Cochleate Formulation Enhances the Stability of Lansoprazole in Acidic Condition

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Lansoprazole (LSP)-loaded cochleates were developed using dimyristoyl phosphatidylcholine (DMPC) and cholesterol in the presence or absence of dicetyl phosphate (DCP). Cochleates containing DCP exhibited unique cylindrical morphologies, resulting in improved LSP loading and enhanced stability in an acidic environment. Thus, we suggest that the use of negatively charged components, such as DCP, is needed to formulate cochleates using a neutrally charged lipid such as DMPC.

Keywords: Lansoprazole, Cochleate, Acidic stability, Dimyristoyl phosphatidylcholine, Dicetyl phosphate

A cochleate is a novel lipid-based formulation used to deliver therapeutic molecules, such as drugs, genes, and vaccines.[1] Cochleates are derived from liposomes whereby the addition of a cation entails fusion and rolling of phospholipids.[2] They present an elongated shape and a carpet roll-like morphology, accompanied by narrowly packed bilayers due to the interaction of phospholipid with divalent cations such as Ca^{2+} as a bridging agent.[2] To form this arrangement, the assembly of bilayers is dependent on the dehydration of the head groups of the phospholipids. Bilayers roll up to minimize their interactions with water, forming cochleates with little or no aqueous phase.[3,4] These multilayered cylindrical structures are stable and can protect encapsulated drugs from external harsh conditions or enzymatic attacks. A previous report described a stable andrographolide-loaded cochleate, which did not show any physical changes even after lyophilization and reconstitution without lyoprotectants.[4]

Lansoprazole (LSP) belongs to proton pump inhibitors, and its chemical designation is 2-{[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methanesulfinyl}-1H-1,3-benzodiazoleis. LSP is one of the effective and well-tolerated treatment options in the management of acid-related disorders.[5] It reduces gastric acid secretion in both animal and human subjects by inhibiting the gastric proton pump (H+, K+-ATPase) in the secretory membrane of parietal cells.[5] Although oral administration is a main route for LSP, oral delivery of LSP is highly limited due to its inherent instability in acidic condition.[6] As shown in Figure 1, LSP has an inherent sulfoxide group and reduction of this group takes place in exposure to acidic environment, resulting in degradation of LSP.[7] Without the protection via enteric coating, LSP is degraded very rapidly under acidic environments, while stable in neutral and alkaline conditions.[6] With this regard, strategies to protect LSP from the destructive effects of gastric acid are necessary. As an alternative approach, in this report, we introduce cochleate formulations to enhance acid-lability of LSP.

Until now, dimyristoyl phosphatidylcholine (DMPC) has been employed to develop a cochleate.

Although DMPC has a negatively charged phosphate group, a choline head group forces it to be electrically neutral. The neutral phospholipid may not form cochleate properly because of a lack of calcium binding. To supplement insufficient negative charge, addition of other lipids with negative charge such as dicetyl phosphate (DCP) may be desirable. LSP-loaded cochleates were successfully prepared by adding $CaCl_2$ to liposomal vesicles composed of DMPC and cholesterol in the presence or absence of DCP as a negatively charged lipid. Developed cochleates were evaluated their characteristics in terms of size, polydispersity index (PDI), encochleation efficiency (EE), and drug loading (DL). As shown in Table 1, size distribution of four cochleates ranged from 251 to 347 nm with large PDI values (0.38–0.46), indicating no homogeneous dispersion. Cochleates tend to have nonuniform size and heterogeneity because they are aggregated and not spherical.[8] In contrast, as a positive control, conventional liposomes were prepared according to the same composition of cochleate B excluding $CaCl_2$ addition, resulted in homogeneous nanodispersion in the size of 121.4 ± 4.1 nm with PDI value of 0.103 ± 0.024.

