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Controlled Release of Simvastatin Acid Using Cyclodextrin Inclusion System

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Simvastatin acid (SVA) has been reported to stimulate bone formation by increasing expression of BMP-2 in osteoblasts. Due to their multi-functional characteristics and bioadaptability, cyclodextrins (CDs) are capable of forming inclusion complexes with many drugs by including a whole drug molecule inside their cavity. In the present study, we prepared SVA/CD inclusion complex solutions with different pH values. These were then used to determine their SVA release behavior after coating on titanium substrates, as well as to clarify the characteristics of SVA/CD complexes *per se*. Results showed that the lower the pH value of the solution, the lower the release kinetics of SVA. Besides, the amount of crystalline complexes in the coatings increased with decrease in pH. These results suggested that the release rate of SVA depended on two factors: pH of the solution and concomitant crystallinity of the coating.

Keywords: Simvastatin acid, Cyclodextrin derivative, Controlled drug release

INTRODUCTION

Simvastatin (SV), a liposoluble statin [inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase], is widely used for lowering cholesterol levels. Recently, it has been reported to stimulate bone formation, both *in vitro* and *in vivo*, in animal osteoporosis models[1,2]. This phenomenon was associated with the increased expression of bone morphogenetic protein-2 (BMP-2) gene in osteoblasts. This suggested that controlled release of simvastatin by means of topical application around dental and maxillofacial implants could promote osteogenesis in surrounding bone tissue[3], thus indicating potential in bone regeneration at bone-deficient areas. Furthermore, another distinct advantage of simvastatin for local stimulation of bone formation lies in its low manufacturing cost compared to that of direct administration of recombinant proteins such as BMPs. Being an alternative to recombinant proteins, SV could help to reduce the possibility of eliciting antibody responses.

In general, SV is administered by gavage and requires hepatic conversion to metabolically active β-hydroxy acid (SVA) to become medicinally active[4,5]. Therefore, for local administration, SV would have to be hydrolyzed to SVA first. On this score, we have reported an immobilization method for SVA around implants in order to promote osteogenesis[6]. Alternatively, a drug delivery system (DDS), in which a carrier is used to deliver SVA to bone-deficient areas, would offer a more powerful strategy for improving bone quality. This would necessitate achieving slow SVA release during the bone formation process after wound healing. Therefore, an effective carrier would be required to express the pharmacological effects of SVA.

Cyclodextrins (CDs), which are recognized as an important group of pharmaceutical excipients, are one such candidate[7]. They are cyclic oligosaccharides consisting of (α-1, 4)-α-D-glucopyranose units, and have a relatively hydrophobic central cavity and hydrophilic outer surface. The hydrophilic exterior surface of CD molecules makes them water-soluble, but the hydrophobic cavity provides a micro-environment for appropriately sized nonpolar molecules. They are capable of forming inclusion complexes with many drugs by including a whole drug molecule inside their cavity. In an aqueous solution, the complexes are readily dissociated, and free drug molecules are in relatively rapid dynamic equilibrium with drug molecules bound within the CD cavity[8-11]. These noncovalent complexes show new physicochemical characteristics when compared with the guest molecules, which include better stability, higher aqueous solubility, increased bioavailability, and fewer undesirable side effects. Therefore, if it were possible to form SVA/CD inclusion complexes and control their solubility, it would be possible to control the release property of SVA.

Since hydrophobic interaction between SVA and CD would be the main mechanism for forming

SVA/CD inclusion complexes, it is important to control the hydrophobicity of the guest molecule SVA. SVA hydrophobicity is influenced by pH value, as the characteristic dissociation of carboxyl groups in SVA molecules depends on pH[8,12,13]. In addition, the crystallinity of the inclusion complexes would also influence the release character of SVA, which would be concomitant to the solubility of SVA/CD complexes.

Consequently, the purpose of the present study was to investigate the possibility of controlling the release rate of SVA. To meet this objective, we prepared SVA/CD solutions with different pH values, and evaluated their SVA release properties from SVA/CD complex coatings on titanium substrates. In addition, we clarified the characteristics of the SVA/CD complexes *per se*.

