 3 days before the start of DSS treatment, reduced pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, and increased the levels of anti-inflammatory cytokines such as IL-4 and -10. Accordingly, LF can exert a protective effect against the development of colitis via modulation of the immune system or cytokine balance. The immunomodulation of LF is also found in adjuvant function for vaccination [40] or protective effect against infection [41]. In addition, bovine LFcin is reported to show immunomodulation by affecting cytokine release [22,42].

2.3 Biological activities related to other healthcare

Moreover, LF has received much attention because it has novel biological activities, such as elevation of the basal metabolic rate, and anti-nociceptive and anti-oxidative stress activities [43,44]. These features are useful for the therapy against obesity and anti-ageing, and also propose LF as a promising agent for the treatment of terminal cancer. Thus, the multifunctional features and mechanisms of LF have been revealed. It is important to know how to apply LF to healthcare or therapeutic treatment. In fact, various trials and examinations have been performed to achieve efficient use of LF, which is stated later.

3. Current problems

The *in vivo* stability of LF is one of the problems for its practical use. As LF is a glycoprotein, it is susceptible to proteolytic enzymes in the body [45,46]. Intravenously injected bovine LF was quickly eliminated from the blood circulation, that is, the plasma half-life is several minutes; therefore, rapid elimination requires frequent administration to obtain a therapeutic effect. Although parenteral administration, such as intravenous (i.v.), subcutaneous and intraperitoneal injections, can achieve high bioavailability, frequent administration is necessary for long efficacy, which is a burden on patients. Oral administration is better for long and safe use. As various biological activities of LF are observed when administered orally, improved oral use may be an important subject for practical use. One of the important targets of LF is the LF receptors on the intestinal mucosal and GALT-related cells. In the oral administration of LF, its protection from protein-digesting enzymes in the gastrointestinal tract is an essential subject.

LF is known to be absorbed from intestinal epithelial cells through specific receptor-mediated transcytosis and nonselective transcytosis [32,47]. LF incorporated in those cells transfers into the lymphatic system and thoracic duct lymph, and enters systemic circulation [47-49]. It is considered to be important for the biological activities that intact LF can reach and transport into intestinal or GALT-related cells.

As to proteolytic degradation of LF after oral administration, various findings were reported. The digestion of LF depends on the animal age and species or diet form. Generally, LF is digested in stomach into several large fragments. After oral ingestion, LF disappears almost completely in the intestine for adult rats [50], but not in suckling miniature pigs [51]. The LF or its large fragments including LFcin, transferred to intestine, were reported to be retained to fair extent [50,51]. Namely, LF and its large fragments can exist in the intestine for a relatively long time (a few hours). In human tests, it was reported that intact LF could enter the small intestine to a fair extent after drinking aqueous solution of LF [52]. From these studies, it is considered that a gastric enzyme, pepsin, influences the degradation of LF greatly, and LF and its large fragments degrade gradually in the small intestine. Furthermore, the intra-duodenal administration of LF gave lymphatic and plasma levels of LF much more as compared with the intra-gastrical administration [49,53]. This supports that the delivery of LF to the intestine without degradation in the stomach is important to increase in LF integrity in the intestine and its biological activities. After LF is digested extensively, the resulting amino acids and small peptides can be absorbed from the intestine [51]. When LF was administered intra-gastrically, an enteric-coated formulation of LF exhibited intestinal absorption of LF much more efficiently

