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# Development of a novel dosage form for intramuscular injection of titrated extract of *Centella asiatica* in a mixed micellar system

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**Abstract**

Titrated extract of *Centella asiatica* (TECA), a drug used in treating systemic scleroderma, is poorly water-soluble. A conventional dosage form for the intramuscular injection of TECA, propylene glycol (PG)-based TECA solution, causes severe pain after intramuscular injection. To improve the solubility of TECA and reduce pain after injection, mixed micellar systems composed of 10% surfactant mixture (Tween 20 and Tween 85) and 90% phosphate-buffered saline, pH 7.0 (PBS) were prepared. As the ratio of Tween 20 to Tween 85 increased from 0:10 to 10:0, the solubility of TECA in the mixed micellar systems increased from 7- to 26-fold compared to that in PBS (pH 7.0). The droplet size of micelles gradually decreased with the increasing ratio of Tween 20 to Tween 85 from 0:10 to 4:6, followed by an abrupt decrease in size above the ratio of 6:4. Furthermore, the micellar systems prepared with Tween 20 and Tween 85 at the ratio of 6:4, 8:2 or 10:0 could solubilize TECA more than 10 mg/ml and the resultant droplet sizes were less than 2 μm. No significant changes were observed in the droplet sizes and asiaticoside contents in these micellar formulations during storage, indicating these systems are stable for at least 60 days. Their osmotic pressures were remarkably lower than those of PG-based TECA solution and similar to that of saline solution, irrespective of dilution ratios. Most importantly, they markedly reduced the number of writhes compared with PG-based TECA solution after injection to mice. All of these results suggest that these three TECA micellar formulations prepared with Tween 20 and Tween 85 improved the solubility of TECA and reduced pain following injection, possibly due to the decrease in osmotic pressure. Thus, these micellar formulations composed of optimum ratios of Tween 20 and Tween 85 may have a potential as dosage forms for the intramuscular injection of a poorly water-soluble TECA. 

*Keywords:* Titrated extract of *Centella asiatica*; Tween 20; Tween 85; Micelle; Solubility; Pain reduction

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## 1. Introduction

Titrated extract of *Centella asiatica* (TECA), a poorly water-soluble drug, contains three principal components such as asiaticoside, asiatic acid and madecassic acid (Nakajima and Ajiyoshi, 1972). These components have been reported to be effective in treating systemic scleroderma, abnormal scar formation and keloids (Kiesswetter, 1964; Tallat and Abbas, 1971) by strongly inhibiting the biosynthesis of acid mucopolysaccharides and collagens in carrageenin granulomas (Sasaki et al., 1972). However, the conventional dosage form for the intramuscular injection of TECA, Madecassol®, formulated by dissolving TECA in propylene glycol (PG), causes severe pain on the injection site, preventing its clinical use. Hence, the alternative dosage form for the intramuscular injection of TECA that can reduce pain on the injection site needs to be developed.

The reason, why the dosage form for injection causes severe pain to patients includes the precipitation of drug at the site of injection, contamination of solution with foreign particles, hypo- or hyperosmolar property of solution and painful property of drug itself (Cannon et al., 1995). Diazepam (Von Dardel et al., 1983), erythromycin (Marlin et al., 1983) and clarithromycin (Lovell et al., 1994; Cannon et al., 1995) have been reported to cause pain on the injection site as a result of the interaction of the drug with nerve endings in the venous wall.

The previous attempt was made to develop a formulation with improved TECA solubility and reduced osmolarity by using a mixture of PG and ethoxylated hydrogenated castor oil (Kim et al., 1997). This formulation could significantly improve the TECA solubility and reduce the pain after injection. However, this formulation could be used only as an extemporaneous form, since it was physically unstable during storage (Kim et al., 1997).

Polyoxyethylene sorbitan fatty acid esters (Tweens) have been used as solubilizing agents in the dosage form for the intramuscular injection of poorly water-soluble drugs (Attwood et al., 1989; Gao et al., 1998). Preparation of micellar system with a mixture of surfactants can have the advantage of controlling the hydrophilelipophile balance (HLB) in proportion to the mixing ratio of surfactants. Thus, in this study, to develop a physically stable mixed micellar system, mixed micellar systems were prepared with 10% surfactant mixture (Tween 20 and Tween 85) and 90% phosphate-buffered saline (pH 7.0, PBS), and then the solubility of TECA, droplet size of micelle, stability, osmolarity and extent of pain in them were evaluated.

