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Kinetic analysis of in vitro and in vivo release of prednisolone from the conjugate of glycol-chitosan and succinyl-prednisolone

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ABSTRACT

Recently, many people have developed rheumatoid arthritis (RA), and prednisolone (PD) is often used for treatment; however, long use and a large dose of PD can cause toxic side effects. In this study, in order to enhance the therapeutic effects and to suppress the toxic side effects, the conjugate (GC–SP) was prepared by coupling between glycol-chitosan (GC) and succinyl-prednisolone (SP). The drug-release properties of GC–SP were examined and analyzed kinetically. The plasma concentration–time profiles of GC–SP and released PD were investigated after i.v. injection to normal rats, and their pharmacokinetic profiles were analyzed. PD was stable and released gradually (ca. 1%/h) from GC–SP at physiological pH, while PD was unstable at basic pH and the release from GC–SP was accelerated at basic pH. GC–SP showed good systemic retention (more than 16-fold area under the plasma concentration–time curve (*AUC*) as compared to PD alone), and released PD gradually in vivo. The in vivo release rate was calculated to be much faster than the in vitro rate. From these results, it is expected that GC–SP will be accumulated at inflammatory sites based on enhanced permeability and retention (EPR) effects, and release PD there effectively.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint synovial inflammation and progressive cartilage destruction (Weyand and Goronzy, 1997). Steroidal and non-steroidal agents are often used in treating joint inflammation (Aghighi et al., 2008; Mekić and Ristić, 2008). Prednisolone (PD) is one of the most widely used steroidal agents for chronic inflammation diseases because it is eliminated fairly rapidly and is not very toxic (Nagahama et al., 2000; Sood et al., 2002; Ito et al., 2005). However, long use and a large dose of PD can cause toxic side effects, such as immune suppression, osteoporosis, adrenal failure and diabetes (Laan et al., 1999; Cağdaş et al., 2008). In order to enhance the therapeutic effects and to suppress the toxic side effects, target drug delivery to the diseased site is suggested to be a useful approach. Nanoparticles or liposomes containing steroidal agents, which could be targeted well to an inflamed joint, showed enhanced anti-inflammatory effects (Metselaar et al., 2003; Higaki et al., 2005). Such targeting is based on the increased permeability of the local vascular endothelium at the inflammatory site, which is the so-called enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986). Namely, as neovascular vessels are highly developed at RA tissues, target delivery systems utilizing the EPR effect are suggested to be useful to treat the disease (Hwang et al., 2008; Liu et al., 2008).

Such targeting is often completed using macromolecular prodrugs because they can be targeted to the inflammatory site due to the EPR effect (Onishi and Machida, 2005; Chau et al., 2006). Water-soluble chitosans, such as succinyl-chitosan and glycol-chitosan (GC), have characteristics to circulate for a long time in the blood stream (Kamiyama et al., 1999). As GC has many reactive amino groups and is not very toxic, it is useful for coupling with agents with carboxy groups (Sato et al., 1996). In this study, the conjugate between GC and succinyl-prednisolone (SP), named GC–SP, was prepared as a macromolecular prodrug for PD in an attempt to develop a delivery system to the RA site. In the present study, the in vitro stabilities or drug release properties of GC–SP were examined and analyzed kinetically. Furthermore, the plasma concentration–time profiles of GC–SP and released PD were monitored after i.v. injection to normal rats, and the in vivo release properties and their pharmacokinetics were investigated.

2. Materials and methods

2.1. Materials

Prednisolone (PD), glycol-chitosan (GC), polyethylene glycol 400 (PEG400) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Prednisolone 21-

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hemisuccinate (SP) sodium salt was obtained from Sigma–Aldrich Co. (St. Louis, USA). All other chemicals were of reagent grade.

