약제학회지(2009), 제 39 권 제 6 호
J. Kor. Pharm. Sci., Vol. 39, No. 6, 417-422 (2009)

Budesonide Microemulsions for Enhancing Solubility and Dissolution Rate

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(Received October 10, 2009 · Revised November 7, 2009 · Accepted November 13, 2009)

ABSTRACT – Budesonide belongs to Class II in the Biopharmaceutics Classification System (BCS) for its high permeability and poor aqueous solubility. The purpose of this study was to improve the solubility and dissolution rate of budesonide using an o/w microemulsion system in order to develop a nasal formulation. Based on the results of the solubility study and pseudo ternary phase diagrams, microemulsions of about 80 nm in mean diameter were formulated using isopropyl myristate and Labrasol® as an oil phase and a surfactant, respectively. Solubility of budesonide in the microemulsions increased up to 6.50 mg/mL, which is high enough for a nasal formulation. *In vitro* release profiles of budesonide significantly increased from the microemulsions compared to that of the budesonide powder. These results suggest that the microemulsions of budesonide could further be developed into a clinically useful nasal formulation.

Key words – Budesonide, Microemulsion, Solubilization, In vitro release

A broad range of medications exists for the treatment of patients with allergic rhinitis which is characterized by symptoms of sneezing, nasal itching, rhinorrhea, and congestion.[1)] Treatments include the use of oral and nasal antihistamines, intranasal corticosteroids, oral and nasal decongestants, topical anticholinergics, leukotriene modifiers, and nasal mast-cell stabilizers.[2)] Among these, intranasal corticosteroids are known to be the most effective treatments, which is most likely related to multiple pharmacologic actions.[3)] Studies have shown that these intranasal corticosteroids down-regulate the recruitment and influx of inflammatory cells, and inhibit the secretion of proinflammatory mediators during the late phase of the inflammatory response.[4)]

Budesonide is a novel glucocorticoid with high topical anti-inflammatory activity and low systemic activity due to its high affinity to the steroid receptor and rapid conversion to metabolites with minimal or no steroid activity.[5)] Budesonide is well absorbed through the nasal mucosa and once it is in the systemic circulation, 88% will be bound to plasma albumin. It is rapidly converted to different metabolites of which 16-hydroxyprednisolone is the major one in human.[6)] Its $t_{1/2}$ after intranasal administration (2.9 hr) is similar to that after intravenous administration (2.3 hr).[7)]

Because budesonide can be easily inactivated in the liver, an alternative route which can avoid the first-pass effect thereby enhancing the cellular uptake in the site of action is necessary. In recent years, the nasal route has received a great deal of attention as a convenient and reliable method for drugs which especially are ineffective as an oral formulation.[8)] This is due to the large surface area, porous endothelial membrane, high total blood flow, avoidance of first-pass metabolism and ready accessibility.[9)] Thus, in modern pharmaceutics, the nose has been considered primarily as a route for local drug delivery. And, although mucociliary clearance can shorten the residence time, low absorption of drugs can be countered by using absorption enhancers or mucoadhesive polymers.[10)]

However, because of the limitation of the nasal cavity volume, high drug content is needed and due to this, solubility could be a problem. Microemulsions have been used to overcome this solubility problem since they can solubilize both polar and nonpolar substances.[11)] Microemulsions are thermodynamically stable and optically isotropic transparent colloidal systems consisting of water, oil, surfactant and co-surfactant. Fortunately, intranasal microemulsions have been reported for allergic rhinitis. These microemulsions could attenuate allergen challenge-induced nasal symptoms and plasma exudation by modifying the allergen-mucosa interaction.[12)] By virtue of their lipophilic nature and low globule size, microemulsions are widely explored as a delivery system to enhance uptake across the mucosa and therefore could be used for the formulation of budesonide.[13)] Herein we report on the development of budesonide microemulsions using GRAS (generally regarded as safe) materials for solubilization of the drug in order to attain a sufficiently high release rate compared to the budesonide suspensions.

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DOI : 10.4333/KPS.2009.39.6.417

Experimental

Materials

Budesonide was purchased from Taihua Natural Plant Pharmaceutical Co., Ltd (Xi'an, China). Soybean oil, cotton seed oil and isopropyl myristate were purchased from Sigma-Aldrich (St.Louise, MO, USA). PEG-8 caprylic/capric glycerides (Labrasol) and polyglyceryl oleate (Plurol Oleique CC497) were donated from Gattefossé (Toronto, Canada). All other chemicals were of analytical reagent grade.