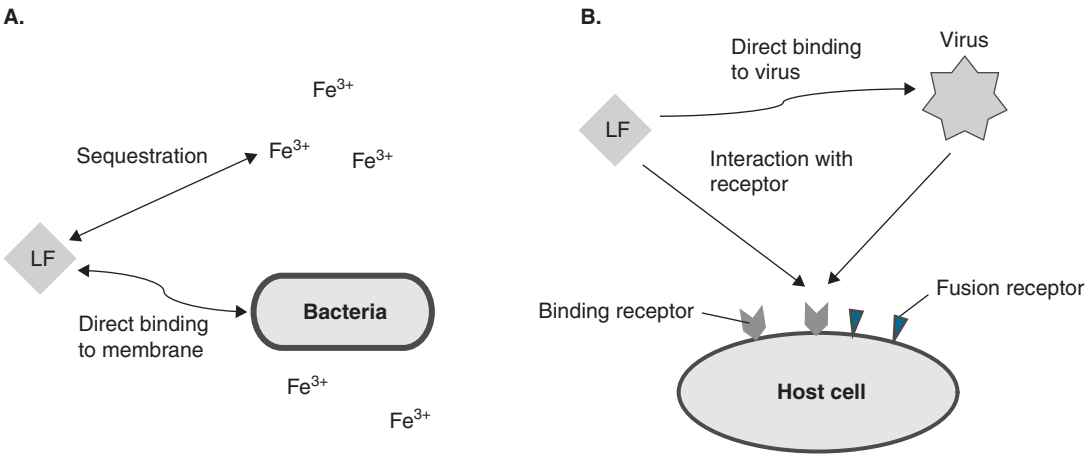

Figure 2. Mechanisms of antimicrobial (A) and antiviral (**B**) actions.

Table 1. Location tissues of LF receptors and classification of cytokines.

A. LF receptor location tissue
Small intestine, liver, monocyte, macrophage, lymphocyte, platelet, fibroblast, bone, brain
B. Classification of cytokines
Pro-inflammatory cytokines
TNF-α, IL-1β, IL-6, IFN-γ, IL-8, G-CSF
Anti-inflammatory cytokines
IL-4, IL-10, IL-11, IL-13, TGF-β

than non-coated LF. Shimizu reported that LF was detected in the blood circulation using enteric-coated LF tablets after oral administration at 15 mg/kg in humans [43], suggesting that the prevention of LF from gastric digestion should be very important for absorption into systemic circulation. Efficient delivery of LF to the target sites, such as an intestinal receptor, is considered to lead to effective use or enhancement of efficacy. The following part provides several efficient delivery systems to improve the use of LF.

4. Delivery systems for more effective use

As stated above, LF is possibly useful for healthcare and disease treatment. Although it is used as a health food, there are some problems to resolve, as described in the former section. The application systems, that is, delivery systems, are considered to be one of the approaches to improve its utility. It is essential to design the systems based on biological features. The delivery systems are applied topically or systemically. In the topical application, the concentration and residence time are important for the efficacy. In the systemic use, efficient delivery to the LF receptors is essential for LF to effectively exhibit the biological functions such as immunomodulation. Several delivery systems of LF, having been reported, are provided below.

4.1 Topical delivery

Intractable stomatitis such as oropharyngeal candidosis is often a problem for patients with low immunity or suffering from AIDS. Antimicrobials including antifungal agents are generally used for the treatment of such infections; however, long and frequent use of these drugs brings about toxic side effects and the appearance of drug-resistant microorganisms. In such cases, other agents, which can be used for a long period and cover the most commonly-used drugs, are promising for pharmacotherapy. LF acts against such diseases through mechanisms different from generally used drugs. As LF has no toxic side effects [5-7] and is a low-cost agent, it is considered a useful candidate.

Recently, it was demonstrated that LF inhibited the growth of clinical *Candida* isolates and exhibited cooperative effects against *Candida* diseases in combination therapy with antifungal drugs [54,55]. It is very important that LF concentration around the fungal isolate reaches an effective level. This treatment must be conducted so that the concentration over the MIC can be achieved for an appropriate period. Kuipers *et al.* developed a mucoadhesive tablet of LF for the treatment of oropharyngeal candidosis [56]. They analyzed the release properties of LF from the tablet and the LF concentration in the saliva *in vivo* (**Figure 3**). The tablet loaded with 250 mg LF maintained an effective level for an extended period (over 3 h). This dosage form is expected to be excellent system supplying LF against oropharyngeal candidosis. Further *in vivo* detailed analysis will elucidate the usefulness of the mucoadhesive dosage form. In addition, a mucoadhesive system aiming at direct action is being attempted for therapy against oral inflammation in animals, because oral inflammation leads to inadequate feeding, preventing growth.