2.2. Animals

Male Wistar rats (7 weeks old, ca. 220 g) were purchased from Tokyo Laboratory Animals Science Co. Ltd. (Tokyo, Japan), and bred on the breeding diet MF supplied by Oriental Yeast, Co., Ltd. (Tokyo, Japan) with water ad libitum at 23 ± 1 °C, relative humidity of 60 ± 5% and a 12 h light–dark cycle. They were used for the experiments soon after purchase. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Japan. The animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

2.3. Preparation of conjugate

The conjugate of GC and SP, named GC–SP, was prepared as follows. GC (200 mg) was dissolved in 40 ml water, and the solution pH was adjusted to 6.5 using a 10% (w/v) HCl aqueous solution. SP sodium salt (100 mg) was added to the solution, and the solution pH was adjusted to 6.5 in the same manner. Then, EDC (1000 mg) was added to the solution, stirred under ice cooling for 3 h, and stirred at room temperature for 21 h. After the resultant mixture had undergone ultracentrifugation at 70,000 × *g* for 1 h, the supernatant was chromatographed with a Sephadex G50 column (4 cm in inner diameter × 20 cm in height) using 1/15 M phosphate buffer of pH 6 as an elution solvent. The fractions of macromolecular weight were taken and dialyzed against water using a cellulose tube with a MW cut-off of 12,000. The solution remaining after dialysis was used as a GC–SP aqueous solution in the following experiments. The content of PD was determined from UV absorption of the GC–SP solution at 246 nm. After the GC–SP solution was mixed with polyethylene glycol 2000 (PEG2000), a little more than the expected amount of GC–SP, the mixture was lyophilized. The obtained powder was dissolved in deuterium oxide, the ^{1}H NMR spectrum was measured with a JNM-GX270 spectrometer (JEOL, Tokyo, Japan).

2.4. In vitro stability tests of PD

A 1/15 M acetate buffer of pH 4, 1/15 M phosphate buffers of pH 6 and 7.4 and a 1/15 M carbonate buffer of pH 9 were used in this study as the buffered solutions. Stability tests of PD were performed as follows. Ten milliliters of PD solution (80 μg/ml) was prepared using the mixture of water and each buffer (3:7, v/v), and incubated at 37 °C by shaking horizontally at 100 strokes/min. The aliquot samples (200 μl) were withdrawn just before incubation and at 7, 24 and 48 h. Immediately after each sampling, 800 μl of 0.1 M acetate buffer of pH 4 was added to each sample (200 μl). The resultant solution was analyzed by HPLC to determine the PD concentration.

2.5. In vitro release tests of PD from GC–SP

The same buffer systems as in the stability tests of PD were used in the in vitro release tests of PD from GC–SP. Ten milliliters of GC–SP solution (80 μg PD eq./ml) was prepared using the mixture of water and each buffer (3:7, v/v), and incubated at 37 °C by shaking horizontally at 100 strokes/min. Aliquot samples (200 μl) were withdrawn at 0.5, 1, 3, 7, 24 and 48 h. Immediately after each sampling, 800 μl of 0.1 M acetate buffer of pH 4 was added to each sample (200 μl). The resultant solution was analyzed by HPLC to determine the PD concentration.

2.6. Pharmacokinetic study after i.v. administration

PD was dissolved in 30% (w/v) PEG400 aqueous solution, and injected intravenously into rats via the tail vein at 2.5 mg/kg (0.5 ml). Separately from this, a GC–SP aqueous solution was administered intravenously to rats via the tail vein at 2.5 mg PD eq./kg (0.5 ml). At 0.25, 0.5, 1, 2, 7, 24 and 48 h after the administration of PD or GC–SP, blood samples (0.5 ml) were withdrawn and centrifuged at 1500 × *g* for 10 min to obtain plasma. Saturated NaCl aqueous solution (100 μl), 5% (w/v) phosphoric acid (100 μl) and 4 ml of the mixture of *t*-tributylmethyl ether and pentane (2:3, v/v) were added to the plasma (100 μl), and shaken vigorously 100 times. After the mixture was centrifuged at 1500 × *g* for 10 min, the supernatant (3 ml) was taken. Three milliliters of the mixture of *t*-tributylmethyl ether and pentane (2:3, v/v) was added to the residue, shaken and centrifuged in the same manner as above. The supernatant (2.2 ml) was added to the previously taken supernatant (3 ml). The resultant organic phase was dried under nitrogen gas, and the residue was dissolved with 200 μl of the HPLC mobile phase. The solution was analyzed for PD concentration by HPLC. For GC–SP, the obtained PD concentration corresponded to the concentration of free PD.

As for the samples for GC–SP administration, the plasma concentration of total PD (=free PD + conjugated PD) was investigated as follows. One hundred microliters of plasma sample, obtained as stated above, and 30 μl of 0.1 M NaOH aqueous solution were mixed, and incubated at 45 °C by shaking horizontally for 10 min. Then, 300 μl of 0.1 M acetate buffer of pH 4 was added to the mixture. The resultant sample (100 ml) was treated in the same manner as above, and analyzed for PD concentration by HPLC. This PD concentration was the concentration of total PD, and the concentration of conjugated PD was calculated by subtracting the concentration of the above free PD from that of the total PD.