To visualize morphological aspects of each cochleate, transmission electron microscopy (TEM) was carried out using energy-filtering transmission electron microscope (LIBRA 120; Carl Zeiss Meditec AG, Jena, Germany). As shown in Figure 2, cochleate A (without DCP) showed simple aggregates of liposomes and no cylindrical structures

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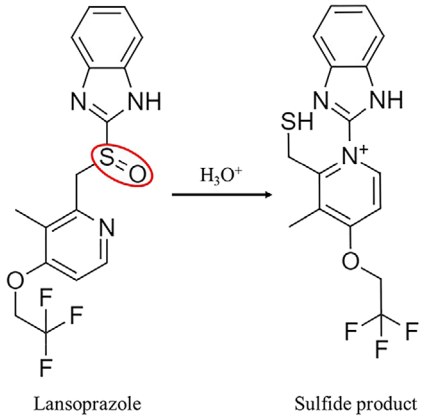

Figure 1. Degradation of lansoprazole in acidic condition.

were found, on the other hand, specific morphologies of cochleates existed in cochleate B, C, and D. These structures were consistent with previous reports, which were fused and rolled-up.[4,9] Because DMPC is a neutral phospholipid, it was difficult to form cylindrical structure without negatively charged component. By adding DCP to DMPC-based cochleate, we were able to find typical cylindrical structures. After mild centrifugation (3000 × g, 10 min), aggregated precipitates were found in cochleates, but not in liposomes possibly due to the solubilizing effect of DMPC and other excipients. The EE and DL values of cochleates ranged from 41.29% to 70.59% and 51.61 μg/mg to 88.24 μg/mg, respectively. These values were greater than those of the liposomes, which had the EE and DL values of 40.85 ± 4.12% and 51.06 ± 0.52 μg/mg, respectively. Because cochleates possess less aqueous phase and more hydrophobic cavities in their structure compared to liposomes, they take advantages of encapsulating hydrophobic drugs. Comparing EE between four cochleates, cochleate A revealed the lowest value. Because cochleate A was aggregated incompletely as shown in the TEM image, it probably reduced the drug entrapment. In comparison, cochleate B, C, and D were successfully formed, thereby enhancing the EE. Comparing three formulations, the order of EE values was cochleate B > cochleate C > cochleate D. This might be attributed to the role of cholesterol. Previous reports figured out that liposomes containing high content of cholesterol stabilized and increased the hydrophobicity of the membrane, which can favor the inclusion of hydrophobic drugs.[10,11]

As depicted in Figure 3, raw LSP immediately degraded after exposure to acidic condition because of its acid-labile property. However, the cochleates protected LSP to a great extent, even though the residual amounts of LSP were decreased with time. Among four formulations, cochleate C and D exhibited the greatest acidic stability, showing over 30% of residual LSP for 2 h. DCP which affords a negative charge to liposomes enabled more rigid interactions with divalent cations than DMPC-only liposomes, thereby resulting in greater stability of LSP against acidic condition. Besides degradation of LSP, the decrease behaviors in four cochleates could be attributed to release of LSP. Thus, in order to investigate how much drugs were released, by replacing the acid medium, a comparative study was carried out in distilled water, in which LSP is expected to be sufficiently stable. On the other hand, residual LSP decreased in four cochleates; however, the values were significantly higher than those of the acidic condition. Further, LSP concentration in supernatant was analyzed: 44.2–57.5% of LSP was released from cochleates for 2 h. Sum of residual LSP and released LSP converged to 100% for all cochleate formulations, suggesting that degradation of LSP did not occur in distilled water. On the contrary, in the acidic condition, LSP was not detected in the supernatant owing to rapid degradation of LSP. Controlled release behavior is disclosed as another important factor. By comparing the residual amount of LSP at 2 h in both media, we found that 42.6% (cochleate A), 48.5% (cochleate B), 61.0% (cochleate C), and 58.9% (cochleate D) of encochleated LSP were protected from external acidic environment.

Alternatively, visual observation in naked eyes was carried out during stability study (Figure 4). Cochleate B was selected as a representative formulation. In distilled water, both raw LSP and cochleate B showed no signs of

Table 1. Composition of various cochleates and their characteristics.