Solubility of Budesonide in Vehicles

An excess amount of budesonide was added to various oils (soybean oil, cotton seed oil, isopropyl myristate) or surfactants (Labrasol, Tween 80, Cremophore RH 40, Poloxamer L-61), and was vortexed for 24 hr at 25ºC. The equilibrated samples were centrifuged at 13,200 rpm for 10 min to remove the undissolved budesonide. The supernatant was properly diluted with isopropyl alcohol and was injected into HPLC to determine the concentration of budesonide.

Construction of Pseudo-ternary Phase Diagrams for Microemulsions

Based on the results of the solubility study (Table I), isopropyl myristate and Labrasol were selected as the oil (O) and the surfactant (S), respectively, to construct the microemulsions by using Plurol Oleique CC497 (CoS 1) and polyethylene glycol 400 (CoS 2) as co-surfactants. In order to determine the existing range of microemulsions, pseudo-ternary phase diagrams were constructed using double distilled water titration method at ambient temperature. Labrasol was first blended with co-surfactant(s) at 2:1 or 3:1 (w/w), and then mixed with oil at various ratios. The mixtures were diluted with double distilled water in a drop-wise manner under vigorous vortexing. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, or emulsions, or gels. Miscibility test using water-soluble methylene blue and fat-soluble sudan III was conducted to determine o/w and w/o microemulsions.

Characterization of Microemulsion

Transmission Electron Microscopy (TEM)

The morphology of the microemulsions was observed by LIBRA 120 Energy-Filtering transmission electron microscopy (Carl Zeiss, Germany). For negative staining, microemulsions were dropped on a formvar-coated copper grid (300-mesh, hexagonal fields) and air-dried for 1 min at room temperature after removing the excessive sample with filter paper. After adhesion of microemulsions, 2% aqueous solution of sodium phosphotungstate was dropped onto the grid. After washing with double distilled water for two times, air-dried samples were directly examined by transmission electron microscopy.

Vesicle Size of Microemulsions

The mean vesicle size and size distribution of microemulsion was determined using dynamic light scattering method with a Nicomp 370 Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA, USA) without dilution.

Viscosity of Microemulsions

The viscosity of the formulations was determined after 3 min rotation to stabilize with a viscometer (Brookfield LVDV-E, Middleboro, MA, USA) using a 16 spindle at a speed of 100 rpm at room temperature.

Solubility of Budesonide in Microemulsions

In order to determine the maximum loading content of budesonide in microemulsions, excess amount of budesonide was dissolved into the oil phase by vortexing for 30 s after which the microemulsions were prepared as mentioned above. It was further mixed in a shaking incubator at 100 rpm (Jeio-Tech, Seoul, Korea) for 24 hr at 25ºC. Excess budesonide was removed by centrifugation at 13,200 rpm for 10 min after which the content of budesonide in the microemulsions was measured by HPLC after appropriate dilution with isopropyl alcohol.

In vitro Drug Release Study

Release of budesonide from microemulsions was observed using the reverse dialysis method,[14] and was compared with that from budesonide powder. The study was conducted using ELECTROLAB TDT - 08L Dissolution Tester (Bombay, India) at the speed of 100 rpm and the temperature was maintained at 37ºC. Dissolution medium (PBS, pH 7.4) contained 0.5% sodium larurylsulfate to maintain sink condition. Dialysis bags (molecular weight cut off 3500) which contained 10 mL dissolution medium were equilibrated with the dissolution medium (500 mL) for about 30 minutes prior to experimentation. An aliquot of each microemulsion (5 mL) or 6.4 mg of budesonide powder was directly dispersed into the dissolution medium. At predetermined time intervals (1, 2, 4, 6, 8, 12 hr), samples (0.1 mL) were taken from the dialysis bag, and was refilled with the same volume of fresh medium. Concentration of budesonide was determined by HPLC after appropriate dilution with isopropyl alcohol without further treatment. The percent of cumulative amount of budesonide released from the microemulsions was calculated as a function of time.

