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Buserelin acetate microparticle dispersion effects drug release and plasma E_1 levels

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Abstract

We investigated the effect of different dispersion methods on release behavior and efficacy onset following microparticle administration of buserelin acetate (BA) sustained-release injection. In this in vitro release study, the initial dispersion of BA increased with increased stirring speed ($p < 0.01$). Stability of BA was studied over 7 days after BA release. The initial BA release rate was higher ($p < 0.01$) after a 1-min vibration dispersion method (VDM) using a test tube mixer (2000 rpm) compared with the standard dispersion method (SDM) by hand. Without shaking, powder aggregation was observed, and BA release was lower than in either the SDM or VDM methods. In this study using 4-week-old Sprague–Dawley female rats, the initial plasma estrone (E_1) concentrations were lower ($p < 0.05$) in the VDM method than in the SDM method. Observations by optical microscope and scanning microscope showed no change in microparticle shape or distribution of size induced by SDM, VDM or the ultrasonication dispersion method. These results suggest that different dispersion methods do not change the shape and distribution of microparticle size, but clearly change the BA release rate and the transition in plasma E_1 concentrations that can affect drug efficacy.

Keywords: Microparticle; Buserelin acetate; Controlled-release; Dispersion method; GnRH agonist; PLGA

1. Introduction

Buserelin acetate (BA) a derivative of the hypothalamic gonadotropin-releasing hormone (GnRH) is widely used to treat endometriosis and uterine leiomyoma. After a transitory elevation in luteinizing hormone, follicle-stimulating hormone, and estrogen, it has inhibitory action on estrogen by inducing the downregulation of GnRH receptors (Cirkel et al., 1989; Regidor et al., 1997; Mori et al., 1999; Takeuchi et al., 2000).

The available preparations for clinical use are nasal spray and microparticle injection. Administration compliance with the nasal spray is generally low, and the absorption of BA is considerably lower from this route (Holland et al., 1986). Microparticle injections are widely used as steady blood concentration of BA can be ensured for about one month after a single injection, low incidence of adverse reactions due to a transient elevation of the blood BA concentration and drug efficacy is maintained (Faisant et al., 2002; Berkland et al., 2002). The microparticle is a type of microsphere. BA is dispersed in the biodegradable carrier, poly-D,L-lactic acid-co-glycolic acid (PLGA). BA is continually released for about one month by gradual hydrolysis of PLGA at the injection site (Aventis Pharma, 2002; Filicori, 1994). The microparticle injection kit consists of microparticles in the front chamber of a double-chamber syringe and water for injection in the back chamber. Microparticles are dispersed in the solution immediately prior to injection. By the standard dispersion method (SDM), water is added to the microparticles and dispersed by tapping the syringe lightly on the palm. However, an alternative method involving vigorous shaking of the syringe has been seen in busy clinical settings.

Until now there have been no investigations as to whether differences in dispersion methods affect release and efficacy of drug. We investigated the effect of the difference in dispersion method on BA release from microparticles and subsequent drug efficacy using rat plasma estrone (E_1) concentrations.

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2. Materials and methods

2.1. Materials

BA standard solution (1.0101 mg/mL, lot no. C616.03) and Suprequr® MP kits (0.75 mL of water for injection and 28.2 mg of PLGA (1:1) including 1.8 mg of BA) were supplied by sanofi-aventis K.K., Tokyo, Japan. Potassium dihydrogenphosphate, disodium hydrogenphosphate, citric acid, and borax for preparation of buffer solutions were purchased from Kanpan Techno Co. Inc., Tokyo, Japan.

2.2. Determination of BA concentration

HPLC conditions are as follows; Pump: LC-10AD (Shimadzu Co., Kyoto, Japan), Detector: SPD-10A (Shimadzu), Column: Mightysil RP-18 ODS column (250 mm × 4.6 mm, particle size 5 μm, Kanpan Techno Co. Inc., Tokyo, Japan), temperature: 33.0 ± 0.1 °C, mobile phase: 30% acetonitrile containing 0.035% trifluoro acetic acid (pH 2.4), flow rate: 0.5 mL/min, retention time: 9.2 min.

2.3. Calibration curve of BA

Standard solution of BA was diluted to 0–8.0 μg/mL by phosphate buffer at pH 7.4 (appropriate mixture of 0.08 mol/L KH_2PO_4–0.0267 mol/L Na_2HPO_4). The calibration curve was made using the least squares method.

2.4. Stability of BA

Standard solutions of BA were diluted to 3.0 μg/mL (ionic strength: 0.086) with water and adjusted to pH and placed in glass-stoppered test tubes. The test tubes were stored in a water bath at constant temperature (37 °C, shaken 50 strokes/min) for 2 weeks.