4.2 PEGylation

As stated above, proteolytic degradation is the largest drawback of LF for its *in vivo* application. Recently, bioconjugation has been performed as a useful approach for many protein

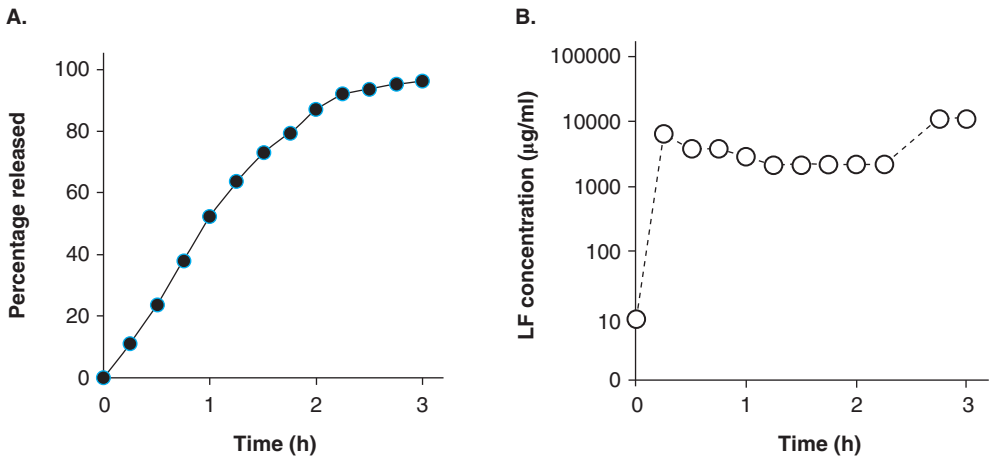

Figure 3. *In vitro* release profiles of LF from mucoadhesive tablet (**A**) and LF concentration in the saliva after *in vivo* application of the tablet (**B**). **A.** mean (n = 2); **B.** one example (volunteer).
Adapted from [56].

drugs [57-61]. The conjugation of proteins with PEG, namely PEGylation, is the most common strategy, and several PEG-protein bioconjuagtes have been successful in enhancing therapeutic effects [62,63]. For example, PEG-introduced IFN-α [64], adenosine deaminase [65] and L-asparaginase [65,66] are clinically available because they exhibit much higher efficacy than intact proteins. PEGylation causes the suppression of immunogenicity of proteins by changing the structure around the antigen region, elongates a biological half-life due to steric hindrance against proteolytic enzymes and inhibits renal excretion with an increase in the molecular mass. These features of PEGylation hold true for LF.

Many PEG derivatives have been developed as bioconjugation reagents. PEG-NHS, an *N*-hydroxysuccinimidil ester of PEG-COOH, is often used. The free amino groups of proteins are derivatized with PEG-NHS without side reactions. PEG-introduced LF, named PEG-LF, was synthesized by the reaction of LF with PEG-NHS. Nojima *et al.* reported mono-derivatized LF with PEG-NHS with a molecular mass of 20,000, which was named 20k-PEG-LF [47]. LF derivatives were produced, substituted by different numbers of PEG molecules. The target of the LF derivative could be obtained by column chromatography and confirmed by SDS-PAGE analysis. It was found that 20k-PEG-LF exhibited iron-binding activity equivalent to that of intact LF. The conjugate showed a relatively high anti-inflammatory effect (about 70% of intact LF) in an *in vitro* stimulation test using cultured cells. Mono-PEGylation appears to change the biological activity of LF slightly. In the *in vitro* degradation test using an artificial gastric fluid containing pepsin, 20k-PEG-LF showed twofold longer half-life than intact LF (**Figure 4A**). The concentration in thoracic lymph was monitored after intragastric administration of 20k-PEG-LF and intact LF in rats. As LF was absorbed in lymphatic tissue from the intestinal membrane and transported into the blood circulation, the thoracic lymph concentration was measured as an index of absorption by ELISA. The lymphatic concentration of 20k-PEG-LF was much higher than that of intact LF and was 10-fold greater at 4 h after administration (**Figure 4B**). In addition, the serum concentration of LF was monitored after i.v. administration to rats at the dose of 1 mg LF eq./kg. The serum half-lifes were 8 and 42 min for intact LF and 20k-PEG-LF, respectively [67]. Overall, 20k-PEG-LF exhibited good retention in the blood circulation, and the AUC of 20k-PEG-LF was approximately nine times greater than that of intact LF. The PEGylation technique is suggested to be useful for the improved systemic delivery system of LF.