HPLC Analysis of Budesonide

The amount of budesonide was determined using an HPLC system equipped with a Waters 515 pump, a Waters 2487 UV detector and a Waters 717 autosampler. The column was a reversed phase C_{18} column (12.5 cm × 4 mm I.D. × 5 μm, LiChroCART, Germany) at room temperature. Mobile phase was a mixture of methanol and double distilled water (70:30, v/v) eluted at a flow rate of 1.0 mL/min. Budesonide was monitored by UV detector at 242 nm with a retention time of 3.5 min.

Data Analysis

All experiments in the study were repeated at least three times and the data was expressed as the mean±standard deviation. A two-tailed Student's *t*-test was performed at $p < 0.05$.

Results and Discussions

Formulation Development

The solubility of budesonide in various microemulsion components at 25ºC is shown in Table I. Since budesonide showed the highest solubility in isopropyl myristate (1.25 mg/mL) and in Labrasol (20.71 mg/mL), they were selected as oil phase and surfactant, respectively, to compose phase diagrams. Labrasol is a non-ionic surfactant used to formulated oral, transdermal and intranasal delivery of drugs. Plurol Oleique CC 497 was chosen as a co-surfactant since it is easily mis-

Table I–*Solubility of budesonide in various vehicles saturated for 24 h at 25°C*

Type	Vehicle	Solubility (mg/mL)
Water	H_2O	0.04 ± 0.00
Oil	Soybean oil	0.94 ± 0.03
Oil	Cotton seed oil	0.70 ± 0.03
Oil	Isopropyl myristate	1.25 ± 0.02
Surfactant	Labrasol	20.71 ± 0.32
Surfactant	Tween 80	11.83 ± 0.14
Surfactant	Cremophore RH 40	10.71 ± 0.39
Surfactant	Poloxamer L-61	3.90 ± 0.14

Each value presents the means ± standard deviation (n=3).