## 2. Materials and methods

### 2.1. Materials

TECA (40.4% asiaticoside, 57.2% asiatic acid, and madecassic acid) was provided from Dong-Kook Pharmaceutical Co. (Seoul, Korea). Polyoxyethylene (20) sorbitan monolaurate (Tween 20, ICI Americas, Wilmington, DE), polyoxyethylene (20) sorbitan trioleate (Tween 85, Sigma Chemical Co., St. Louis, MO.), PG (Yakuri Pure Chemical Co., Osaka, Japan) and 0.45 μm membrane filter (Gelman Science, MI) were used. All other chemicals were of analytical grade and used without further purification.

### 2.2. Determination of solubility of TECA and droplet size of micelle in the micellar systems

Homogeneous surfactant mixtures with varying ratios of Tween 20 and Tween 85 were prepared. Excessive amount of TECA (100 mg), 1 ml of surfactant mixture and 9 ml of PBS (pH 7.0) were mixed together and heated at 50°C for 30 min while stirring. The mixture was cooled to room temperature for three days to reach the equilibrium and filtered through the membrane filter (0.45 μm). The amount of asiaticoside (as a standard reference) in the samples was analyzed by HPLC (Kim et al., 1997). Each sample (20 μl) was directly injected onto a μ-Bondapak $C_{18}$ column (Waters, 10 μm, $30 \times 0.39$ $cm^2$ i.d.). The chromatograph consisted of a high-performance liquid chromatograph (Waters TM 717) and a variable ultraviolet spectrophotometric detector (Model SPD-6A). The mobile phase consisted of acetoni-

trile, methanol and water (1:1:2, vol. ratio). The eluent was monitored with UV–Vis detector set at the wavelength of 214 nm with a flow rate of 1.0 ml/min.

The particle size distribution and the average droplet size of micelles were measured by photon correlation spectroscopy. A particle size analyzer (LPA-3000, Otsuka Electronics, Kyoto, Japan), was used to characterize the particle size in the 3–5000 nm range using the dynamic light scattering method.

### 2.3. Stability test

TECA micellar formulations were kept at room temperature for 60 days. At designated time intervals, the droplet sizes of micelles were measured using a laser particle size analyzer (LPA-3000, Otsuka Electronics, Kyoto, Japan). The micellar formulations were filtered through the membrane filter (0.45 μm) and the contents of asiaticoside were determined using HPLC as described above.

### 2.4. Measurement of osmotic pressure

The osmotic pressures of TECA micellar formulations were measured using a microosmometer (Precision System Inc. Natick, MA) by diluting with PBS, and then compared with that of PG-based TECA solution and PG alone, respectively.

### 2.5. Writhing test

ICR mice weighing $30 \pm 5$ g were obtained from the Experimental Animal Breeding Center of Seoul National University (Seoul, Korea). All experiments were performed according to the Seoul National University guideline of experimental animal care.

The writhing test in mice was performed to obtain the pain score by micellar formulations and PG-based TECA solution (Porreca et al., 1987; Kim et al., 1997). Phenyl paraquinone was known to cause pain when injected intraperitoneally and produce the constant number of writhes in mice depending on its injection amount. Thus, for control experiment, phenyl paraquinone (4.5 mg/kg) was injected into the peritoneal cavity of a group of mice and writhes/10 min was counted as an indication for pain. In another group of mice, morphine (5 mg/kg) was also injected subcutaneously 30 min prior to the administration of phenyl paraquinone to make sure, if the number of writhes by phenyl paraquinone was reduced by morphine pretreatment.

For test groups, TECA micellar formulations or PG-based TECA solution appropriately diluted with PBS was injected to peritoneal cavity of mice and the number of writhes in each group was counted per 10 min as an indicator of pain. The number of writhes due to TECA micellar formulations (0.34 mg/kg) was compared with that obtained by PG-based TECA solution. The statistical significance of the difference between the number of writhes was determined by the Student's $t$-test.

## 3. Results and discussion

### 3.1. Solubility of TECA and droplet size of micelle in the micellar systems

To improve the solubility of a poorly water-soluble TECA, varying ratios of two surfactants, Tween 20 (HLB = 16.7) and Tween 85 (HLB = 11.0), were used to prepare micellar systems (Table 1). The concentration of surfactant mix-