2.7. HPLC assay

HPLC was used to determine the PD concentration in each sample solution. The following apparatus and conditions were used. The HPLC system consisted of an LC-6AD pump, an SPD-10AV spectrophotometric detector, and a C-R7A plus chromatopac (Shimadzu Corp., Kyoto, Japan). A Supelcosil LC-18-DB column (4.6 mm in inner diameter × 150 mm in length, particle size 3 μm; SUPELCO, Bellefonte, USA) was used as the analytical column. The detector was set at 246 nm, and the column was set at 30 °C using a column oven. A 20% (v/v) 2-propanol aqueous solution containing 0.1% (v/v) trifluoroacetic acid was used as the mobile phase, and the flow rate was 1.2 ml/min. The absolute calibration curve method was applied for quantification analysis.

2.8. Statistical analysis

Statistical evaluation of the experimental data was performed using the unpaired *t*-test. Significant difference was set as $p < 0.05$.

3. Results and discussion

3.1. Characterization of GC–SP

The content of PD in GC–SP was 8.5% (w/w) from the UV absorption study. The chemical structure of GC–SP was confirmed from its ^{1}H-NMR spectrum. Namely, since it was composed of only the signals of GC and SP, GC–SP was found to be a conjugate between GC and SP. Furthermore, the comparison of the integrated intensities of the protons of SP and those of the protons of GC showed PD content similar to that obtained by UV absorption analysis. The proposed structure of GC–SP is described in Fig. 1.

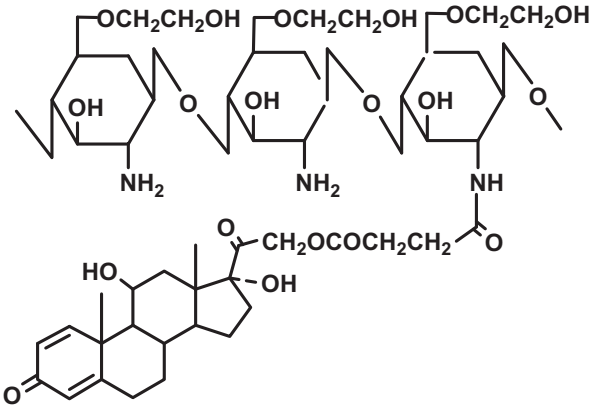


Fig. 1. Proposed structure of GC–SP.

3.2. Stability of PD and release of PD from GC–SP at different pHs

The profiles of the remaining PD during incubation of PD in the tested buffered solutions are shown in Fig. 2. PD was very stable in acidic and neutral media, but the decomposition was found markedly at pH 9. The ratio of the remaining PD at pH 9 was 40% 24 h after incubation. PD release from GC–SP was investigated under the same conditions. The PD release was accelerated with the increase in pH (Fig. 3). The ratios of free PD at 24 after incubation were 1.4, 9.9, 24 and 49% at pHs 4, 6, 7.4 and 9, respectively.

3.3. Kinetic analysis for in vitro stability of PD and release of PD from GC–SP

The degradation of PD at different pHs almost followed the pseudo-first-order kinetics; the degradation rate constant (h_0) was calculated with the pseudo-first-order kinetics in the following equation:

$$A(t) = A(0) \times \exp(-h_0 t) \quad (1)$$

$A(t)$ represents the ratio of the remaining PD at time t. As some data were over 100% for remaining PD, the mean values were used

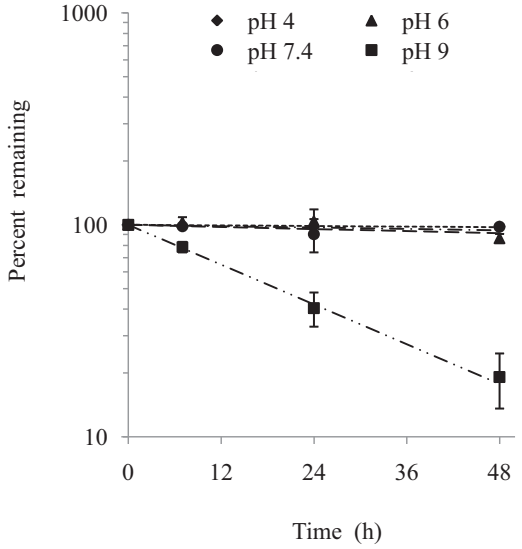


Fig. 2. Stability of PD in aqueous buffered solutions of different pHs at 37 °C. Each point represents the mean ± S.D. of the observed data (n = 3). The dotted, broken, dashed and dashed double-dotted lines represent the calculated values at pHs 4, 6, 7.4 and 9, respectively.