MATERIALS AND METHODS

Preparation of SVA

Commercially available simvastatin {(+)-(1 S, 3 R, 7 S, 8 S, 8a R)-1, 2, 3, 7, 8, 8a-hexahydro-3,7-dimethyl-8-[2-[(2R, 4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1-naphthyl 2, 2-dimethyl-butanoate; S3449, Wako Pure Chemical Industries, Osaka, Japan} was used in this study. The chemical structures of simvastatin (SV, pro-drug; lactone) and simvastatin acid (SVA, open-acid; β-hydroxy acid) are shown in Fig. 1. Since only the open- ring, or β-hydroxy acid forms exhibit the efficacy of this medication, SV was hydrolyzed to β-hydroxy acid in the following manner. To open up the lactone ring, 4 mg of SV was dissolved in 0.1 mL of ethanol (95−100%) and then 0.15 mL of 0.1 M NaOH. After being heated at 50℃ for two hours, the resulting solution was neutralized with HCl to a pH of approximately 7.2 and brought up to a volume of 1 mL with distilled water.

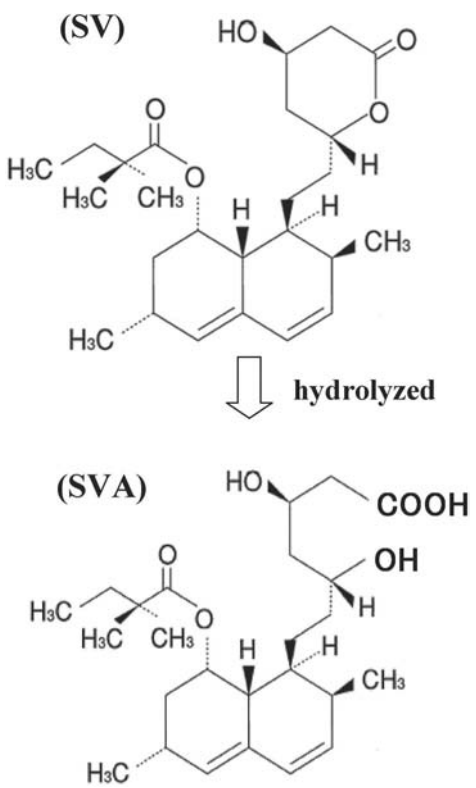

Fig. 1 Chemical structures of simvastatin (SV) and simvastatin acid (SVA).

Preparation of SVA/CD solutions

β-cyclodextrin (CD, Wako Pure Chemical Industries, Osaka, Japan) was used to prepare the inclusion complexes (Fig. 2). A CD content of 25 mg was added to 5 mL of SVA solution (1000 ppm)[6] at 65℃ and stirred. Then, the following three solutions — each with a different pH — were prepared by adding 0.1 N HCl solution. SVA solution (1000 ppm) was also used as a control.

1) SVA/CD 6.8 pH 6.8 (without HCl)
2) SVA/CD 5.2 pH 5.2 (added 70 μL of 0.1 N HCl)
3) SVA/CD 4.2 pH 4.2 (added 160 μL of 0.1 N HCl)
4) SVA (control) pH 7.2

pH 5.2 and pH 4.2 were chosen as occurring just before clouding of the solution at 65℃, and as slight clouding at 65℃, respectively.

SVA release assay from coatings

Surfaces of cp-titanium (Ti) plates (diameter: 30 mm,

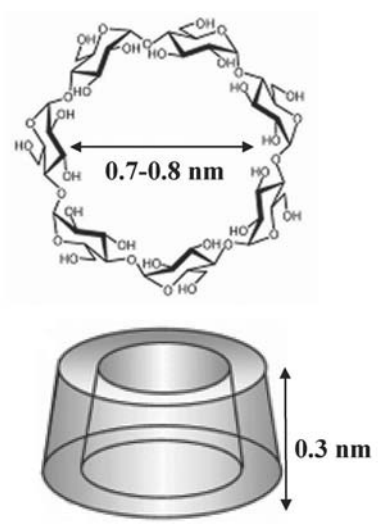

Fig. 2 Chemical structure (upper) and toroidal shape (lower) of β-cyclodextrin molecule.

thickness: 2 mm) were blasted with 50-μm alumina, then ultrasonically cleaned with acetone and distilled water for 20 minutes. Surfaces were subjected to ultraviolet radiation before coating to improve wettability. Subsequently, 50 μL each of SVA/CD solution and SVA solution were heated to 65℃ and coated onto the titanium surfaces under air-drying at 25℃.