Table 1
Solubility of TECA and droplet size of micelle in the micellar formulations composed of 10% surfactant mixture and 90% PBS (pH 7.0)[a]

| Tween 20/Tween 85 | Solubility (mg/ml) | Droplet size (nm) |
|---|---|---|
| PBS(pH7.0) | 0.7 | – |
| 0:10 | $4.8 \pm 0.2$ | $251.6 \pm 42.3$ |
| 2:8 | $5.9 \pm 0.1$ | $235.8 \pm 30.7$ |
| 4:6 | $9.8 \pm 0.1$ | $216.5 \pm 4.0$ |
| 6:4 | $14.2 \pm 0.1$ | $41.5 \pm 0.9$ |
| 8:2 | $16.1 \pm 0.1$ | $45.8 \pm 3.0$ |
| 10:0 | $18.1 \pm 0.2$ | $79.4 \pm 7.6$ |

[a] Data are expressed as mean $\pm$ SD ($n = 4$).

ture and PBS (pH 7.0) in micellar systems was fixed to 10 and 90%, respectively, to adjust the osmolality and pH similar to those of physiological condition (Ismail et al., 1970; Attwood et al., 1989).

In the absence of surfactant, the solubility of TECA was about 0.7 mg/ml in PBS, which was insufficient to provide the desired amount in a solution for intramuscular injection. As the ratio of Tween 20 to Tween 85 increased from 0:10 to 10:0, the solubility of TECA in the mixed micellar systems increased from seven to 26-fold compared to that in PBS (Table 1). Especially, the micellar systems with the surfactant ratios of 6:4, 8:2 and 10:0 of Tween 20 and Tween 85 showed 20-fold higher solubility of TECA, compared with PBS solution.

The mean droplet size of micelles gradually decreased until the ratio of Tween 20 to Tween 85 increased from 0:10 up to 4:6 (Table 1). At above the ratio of 6:4 of Tween 20:Tween 85, the droplet size was dramatically decreased (Table 1). The smallest droplet size was obtained with micelles composed of 6:4 or 8:2 mixture of Tween 20 and Tween 85.

The increased hydrophilicity conferred by the relatively short alkyl chain length of Tween 20 may result in smaller aggregation number of more hydrophilic micelles, compared with micelles prepared with lower ratio of Tween 20 and Tween 85. Thus, the improved solubility and smaller size of micellar system prepared with higher ratios of Tween 20 to Tween 85 ratio might be explained by the increased hydrophilicity conferred by the relatively short alkyl chain length of Tween 20. However, the droplet sizes of single micelles prepared with Tween 20 alone were larger than those of mixed micelles in the micelle system prepared with 6:4 or 8:2 of Tween 20 and Tween 85 mixture (Table 1). It might be due to the larger aggregation number of the mixed micelle system, compared with single surfactant micelles, which causes lower micellar concentration than that of single surfactant micelles (Uchiyama et al., 1989; Abe et al., 1992; Rosen et al., 1993).

The conventional dosage form for the intramuscular injection of TECA, PG-based TECA solution (Madecassol®), contains 10 mg/ml of TECA (Kim et al., 1997). Generally, dispersed system as a drug carrier may induce the embolism and does not deliver drug to the target area unless the size of the dispersed component is less than 2 μm, since the system is preferably accumulated to the reticuloendothelial system by the phagocytosis (Kim et al., 1997; Park and Kim, 1999; Park et al., 1999). In our study, the micellar systems with 6:4, 8:2 and 10:0 ratios of Tween 20:Tween 85 could solubilize TECA at more than 10 mg/ml of concentration and the resultant droplet sizes were less than 2 μm (Table 1). Thus, micellar formulations I–III with 10 mg/ml of TECA were selected as optimal dosage forms for the intramuscular injection of TECA (Table 2). The droplet size of TECA micellar formulation I, II, and III was $35.5 \pm 2.0$, $36.9 \pm 1.7$ and $65.1 \pm 8.7$ nm, respectively.

Table 2
Compositions of TECA micellar formulations

| Formulation | I | II | III |
|---|---|---|---|
| TECA (mg/ml) | 10 | 10 | 10 |
| Tween 20 | 6% | 8% | 10% |
| Tween 85 | 4% | 2% | – |
| PBS (pH 7.0) | 90% | 90% | 90% |

### 3.2. Stability of TECA micellar formulations

The contents of asiaticoside and droplet size of micelle in the micellar formulations were evaluated over 60 days at room temperature. The content of asiaticoside was measured after filtering the micellar formulation in order to determine the precipitation of TECA in them (Kim et al., 1997). The droplet size of micelles in micellar formulation was also monitored during storage, since the droplet size of micelles might change with time due to the possible interaction between surfactants and drugs within core or mantle site (Trenier et al., 1990; Abe et al., 1992).