The following buffers were prepared and adjusted to pH; McIlvaine buffer (appropriate mixture of 0.08 mol/L citric acid–0.0267 mol/L Na_2HPO_4), pH 3.0, 4.0, 5.0, 7.4; phosphate buffer (appropriate mixture of 0.08 mol/L KH_2PO_4–0.0267 mol/L Na_2HPO_4), pH 6.0, 7.4; potassium phosphate-borate buffer (appropriate mixture of 0.08 mol/L KH_2PO_4–0.0267 mol/L $Na_2B_4O_7$), pH 7.4.

2.5. Release apparatus

The apparatus for the release test was a double-jacketed beaker (internal diameter: 50 mm; height: 65 mm) maintained at 37.0 ± 0.5 °C by a circulatory flow system from a constant temperature water bath.

The paddle (34 mm long and 1.8 mm in diameter) generally used as the suppository release-testing apparatus (TMS-103, Toyama Sangyo Co. Ltd., Osaka, Japan) was rotated at 10 or 100 rpm. Fifty milliliters of phosphate buffer (pH 7.4, 0.08 mol/L KH_2PO_4–0.0267 mol/L Na_2HPO_4, ionic strength: 0.08) was used as the test solution. Microparticles (containing 7.2 mg BA) were dispersed in solution.

One milliliter of test solution was withdrawn every 10 min for the first 60 min then daily for 7 days and filtrated (membrane filter: Miller-LH, pore size 0.45 μm, Nihon Millipore Ltd., Yonezawa, Japan). The BA concentrations in the diluted solution were determined by HPLC. The same volume of test solution was added to the solution to keep the fluid volume constant.

2.6. BA release by different dispersion methods

(1) After inflow of water for injection to the anterior chamber, microparticles were sufficiently dispersed in water by oscillation of the syringe on the palm (standard dispersion method, SDM).
(2) We arranged two other dispersion methods. The first one was dispersion with oscillation at 2000 rpm using a test tube mixer (Voltex-2, Scientific Industries Inc., NY, USA) for 1 min (vibration dispersion method, VDM).
(3) The second one involved very slow shaking to sink the microparticles in water without oscillation (without oscillation method, WOM). The suspension was kept at 37 °C. The BA concentrations in the solution were determined at 0.1, 1, 3, 5 and 24 h.

2.7. Microscopic observation of morphology

In addition to the dispersion methods described above, we dispersed the microparticles in water for 3 min using an ultrasonic machine (5210; Branson Ultrasonic Co., CT, USA) (ultrasonic dispersion method, UDM).

Morphological change was observed for 21 days using scanning electron microscopy (S-3500N; Hitachi High-Technologies Co., Ibaraki, Japan) and optical microscopy (Leica DM IRB; Leica Co., Tokyo, Japan).

For scanning electron microscopy observation, a suspension of microparticles was painted on an aluminum sample table (15 × 10 m/mH, Okenshoji Co. Ltd., Tokyo, Japan). The moisture in the sample was removed and evaporated in a plate covered with silica gel and coated with platinum–palladium using Ion Coater (IB-3, Eiko Engineering Co. Ltd., Ibaraki, Japan).

The surface and morphology of microparticles observed by scanning electron microscopy were exposed on Polaroid film (Polapan 572 B&W 4 × 5 in.; Polaroid Co., USA).

After measurement of particle size by Green diameter, the mean particle size and size distribution were calculated by optical microscopic observation.

2.8. Concentration of estrone after injection of microparticle in rats

The experiments were performed in accordance with the guidelines for animal experimentation, which were approved by the Japanese Association for Laboratory Animal Science in 1987.

Female rats (Sprague–Dawley, 4 weeks old, body weight 193 ± 6.7 g) that were given food and water ad libitum were used. Microparticle injection following dispersion by one of

SDM, VDM or WOM was used. Microparticles (3.1 mg/kg of BA) were subcutaneously injected at the back of the neck. Using a heparin sodium syringe, blood was collected at 0, 3, 6 h for the first day, then on days 1, 2, 4 and 7 (0.5 mL each time) from the external jugular vein via the subclavian sternocleidomastoid muscle. No anesthesia was given. Blood was centrifuged for 10 min at 3000 rpm to separate the plasma. As estrone (E_1) does not change significantly as a result of the estradiol cycle, plasma E_1 concentrations were determined using the RIA double antibody technique at SRL Hokkaido Inc., Sapporo, Japan.

2.9. Statistical analysis

Results were expressed as mean ± standard error (S.E.), or mean ± standard deviation (S.D.), and significance between more than two groups was determined by analysis of variance (ANOVA). p values of 0.05 or less were considered significant.

3. Results

3.1. Determination of BA

Good linearity was observed ($y = 958.63x - 115.24$, $r = 0.996$) for the standard curve of BA. The detection limit was 150 ng/mL. The diurnal variation for HPLC was low at 0.71% ($n = 7$), and highly reproducible results were obtained. Under the HPLC conditions used in this study, the long retention time of the conventional methods (Kertscher et al., 1995; Matsubara et al., 1997; Hoitink et al., 1998) was shortened to 9.2 min.

