In vivo fate of lipid-based nanoparticles

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The *in vivo* fate of lipid-based nanoparticles (LBNs) is essentially determined by the properties of their lipid compositions. LBNs are rapidly degraded via lipolysis wherever lipases are abundant, especially in the gastrointestinal tract. LBNs that survive lipolysis can be translocated through the circulation to reach terminal organs or tissues. Lipid composition, particle size, and surface decoration, as well as the formation of protein corona, are the main factors influencing the *in vivo* fate of LBNs. As we discuss here, elucidation of the *in vivo* fate of LBNs helps weigh the balance between lipolysis and biorecognition, and is emerging as a new field of research.

Introduction

LBNs have received much attention from the drug delivery community recently because of their excellent biocompatibility, efficient permeation enhancement, ease of scaling-up, and wide applicability. Typical LBN formulations include solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid–drug conjugates (LDCs), and nanoemulsions (NEs), all of which primarily comprise physiological lipid analogs with surfactants as stabilizers [1]. Lipid hybrids of either organic or inorganic materials are recent additions to the LBN family [2,3]. LBNs have shown potential for drug delivery via various administration routes, especially for the oral delivery of poorly water-soluble drugs, because of their capability to mimic the digestive process of food lipids [4,5]. In addition, LBNs can also work as nanocarriers to deliver drugs to specific tissues, such as tumors, through either passive or active targeting [6].

The underlying mechanisms of lipid-based drug delivery systems have been surrounded by controversy for several years. Some researchers ascribe improved oral absorption mainly to the permeation of LBNs as integral particles across intestinal epithelia [7], whereas others favor an explanation of lipolysis and the subsequent transformation of LBNs into easily absorbable mixed micelles [8]. The accurate identification of integral LBNs *in vivo* is important to shed light on this controversy and, therefore, exploration of the *in vivo* fate of LBNs is emerging as a new field of research, as is true for other types of nanoparticles (NPs).

Some recent studies have focused on the *in vivo* fate of NPs using various biosensing tools, such as fluorescent imaging, computed tomography (CT), and magnetic resonance imaging (MRI) [9,10]. However, it has been difficult to compare the evidence obtained in many cases because of differences in experimental conditions and imaging technologies, as well as inaccurate deductions from unclear results. In this review, we summarize and analyze recent findings on the *in vivo* fate of LBNs to clarify some of the fundamental concepts relating to these important nanocarriers.

Overview of the *in vivo* fate of LBNs

Lipolysis by lipases

Aliphatic esters in LBNs can be readily degraded by lipases, especially in the small intestine, where pancreatic lipases are abundant [11]. Triglycerides, which are common components of LBNs, are first broken down into diglycerides, then to monoglycerides, and finally to fatty acids, in the gastrointestinal tract (GIT) [12]. Then,

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both lipolysates and encapsulated drugs are transferred via secondary carriers, such as vesicles and cubic nanocarriers, to epithelial surfaces for absorption [1]. Although lipolysis has been well accepted as the primary mechanism that governs the *in vivo* fate of LBNs, most results relating to lipolysis have been obtained by using either *in vitro* or *ex vivo*, rather than *in vivo*, models because it is difficult to identify LBNs or lipolysates in the GIT [1]. Recently, Hu *et al.* developed an environment-responsive dye and used it to visualize the *in vivo* lipolysis of SLNs [13]. *In vivo* live imaging indicated predominant digestion within 2 h in the GIT of mice, correlating well to *in vitro* data (Fig. 1) [13]. However, it is difficult to determine whether lipolysis should be fast or slow. In the case of oral bioavailability enhancement for poorly water-soluble drugs, faster lipolysis is more favorable because of the faster transformation into mixed micelles and thereby faster absorption. However, whenever the integrity of LBNs is important, the rate of lipolysis should be slowed. It has been reported that incorporating nonionic surfactants with PEG tails attenuates the lipolysis of LBNs [1]. The inhibitory effect of PEGylated surfactants on lipolysis increases in tandem with an increase in PEG chain length [14]. Recently, an *in vitro* digestion – *in vivo* absorption model was developed and used to better understand the interplay between drug solubilization, supersaturation, precipitation, and absorption [8].

