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DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 29, No. 8, pp. 903–908, 2003

RESEARCH PAPER

Potential Use of Cyclodextrins to Enhance the Solubility of YM466 in Aqueous Solution

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ABSTRACT

Various solubilizing agents for YM466, a new Factor Xa inhibitor, were investigated to begin designing the aqueous formulation for subcutaneous administration. The tentative target concentration was 5 mg/mL. First, three kinds of buffer solutions (glycine–HCl, citrate, and lactate) were examined for their solubilizing effects. The dissolution rate of YM466 in lactate buffer was the fastest, as determined by visual examination at room temperature. The dissolution rate of YM466 in lactate buffer was enhanced, without degradation, by heating at 40°C, and YM466 solution at a concentration of 1 mg/mL became transparent 10 min after the start of heating. The solubility of YM466 increased along with lactate concentrations ranging from 50 mM to 200 mM and reached a high of 1.3 mg/mL after increasing lactate concentration to 200 mM at 5°C. The addition of cyclodextrins β-cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), and γ-cyclodextrin (γ-CD), but not α-cyclodextrin (α-CD), had remarkable impact on its solubility, and 7–8 mg/mL of YM466 was dissolved by the addition of HP-β-CD or γ-CD. These results demonstrated that YM466 was included in cyclodextrins and that the inclusion formations required a cavity size larger than α-CD. Based on the calculation from the linear portion of the phase solubility diagrams, apparent stability constants of α-CD, β-CD, HP-β-CD, and γ-CD at 5°C were estimated to be $2\,M^{-1}$, $206\,M^{-1}$, $143\,M^{-1}$, and $276\,M^{-1}$, respectively. Therefore, we found that γ-CD has the largest inclusion capacity.

Key Words: YM466; Factor Xa; Aqueous solution; Formulation; Solubility; Cyclodextrin.

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DOI: 10.1081/DDC-120024186
0363-9045 (Print); 1520-5762 (Online)

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INTRODUCTION

In designing aqueous drug formulations, the important properties to be examined are whether a drug compound demonstrates good stability and sufficient solubility in the formulation without precipitation. If good stability is not seen, stabilizing agents for the compound are investigated. Likewise, if the drug is not soluble enough to formulate the solution, solubilizing agents are required.

YM466,[#] {[*N*-[(7-carbamimidoyl-2-naphthyl)-methyl]-*N*-(4-{[1-(1-iminoethyl)-4-piperidyl]oxy}phenyl) amino]sulfonyl}acetic acid monomethanesulfo nate (Fig. 1), is a new anticoagulant that inhibits Factor Xa.[1] It was shown to be a safe antithrombotic agent with low bleeding risks in animal models. Therefore, it is expected to prove clinically effective in the treatment of thrombotic disorders, including deep-vein thrombosis and disseminated intravascular coagulation.

The purpose of this study was to examine the dissolution characteristics of YM466 into aqueous solution and discover suitable ingredients for solubilizing YM466. The first step was to evaluate three kinds of buffers (glycine-HCl, citrate, and lactate). Next, to enhance the solubility, the effects of cyclodextrins on the solubility were investigated because YM466 is expected to be administered to patients through subcutaneous administration, which limits the dosage volume to at most a few milliliters. The tentative target concentration was 5 mg/mL. Cyclodextrins are cyclic oligosaccharides consisting of glucose units, which have often been used to form inclusion complexes with various drug molecules and improve their solubility.[2,3] In this study, four kinds of cyclodextrins were used to evaluate their individual capacities in solubilizing YM466.

MATERIALS AND METHODS

Materials

YM466 was synthesized by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Glycine, lactic acid, and hydrochloric acid were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan); citric acid from Komatsuya Chemical Co., Inc. (Wakayama, Japan); and sodium hydroxide from Tokyo Ohka Kogyo Co., Ltd. (Kanagawa, Japan). Among cyclodextrins used, α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan); and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) with 5.3 degree of substitution from Japan Maize Products Co., Ltd. (Tokyo, Japan). All other ingredients and reagents were of analytical grade. Water for injection was used as the solvent to formulate drug solutions.