3.2. Stability of BA

The degradation of BA in aqueous solution followed to first-order kinetics. The logarithm of rate constant (k) of BA degradation at various pH values at 37 °C is shown in Fig. 1. The degradation rate constant, half-life ($t_{50\%}$), and time required for 10% degradation ($t_{10\%}$) are shown in Table 1. In MacIlvaine buffer solution or phosphate buffer solution, the rate constants

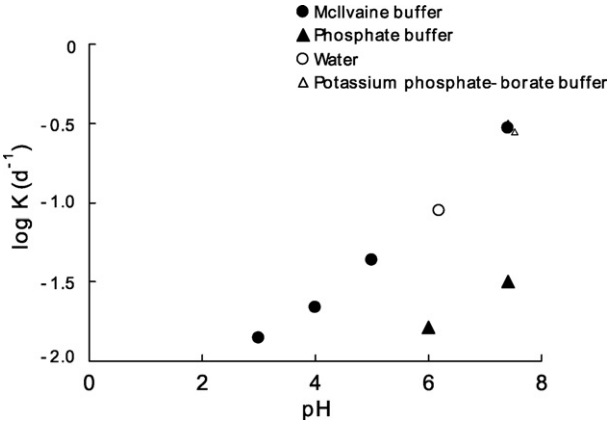

Fig. 1. pH–rate profile of buserelin acetate (BA) degradation.

Table 1
Rate constant of degradation, half-life ($t_{50\%}$), and time required for 10% degradation ($t_{10\%}$)

pH	k ($\times 10^{-2}$ d^{-1})	$t_{50\%}$ (d)	$t_{10\%}$ (d)
3.0[a]	1.41	49.1	7.4
4.0[a]	2.18	31.8	4.8
5.0[a]	4.31	16.1	2.4
6.0[b]	1.62	42.8	6.5
7.4[a]	29.4	2.4	0.4
7.4[b]	3.19	21.7	3.3
7.4[c]	30.9	2.2	0.3
H_2O	8.80	7.9	1.2

[a] McIlvaine buffer.
[b] Phosphate buffer.
[c] Phosphate-borate buffer.

of BA degradation were larger in higher pHs, however, they did not seem to be the specific base-catalyzed hydrolysis, because the slope was not +1 (Martin, 1993). The k value of the degradation in water (pH 6.1) was on the line obtained in MacIlvaine buffer solution. Among three buffer solutions at pH 7.4, k value obtained in phosphate-borate buffer solution and MacIlvaine buffer solution was almost ten times lager than that obtained in phosphate buffer solution (Fig. 1). At pH 7.4, k value in phosphate-borate buffer was almost the same as that of MacIlvaine buffer. These results indicated that the components of the buffer solutions might affect the rate of degradation and that BA is more stable in acidic solution.

3.3. Release of BA

Fig. 2(a) shows the BA release from microparticles over 60 min stirred at 10 and 100 rpm in the beaker. At the 100 rpm stirring speed, significantly higher BA release was observed compared to that at 10 rpm.

Fig. 2(b) shows BA release over 7 days. BA concentrations released from microparticles initially increased for 60 min after start of stirring, but decreased gradually in both groups day by day. The significance between two groups during 7 days was $p < 0.01$ by ANOVA. At the 100 rpm stirring speed, higher BA release was observed at the initial stage compared to 10 rpm, however, lower BA release was observed after 3 days at 100 rpm since the concentration of BA might be decreased by degradation in solution. At 10 rpm, initial concentrations were lower, however, as BA was continuously released from microparticles, BA concentrations increased at day 3 then decreased gradually in a similar manner to the solution at 100 rpm. Higher concentrations were observed 3 days after start of dispersion at 10 rpm.

3.4. BA release by three different dispersion methods of microparticle

Fig. 3 shows the BA release in the syringe after microparticle dispersion from the injection kit by three different methods (SDM, VDM, WOM). Initial concentrations of BA were higher