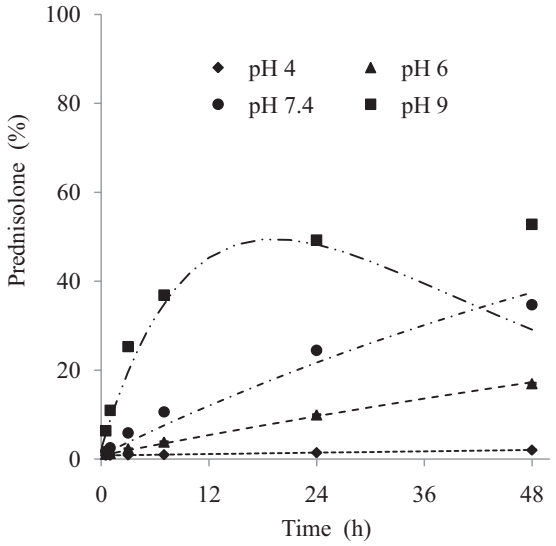


Fig. 3. PD release profiles from GC–SP in aqueous buffered solutions of different pHs at 37 °C. Each point represents the mean ± S.D. of the observed data (n = 3). The dotted, broken, dashed and dashed double-dotted lines represent the calculated values at pHs 4, 6, 7.4 and 9, respectively.

for profile fitting or calculation, in which non-linear least squares program MULTI (Yamaoka et al., 1981) was used.

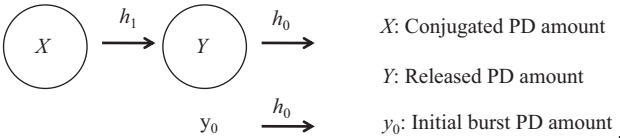
The conversion for GC–SP was analyzed with the kinetic model shown in Table 1, in which the percentage of released PD in the media ($Y(t)$) was given by the following equation:

$$Y(t) = (100 - y_0) \times h_1 \times \frac{(\exp(-h_1 t) - \exp(-h_0 t))}{(h_0 - h_1)} \quad (2)$$

The value of h_0, obtained from the stability studies of PD (Eq. (1)), was used as the fixed value. The value of the initial ratio of free PD (y_0) was determined by extrapolation of the first curve of the conjugated PD to y axis. Then, the parameter h_1 was calculated by fitting the equation ($Y(t) + y_0 \times (\exp(-h_0 t))$) to the observed profile. The obtained parameters are shown in Table 1. The h_0 value was calculated using the mean value of PD remaining, and the obtained value was as a fixed one. Therefore, the mean values of released PD were also used for this analysis, in which non-linear least squares program MULTI (Yamaoka et al., 1981) was used.

In addition, the observed PD release rate from GC–SP could be obtained by deconvolution using the profile of the released PD ($Y(t)$) (not including that by initial burst) and the PD conversion profile in each medium. In Fig. 4, the observed release rate is described as a step function (solid line), and the broken line shows the conversion rate given by the first-order rate constant, which is expressed as $h_1 \times X(t)$. As for the conditions of pH 4 and 6, the observed rates were almost the same as the calculated rates, and were almost con-

Table 1
In vitro conversion rate constants of GC–SP and PD at different pH media.



pH	y_0 (%)	h_0 (h^{-1})	h_1 (h^{-1})
4	0.82	0.0006	0.0003
6	0.79	0.0019	0.0040
7.4	0.85	0.0012	0.0100
9	2.28	0.0360	0.0702

The mean values were used for profile fitting or calculation. h_0 and h_1 are first-order rate constant (h^{-1}).