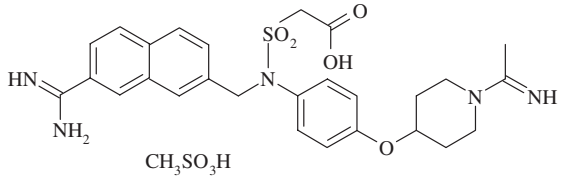

Figure 1. Chemical structure of YM466.

Preparation of YM466 Solution

The preparations were conducted as follows. First, the necessary amount of the ingredient required to make any given final buffer was dissolved in water for injection at approximately 50% of the final volume. Sufficient YM466 was added to the buffer solutions. A 1 M hydrochloric acid or sodium hydroxide solution was used to adjust the pH of the solutions to the desired value, and then the solutions were brought to final volume with water. The concentration that would reach if all the YM466 weighed were to be completely dissolved is referred to as ''theoretical concentration'' in this study. The ''actual concentration'' was obtained by measuring each sample after filtering through a 0.45 μm filter to remove undissolved YM466 before high-performance liquid chromatography (HPLC) analysis.

Regarding the investigation of cyclodextrin solubilizing capacities, lactate solutions (50 mM, pH 3.5), including various cyclodextrins (except β-CD), ranging from 1 to 50 mM were prepared as test solutions. Due to the limited solubility of β-CD, it ranged from 1 to 15 mM. Two hundred milligrams of YM466 was added to 5 mL of each test solution. The solutions were shaken at room temperature for one day and then allowed to stand at 5°C for one month. After filtration of each solution, it was measured for HPLC assay.

HPLC Analysis

HPLC was conducted at a constant temperature of 40°C using a TSK gel ODS-80TsQA column (150 mm in length × 4.6 mm in inside diameter, TOSOH Corporation, Tokyo, Japan) to evaluate

the solubilizing capacities. The mobile phase consisted of acetonitrile –0.68% potassium dihydrogenphosphate, which was adjusted to pH 2 with phosphoric acid (15:85 v/v). All separations were carried out at a flow rate adjusted so that the retention time of YM466 was approximately 10 min. Detection was made at the wavelength of 240 nm.

RESULTS AND DISCUSSION

Solubility of YM466 in Three Buffer Systems

It has often been reported that basic substances are better solubilized with organic acids rather than inorganic acids.[4] Since YM466 is the mesilate of the free base, the solubilization in acidic range was first examined. Three kinds of buffers (glycine-HCl, citrate, and lactate) that are usually used for injectable ingredients were selected. Regarding pH of the solutions, 3.5 and 5.0 were tentatively chosen among clinically acceptable values for the solubility evaluation. Each solution was continuously stirred at room temperature after the addition of YM466.

Visual appearances in each buffer are summarized in Table 1. In glycine-HCl (sample A) or citrate (sample B) buffer with pH of 3.5, YM466 was partially undissolved after storage at room temperature for 4 h. This indicates that the concentrations in the two buffers did not reach 0.75 mg/mL over the course of this observation. Regarding pH 5.0 citrate (sample C), the undissolved portion appeared to be much larger than in pH 3.5 citrate and the solution was cloudy. Thus, YM466 was apparently more difficult to dissolve at higher pH values. In contrast, YM466 was completely dissolved in lactate buffer (sample D) and the concentration reached at least 0.75 mg/mL. In order to evaluate how soluble YM466 is, a lactate solution was prepared such that the YM466 concentration would have reached 1.5 mg/mL if all the YM466 added were to be completely dissolved (sample E). However, YM466 in 50 mM lactate buffer could not be completely dissolved.