Lipolysis of LBNs can also occur in tissues other than the GIT, even within cells. Triglycerides in LBNs can be degraded by lysosomal acid lipase (LAL) following endocytosis [15]; an *in vitro* lipolysis test indicated that most lipids can be degraded within 2 h by LAL [15]. Fluorescently labeled LBNs have been found to accumulate in late endosomes and lysosomes following endocytosis and then to be degraded and cleared rapidly. In addition, most degraded lipids in cells can be excreted within 24 h with approximately 50% of the original level detected in both urine and feces [16].

Erosion of the lipid matrix

In addition to lipolysis, surface erosion because of hydrolysis or dissolution of the lipid matrix also contributes to the overall degradation profile of LBNs, especially for lipid matrices based on fatty acids, which are not sensitive to lipolysis. The erosion rate is usually slow and declines along with the increase in chain length of fatty acids [17]. As a result, the drug release rate is generally slow. Surfactants in the medium, such as sodium dodecyl sulfate (SDS) and bile salts, significantly enhance the erosion rate of the lipid matrix, thereby accelerating drug release [18]. In general, surface erosion is less important than is lipolysis in determining the *in vivo* fate of LBNs.

Pharmacokinetics and biodistribution of integral particles

Most reports on the pharmacokinetics and biodistribution of LBNs are based on the monitoring of drug levels [5,19]. A few pilot studies have investigated the behavior of LBNs by using molecular imaging. However, probes can easily escape from the NP matrix but still retain the same fluorescent patterns. Nevertheless, it is assumed that as long as the probes are tightly embedded in the lipid matrix, their signals can be used to represent integral NPs. Recent work by Mérian *et al.* compared two different cyanine dyes (DiD and ICG) and found that ICG leaked quickly in blood whereas there was almost no leakage for DiD from LBNs [20]. This disparity led to highly differentiated biodistribution profiles (Fig. 2), with that of DiD considered reliable enough to represent integral LBNs. Another report also demonstrated the discrepancy between radioactive and fluorescent signals of the biodistribution patterns of lipid/calcium/phosphate NPs [21]. By securing radioactive probes in the lipid matrix, the pharmacokinetics and biodistribution of integral LBNs can also be evaluated. Following intravenous administration, there was little difference in the pharmacokinetics and biodistribution of radio-labeled NLCs of different particle sizes (ranging from 150 to 480 nm) and surface charges (ranging from –20 to 44 mV) [22]. Inhaled LBNs labeled with ^{99m}Tc were prone to be translocated through the interstitial space to the lymphatic system [23]. In addition, the concept of physiologically based pharmacokinetics (PBPK) has been introduced, although this still requires experimental verification [24].

Uptake and translocation of integral LBNs

Whether integral LBNs can be taken up and transported across biomembranes remains a matter of debate. Yuan *et al.* used FITC-conjugated lipids to label SLNs and found that integral SLNs could be absorbed orally into the systematic circulation via lymph, reaching levels of 77.9% absorption [25]. By contrast, results from Chen *et al.* suggested that integral NPs was not taken up by the intestinal epithelia, although the drug (exendin-4) was [26]. This disparity results from the use of undiscriminating probes, such as FITC, which still emit fluorescence even after escaping from NPs. A recent study used a more accurate strategy to detect signals of integral SLNs using a novel environment-responsive near-infrared fluorescent probe. Evidence indicated that the translocation of integral SLNs across intestinal epithelia was negligible [27].

Factors influencing the in vivo *fate of LBNs*

Based on the above discussion, it is assumed that factors influencing either lipolysis, biorecognition, or erosion can affect the *in vivo* fate of LBNs.

Lipid composition

Lipid composition affects the surface properties and degradation rate of LBNs and, therefore, their *in vivo* fate. For example, LBNs made from Compritol 888 ATO noticeably promoted lymphatic transport compared with LBNs based on stearic acid, monostearin, and tristearin [28]. In another study, transdermal delivery was found to be significantly affected by the chain lengths of fatty alcohols in Precirol®-based LBNs [29]. Similarly, although cationic lipids did not influence cell uptake dramatically, small interfering (si)RNA-loaded LBNs comprising cationic lipids that were insensitive to digestion by lipases exhibited higher potency of gene silencing compared with digestible lipids [30]. Thus, it appears that screening of lipids is necessary during the early stages of formulation optimization.