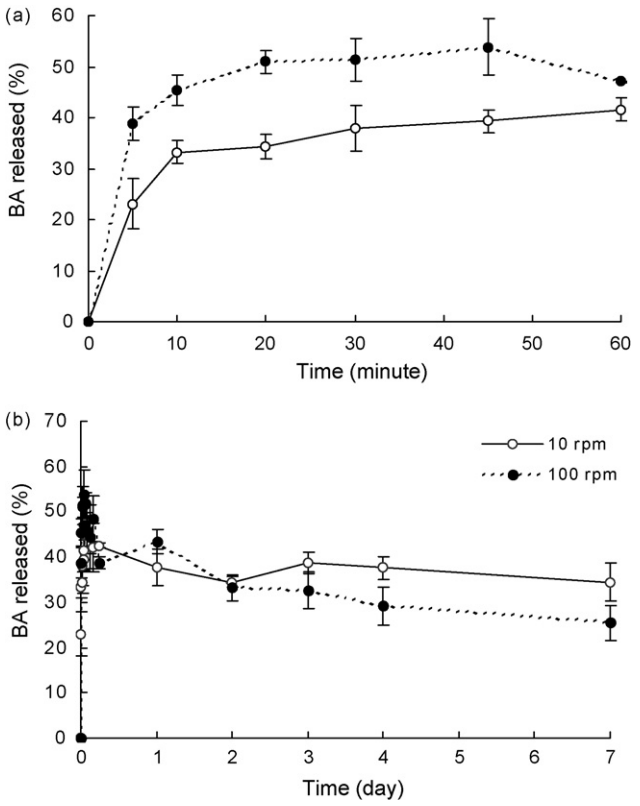

Fig. 2. Time course of buserelin acetate (BA) release from microparticle in pH 7.4 buffer over 60 min (a) and over 7 days (b), mean ± S.E. ($n = 4$); ANOVA: $p < 0.01$. Microparticles (containing 7.2 mg BA) were dispersed in 50 mL of phosphate buffer (pH 7.4). About 1 mL of test solution was withdrawn every 10 min for the first 60 min then daily for 7 days and filtrated. The BA concentrations in the diluted solution were determined by HPLC. The ratio of BA released (%) was calculated by the division process to the BA concentration of the complete dissolution (144 μg/mL).

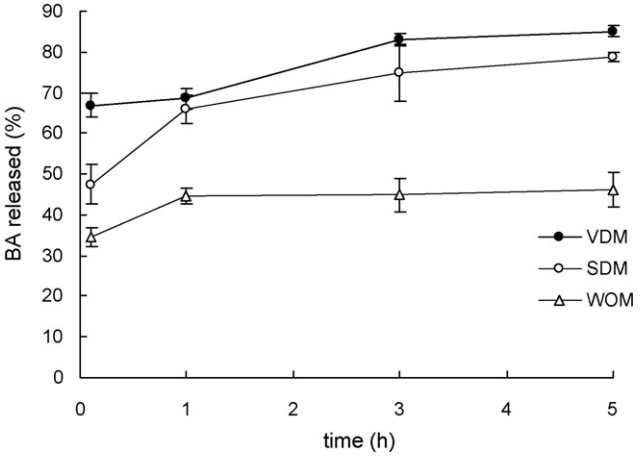

Fig. 3. Buserelin acetate (BA) release from microparticle by three different dispersion methods, mean ± S.E. ($n = 3$); ANOVA: $p < 0.01$ (VDM vs. SDM). SDM, standard dispersion method; VDM, 1 min vibration dispersion method; WOM, without oscillation method. The BA concentrations in the syringe were determined by HPLC at 0.1, 1, 3, 5 and 24 h. The ratio of BA released (%) was calculated by the division process to the BA concentration of the complete dissolution in the syringe (2400 μg/mL).

in VDM (ANOVA, $p < 0.01$) than in SDM, and the average value was almost 1.5 times higher than that in SDM. The BA release reached around 80% 5 h after dispersion by VDM and SDM. On the other hand, the initial average concentration was only 35% in WOM, and the BA concentration was only 45% even 5 h after dispersion. The apparent microparticle aggregation was observed in the syringe in WOM.

3.5. Change in shape and distribution of microparticle size by three different dispersion methods

Since the concentrations of BA released from microparticles were affected by the different dispersion methods, we investigated the morphological changes and the distribution of microparticle size by these methods. Microparticles were dispersed in water by three different methods SDM, VDM and ultrasonication (UDM) and microparticle shape was observed by scanning electron microscopy. As WOM is a weaker dispersion method than SDM we did not study the observation of the shape or size distribution of microparticles. The results are shown in Fig. 4(a). No clear difference was seen in microparticle shape among the three dispersion methods. No cracks or erosions were seen on the surface of the particles. The distribution of microparticle size determined by an optical microscope is shown in Fig. 4(b). Both distribution of size and mean particle size were similar in the three different dispersion methods. These results indicated that microparticles are relatively stable even if subjected to strong physical forces to prepare the suspension and that the difference in BA release was not due to changes in shape of microparticles induced by the dispersion methods.

3.6. Morphological change in microparticle 3 weeks after the dispersion

Morphological changes of microparticles dispersed in water by SDM is shown in Fig. 5(a). Microparticles were spherical immediately after dispersion, but the shape changed 7 days after dispersion, with pits seen at several places on the polymer surface. Further deformation and a reduction in particle size were observed 21 days after the dispersion. The distribution of microparticle size immediately and 7 days after dispersion are shown in Fig. 5(b). Modal diameter was found to be 5–10 μm after dispersion, but shrunk to 0–5 μm in 7 days, and the mean particle size also decreased by about 3 μm (35%). Based on these findings, it appears that the particle size decreases due to hydrolysis of polymers from the surface of microparticles in aqueous solution.