PEGylated LF was evaluated *in vivo* for a hepatoprotective effect using rats with CCl_4-induced liver injury [68]. CCl_4 causes an oxidative liver injury due to its rapid metabolism to trichloromethyl radical metabolite followed by lipid peroxidation, that is, CCl_4-mediated peroxidation, leading to fatty liver, cell necrosis, lipid peroxidation, membrane damage and enzyme activity loss [69]. ALT and AST leaked into the blood from liver by damage to the hepatocytes, and are often used as indices of liver damage. The serum levels of these enzymes were investigated among intact LF, 20k-PEG-LF and 40k-PEG-LF for pretreatment of the CCl_4-induced liver injury model. The protective effect by intraperitoneal injection on consecutive 3 days is in the order of 40k-PEG-LF > 20k-PEG-LF > intact LF after intraperitoneal administration, suggesting that PEGylation would be a useful approach for protection against peroxidation damage [68].

4.3 Liposome formulations

Oral ingestion of LF is a promising administration manner because it allows using LF safely for a long period. A major obstacle is proteolytic degradation in the gastrointestinal tract, especially by pepsin in the stomach. PEGylation is a useful technique against the proteolytic degradation as described

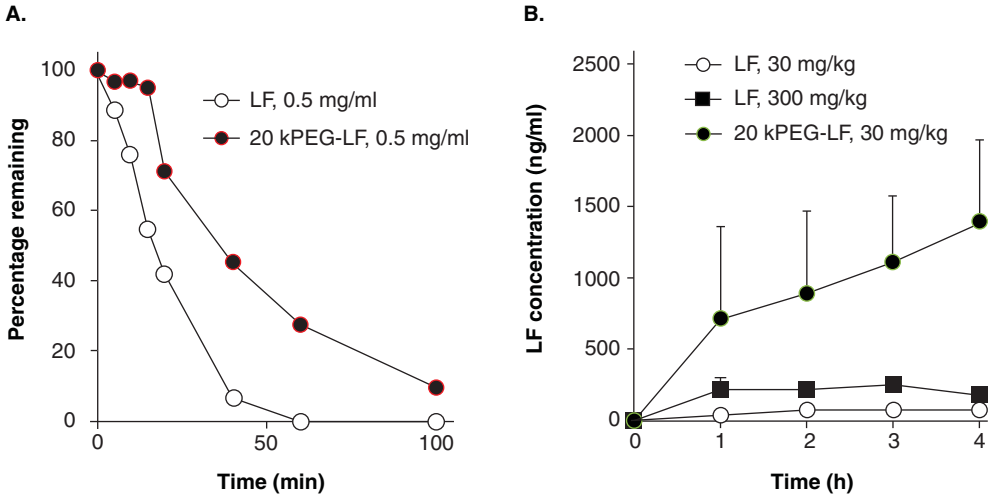

Figure 4. *In vitro* degradation of LF and 20k-PEG-LF by pepsin (20 ng/ml) (**A**) and LF concentration in thoracic lymph after their intragastric injection (**B**). **A.** one datum for each point; **B.** mean ± s.e. (n = 4 – 5).
Adapted from [47].

above. In addition, encapsulation or coating of proteins with appropriate materials has been applied to deliver them to target sites and/or protect their stability from harsh environmental conditions such low pH and digestive enzymes [70-72].