Particle size

Although the *in vivo* behavior of LBNs is dominated by a variety of factors, particle size and surface properties are among the most studied. Generally speaking, NPs smaller than 100 nm are able to circulate longer in the circulation because of their enhanced ability to escape capture by macrophages [31]. However, Hirsjärvi

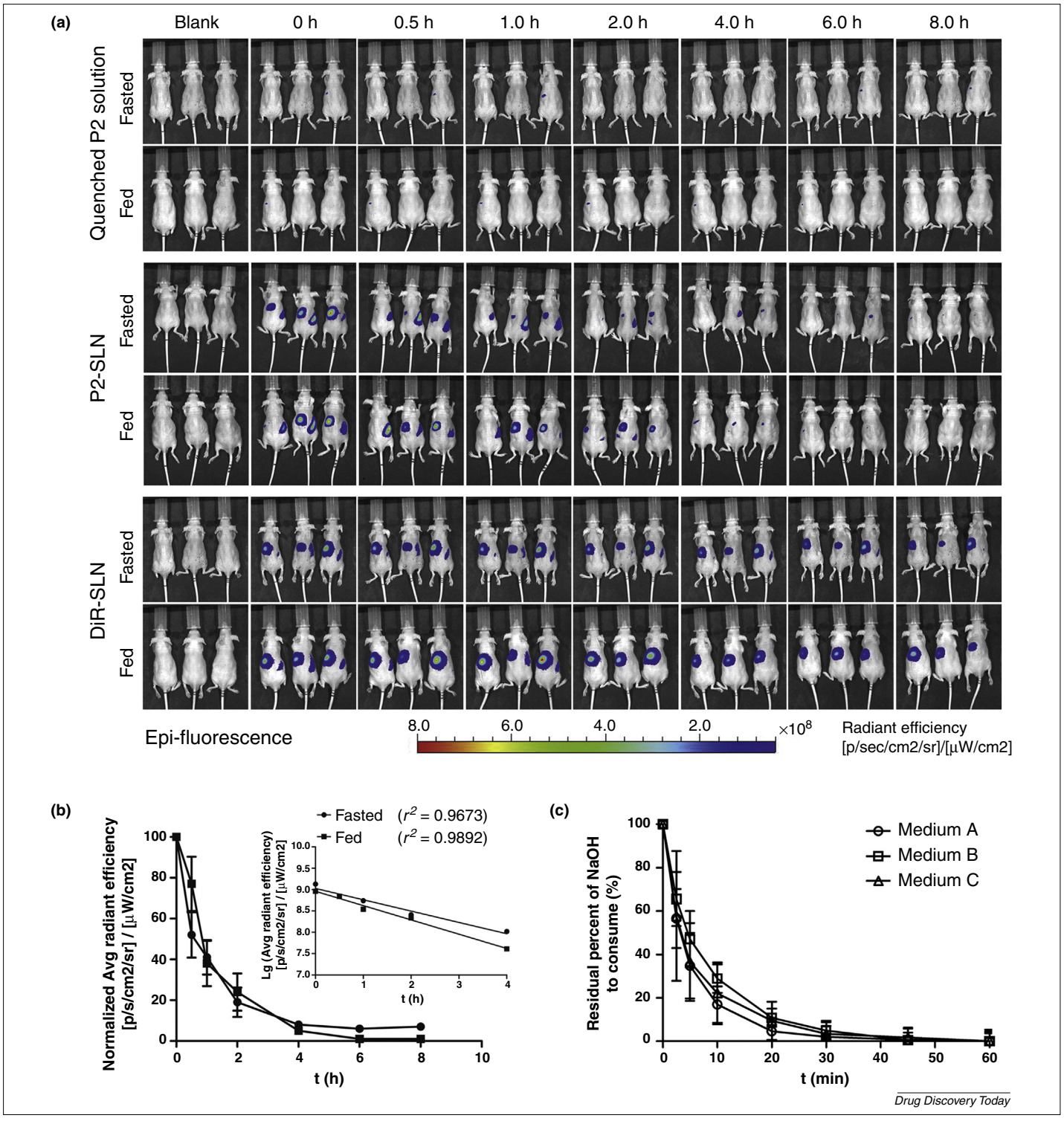

FIGURE 1

Lipolysis predominates the in vivo fate of LBNs in the GIT. **(a)** *In vivo* live-images of nude mice after gavage administration of solid lipid nanoparticles (SLNs) labeled with the environment-responsive dye P2 compared with quenched P2 solution and SLNs labeled with a conventional dye DiR. P2 emits fluorescence when embedded in SLNs but quenches immediately upon release into ambient aqueous phases. **(b)** Quantification of average radiant efficiency (ARE) of live images reveals first-order lipolysis kinetics *in vivo*. **(c)** Good *in vitro in vivo* correlation (IVIVC) occurs between ARE and residual NaOH consumption during *in vitro* lipolysis. *Source*: Adapted, with permission, from Ref. [13].

