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Review Article

Macromolecular Ester Prodrug of Prednisolone with Chondroitin Sulfate for the Treatment of Rheumatoid Arthritis

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Abstract

This paper introduces a drug delivery system candidate for the treatment of rheumatoid arthritis (RA). It was designed as a conjugate between prednisolone (PD) and chondroitin sulfate (CS) via a glycine linker. The obtained conjugate, named CS-GP was examined for the property and feasibility as the DDS for the treatment of RA. First, the release characteristics were investigated under different pH conditions. PD was released directly from CS-GP, it was gradually released at physiological pH (pH 7.4), and more slowly regenerated at acidic RA conditions (pH 6 – 7.3). The efficacy was evaluated using rats with adjuvant-induced arthritis. CS-GP suppressed the swelling of the joints more than PD alone and the mixture of PD and CS, which indicated that the conjugate improved the efficacy of PD. The pharmacokinetic analysis using normal rats exhibited the well-retained manner of CS in blood circulation, and organ distribution was small with i.v. injection of CS-GP. Furthermore, CS-GP showed higher localization to the inflammatory joints than normal ones, which supported that CS-GP should have a targeting potential to the inflammatory sites. These resultant physicochemical characteristics, biological functions and pharmacokinetic features of CS-GP demonstrated the possible usefulness of CS-GP as an anti-arthritic delivery system.

INTRODUCTION

One of the most common autoimmune diseases is rheumatoid arthritis (RA). Population of RA is reported to be about 70 million in the world [1]. As it causes various complications andreduces healthy life-span, its treatment is the most important medical problem [2-4].

The etiology of RA has been studied by many researchers, and now both genetic background and environmental factors have been associated with the disorder of immune tolerance and rising autoimmunity [5,6]. Namely, arthritis-generating antigens or immune complexes are caused around synovium from genetic risk conditions and disordered environments related to bacteria or virus [7]. As a result, the synovial inflammation develops; that is, immunological cells, synoviocytes and neo vasculatures proliferate, leading to persistent pain, stiffness and joint swelling,which caused deterioration of the quality of life (QOL) [8-10]. Furthermore, chronic diseased conditions activate inflammatory cells via cytokines and other chemical mediators. Finally, RA causes destruction of cartilage and bone [11,12]. The onset and progressive pathway of RA are described in Figure (1).

There are several approaches in the treatment of RA.Surgery improves joint function, and physiotherapy is performed to recover motor function. Pharmacotherapy is important to ameliorate inflammation and joint destruction.As to the medical therapy, non-steroidal anti-inflammatory drugs (NSAIDs) [13,14], disease-modifying anti-rheumatic drugs (DMARDs) [15] and glucocorticoids [13,14,16] have been used to manage various disease states.Recently, biologics (antibody drugs) have been developed actively, and their use is spreading progressively because of their high potency and low toxicity [17,18]. Since biologics are high costly and can cause serious toxic side effects in rare cases [19], conventional small molecular drugs still play an important role in the RA medication.

Recently, in order to improve the therapeutic activity of conventional drugs, drug delivery systems (DDS) have been developed.As to the treatment of rheumatoid arthritis (RA), various drug delivery systems have been developed for conventional anti-inflammatory agents [20-24]. In particular, a targeted delivery system is one of the best ways in order to improve the therapeutic effect because it enables high drug concentration at the target site and reduces the drug distribution in other parts [24-26]. Such a delivery system is expected to display a high potency at low dosage or less administration frequency, leading to the reduction of the toxic side effects. As to the RA histological features, the synovial membrane located