Liposome encapsulation (liposomalization) of LF is used frequently as a drug delivery approach. LF can bind iron which is potentially toxic because of its acting as a catalyst to produce free radicals and related to inflammation. It was demonstrated that LF, injected intra-articularly, could reduce inflammation in mice with collagen-induced arthritis [73]; however, LF is eliminated relatively quickly from the injected site. Once LF enters the systemic circulation, it is cleared rapidly, in particular by the liver [74,75]. Liposomal formulations of LF, injected intra-articularly, were prepared and investigated for their pharmacokinetic profiles by Trif *et al.* [75]. At that time, positively-charged liposomes were found to be useful to increase residence in the inflamed joint. On the other hand, negatively-charged LF exhibited less retention than intact LF. As LF is released gradually over 24 h, positively-charged liposomes can supply LF for a long period around the joint. Thus, as to the parenteral application of liposomes loaded with LF, the intra-articular administration to rheumatoid arthritis is considered to be a useful approach for enhancement of LF anti-inflammatory potential.

Liposome formulations are also useful to enhance LF activity in oral administration. The stability of LF against gastric digestion can be improved by liposomalization. Ishikado *et al.* reported the effect of liposomalization on LF potential in oral administration using rats with CCl_4-induced hepatitis [76]. LF-containing liposomes composed of phosphatidylcholine and phytosterol (L-LF) were prepared by production of the lipid membrane and subsequent aqueous suspension, and investigated *in vivo* for liver protection. In oral administration, L-LF (580 nm in size) showed a better suppressive effect against liver injury than intact LF (**Figure 5**). Furthermore, when the intestinal absorption was measured following the infusion of LF and L-LF (70 nm in size) into the duodenum of rats, no difference was observed for intestinal absorbability between LF and L-LF. Liposomalization appeared not to enhance the permeability of LF by the intestinal membrane. In fact, LF was absorbed from the intestine much better by intra-duodenal infusion than intra-gastrical one. Namely, LF could be detected more clearly and highly in thoracic lymph and plasma when administered intra-duodenally [49,53]. This indicated that LF could be absorbed easily once it reached the intestine in the intact form. Liposomes seem not to influence the permeability or absorption rate of LF. Rather, liposomalization is presumed to facilitate the LF action by the improvement of stability against gastric degradation and interaction with the intestinal membrane.

Recently, the regulation of cell growth by LF has been reported [35,77]. The binding and internalization of LF lead to the following signaling pathways in the cells. Roseanu *et al.* reported that positively-charged liposomes containing apo-LF inhibited the cell growth of murine melanoma cells *in vitro* [35]. LF affected the cell viability, cell cycle, morphology and so on. Entrapment of LF into positively-charged liposomes enhanced the cytotoxic action of LF. Positively-charged liposomes increased the intracellular accumulation of LF. Furthermore, the liposomes taken up were considered to protect LF from its lysosomal digestion. The increase in uptake and protective effect are associated with the capacity of positively-charged liposomes to promote the antitumor potential of LF.