As shown in Fig. 1, no significant changes were observed in the asiaticoside contents in the micellar formulations during storage, suggesting that TECA in the micellar formulations were hardly precipitated for at least 60 days. Furthermore,

TECA micellar formulations had no detectable change in the mean droplet size and size distribution of micelle after 60 days of storage, compared to those at initial days (Formulation I, $35.5 \pm 2.0$ vs. $36.8 \pm 1.3$; Formulation II, $36.9 \pm 1.7$ vs. $38.4 \pm 0\ 8$; Formulation III, $65.1 \pm 8.7$ vs. $71.7 \pm 9.5$ nm). Thus, TECA micellar formulations were stable for at least 60 days.

### *3.3. Osmotic pressure of TECA micellar formulations*

Hypo- or hyper-osmolality of parenteral formulations are known to cause pain, morphological change of erythrocytes and tissue damage at the injection site (Klement and Arndt, 1991). The osmotic pressures of saline and red blood cell in serum are about 308 and 306 mOsm/kg, respectively. Although, the ideal osmotic pressure of injectable solutions ranges 250–350 mOsm/kg, it is hardly obtained from the formulation of water-insoluble drugs (Demorest, 1984). PG-based TECA solution (Madecassol®), the conventional dosage form of TECA for the intramuscular injection, causes swelling, pain and stiffness at the injection site due to its hypertonicity after injection (Kim et al., 1997).

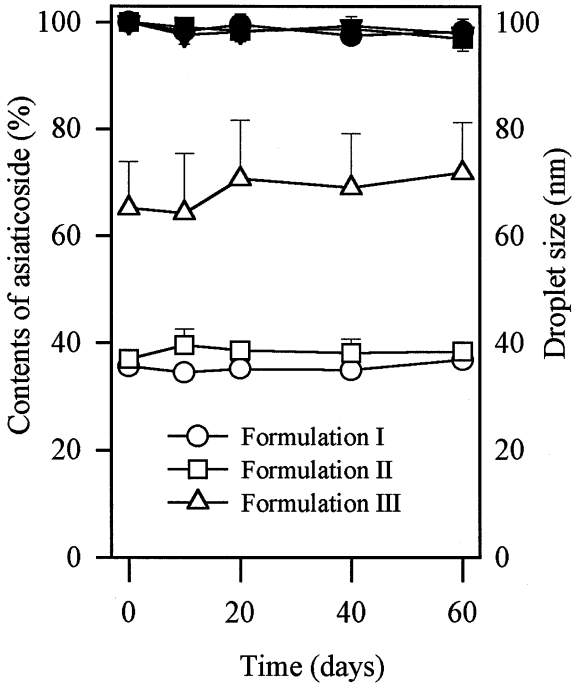


Fig. 1. Content of asiaticoside (black circle) and droplet size (white circle) of micelle in various micellar formulations during storage. Data are expressed as mean ± SD ($n = 3$).

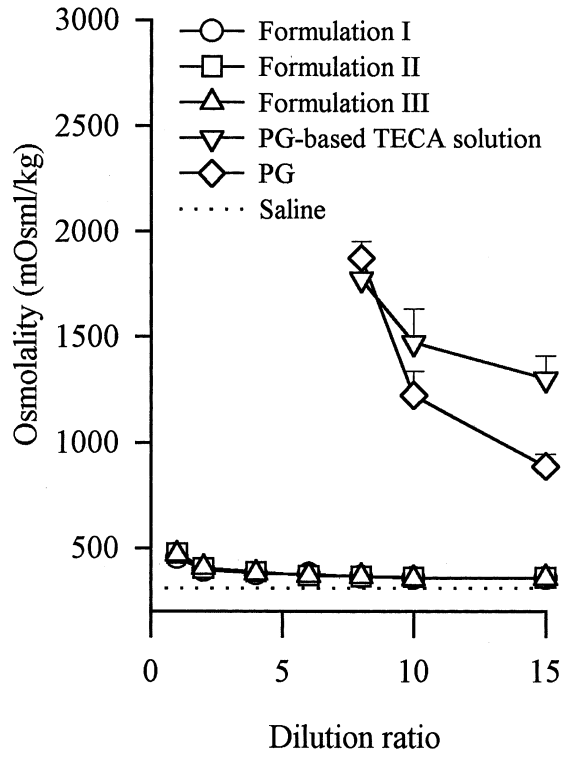


Fig. 2. Osmolarity of various micellar formulations and PG-based TECA solution as a function of dilution ratio. Data are expressed as mean ± SD ($n = 3$).