et al. showed that LBNs of 25 nm disappeared from the circulation faster compared with larger LBNs of 50 nm and 100 nm because of faster elimination and extensive tissue distribution [9]. This discrepancy reminds us that other factors, such as surface properties, can have a more important role in determining the *in vivo* fate of LBNs. The digestion rate of LBNs increases in parallel with particle size reduction because of the significantly increased surface area available for lipolysis [32]. Furthermore, smaller LBNs more easily

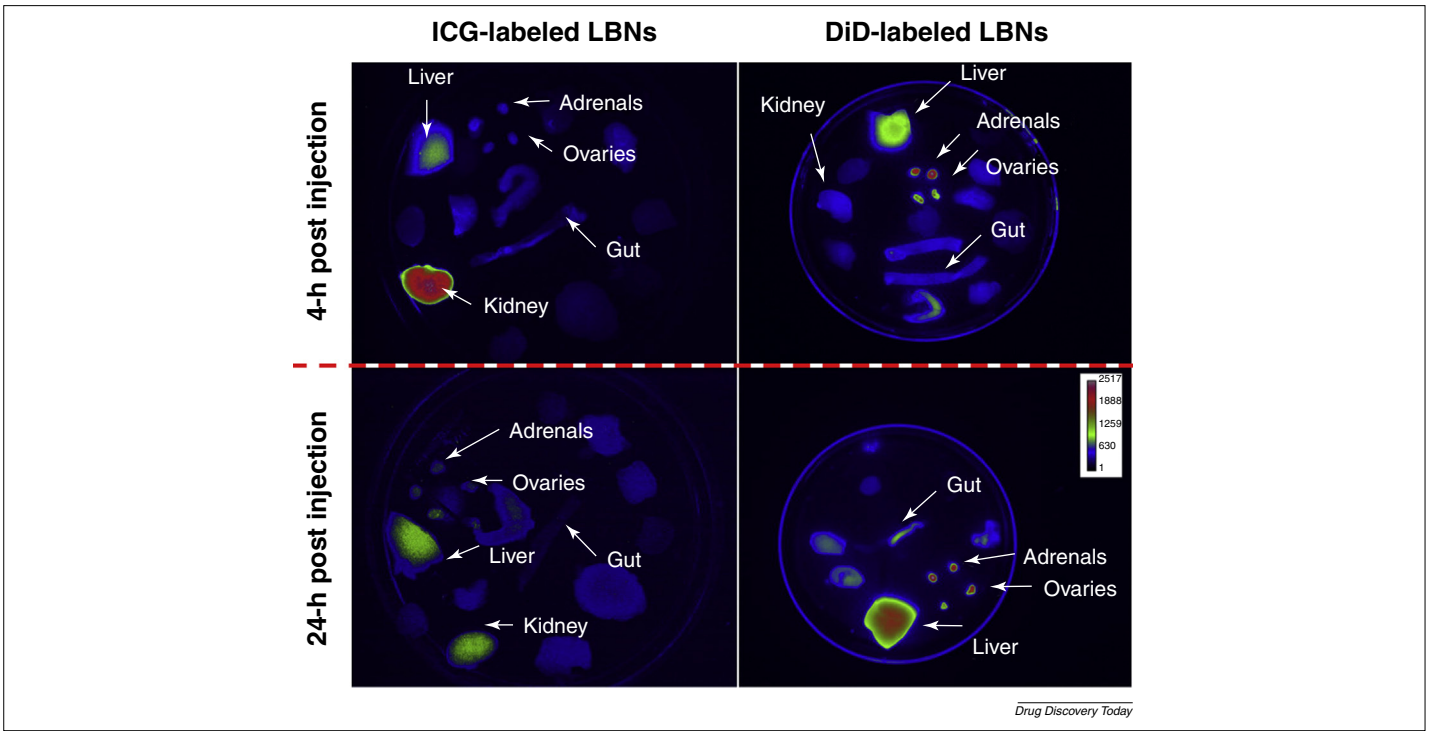

FIGURE 2

Fluorescent images of dissected organs obtained 4 h and 24 h after tail vein injection of ICG- and DiD-labeled lipid-based nanoparticles (LBNs), highlighting the different biodistribution profiles that result from the distinct properties of the fluorescent probes used.
Source: Adapted, with permission, from Ref. [20].

penetrate the pulmonary mucus, increasing their chances of being transporting across the epithelia as integral particles [33].