4.4 Microparticulate formulations

Small particulate enteric formulation of LF (EF-LF) was examined for absorption from the intestine by Takeuchi

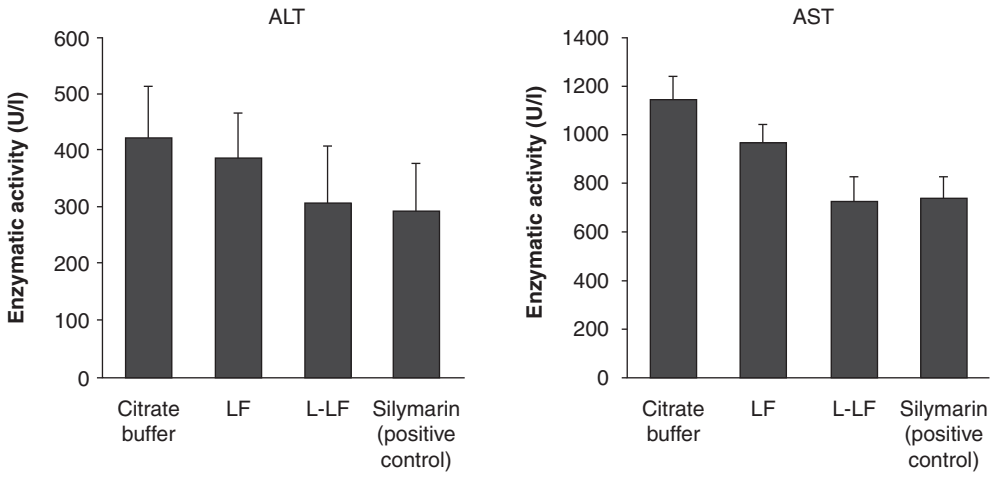

Figure 5. Effect on serum levels of ALT and AST on CCl_4-induced liver injury in rats. Citrate buffer, LF and L-LF were administered orally at 80, 56, 32 and 8 h before CCl4 injection. Silymarin was administered orally 0.5 h before and 4 and 8 h after CCl4 injection. Mean ± s.d. (n = 5).
Adapted from [76].

et al. [53]. EF-LF protected LF from digestion in the stomach. Generally, microcapsules with controlled release ability are useful for resistance to gastric digestion. When LF is administered intra-gastrically, it undergoes degradation enzymatically to a large extent; however, LF is absorbed successfully from the intestine after intra-duodenal administration. LF, having escaped peptic digestion, can reach LF receptors on the intestinal membrane and Peyer's patches. Enteric formulations including an enteric coating are useful to enhance LF activities. When administered intra-gastrically, the transportation of LF to the thoracic lymph is very different between EF-LF and intact LF. LF amount in thoracic lymph is much higher with EF-LF than intact LF. In contrast, after intra-duodenal administration, lymphatic LF levels are almost the same between EF-LF and intact LF, suggesting that the protection against gastric digestion is important for the delivery of LF to the intestinal receptors. EF-LF is an excellent oral administration system to efficiently deliver LF to the intestinal membrane.

The author has developed a microparticulate delivery system of LF as an appropriate LF therapeutic system. Although various microparticles could be prepared for encapsulation of LF, their particle characteristics, such as size, physical stability and release rate, are important to enhance LF activity. It is important to optimize the conditions of microparticles so that they can deliver LF efficiently to the target, for example, intestinal LF receptors. Chitosan microparticles with a high content of LF were developed [78]. They may be degraded or dissolved at acidic pH in the stomach because chitosan can swell and be dissolved at acidic pH; therefore, these microparticles may be appropriate for topical application due to their high LF content, rather than oral administration.

In order to complete the effective delivery of LF to intestinal LF receptors, the microparticulate delivery system must be stable in the stomach, and the release of LF has to be controlled. We focus on LF-loaded chitosan/aligane/Ca complex microparticles (Ch/Al/Ca-MP). Their size ranged from 1 to 2 µm. Ch/Al/Ca-MP exhibited good physical stability at both stomach and intestinal pHs [79]. The release rate of LF from Ch/Al/Ca-MP was controlled by the concentration of chitosan in the preparation of the microparticles. Treatment with chitosan at the concentrations of 0.25 – 0.5% (w/v) was adequate for the gradual release. A high concentration of chitosan accelerated the release of LF probably because chitosan should compete with LF for interaction with alginate due to their same electric charge. These physicochemical properties proposed that LF should be protected from stomach harsh environment and released in the intestinal region. Ch/Al/Ca-MP (mean size = 1.65 µm, mean LF content = 22% (w/w)) were evaluated *in vivo* for its anti-inflammatory therapeutic potential by the pretreatment using rats with carrageenan-induced edema [80]. As a result, the anti-inflammatory potential was greater in the order of Ch/Al/Ca-MP > LF solution > saline (control) (**Table 2**) [81]. The promotion of anti-inflammatory effect with Ch/Al/Ca-MP is considered to be due to the effective delivery based on their protective effect and close approach to the intestinal membrane. Microparticulate formulations are suggested to be useful candidates to improve the activity of LF in oral administration.