These coated specimens were immersed in distilled water and stored for 0.5, 1, 3, 7, and 21 days at 37℃. At each time interval, absorbance of released SVA in the solution was measured using a UV-Visible spectrophotometer (V-660, Jasco Corp., Tokyo, Japan) at a wavelength of 239.1 nm[14,15]. The cumulative concentration of SVA was calculated using a previously determined working curve.

Estimation of degree of substitution

Degree of substitution[16], that is, the ability to form inclusion complexes, was estimated in order to understand the release character of SVA from the coatings. SVA/CD solutions with different pH values were centrifuged at 3600 rpm at 4℃ for 30 minutes to separate the precipitates. After filtering of supernatant fluid with a Millipore filter with a pore size of 0.2 μm, SVA concentration in the solution was measured using the UV-Visible spectrophotometer as described above. Degree of substitution was estimated according to the following equation:

Degree of substitution (%) = 100 × (concentration of SVA in original solution — concentration of SVA in supernatant fluid) / (concentration of SVA in original solution)

Characterization of coatings

The crystallinity of films coated onto the mirror-polished titanium was determined by X-ray diffraction using a thin film attachment (XRD, RINT-2500, Rigaku, Tokyo, Japan), with an X-ray source of Cu Kα1 and at a power of 50 kV/300 mA. These films were coated with the SVA/CD solution, as described in SVA release assay.

Surface morphology of the coatings on the blasted titanium surfaces was observed under a scanning electron microscope (SEM; JSM-6340F, JEOL, Japan). The specimens were coated with Au-Pd alloy. Accelerating voltage was set at 15.0 kV.

Statistical analysis

Data (n=5) were analyzed for statistical significance using an analysis of variance (ANOVA), followed by Scheffe's test for multiple comparisons.

RESULTS

Release behavior of SVA from coatings on titanium substrates

Figure 3 shows the SVA release profiles of the films coated onto the titanium substrates using SVA/CD

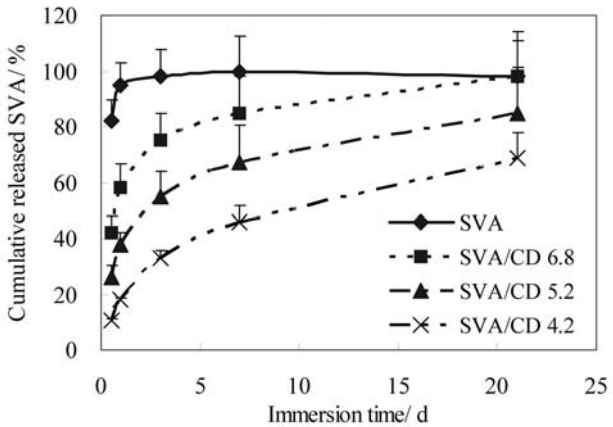

Fig. 3 Release profiles of SVA from films coated onto titanium substrates using SVA/CD solutions with different pH values (Average + SD). Significant differences in the SVA released were recognized among specimens, with SVA > SVA/CD 6.8 > SVA/CD 5.2 > SVA/CD 4.2 in this order at 0.5, 1, 3, 7 days ($P<0.05$). At 21 days, there was no significant difference in the SVA released between SVA and SVA/CD 6.8 ($P>0.05$).