Surface decoration

To modulate the performance of LBNs both *in vitro* and *in vivo*, LBNs are commonly coated with polymers or other materials. Surface engineering helps to improve the stability of LBNs or to impart special surface properties: coating NEs with chitosan, alginate, or proteins improved their physical stability [34]; coating SLNs with chitosan improved their stability, mucoadhesiveness, and cellular uptake in the GIT [35]; and coating NLCs with hyaluronic acid not only prolonged circulation time, but also enhanced accumulation in tumors overexpressing CD44 [36]. Surface coating with PEGs also provides good protection, because it slows down the lipolysis rate of LBNs in the GIT [14] or enables them to evade uptake by macrophages, resulting in prolonged circulation time [37]. However, PEGylation is a two-edged sword; as well as camouflaging the particles, it also inhibits their cellular uptake and subsequent endosomal escape [38]. In agreement with conventional vesicles, such as liposomes, PEGylation of SLNs leads to the accelerated blood clearance (ABC) phenomenon upon repeated injection [39]. Interestingly, Yuan *et al.* demonstrated that PEGylated SLNs can penetrate the mucus rapidly, be absorbed through enteric epithelia, and achieve a prolonged effect *in vivo* [7,25].

However, some factors might not affect the performance of LBNs directly. Some formulation variables work by affecting the formation of the 'protein corona'. The formation of the protein corona has been regarded as a crucial intermediate procedure in determining the *in vivo* fate of various NPs. The switch from the clathrin- to the caveolae-mediated endocytosis pathway might result from the formation of larger aggregates of cationic LBNs because of the adsorption of plasma proteins [40]. In some instances, the adsorption of certain proteins might act as a triggering factor for more efficient biorecognition; for example, a vitronectin corona mantling DOTAP/DNA LBNs significantly improved the uptake efficiency by cancer cells expressing high levels of the vitronectin $\alpha_v\beta_3$ integrin receptor [41]. There is evidence that components or surface properties of LBNs have an important role in determining the type of protein corona. Replacing cationic DOTAP with neutral DOPE reduced the adsorption of fibrinogen, prothrombin, vitamin K, and vitronectin onto the surfaces of LBNs; DOPE favored the adsorption of apolipoproteins and serum albumin, whereas cholesterol favored immunoglobulins and complement proteins [42].

Physiological factors

A variety of physiological factors can affect the *in vivo* behavior of LBNs. However, because of physiological complexity, especially in the GIT, and a lack of reliable models, the study of physiological factors is difficult. After ligation of the biliary duct in a rat model, the degradation of a microemulsion formulation was inhibited completely, and the oral absorption of the drug payloads was impeded significantly [43]. By active manipulation of GIT movement, the survival duration of SLNs in the GIT was prolonged, as observed in rats fed high-fat food to slow down gastric empting or orlistat to inhibit lipases [27]. Physiopathological conditions can sometimes significantly alter the fate of NPs. For transdermal delivery, SLNs can penetrate deeper and in greater amounts through damaged skin following treatment with 25 successive

tape strippings to partially remove the stratum corneum [44]. By contrast, the stratum corneum is thickened in patients with psoriasis and can notably hamper the penetration of topically applied materials [45].