	Cochleate A	Cochleate B	Cochleate C	Cochleate D
Composition (mg)				
LSP	1	1	1	1
DMPC	6	6	6	6
Cholesterol	2	1.5	0.5	0
DCP	0	0.5	1.5	2
$CaCl_2$	Lipid:$CaCl_2$ = 1:4 (molar ratio)			
Characteristics				
Size (nm)	251.6 ± 142.2	278.2 ± 97.4	311.1 ± 112.7	347.6 ± 157.2
PDI	0.457 ± 0.214	0.377 ± 0.154	0.437 ± 0.212	0.385 ± 0.102
EE (%)	41.29 ± 0.58	70.59 ± 1.60	57.14 ± 1.34	53.12 ± 1.63
DL (μg/mg)	51.614 ± 0.06	88.24 ± 0.17	71.42 ± 0.14	66.40 ± 0.17

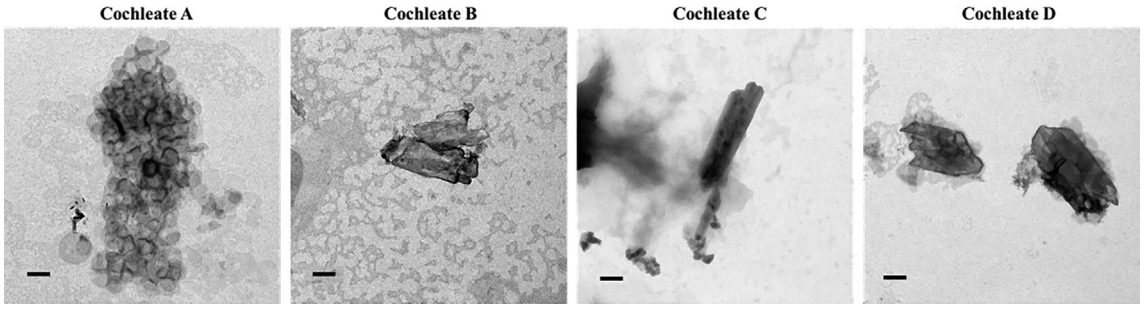

Figure 2. TEM images of four cochleates. Size bars represent 0.1 μm.

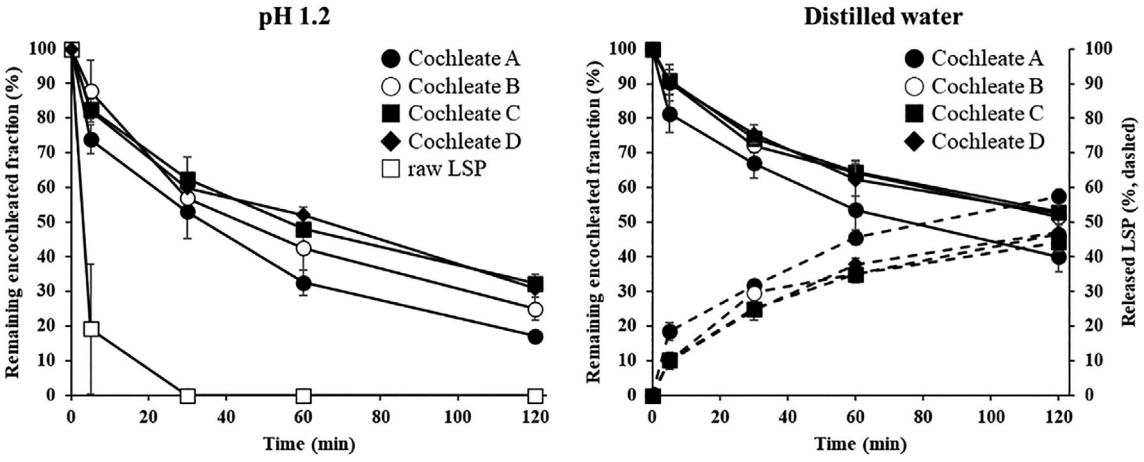

Figure 3. Plots of the amount of LSP against time in pH 1.2 medium and distilled water. Solid and dashed lines represent the residual amount of LSP and released amount of LSP, respectively.

degradation, maintaining their initial color and transparency (raw LSP) or turbidity (cochleate). In contrast, in the pH 1.2 medium, color changes were observed after 1 h, indicating the degradation of LSP. Especially, raw LSP turned into deep yellow color, whereas cochleate B revealed a mild color change, suggesting the relevant stabilization effect of cochleation. These results were consistent with outcomes of acidic stability test, in which LSP loss was significantly reduced by cochleate formulation.