In this study, the osmotic pressures of PG-based TECA solution and PG alone could not be determined, since their osmotic pressures were more than 2000 mOsm/kg, beyond the threshold for the determination of osmotic pressures. Thus, the osmotic pressures of TECA micellar formulations were evaluated as a function of dilution ratio in comparison to PG-based TECA solution and PG alone (Fig. 2). The osmotic pressures of TECA micellar formulations were significantly lower compared with those of the PG-based TECA solution and PG alone, irrespective of dilution ratios. Furthermore, there was no signifi-

cant difference in the osmotic pressures between TECA micellar formulations. Notably, the osmotic pressures of TECA micellar formulations were similar to that of saline, irrespective of dilution ratios. Thus, our results indicate that TECA micellar formulations would exert much less swelling, pain and stiffness at the injection site after intramuscular injection than PG-based TECA solution.

### 3.4. Writhing test

The writhing test (Porreca et al., 1987; Kim et al., 1997) in mice has been developed and used to test analgesics by counting the number of writhes per unit time induced by a certain treatment. Many algogenic substances such as acetic acid or phenyl paraquinone are used to evoke a peculiar pain reflex, writhe. This pain reflex is adopted not only for analgesic response but also for hyperalgesic response like many other pain reflex tests such as mouse scratch test, rat paw-lick test, rabbit ear vein test and rat tail vein irritation test (Lovell et al., 1994; Cannon et al., 1995). For example, hyperalgesia can be elicited in writhing test by the intraperitoneal injection of prostaglandins (Smith et al., 1985; Akarsu et al., 1989), bradykinin (Emele and Shanaman, 1963) or endothelin (Raffa and Jacoby, 1991). Similarly, in the present study, we injected PG-based TECA solution intraperitoneally to determine whether this formulation is painful (Kim et al., 1997).

The pain score after the injection of TECA micellar formulation was assessed by a writhing test and compared with that of PG-based TECA solution. To address whether the writhing test is suitable for test for analgesic response, phenyl paraquinone (4.5 mg/kg), a conventional pain causing substance, was injected intraperitoneally. As shown in Table 3, the intraperitoneal injection of phenyl paraquinone produced a high incidence of writhe. In other group of mice, morphine (5 mg/kg) was injected subcutaneously 30 min prior to the injection of phenyl paraquinone. The pretreatment of morphine prevented the writhing response to phenyl paraquinone in mice (Table 3). Similar protocol was used to test whether the injection of PG-based TECA was hyperalgesic. As shown in Table 3, the intraperitoneal injection of PG-based TECA solution produced an appreciable number of writhes in mice, indicating that the solution was hyperalgesic (Klement and Arndt, 1991). Furthermore, there was no significant difference in the number of writhes in mice injected with PG and PG-based TECA solution, indicating that TECA hardly produced hyperalgesia. In contrast, the injection of TECA micellar formulations markedly reduced the number of writhes in mice regardless of surfactant ratios. For a control test, PBS (pH 7.0) in the same volume was injected intraperitoneally. The injection of PBS did not exhibit a single writhe in mice (Table 3). This observation was well correlated with the result of osmotic pressure measurement. Our results suggest that the micellar solutions of TECA with the osmolality similar to that of physiological condition would induce much less pain on injection compared with PG-based TECA solution.

## 4. Conclusions

Micellar formulations of TECA composed of high ratios of Tween 20 and Tween 85, which showed the improved solubility of TECA with the

Table 3
Hind-paw writhing test[a]

| Drugs | Number of writhes |
|---|---|
| PBS (pH 7.0) | 0 |
| Phenyl paraquinone[b] | $36.75 \pm 8.19$ |
| Phenyl paraquinone + morphine[c] | 0 |
| PG | $8.58 \pm 2.78$ |
| PG-based TECA solution[d] | $9.92 \pm 3.65$ |
| Formulation I[e] | $0.33 \pm 0.82$[f] |
| Formulation II[e] | $0.17 \pm 0.41$[f] |
| Formulation III[e] | $0.58 \pm 1.02$[f] |

[a] Data are expressed as mean ± SD ($n = 6$).
[b] Peritoneal injection of 4.5 mg/kg phenyl paraquinone.
[c] Subcutaneous injection of 5 mg/kg morphine 30 min prior to phenyl paraquinone.
[d] peritoneal injection of 0.34 mg/kg TECA diluted with PBS.
[e] peritoneal injection of 0.34 mg/kg TECA.
[f] $P < 0.05$ by the Student $t$-test when compared to PG-based TECA solution.

osmotic pressure similar to that of physiological condition, gave much less pain upon injection than did the commercially available product. Thus, these micellar formulations may have a potential as a dosage form for the intramuscular injection of a poorly water-soluble TECA.

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