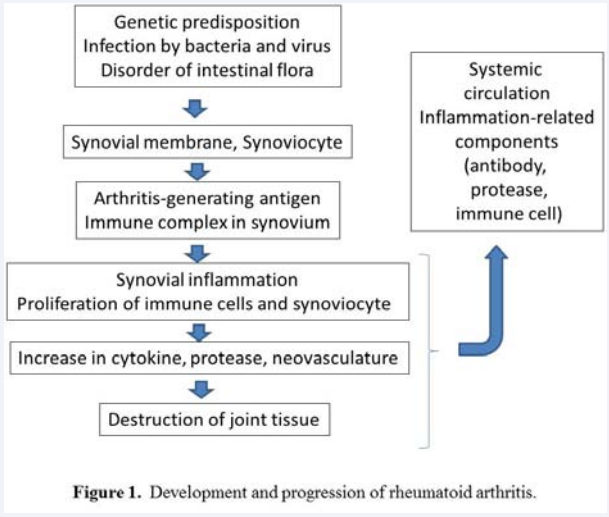

Figure 1. Development and progression of rheumatoid arthritis.

inside the articular capsule are inflamed, inflammatory cells were infiltrated into the joint cavity and neo vascular vessels grow abnormally [16,27]. In these inflammatory conditions with the development of neo vascular vessels [28, 29], passive targeting based on enhanced permeability and retention (EPR) effect is feasible. The several targeting systems for the treatment of RA have been investigated; liposomes, nanoparticles and polymer-drug conjugates have been developed [16,20,30,31]. These systems can deliver the drug to the target site efficiently because of the EPR effect by the higher plasma systemic retention.

Glucocorticoids are very highly potent and fast-acting agents in the treatment of inflammatory diseases [13] however, their chronic use often causes severe systemic side effects such as diabetes, osteoporosis and adrenal failure, resulting in their limited use [24, 32-34]. The DDSs to improve glucocorticoid drugs are suggested to be highly useful, and the DDSs related to glucocorticoids should be expected for the medicationof RA. In our research, prednisolone (PD), often used for the RA therapy [35-37], was focused on in the development of the DDS for the RA therapy.

Design of CS-GP conjugate

The present DDS was produced as a macromolecular prodrug of PD, in which an ester linkage was used to achieve the adequate drug release rate. As chondroitin sulfate (CS) is a very safe macromolecule [38,39], allowed to be injected parenterally [40], it was chosen as a drug carrier. The production pathway of the present DDS was performed as shown in Figure (2). The design concept was constructed based on the CS biological features and conversion properties of the ester chemical bond. The background of the concept is described as follows.

Although CS, injected intravenously, is excreted into urine to a large extent, some of the excreted CS-related molecules exhibit a high molecular weight similar to that of the original polymer, and some of them appear in the degradation form of oligosaccharides or inorganic sulfate ions [41,42]. From these pharmacokinetic features, CS is considered to behave as a polymer to a certain extent in the systemic circulation. Namely, CS is expected to complete the systemic retention. In fact, prolonged systemic circulation and elevation of the area under the plasma concentration–time curve (AUC) were reported in the CS–cisplatin conjugate [43] .The safety and pharmacokinetics of CS suggest that CS should be anappropriate drug carrier for the DDS of RA treatment.

Hitherto, very few conjugates between CS and glucocorticoids for the treatment of RA have been reported. Regarding CS–drug conjugates for anti-inflammatory therapy, Peng et al. reported conjugates of CS and NSAIDs, which were tested for their effect on carrageen-induced edema [44]. The conjugates were found to exhibit a prolonged effect. In the present study, prednisolone (PD), used often for the treatment of RA, was chosen as the glucocorticoid agent. The design of the conjugate of PD with CS was performed by taking into account the following matters. In order to enable the hydrolysis at the inflammatory acidic pH, the chemical bond such as hydrazone [16,21,46,47] and cis acotinyl group [48] could be proposed as an adequate linker because they are susceptible to hydrolysis at acidic pH with the fairly high stability at physiological pH. Otherwise, a peptide linker is another choice because they can be hydrolyzed by various peptidases in the tissues or cells after the localization there [49]. In the case of PD, being one of the glucocorticoid drugs, the ester linkage is the most simple because it possesses hydroxyl groups [50-52]. In other reports, ester prodrugs for glucocorticoid drugs, including macromolecule–glucocorticoid conjugates, have been examined by many researchers [53-56]. In the design of macromolecule–glucocorticoid conjugates, it is required that they are stable to a fair extent at the systemic physiological condition and that they can release the active drug at an adequate rate at the target site. Although an ester bond is generally more susceptible to hydrolysis at higher pH, such requirements are considered to be achieved to a fair extent with the ester linkage of the macromolecular prodrugs. Namely, the ester bond is generally fairly stable at the systemic pH of 7.4, though it depends on the chemical structure [52]. Furthermore, the ester linkage in the macromolecular prodrugs is generally stable against enzymatic hydrolysis due to the steric hindrance to the esterase [57]. These features of the ester bond in the macromolecular prodrugs suggest that the pH-dependent drug release might be caused simply at the tissues, which enables in vivo release to be more predictable as compared to other designs of prodrugs. In addition, the pH