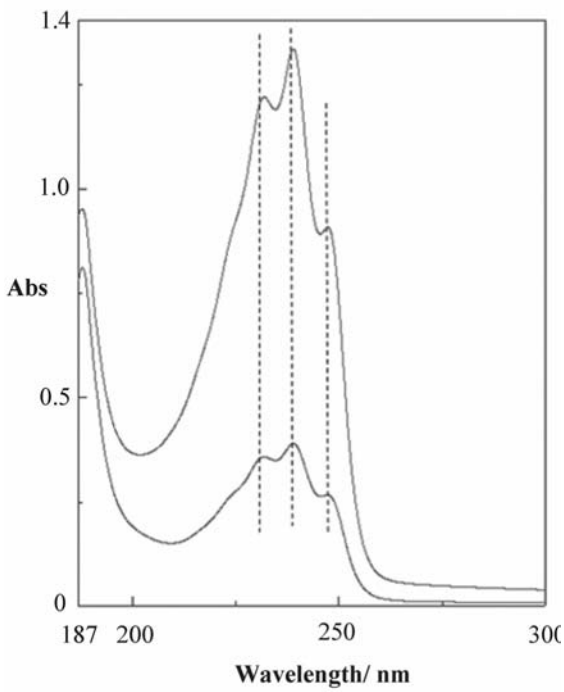

Fig. 4 UV spectra of SVA in original solution (upper) and SVA in supernatant fluid at pH 4.2 (lower).

solutions with different pH values. Two-way ANOVA revealed significant differences depending on pH and immersion period ($P<0.05$). Significant differences in the SVA released were recognized among the specimens, with SVA > SVA/CD 6.8 > SVA/CD 5.2 > SVA/CD 4.2 in this order at 0.5, 1, 3, 7 days ($P<0.05$). At 21 days, there was no significant difference in the SVA released between SVA and SVA/CD 6.8 ($P>0.05$). SVA from SVA-only coating

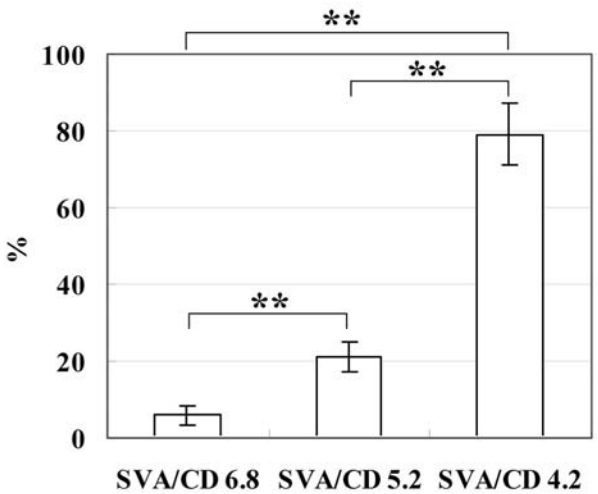

Fig. 5 Estimated degree of substitution of each solution with different pH values (Average ± SD).

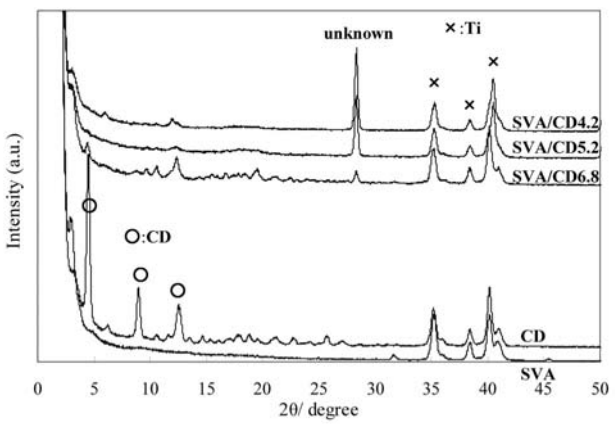

Fig. 6 Thin film X-ray diffraction profiles of coatings on titanium substrates.