BA release from microparticles is proposed as three stages, as shown in Fig. 6 (Chiu et al., 1995; Sansdrap and Moës, 1997; Matsumoto et al., 2005): first, rapid BA dissolution on the polymer surface and in the space near the surface layer as a result of the microparticle dispersion in the aqueous solution (surface route). Second, gradual BA release from the intricate pores inside the polymer (pore route). Finally, release of BA distributed internally as a result of hydrolysis of the carrier polymer (matrix route).

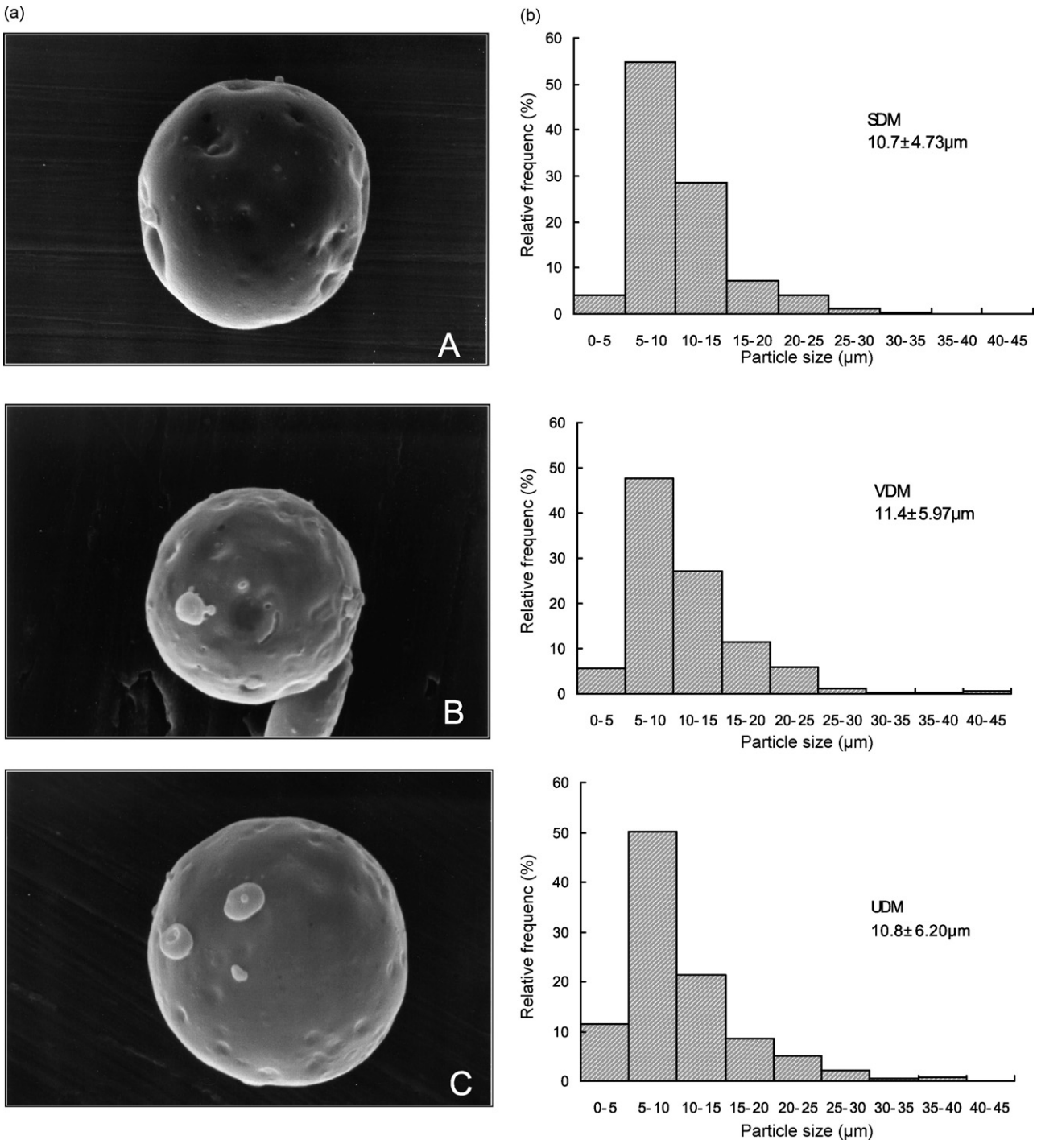

Fig. 4. Photographs of scanning electron microscope immediately after dispersion of microparticle (a) and distribution of microparticle size and mean particle size (b) in standard dispersion method (SDM), 1 min vibration dispersion method (VDM), and 3 min ultrasonic dispersion method (UDM). mean diameter ± S.D. (A) Standard dispersion method (SDM); (B) 1 min vibration dispersion method (VDM); (C) 3 min ultrasonic dispersion method (UDM).