All aforementioned samples were allowed to stand at 5°C in consideration of the extreme storage conditions to which the drug product was exposed to during storage. Observation on day 26 showed that samples with theoretical concentrations of 0.75 mg/mL became clear, except the pH 5.0 citrate sample, and their actual concentrations reached the same values as their theoretical concentrations (Table 2).

These results indicated that, among buffers tested in this study, the speediest dissolution of YM466 can be achieved in lactate. Regarding pH, lower pHs seem to be more effective in solubilizing YM466.

Effects of Lactate Concentration and pH on YM466 Solubility

For further evaluation of lactate solubilizing capabilities, the effects of the concentration and pH on YM466 solubility were examined. The lactate concentration varied from 50 mM to 200 mM, and pH was adjusted to 3.5 and 4.5 (Table 3). Each sample solution was stored at 5°C for 26 days. The solubility of YM466 tended to be slightly enhanced with increased lactate concentration. It was thought that YM466 formed a soluble complex with lactic acid. Although we expected a lower pH to allow slightly higher concentration, no marked difference in the solubility of YM466 was seen between 3.5 and 4.5.

Solubilizing Method for YM466

Since the complete dissolution of YM466 requires a few hours at room temperature even if lactate is used, methods for shortening the formulation time were investigated. Two procedures were as

Table 1. Visual appearances of various YM466 solutions 4 h after the addition of YM466.

Sample	Buffer	Buffer conc. (mM)	pH	Theoretical conc. (mg/mL)	Visual appearance
A	Glycine-HCl	25	3.5	0.75	Slight precipitate
B	Citrate	50	3.5	0.75	Slight precipitate
C	Citrate	50	5.0	0.75	White opaque
D	Lactate	50	3.5	0.75	Clear
E	Lactate	50	3.5	1.50	White opaque

All solutions were stored at room temperature.

Table 2. Visual appearances of various YM466 solutions after storage at 5°C for 26 days.

Sample	Buffer	Buffer conc. (mM)	pH	Actual conc. (mg/mL)	Visual appearance
A	Glycine-HCl	25	3.3	0.75	Clear
B	Citrate	50	3.6	0.76	Clear
C	Citrate	50	5.1	0.72	Slight precipitate
D	Lactate	50	3.5	0.76	Clear
E	Lactate	50	3.5	1.28	White opaque

Table 3. Effects of lactate concentration and pH on YM466 solubility.

Conc. (mM)	pH	Visual appearance	Theoretical conc. (mg/mL)	Actual conc. (mg/mL)
50	3.5	White opaque	1.5	1.26
50	4.5	White opaque	1.5	1.25
100	3.5	White opaque	2.0	1.42
100	4.5	White opaque	2.0	1.32
200	3.5	White opaque	2.0	1.56
200	4.5	White opaque	2.0	1.56

All solutions were stored at 5°C.

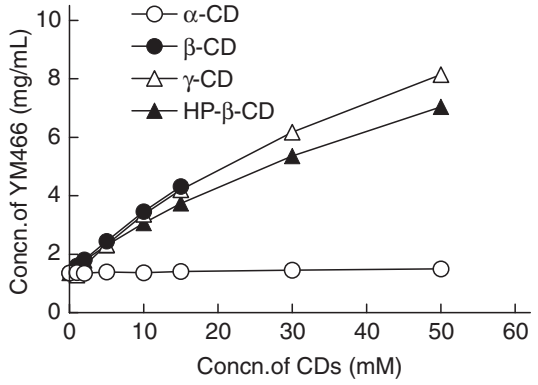

Figure 2. Phase solubility diagrams for YM466/cyclodextrins in 50 mM lactate (pH 3.5) at 5°C. Open circle, closed circle, open triangle and closed triangle, represent α-CD, β-CD, HP-β-CD, and γ-CD, respectively.

follows: one, to use YM466 manually comminuted with a mortar and pestle; two, to heat the solution to 40°C. YM466 was weighed so that the concentration would have reached 1 mg/mL if it were completely dissolved. In this study, the buffer system was 50 mM lactate with a pH of 3.5.