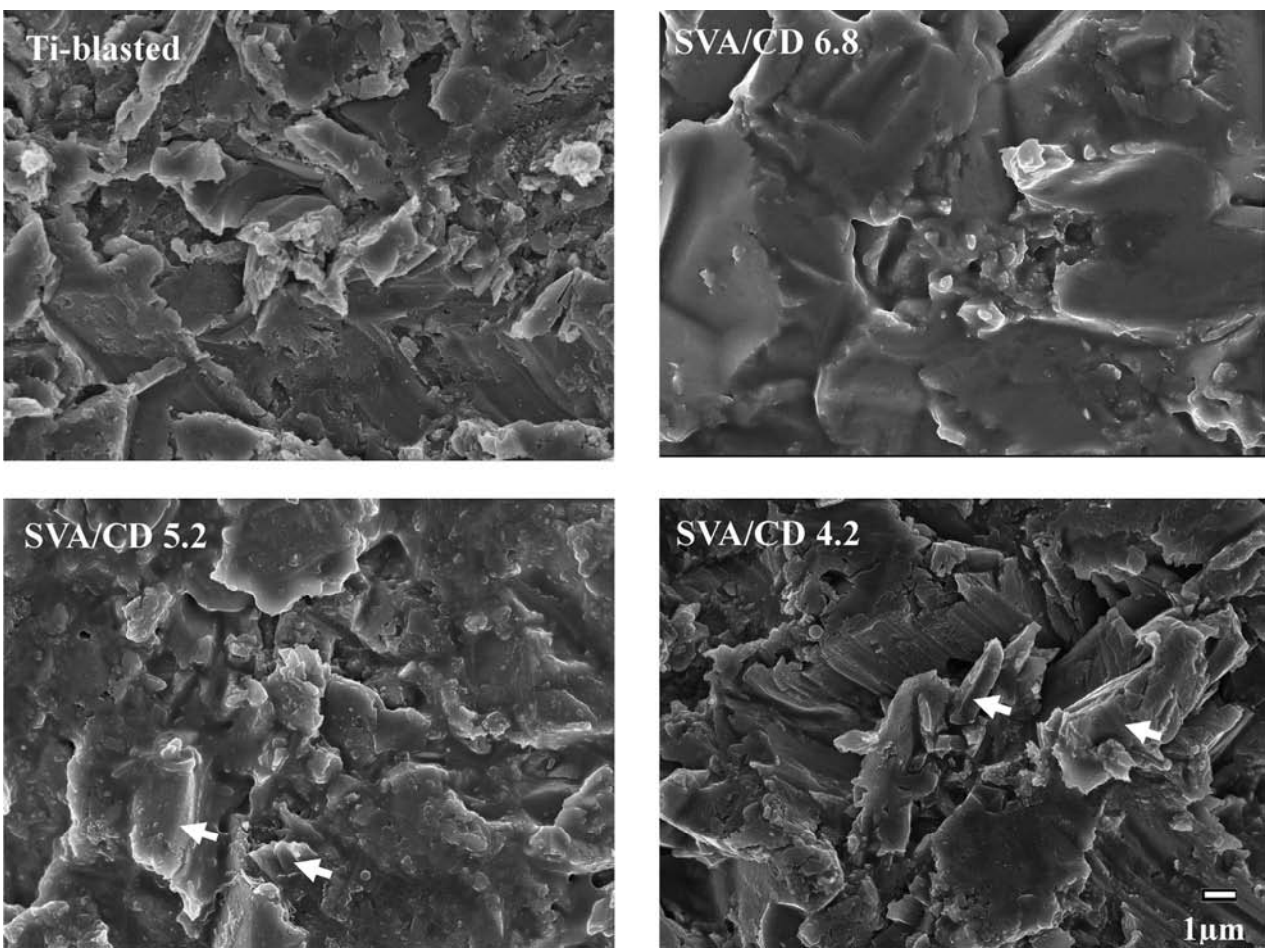

Fig. 7 SEM images of titanium substrate (Ti-blasted) and coatings of SVA/CD solutions with different pH values on titanium substrate. Crystalline-like features were recognized in the SVA/CD 5.2 and SVA/CD 4.2 specimens, as compared to SVA/CD 6.8 specimen (arrows).

was almost completely released after one day. Release from SVA/CD coatings depended on the pH of the solution. A lower pH not only yielded a lower amount of SVA, but also a tendency toward a slower release. Typically, the concentration of SVA released from the SVA/CD coatings with a pH of 4.2 showed a rate of less than 70% of the entire release on day 21.