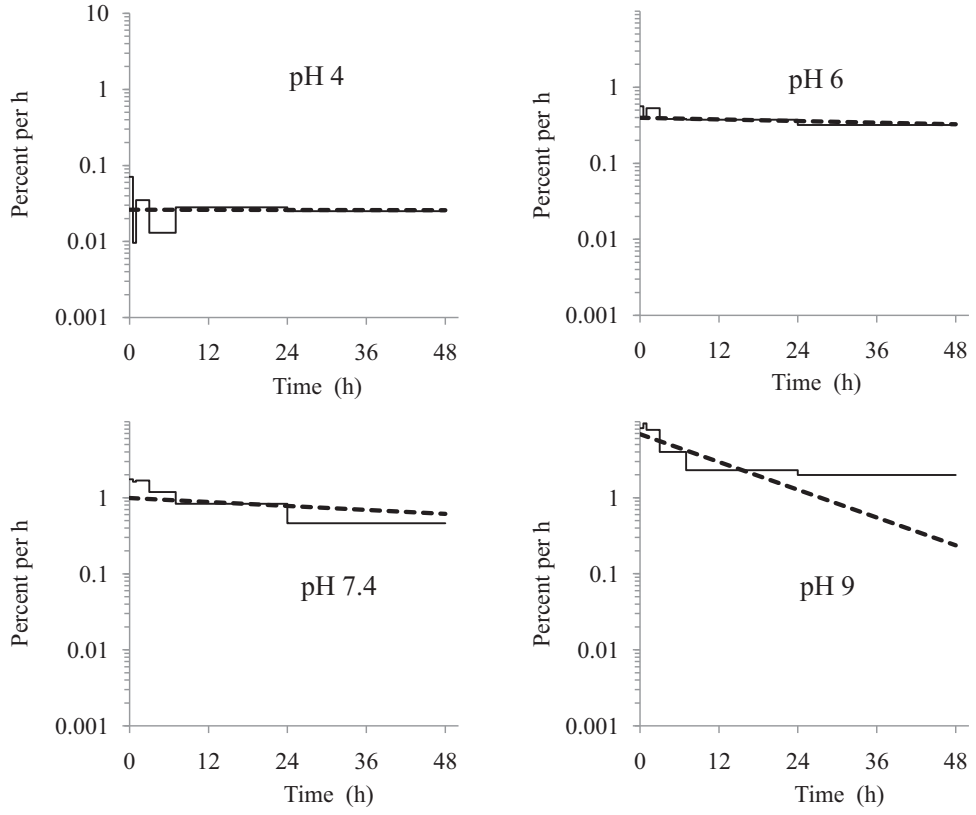


Fig. 4. In vitro conversion rate from GC–SP to PD in aqueous buffered solutions of different pHs at 37 °C. The solid line represents the observed rate, and the broken line represents the calculated rate. The mean values were used for calculation.

stant, 0.03%/h and 0.4%/h, respectively. On the other hand, with pH 7.4 and 9, the observed release rates were different from the calculated rates, which were estimated from the results in Fig. 3. In particular, at pH 9, the calculated profile was very different from the observed one. The conversion analysis shown in Fig. 4 indicated that the observed conversion rate was higher in the initial and latter stage than the rate calculated from the first-order rate constant. Also, the calculated rate appeared to be greater in the middle stage (around 3–12 h). Probably, this means the conversion mechanism is not simple; for example, other phenomena, such as acyl migration (Anderson and Taphouse, 1981; Anderson et al., 1984; McLeod et al., 1993), would be related, resulting in the discrepancy between the observed and calculated profiles.

3.4. Plasma concentration–time profiles after i.v. administration

The plasma concentration of PD was investigated after i.v. injection of the PD solution. As shown in Fig. 5A, the concentration–time profile could be fitted to the curve of the 2-compartment model. The concentration, C_{PD}, was expressed as follows:

$$C_{PD}(t) = 5.567 \times \exp(-6.4230t) + 0.739 \times \exp(-0.0412t) \quad (3)$$

The related kinetic parameters, V_p, r, l_1 and l_2, were calculated from Eq. (3). They are shown in Table 2 and the calculated curve is shown in Fig. 5A as a broken line. In some cases, the plasma level or obtained parameter value was deviated too largely. Considering such situations, the mean values were used for profile fitting