The in vivo *fate of LBNs administered via different routes*

Oral administration

Fig. 3 depicts the processes that can affect either LBNs or drugs in the GIT after oral administration. Most LBNs can retain their structural integrity because of the lack of lipases in the stomach, where they are rarely absorbed [27]. Following gastric emptying, LBNs are transported to the small intestine, where they are subsequently broken down by lipases [8]. However, the full details of what happens to LBNs in the small intestine have not been fully elucidated. It is hypothesized that lipolysates of LBNs form secondary nanocarriers, such as liquid crystal vesicles, multilamellar vesicles, and mixed micelles. The drugs can be released as free molecules or can be transferred to secondary nanocarriers before absorption. There is the possibility of precipitation of the drug during either lipolysis or the transferring process. However, there is no direct evidence of the precipitation of drugs in the GIT. Whether drugs can be absorbed as free molecules by passive diffusion, through fusion of the secondary nanocarriers with enteric epithelia, or through uptake as integral LBNs needs to be clarified. Evidence shows that both LBNs and simulated mixed micelles can be trapped within the mucus lining, whereas micelles have the ability to penetrate the enteric epithelia because of their relatively small size [27]. Bioimaging evidence both *in vitro* and *ex vivo* has shown the negligible absorption of LBNs as integral particles [27].

Intravenous administration

Intravenously administered LBNs follow the common fate of noncamouflaged particles, that is, adsorption of opsonins, recognition by the reticuloendothelial system (RES), and accumulation in RES organs, such as liver, spleen, lung, and kidney [38]. Most LBNs that reach the liver are broken down there and then eliminated [16]. PEGylation helps to prolong the circulation time and evade uptake by the RES [38], diverting LBNs to nonRES tissues, such as the brain [46]. The adsorption of blood proteins, such as apolipoproteins, facilitates the adherence of SLNs to endothelial cells of the blood–brain barrier (BBB) [47]. Although Kakka *et al.* reported 'proof-of-concept' for the translocation of SLNs to the brain, their conclusions were significantly weakened by the fact that the fluorescent or radioactive probes used to label SLNs can dissociate, resulting in pseudo-positive interferences [48]. Intracellular fate studies both *in vivo* and *in vitro* have indicated that LBNs could be taken up into cells by clathrin-mediated endocytosis as well as macropinocytosis, and then become embedded in lysosomes [49], where most LBNs would be degraded by LAL to produce lipolysates [50]; these would then be released into the cytosol, metabolized, eliminated from cells, and finally excreted from the systemic circulation [51].