5. Expert opinion

LF is proposed to be a possibly excellent therapeutic agent because it is exerts various useful biological functions and is non-toxic and low-cost; however, as its potential is not necessarily high and it degrades rapidly in the body, frequent dosing for a long period is required in order to achieve good

Table 2. Anti-inflammatory effects of LF solution and Ch/Al/Ca-MP on carrageenan-induced edema in rats.

Time (h) after carrageenan injection	Ratio to the initial hind paw volume (mean ± s.e.)*		
	Control (saline)	LF solution	Ch/Al/Ca-MP‡
0	1 ± 0	1 ± 0	1 ± 0
0.5	1.144 ± 0.072	1.318 ± 0.071	1.301 ± 0.076
1	1.240 ± 0.103	1.339 ± 0.027	1.320 ± 0.097
2	1.555 ± 0.058	1.510 ± 0.010	1.415 ± 0.048
3	1.595 ± 0.072	1.511 ± 0.042	1.414 ± 0.101
4	1.559 ± 0.072	1.472 ± 0.016	1.377 ± 0.034
5	1.502 ± 0.041	1.379 ± 0.056	1.284 ± 0.059§
7	1.354 ± 0.052	1.303 ± 0.025	1.188 ± 0.017§

Adapted from [81].
*n = 3 for each group.
‡Ch/Al/Ca-MP (thoroughly washed) was used.
§$p < 0.05$ vs control (Dunnett's *post hoc* test).
Ch/Al/Ca-MP: Chitosan/aligane/Ca complex microparticle.

efficacy. Generally, in repeated or long use, non-parenteral administration is better than parenteral dosing because the burden on patients is expected to be much lower with the former administration. In particular, the oral dosage form is promising as the dosing can be conducted easily and safely. In the case of LF, oral administration is effective as its target receptors are distributed in the intestinal membrane. Recently, it has been realized that the therapeutic potential of LF is importantly associated with the activation of GALT. Many immune system-related cells, such as M cells, monocytes, macrophages and lymphocytes, have LF-specific receptors. The binding and uptake of LF via its receptors trigger the activation of various immune responses; therefore, efficient delivery of LF to those intestinal target sites is proposed to be useful to improve the therapeutic potential. As LF is susceptible to peptic digestion in the stomach and not easily access the intestinal target site due to a high viscous mucus layer, the delivery systems with the ability to overcome these obstacles are required. Microparticulate systems such as polymeric microparticles and liposomes are suggested as a useful candidate. Microparticles loaded with protein drugs such as insulin appear to enhance absorption by protecting it from digestive enzymes and facilitating the accessibility of the protein to the mucosal membrane; small particles can penetrate the mucus layer more easily and are retained there long in addition to avoiding gastric digestion. Also, such high targeting ability and controlled release can lead to a reduction of the dosing amount and frequency. Moreover, healthcare functions of LF such as anti-oxidative stress effect are very useful in the current ageing society. LF is also useful as an agent to support conventional drugs because LF is very safe and strengthens the body defense systems. Oral delivery systems such as microparticles, as stated above, which can enhance the activity and improve the administration manner, can be provided as better formulations for LF.

Declaration of interest

The author states no conflict of interest and has received no payment in the preparation of this manuscript. The author alone is responsible for the content and writing of this paper.

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