In the case of a general dissolution procedure without any modification, the clarity of solution increased 40 min after the addition of YM466. Thereafter, complete dissolution took 4 hours. When comminuted YM466 was used, the clarity of solution increased 25 min after the addition of YM466. However, complete dissolution eventually required nearly the same time as was the case with intact YM466, indicating that making the particle size smaller is not effective in shortening the total dissolution time. In contrast, when the solution was heated to 40°C, YM466 completely dissolved in 10 min. To evaluate the YM466 stability in solution at 40°C, the samples after dissolution were allowed to stand at 40°C for 26 days and the YM466 remaining was measured. The results showed that the amount of YM466 remaining was 99.2% of the initial value. Therefore, it appears that a 10 min heating for dissolution is not problematic to YM466 stability. Heating this solution to 40°C is helpful in shortening the time to formulate YM466 solution.

Effect of Cyclodextrins on the Solubility of YM466 in Aqueous Solution

YM466 is expected to be administered to patients through subcutaneous administration, which limits the dosage volume to at most a few milliliters. To enhance the solubility of YM466 in aqueous solution, effects of various cyclodextrins were examined. Figure 2 shows phase-solubility diagrams of YM466-cyclodextrin systems. When β-CD, γ-CD, or HP-β-CD was included in the solutions, the

concentration of YM466 increased along with that of the cyclodextrin. However, interestingly enough, no increase in YM466 concentration was found even when α-CD was increased. It is known that α-, β-, and γ-CD consist of 6, 7, and 8 D-glucopyranosyl units, respectively, and each cyclodextrin has a cavity with an internal diameter dependent on the cyclodextrin type.[2,5] In the solubilization, steric considerations are important in that the drug molecule must fit wholly or at least partially into the cyclodextrin cavity.[5] Among cyclodextrins, α-CD has the smallest cavity (4.7–5.2 Å), while β-CD and γ-CD have larger cavity sizes, 6.0–6.4 and 7.5–8.3 Å, respectively.[2] Thus, poor solubilization capacity of α-CD may be attributed to the internal cavity size, which may not be large enough to include the YM466 molecule. In contrast, β-CD and γ-CD may have a large enough cavity to form inclusion complexes with YM466. The most frequently used cyclodextrin is β-CD because of its availability.[2] However, the used amount of β-CD was limited in this study due to its poor solubility. Thus, the maximum of the YM466 concentration attained by the addition of β-CD was just almost 4 mg/mL at the β-CD concentration of 15 mM. However, this limitation was overcome by use of HP-β-CD and γ-CD. HP-β-CD is a modified β-CD and has much higher solubility in aqueous solution than the parent β-CD.[6] By virtue of this property, HP-β-CD was added more often in the formulation than β-CD. As a result, YM466 was more solubilized by increasing the HP-β-CD concentration. Among cyclodextrins tested, γ-CD indicated the beneficial inclusion attributes in terms of solubilizing YM466. This may be because γ-CD has the largest cavity that is the most suitable for the inclusion of YM466. In particular, YM466 in 50 mM γ-CD solution was about eight times as soluble as in solution with no cyclodextrins.

Judging from the phase solubility diagrams in the γ-CD and HP-β-CD systems, the systems exhibited negative curvature and might be categorized as A_N type,[7] showing that the complexation is rather complicated. However, in order to compare the inclusion capacities of these cyclodextrins, apparent stability constants (K') were calculated from the linear portion of phase solubility diagrams (corresponding to concentrations of 0–15 mM cyclodextrins) using the Higuchi and Connors equation.[7] The calculated values are listed in Table 4. It was thought that γ-CD has the highest affinity for YM466 because it showed the highest value (276 M^{-1}) among them. Since γ-CD has not been used in injectable formulations, HP-β-CD, which showed the same solubilizing capacity as γ-CD, would be more suitable for making injectable YM466 solution.