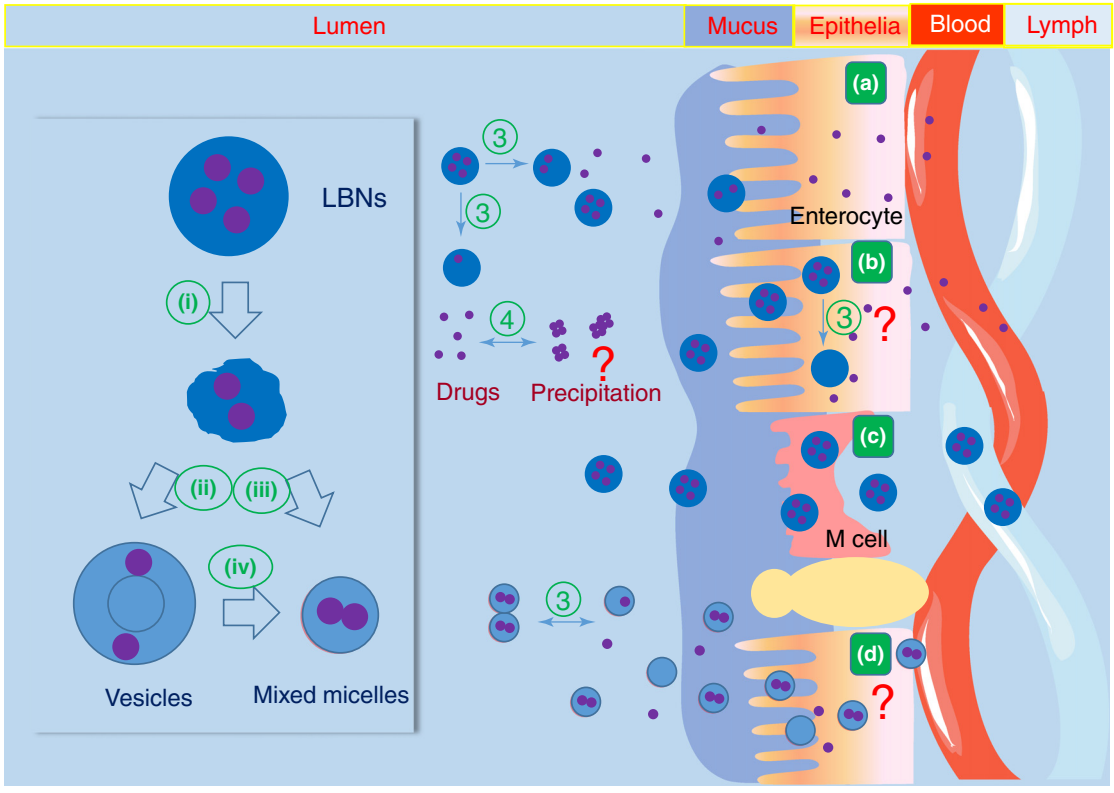

FIGURE 3

Schematic delineation of the *in vivo* fate of lipid-based nanoparticles (LBNs) and encapsulated drugs via oral delivery. **(a)** Absorption of drugs by passive diffusion; **(b)** absorption of integral LBNs through enterocytes; **(c)** translocation through M cells; **(d)** absorption through mixed micelles: ① lipolysis; ② transformation into secondary carriers; ③; release of the drugs; and ④ precipitation of the drugs. '?' refers to processes that await verification.

Pulmonary administration

LBNs with an optimum aerodynamic size of approximately 1–5 μm can be deposited in alveoli via pulmonary administration [52]. Particles that fail to deposit in one breathing cycle are exhaled. There are several possibilities regarding the pulmonary fate of LBNs, including degradation at the site of sedimentation, mucociliary clearance, uptake by alveolar macrophages, and subsequent lymphatic drainage [53]. Drugs can be absorbed after release from LBNs or can be taken up while encapsulated in LBNs. The pulmonary delivery of LBNs results in the extended residence of LBNs and a relative high drug concentration in the lung compared with intravenous administration [54]. However, scavenging capacity of pulmonary macrophages can clear particles rapidly from the lung via the lymphatic system.

Ophthalmic administration

LBNs administered to the eye might interact with the lipid layer of tear films and spread over the corneal surfaces rapidly because of the presence of emulsifiers [55]. Cationic LBNs can further reduce the contact angle and improve their residence time. Some LBNs can penetrate the lipid layer of tear films to the mucus layer, whereas others are prone to becoming trapped in the mucus [56]. In addition, thiol-NLCs significantly increase the adhesion with mucin and further improve their retention time [57]. LBNs can improve the transport of NPs across the cornea by increasing the fluidity of the cell membrane, opening tight junctions, or inhibiting the activity of P-gp on epithelia [58]. However, there is no evidence showing that integral LBNs can be transported to posterior cavities.

Transdermal administration

There are two pathways involved in transdermal delivery: the transepidermal and follicular pathways. Integral particles are generally not considered to be able to permeate the horny layers of the skin. Yet, in one study, NLCs were detected 200 μm into the skin, suggesting the absorption of integral NLCs, although this result is strongly weakened by the fact that free probes also emit fluorescence [59]. A comparison with the control group of free probes might clarify this issue. In addition, particles smaller than 10 μm can be transported efficiently through the follicular pathways because of their high affinity with lipophilic entities therein [60].

Concluding remarks

Although there are now many published observations of the efficacy of LBNs, the paucity of understanding of their *in vivo* behavior is an impediment to their further development and clinical application. In recent years, researchers have begun to appreciate the importance of the *in vivo* fate of various NPs, including LBNs; thus, some light is now being shed on the use of LBNs as drug carriers. Most importantly, LBNs degrade in the body via lipolysis, whose rate depends on the lipid composition, particle size, surface decoration, presence of lipases, and so on. It is also certain that LBNs might not be broken down instantly and can survive for some time, which provides opportunities for them to be used as nanocarriers for drug delivery. However, for any study with LBNs, it will be important to find a balance between the degradation and translocation of LBNs. For oral delivery, lipolysis has been shown to be the governing mechanism for enhanced oral absorption of poorly water-soluble drugs, whereas evidence does not support the absorption of integral LBNs. As for other routes, there is a good chance of the interaction of LBNs with biological tissues and, thus, their translocation throughout the body.

Owing to the readily biodegradable nature of LBNs, it is difficult to secure probes within the LBN matrix. The dissipation of the probes imposes significant interference with the identification of integral LBNs. If undiscriminating probes are used, the released probes exhibit the same signals as the NPs, resulting in interference. Thus, environment-responsive probes are emerging as novel tools to explore the *in vivo* fate of LBNs with more accuracy.

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