3.7. Effect of three different dispersion methods on plasma estrone concentrations in rats

Plasma E_1 concentrations after the administration of microparticle dispersed by SDM, VDM and WOM to female rats is shown in Fig. 7. The E_1 concentrations decreased immediately following administration of all suspensions. In WOM, E_1 concentration decreased to 80.5% of baseline 2 days after administration and gradually recovered at 7 days. In SDM, E_1 concentration decreased to 63.5% of baseline 4 days after administration and recovered gradually. The decrease of E_1 concentrations was more rapid in VDM (43.1% of baseline 2 days after administration) than SDM (ANOVA, $p < 0.05$). Subsequent recovery began 2 days after administration, which was earlier than SDM.

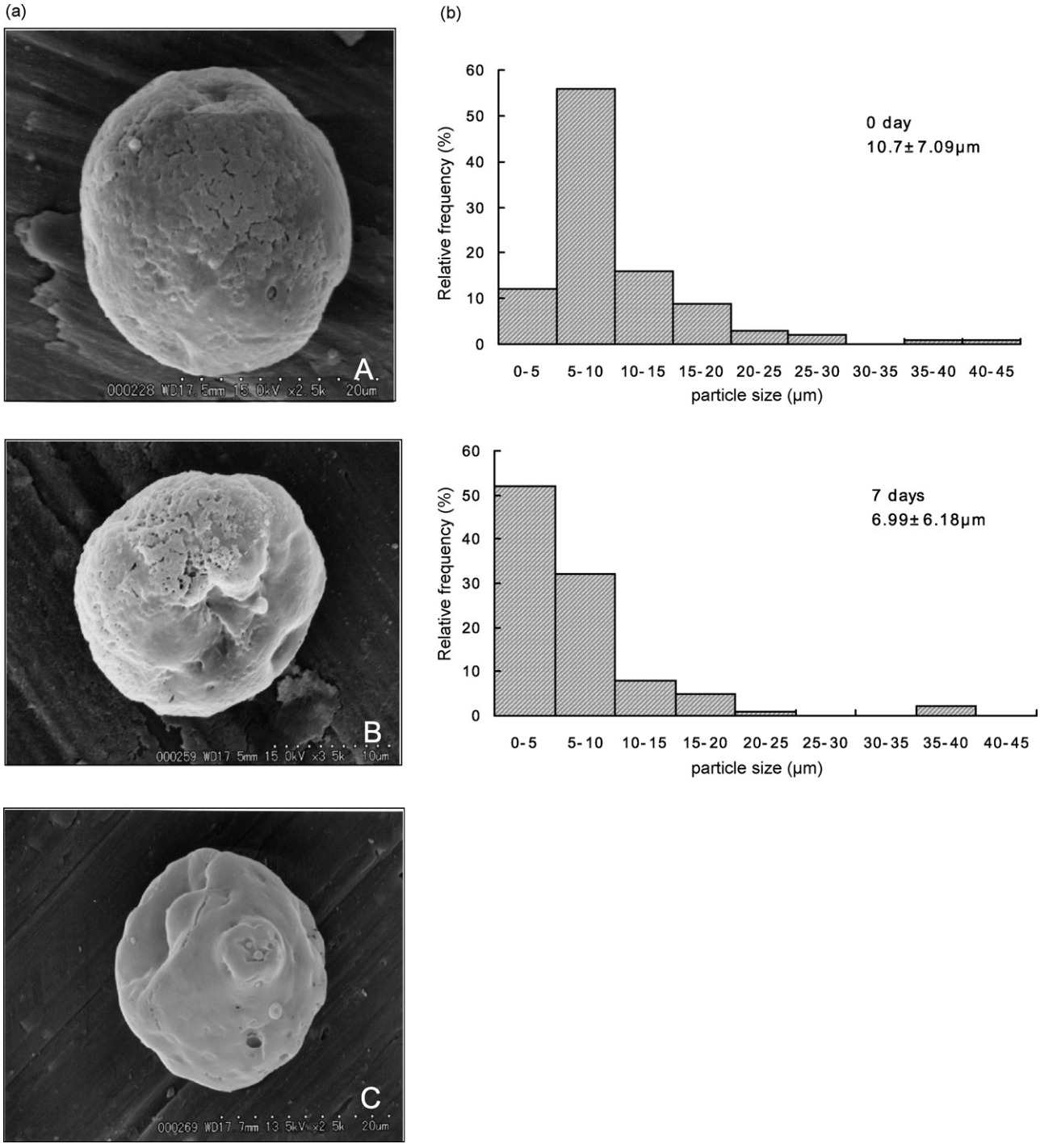

Fig. 5. Time course of morphological change in microparticle (a) and distribution of microparticle size after dispersion (b) by standard dispersion method (SDM). (A) immediately; (B) 7 days; (C) 21 days after the suspension.