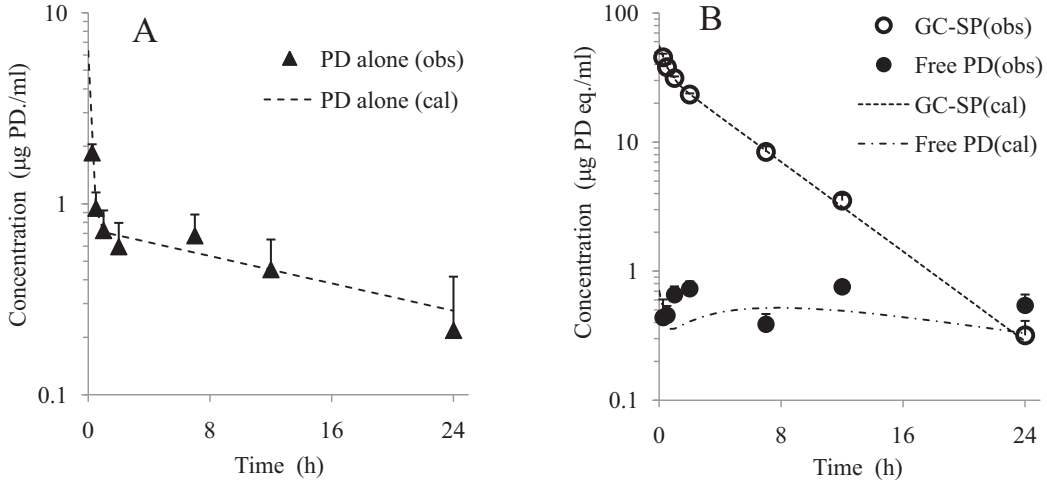
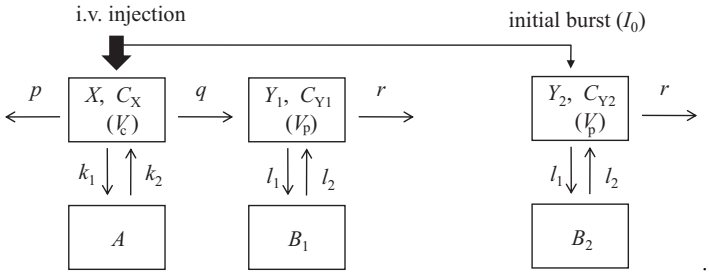


Fig. 5. Plasma concentration–time profiles after i.v. bolus administration of PD alone (A) and GC–SP (B) to normal rats. The results are expressed as the mean ± S.E. (n = 4). The broken, dotted and dashed lines represent the calculated concentrations for PD for PD alone, conjugated PD and free PD for GC–SP. The mean values were used for calculation.

Table 2
Pharmacokinetic and conversion parameters for PD and GC–SP after i.v. injection of GC–SP at 2.5 mg PD eq./kg in normal rats.



V_c (ml/kg)	V_p (ml/kg)	k_1 (h^{-1})	k_2 (h^{-1})	l_1 (h^{-1})	l_2 (h^{-1})	p (h^{-1})	q (h^{-1})	r (h^{-1})	I_0 (mg PD/kg)
39.7	396.4	0.657	1.461	5.340	0.789	0.000	0.304	0.304	0.280

The mean values were used for profile fitting or calculation. X, Y_1 and Y_2 are plasma amounts for GC–SP, released PD and initial burst PD, respectively. C_X, C_{Y_1} and C_{Y_2} are plasma concentration for GC–SP, released PD and initial burst PD, respectively. V_c and V_p are central compartment distribution volumes of GC–SP and free PD, respectively. k_1, k_2, l_1, l_2, p, q and r are first-order rate constants (h^{-1}). I_0 is in vivo initial burst at the dose of 2.5 mg PD eq./kg.

or calculation, in which non-linear least squares program MULTI (Yamaoka et al., 1981) was used.