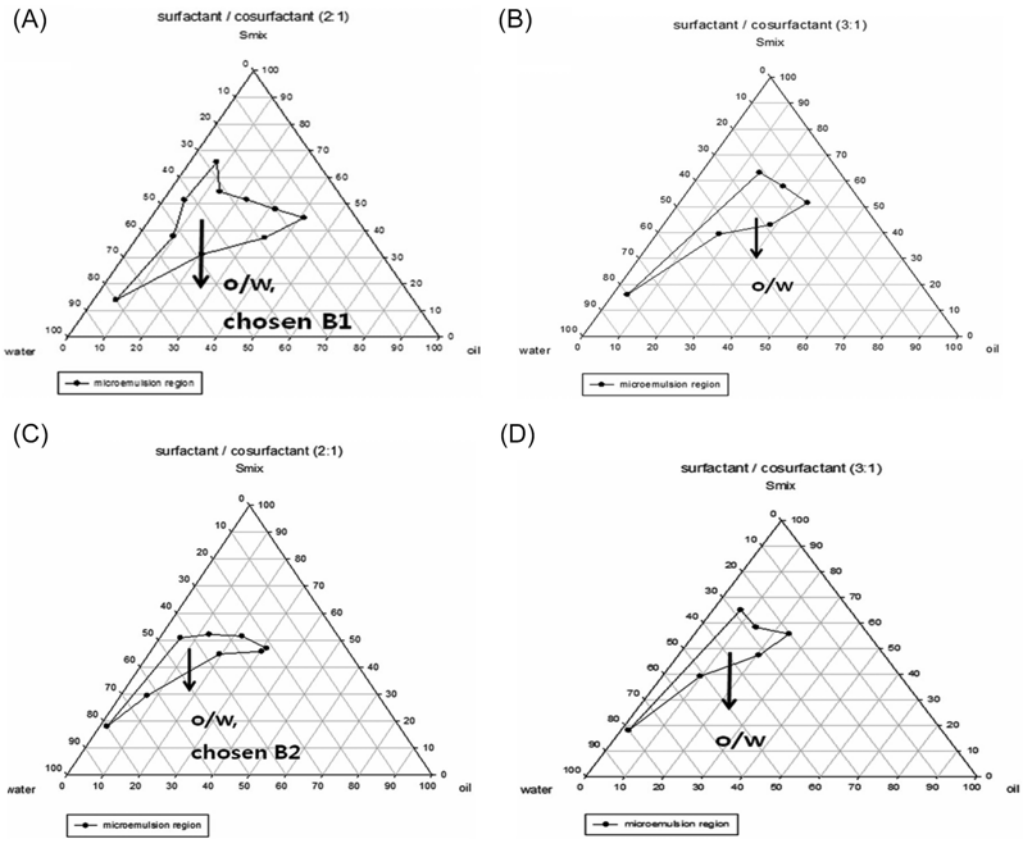

Figure 1–Pseudo-ternary phase diagrams of microemulsions composed of Labrasol and Plurol Oleique CC497 as surfactant and co-surfactant, respectively, at (A) 2:1 (w/w) and (B) 3:1 (w/w). Co-surfactants were composed of Plurol Oleique CC497 and PEG 400 at 1:1 (w/w) for (C) and (D), while surfactant: co-surfactant ratios were (C) 2:1 (w/w) and (D) 3:1 (w/w). Isopropyl myristate was used as an oil phase for all microemulsions.

Table II– *Formulations of microemulsions*

Formulation	O	S	CoS 1	CoS 2	Water
B1 (%)	6	36	18	-	40
B2(%)	6	36	9	9	40

O, oil phase (isopropyl myristate); S, surfactant (Labrasol); CoS 1, co-surfactant (Plurol Oleique CC497); CoS 2, co-surfactant (Polyethylene Glycol 400).

cible with both Labrasol and water.[15] Moreover, polyethylene glycol 400 (PEG 400) is known to enhance the dissolution rate by increasing the wettability.[16] Thus, a total of four phase diagrams were examined by fixing the ratio of surfactant and co-surfactant at 2:1 or 3:1 (w/w), where co-surfactant was either Plurol Oleique CC497 alone or the mixture of Plurol Oleique CC497 and PEG 400 (1:1, w/w).

A pseudo-ternary phase diagram of the investigated quaternary system of isopropyl myristate/Labrasol/Plurol Oleique CC 497/water is presented in Figure 1 (A) and (B), where the ratio of surfactant and co-surfactant was 2:1 and 3:1 (w/w), respectively. The transparent and low viscosity microemulsion area was presented in the phase diagrams. Methylene blue and sudan III were conducted to distinguish o/w and w/o microemulsions. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. No liquid crystalline structure was observed. Pseudo-ternary phase diagrams added with PEG 400 were showed in Figure 1 (C) and (D). When the surfactant and co-surfactant were blended at the ratio of 2:1 (w/w), the microemulsion region was much more increased than that of 3:1 (w/w). Thus, two formulations were selected from Figure 1 (A) and (C) for further studies, and were named as B1 and B2, respectively (Table II).

Physicochemical Characterization of Microemulsions

Solubility of budesonide in the microemulsion is shown in Table III, together with the mean droplet size and the viscosity. For nasal delivery of budesonide, one challenge to overcome was the solubilization of drug in small volume (less than hundred microliter) to be suitable for application in the nasal cavity. As shown in Table 3, The solubility of budesonide in B1 and B2 formulations was 6.50 mg/mL and 6.12 mg/mL which is about 163 and 153 times higher than that in the aqueous phase (0.04 mg/mL). The solubility was considered high enough for nasal administration (50-200 μg/dose, Pulmicort® Nasal AQ). On the other hand, the droplet size of B1 and B2 were 83.5 nm and 73.6 nm, and was consistent with the results of TEM study (Figure 2).

Table III–*Physicochemical properties of budesonide microemulsion*

Formulation	Solubility (mg/mL)	Size (nm)	Viscosity (cp)
B1	6.50 ± 0.15	83.5 ± 2.0	78 ± 1
B2	6.12 ± 0.69	73.6 ± 2.0	67 ± 6

Each value presents the means ± standard deviation (n=3).

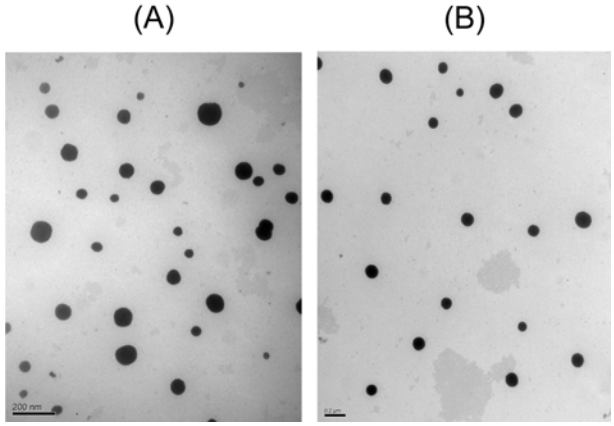

Figure 2–Transmission electron microscopy (TEM) of budesonide loaded microemulsion of (A) B1 and (B) B2 formulations. The scale for bar for all images represents 200 nm.