These results demonstrate that the difference in the initial amount of BA released affects the rate and the degree of decrease in E_1 concentrations, which subsequently affects the recovery rate.

4. Discussion

The results of this study suggest that different dispersion methods do not change the shape and distribution of microparticle size but do change the BA release rate from microparticles and subsequently drug efficacy following administration in rats.

The co-polymer that makes up microparticles is stable when subjected to external physical stimulation. Hence, the changes in increase in the initial release rate caused by the difference in the dispersion methods were not due to hydrolysis of the co-polymer.

In this release study using a double-jacketed beaker, the initial release rate increased in association with an increase of the rate

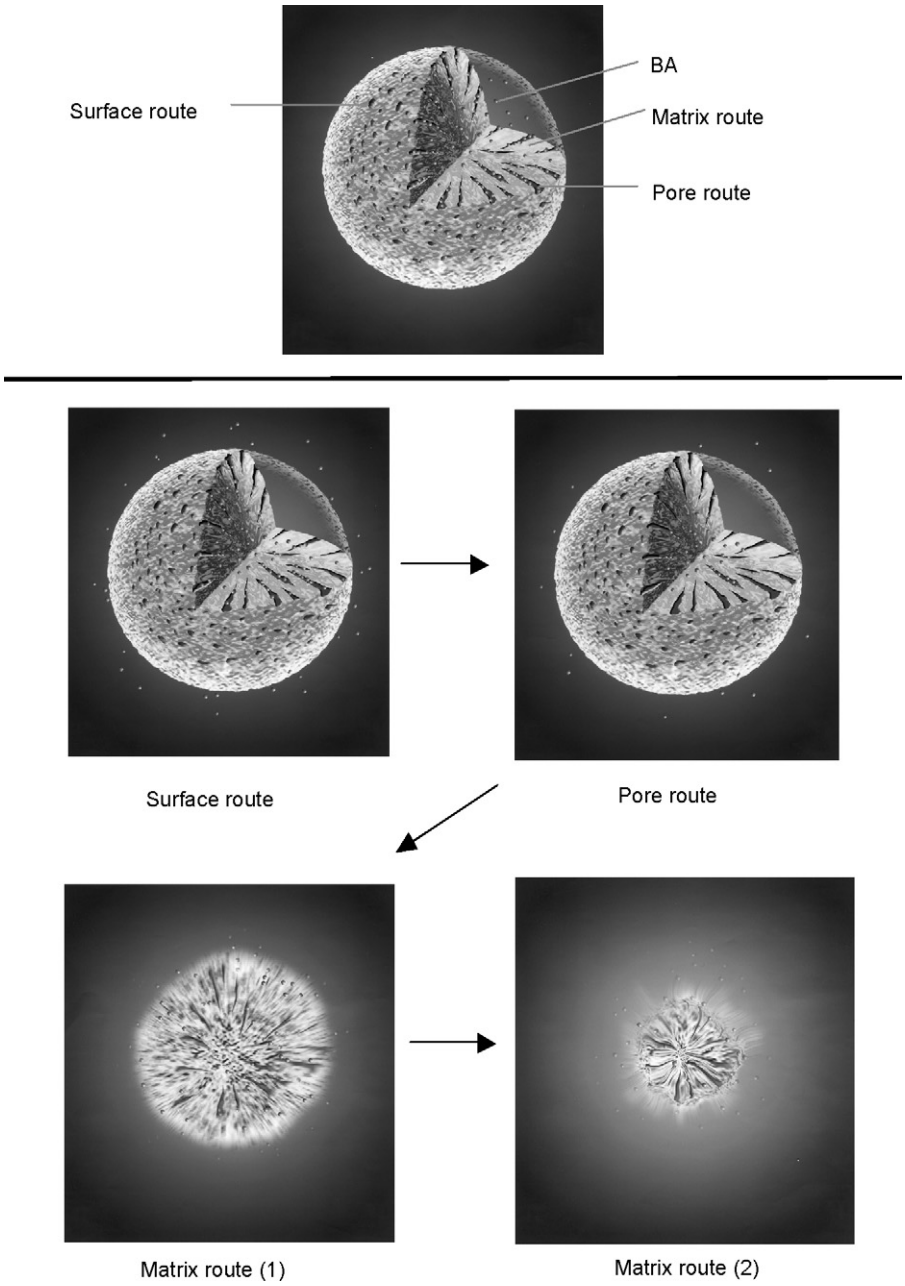

Fig. 6. Conceptive diagram for buserelin acetate (BA) release mechanism from microparticle.

of agitation. This is believed to have occurred mainly by the BA dissolution in a diffusion-controlled manner according to Fick's law from the surface route.