The concentrations of free PD and conjugated PD were examined in the case of i.v. administration of the GC–SP solution. Their conjugated PD was eliminated gradually from the systemic circulation, and the free PD was maintained at 0.3–0.8 μg/ml for 24 h (Fig. 4B). The plasma concentration of the conjugated PD (C_X) was fitted to the curve of the 2-compartment model (Table 2). Also, the in vivo kinetic model is shown in Table 2. Y and C_Y represent the distributed amount and concentration of free PD in the central compartment. Y contained the PD released from the conjugate (Y_1) and the PD derived from initial burst (Y_2). Likewise, C_Y contained the PD concentration caused by the release (C_{Y_1}) and the PD concentration based on initial burst (C_{Y_2}). The plasma level of the free PD (C_Y) was given as ($C_{Y_1} + C_{Y_2}$). The elimination of conjugated PD was divided into direct disappearance (p) and the input of free PD (q). The kinetic parameters obtained above for free PD, V_p, r, l_1 and l_2, were fixed as they were, and then the conversion parameters for conjugated PD and in vivo initial burst (I_0) were calculated by the profile fitting of the model-calculated equations to the observed concentrations. The parameters for PD were calculated using the mean value of the PD plasma concentrations for i.v. injection of free PD as described above. These obtained values of PD were fixed and used in this analysis. Therefore, the mean values of plasma levels of remaining GC–SP and released PD were also used for this analysis, in which non-linear least squares program MULTI (Yamaoka et al., 1981) was used. The similar approach had been reported by Hashida et al. (1984).

As the results, as p was converged to almost zero or minus, p was fixed to zero. As the calculated profiles obtained by including I_0 as a variable parameter were improved slightly, I_0 was used as a variable parameter. The final equations obtained by these profile fittings were expressed as follows:

$$C_X(t) = 21.065 \times \exp(-2.2226t) + 34.586 \times \exp(-0.2001t) \quad (4)$$

$$C_Y(t) = C_{Y_1}(t) + C_{Y_2}(t) \quad (5)$$

$$C_{Y_1}(t) = 0.100 \times \exp(-2.2226t) - 0.633 \times \exp(-0.2001t) - 0.286 \times \exp(-6.4230t) + 0.819 \times \exp(-0.0412t) \quad (6)$$

$$C_{Y_2}(t) = 0.624 \times \exp(-6.4230t) + 0.083 \times \exp(-0.0412t) \quad (7)$$

Accordingly, the parameters related to the conversion of the conjugated PD and distribution volumes are obtained as shown in Table 2. The calculated curves given by C_X and C_Y are shown in Fig. 5B as broken and dashed lines, respectively. The area under the plasma concentration–time curve (AUC) of PD in Fig. 5B is similar to that of PD itself in Fig. 5A (see Table 3). This suggested that the direct disappearance of GC–SP should be regarded as slight or negligible, which was consistent with the result that $p = 0$ in the kinetic model analysis (Table 2). The release rate of PD in vivo was much faster than that in vitro at pH 7.4. As the addition of rat plasma to the GC–SP solution of pH 7.4 at 1:1 (v/v) ratio was found to almost double the release rate of PD (data not shown), the release of PD was considered to be accelerated in vivo, which might be mostly due to enzymatic contribution.

In addition, the observed conversion rate was calculated by deconvolution using the value of subtracting initial burst contribution from the observed concentration of free PD, that is (the observed free PD concentration – C_{Y_2}), and the function in the i.v. bolus injection (C_{PD}). The result is shown in Fig. 6. As the concentration at 7 h was fairly lower than that at 2 h, the observed rate for 2–7 h became extremely low. On the other hand, the calculated conversion rate was given with $q \times X$, which were parameters in Table 2. Overall, the observed conversion rate was faster than the calculated rate to some extent, which led to the free PD profile in Fig. 5B.

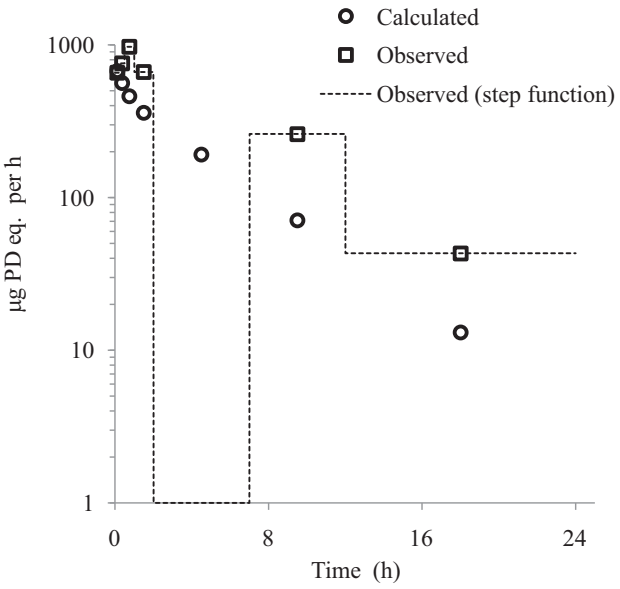


Fig. 6. In vivo conversion rate from GC–SP to PD after i.v. bolus administration of GC–SP in normal rats. The dotted line represents the observed rate calculated by deconvolution. The mean values were used for calculation.