Degree of substitution

Figure 4 shows the UV spectra of SVA in the original solution and in the supernatant fluid at pH 4.2. No differences in wavelength were observed, with the highest peak occurring at 239.1 nm.

Degree of substitution in each solution is shown in Fig. 5. It was estimated that the degrees of substitution were 6%, 21%, and 79% for SVA/CD 6.8, SVA/CD 5.2, and SVA/CD 4.2, respectively. Significant differences in the degree of substitution between SVA/CD 6.8 and SVA/CD 5.2 or SVA/CD 4.2 ($p<0.01$) were observed.

Characterization of coatings

X-ray diffraction profiles are shown in Fig. 6. The diffraction peaks between 35 and 45 degrees in all specimens were attributed to the titanium substrate. The SVA coatings were almost completely amorphous, whereas the CD coatings showed crystalline patterns at a range of 3 to 15 degrees with raw β-CD. A clear new crystalline peak, which was not identified by JPCDS cards, was observed at about 28.3 degrees on the SVA/CD coatings. This peak increased with decrease in pH value, whereas the peaks attributed to β-CD decreased with decrease in pH value. This behavior might be explained by the formation of inclusion complexes.

Figure 7 shows the SEM images of titanium substrate (Ti-blasted) and SVA/CD solution coatings with different pH values on the titanium substrates. An irregular morphology was observed in the Ti-blasted specimen. Amorphous-like films covering the Ti-blasted surface were observed in the SVA/CD 6.8 specimen. These films changed to crystalline-like features in the SVA/CD 5.2 and SVA/CD 4.2 specimens. This tendency was especially marked in the SVA/CD 4.2 specimen.

DISCUSSION

The objective of the present study was to clarify the release character of SVA from SVA/CD coatings with different pH values. Results showed that the lower the pH value of the solution, the lower were the release kinetics of SVA, indicating that the release character of SVA could be controlled by adjusting the pH value. In general, release character is influenced by two factors: degree of substitution and crystallinity of coating.

Degree of substitution plays an important role in balancing CD water solubility and its ability to form complexes. Raising degree of substitution induces binding of guests to CDs by increasing the surface area available for binding. In this study, the results showed that the lower the pH value of the SVA/CD solution, the higher was the degree of substitution. This was a consequence of the different hydrophobic character of the guest molecule, SVA. SVA has a carboxylic acid ($pKa = 4.18$) which is almost completely dissociated at pH 6.8, with the carboxylic group being gradually deionized with decrease in pH[8,16]. Therefore, at a low pH value, SVA becomes stable and hydrophobic, resulting in enhanced hydrophobic interaction between SVA and CD.

Temperature changes can also affect drug/CD complexes, in terms of the degree of substitution. In most cases, increasing the temperature decreases the magnitude of the apparent stability constant of the drug/CD complex, possibly due to subsequent reduction in drug/CD interaction forces such as van der Waals and hydrophobic forces[17,18]. In this study, we believed that few SVA/CD complexes occurred at 65℃. However, the number of complexes increased with decrease in temperature during the drying process.

The crystallinity of the SVA/CD complexes influenced the solubility of the coatings[8]. A clear crystalline peak was observed by XRD analysis, which increased with decrease in pH value. This peak appeared to originate in the inclusion complexes of SVA/CD formed in the coatings. These crystalline structures might have retarded the dissolution rate of the coatings, resulting in delayed release of SVA.

In this study, β-CD was selected as first choice to form inclusion complexes as it has the least solubility in water among the most abundant natural CDs. Further study is necessary to evaluate other CDs that promote a slow release of SVA. Hydrophobic CDs, such as alkylated and acylated derivatives, are useful as slow-release carriers in prolonged release of water-soluble drugs[19-21].

In conclusion, the results of the present study indicated that the number of SVA/CD complexes formed depended on the pH of the solution, and that subsequent release of SVA from the coatings depended on the number of complexes and resulting crystallinity of the coatings. These results suggested that SVA/CD complexes exhibited potential in bone regeneration with a drug delivery system in bone-deficient areas as well as in promoting osteogenesis surrounding bone tissue.

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