Additionally, the initial release rate also increased when the syringe was vibrated using the VDM method. This result is consistent with the results of the afore-mentioned study using two stirring speeds. This might be due to increased BA dissolution from surface route after physical force by the vibration. Moreover, the initial release rate was higher compared to the release study using a double-jacketed beaker. The vibration promotes water penetration into the microparticle pores. Dissolved BA at pores is promoted to the microparticle surface by the vibration. Compared to the surface route, the rate of water penetration into microparticles at the injection site could be smaller because the extracellular fluid is limited. Flow of extracellular fluid around the microparticle might also be considerably slowed. The rate of BA release via pores in vivo might be much smaller than in this in vitro study. In the case of VDM, BA in the deep region of the pore might be released rapidly via the pores in addition to surface release. In contrast, in the WOM method, microparticle aggregation occurred and the rate of water penetration into the pores might be small and BA release from microparticles might be mainly from the surface.

Observation of microparticle shape showed erosion on the microparticle surface after several days. BA contained in the

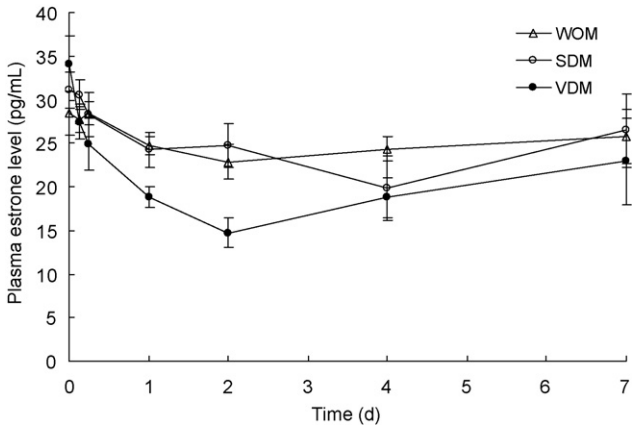

Fig. 7. Time courses of E_1 concentrations after microparticle administration using three different microparticle dispersion methods in rats. SDM, standard dispersion method; VDM, 1 min vibration dispersion method; WOM, without oscillation method, mean ± S.E. ($n = 4$); ANOVA: $p < 0.05$ (SDM vs. VDM). Using a heparin sodium syringe, blood was collected at 0, 3, 6 h for the first day, then on days 1, 2, 4 and 7 (0.5 mL each time) from the external jugular vein via the subclavian sternocleidomastoid muscle. Plasma E_1 concentration was determined using the RIA double antibody technique.

interior region of microparticle may be continuously released by hydrolysis of the polymer. Polymer route could provide BA after the BA release from the surface route or the pore route. It is conjectured that differences in dispersion methods affect dissolution or BA release via the surface route and the pore route at a relatively early stage, i.e., before polymer hydrolysis occurred.

The release of the drug did not fit to Higuchi's equation. Higuchi's equation applies to the release of drug from insoluble matrices (Higuchi, 1963). In this study, the release of the drug from microparticles was considered to be dissolution or penetration from surface and pore routes.

There is transient increase of E_2 concentration for about the first week which is followed by downregulation of GnRH receptors which begins and continues for 4 weeks (Aventis Pharma, 2002; Mizuguchi et al., 1996) when a microparticle preparation is administered to humans. In the present study, the concentration of estrogen, E_1 was determined, as it does not change greatly as a result of the estradiol cycle, although compared to E_2, E_1 has been reported to show weaker physiological activity and lower drug susceptibility. Therefore, we administered a dose that was about 80 times higher than that used in humans. As a result, the E_1 concentrations decreased immediately after administration in rats. In other words, downregulation of GnRH receptors occurred more quickly without an initial increase in E_1 concentrations. By the determination of E_1 concentrations in rats, we found that their decrease were significantly larger using VDM compared with SDM at an early stage. An increase in the initial BA release might enhance the decrease of E_1 concentration. We should investigate E_2 concentrations that have stronger physiological activity in the future in order to predict the effect in humans.

Recovery of the E_1 concentration began earlier in the VDM method compared to SDM. These results also demonstrated that remaining BA in microparticles might affect the degree of decrease in E_1 concentrations at the terminal stage.

5. Conclusion

The BA release from microparticle takes place via three release pathways. Strong physical stimulation for dispersion increases initial BA release, especially from the surface and pore routes, in both of which BA is dissolved in a diffusion-controlled manner. The increase in initial BA release enhances the decrease in E_1 concentrations after administration in rats due to early downregulation of GnRH receptors, and speeds up the subsequent recovery of the E_1 concentrations.

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