Table 3
Pharmacokinetic parameters for plasma concentration–time profiles after i.v. injection of PD alone and GC–SP at 2.5 mg PD eq./kg in normal rats.

Sample injected	Species in plasma	C_{max} (mg/ml)	T_{max} (h)	AUC_{0-24h} (mg h/ml)	MRT_{0-24h} (h)	VRT_{0-24h} (h^2)
PD alone	PD	3.99 ± 0.92	0.00 ± 0.00	12.19 ± 1.29	8.64 ± 0.42	45.03 ± 4.02
GC–SP	GC–SP	49.18 ± 6.98[#]	0.00 ± 0.00	200.91 ± 8.13[$]	4.26 ± 0.24[$]	19.98 ± 1.34[$]
	PD released	0.77 ± 0.06[#]	4.25 ± 2.59	14.52 ± 1.64	11.86 ± 0.44[#]	55.85 ± 2.82[*]

The results are expressed as the mean ± S.E. ($n = 4$).
[*] $p < 0.05$ vs. PD (PD alone).
[#] $p < 0.01$ vs. PD (PD alone).
[$] $p < 0.001$ vs. PD (PD alone).

Moment analysis was performed using the observed plasma concentrations. The *AUC*, mean residence time (*MRT*) and variance of residence time (*VRT*) were calculated by the trapezoidal method using the program MULTI (Yamaoka et al., 1981). The results are shown in Table 3. GC–SP exhibited much greater *AUC* significantly ($p < 0.001$) than that by PD solution, indicating that GC–SP showed good systemic circulation. After i.v. administration of GC–SP, the initial concentration of free PD was suppressed as compared with that after i.v. administration of PD alone; that is, the C_{max} of free PD was much lower with PD alone than with GC–SP ($p < 0.01$). This was because PD was released gradually in vivo. Also, as stated above, the conversion of GC–SP to PD was considered to be good, because the *AUC* of the released PD was almost equal to that by the PD solution. These results suggested that GC–SP might act as a polymeric prodrug of PD with passive targeting ability due to the good systemic retention.

The potency of steroids is determined by the concentration, and efficacy depends on potency and duration at the target site. Various effects are included in the steroidal responses, and the concentrations producing the half-maximal effects (sensitivities) are varied among kinds of responses (Czock et al., 2005). As to PD, lymphocyte suppression appeared to be achieved in the range of several dozen to one hundred ng/ml of a plasma level (Czock et al., 2005). These responses and effective levels are different among the kinds of steroids, in which differences for the receptor-binding affinity or subsequent response mechanism are involved. Therefore, the prolonged supply of PD in the systemic circulation (Fig. 5) implies elongation of PD effect, which might be an advantage of GC–SP. However, the maintenance of the plasma level is not directly associated with enhancement of efficacy and suppression of toxic side effect. The strong point of GC–SP is its high systemic retention rather than the elongated plasma level of free PD. That is, GC–SP is considered to be useful for the treatment of local inflammatory disease such as arthritis because a good systemic circulation of macromolecules can generally facilitate drug passive targeting to the inflamed site where enhanced permeability and retention (EPR) effect is observed as a physiological property (Matsumura and Maeda, 1986; Hwang et al., 2008; Liu et al., 2008). Then, efficacy is considered to emerge by the release of PD from GC–SP localized in the inflammatory site. The targeting ability of GC–SP and its efficacy will be elucidated in the following studies using the diseased model of inflammation.

4. Conclusions

The macromolecular conjugate (GC–SP) was prepared by coupling GC and SP using water-soluble carbodiimide. The stability of PD and its release from GC–SP were examined in vitro at different pHs. PD was stable and was released gradually in aqueous buffer of physiological pH. The actual release rate was analyzed by the deconvolution technique, and was slightly different from the first-order kinetic rate. After i.v. injection to normal rats, the plasma concentration–time profiles were investigated. The profiles of PD and GC–SP could be analyzed with a two-compartment model. GC–SP exhibited good systemic retention, and released PD gradually in vivo. Thus, GC–SP might be useful as a targeted delivery system to RA sites based on EPR effect because neovascular vessels are highly developed at RA tissues. This will be elucidated in the following study.

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