In summary, four cochleates showed unique structures, which possess protective effect against external acids. Particularly, DCP effectively interacted with Ca^{2+}, thereby cochleate containing DCP reflected cylindrical morphologies, higher EE, and improved acid-protective effect. Thus, we suggest that the use of negatively charged component such as DCP is needed to formulate cochleates using neutrally charged lipid such as DMPC. However, to apply LPS-loaded cochleate for practical product development, further optimization is still needed for not only stabilizing acid-labile drug molecules but also controlling the release of drugs.

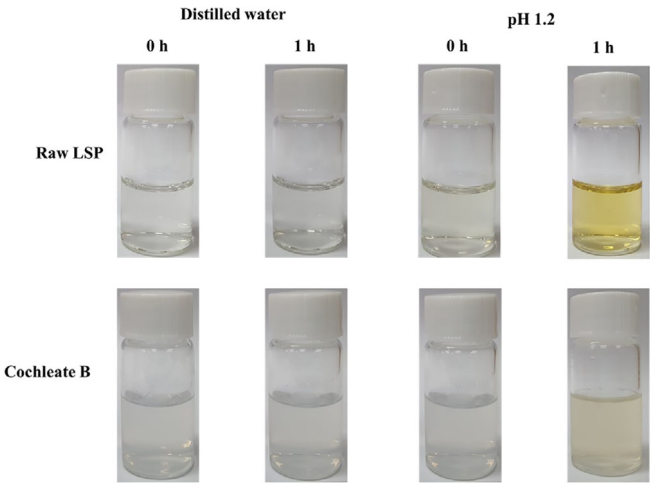

Figure 4. Visual comparison of raw LSP and cochleate B during acid-stability study.

Experimental

A thin lipid film hydration method was used to prepare liposomal vesicles.[12] Briefly, the specific amount of DMPC, cholesterol, and DCP were dissolved in chloroform, and LSP was dissolved in methanol. After blending these mixtures, organic solvent was removed via rotary vacuum evaporation at 40 °C and solvent traces were completely excluded under a nitrogen gas stream. Then,

thin film was hydrated with 2 mL of distilled water and sonicated using a probe type sonicator (Sonoplus, HD 2070; Bandelin Electronics, Berlin, Germany) operating at 50% power for 3 min under cooling with ice. Successively, cochleates were prepared by the trapping method as previously reported.[9] Accordingly, calcium chloride solution (100 mM) was slowly added to LSP-loaded small unilamellar vesicles under vigorous stirring until the vesicle phase turned turbid owing to cochleate formation.

Droplet size and PDI were measured using a dynamic light-scattering particle size analyzer (Zetasizer Nano-ZS, Marlvern Instruments, Worcestershire, UK). For EE and DL measurement, each LSP-loaded cochleate suspension was centrifuged at 16 000 × g for 20 min to separate cochleates from the supernatant. The sediment vesicles were disrupted with methanol to break up vesicles and release the entrapped drugs. Separately, LSP-loaded liposome suspension was subjected to ultrafiltration using Amicon® ultra-centrifugal filter (MWCO 100 000). The percent EE and DL were calculated as follows: EE (%) = (amount of drug entrapped in cochleates or liposomes/total amount of the drug initially added) × 100; DL (μg/mL) = amount of drug entrapped in cochleates or liposomes/total amount of lipid.[7,13] Analysis of LSP was carried out using high-performance liquid chromatography (HPLC) as previously reported.[14] The isocratic mobile phase consisted of 10 mM phosphate buffer and methanol (45:55 vol/vol), then LSP detection was achieved at 285 nm.

For acid-protective study, specific amount of cochleates equivalent to 0.5 mg LSP was dispersed into 5 mL of distilled water or pH 1.2 medium with gentle shaking at 37 °C, and sampled at 5, 30, 60, and 120 min, followed by centrifugation. In addition, the residual amount of LSP in pellets and the released amount of LSP in supernatant were quantified using HPLC.

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