A drug applied into the nasal cavity is translocated to the nasopharynx and thereafter to the gastrointestinal tract by the coordinated beat of the cilia of respiratory epithelial cells. This is an important nonspecific defense mechanism of the respiratory tract and is called mucociliary clearance. Thus, the formulation with high viscosity is preferred in order to increase the mean residence time in the nasal cavity. However, the penetration rate of the formulation into mucus would decrease with the increase in the viscosity of applied formulation, resulting in a delay of the drug's approach to the cell surface. Furthermore, the viscosity of the formulation can influence the surface area where the drug can spread in the nasal cavity.[17] Although the optimum viscosity for nasal administration has not been reported, 60-70 cp of B1 and B2 microemulsions in this study seems to be appropriate for clinical application.

***In vitro* Release Kinetics**

In order to be an effective and reliable dosage form, submicron-sized emulsions should have predictable drug release profiles. It is technically difficult to characterize *in vitro* drug release from submicron-sized emulsions due to the physical obstacles associated with separation of dispersed and continuous phases. The most commonly used technique for assessing the *in vitro* release kinetics from submicron emulsions is

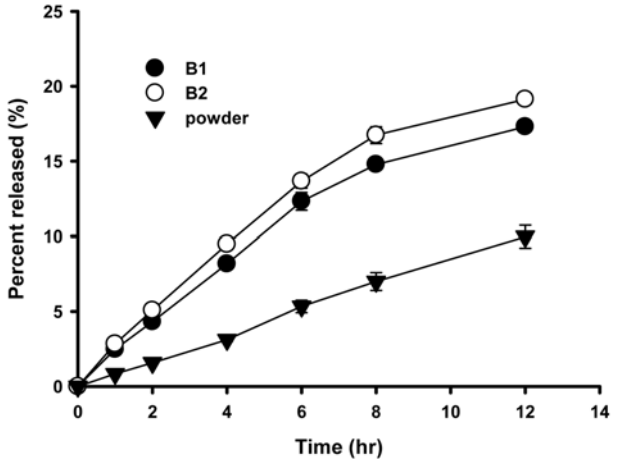

Figure 3—The *in vitro* release profiles of budesonide from microemulsions and powder determined by the reverse dialysis method at 37°C. Each data represents the mean ± standard deviation (n=3).

the dialysis method. However, the major limitation of this method is the potential for violation of sink conditions. The reverse dialysis bag technique chosen in this dissolution study can overcome these drawbacks by diluting the submicron-sized emulsion in the donor chamber and by increasing the surface area of the permeating membrane (dialysis bags).[14]

The *in vitro* release profile of budesonide from the microemulsions is plotted in Figure 3. A biphasic release profile was obtained using the reverse dialysis bag technique. The initial fast release rate may be due to the release of free drug and/or solubilized drug in micelle. The latter slower release rate may be due to the drug release from the oil droplets to the dissolution medium. Microemulsions of B1 and B2 released about 20% of budesonide for 12 hr, while budesonide powder suspended in the dissolution medium released only 10% of budedonide within 12 hr. Moreover, microemulsion B2 formulation which used Plurol Oleique CC 497 and PEG 400 (1:1, w/w) as co-surfactant showed higher release of budesonide than that of B1. Increased dissolution rate seems to be due to the enhanced wettability of PEG 400[16], which needs further investigation to understand the exact mechanisms.

Conclusions

Microemulsion formulation could be employed to solubilize the water-insoluble drug, budesonide. The Budesonide microemulsions composed of isopropyl myristate and Labrasol as an oil phase and as a surfactant, respectively, showed appropriate physical properties and increased the drug solubility up to 163-fold higher than that in water. Moreover, the *in vitro* dissolution rate also significantly increased from the microemulsions compared to the powder formulation. Therefore, microemulsions of budesonide could further be developed into a clinically useful nasal formulation.

Acknowledgements

The authors are thankful to Masung & Co. Ltd. (Seoul, Korea) for supplying the surfactants (Gattefosse, Toronto, Canada). This work was supported by the Industrial Source Technology Development Program funded by the Ministry of Commerce, Industry and Energy (MOCIE) in Korea (Grant No. 10031825).

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