Table 4. Apparent stability constants (K', M^{-1}) of complexes of cyclodextrins with YM466 in lactate buffer (pH 3.5) at 5°C.

	α-CD	β-CD	HP-β-CD	γ-CD
Stability constant(K', M^{-1})	2	206	143	276

Our study showed that lactate/CD system is good for the solubilization of YM466. It is reported that citrate buffer can induce myotoxicity, which might cause local irritation after administration.[8] To reduce this possibility, lactate buffer may be more suitable. Furthermore, the inclusion of YM466 with CDs may reduce local irritation. This in vivo study is ongoing.

CONCLUSIONS

Among glycine-HCl, citrate, and lactate, lactate buffer appeared to be the most efficient in solubilizing YM466 in aqueous solution. The dissolution rate of YM466 in lactate buffer was enhanced, without degradation, by heating at 40°C. The solubility of YM466 increased along with lactate concentrations ranging from 50 mM to 200 mM: however, the YM466 solubility at 5°C reached a maximum of 1.3 mg/mL when the lactate concentration was increased to 200 mM. In contrast, the addition of cyclodextrins β-CD, HP-β-CD, and γ-CD, but not α-CD, greatly impacted the solubility of YM466. Significantly, 50 mM of HP-β-CD or γ-CD dissolved YM466 to approximately 7 and 8 mg/mL, respectively. These results demonstrated that YM466 was included in cyclodextrins and the inclusion formations need a cavity size larger than α-CD. Based on the calculation from the linear portion of phase solubility diagrams, apparent stability constants of α-CD, β-CD, HP-β-CD, and γ-CD at 5°C were estimated to be 2 M^{-1}, 206 M^{-1}, 143 M^{-1}, and 276 M^{-1}, respectively, indicating that γ-CD has the largest inclusion capacity. In light of application of CDs to injectables, HP-β-CD, which showed the same solubilizing capacity as γ-CD, would be more suitable for a YM466 system than γ-CD, because HP-β-CD has been used in injectable formulations.

REFERENCES

1. Iwatsuki, Y.; Kaku, S.; Sakai-Moritani, Y.; Taniuchi, Y.; Mano, Y.; Usui, T.; Iizumi, Y. Antithrombotic effect of YM466, a synthesized factor Xa inhibitor, in arterio-venous shunt thrombosis model in squirrel monkeys. Jpn. J. Pharmacol. **2002**, *88* (suppl 1), 76.
2. Uekama, K. Pharmaceutical applications of cyclodextrin complexations. Yakugaku Zasshi **1981**, *101*, 857–873.
3. Loftsson, T.; Brewster, M. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. **1996**, *85*, 1017–1025.
4. Ueda, M. Medicine and Drug Journal **2000**, *36*, 2763–2771.
5. Brewster, M.E.; Simpkins, J.W.; Hora, M.S.; Stern, W.C.; Boder, N. The potential use of cyclodextrins in parenteral formulations. J. Parenter. Sci. Technol. **1989**, *43*, 231–240.
6. Yoshida, A.; Arima, H.; Uekama, K.; Pitha, J. Pharmaceutical evaluation of hydroxyalkyl ethers of beta-cyclodextrins. Int. J. Pham. **1988**, *46*, 217–222.
7. Higuchi, T.; Connors, K.A. Phase-solubility techniques. Adv. Anal. Chem. Instrum. **1965**, *4*, 117–212.
8. Napaporn, J.; Thomas, M.; Svetic, K.; Shahrokh, Z.; Brazeau, G. Assessment of the myotoxicity of pharmaceutical buffers using an in vitro muscle model: effect of pH, capacity, tonicity, and buffer type. Pharm. Develop. Technol. **2000**, *5*, 123–130.