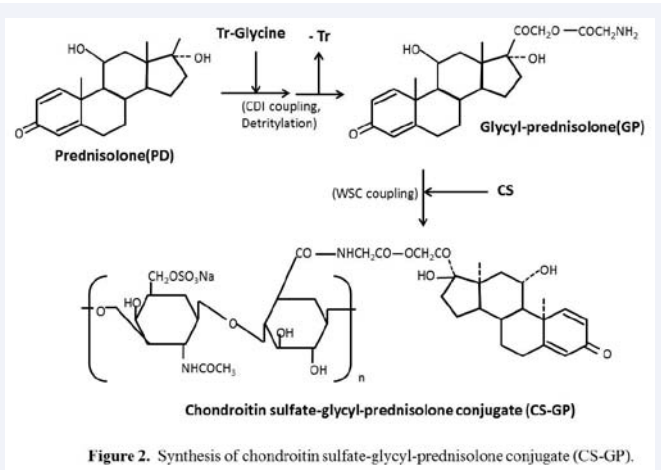

Figure 2. Synthesis of chondroitin sulfate-glycyl-prednisolone conjugate (CS-GP).

value tends to be acidic at the arthritic inflammatory site, being reported to vary from pH 7.4 to 6.0, dependent on the diseased states [58]. As the ester bond is subjected to hydrolysis widely at weakly basic, neutral and weakly acidic pH, it might be adequate as a linker for the activation. In the case of design of the ester prodrug of PD with CS as a carrier, a bi-functional linker with a carboxy group and an amino group is considered to be useful for the combination with the hydroxyl group of PD and the carboxy group of CS. Therefore, amino acids and peptides are proposed as an available linker. A simple amino acid, glycine, is suggested as a candidate for the linker. Conover et al. produced the conjugate of polyethylene glycol and camptothecin using a glycine linker, and investigated the pharmacokinetics and antitumor effect; the conjugate showed the gradual release in the conditions of physiological pH and rat plasma and achieved the fairly fast delivery to the diseased site, resulting in high antitumor effect [59]. Considering these chemical and biological conditions on the arthritis pharmacotherapy, glycine was employed as a linker in the present study. Thus, the preparation of the conjugate was attempted as shown in Figure (2). Namely, in the first step, PD was derivatized to a glycine ester of PD, named GP, and then CS and GP were combined to obtain the conjugate of GP with CS, called CS-GP. The chemical structures of GP and CS-GP were checked by using ^{1}H-NMR spectra, [60] which are shown in Figure (3), in which GP and CS-GP were dissolved in DMSO-d_6 and D_2O, respectively. For GP, the protons at the C21of PD showed the low field shift as compared with original PD (PD: 4.04–4.09 and 4.47–4.51 ppm, GP: 4.84-4.87, 4.97-5.00 ppm. This change in the chemical shifts indicated ester formation between the carboxy group the hydroxyl group at the C21 position of PD. The ^{13}C-NMR and mass spectra also indicated the mono glycine ester formation of PD at the C21 position. Furthermore, the conjugation of GP with CS was observed from the bottom spectrum of Figure (3) [60]. The PD content calculated from the integrated intensities such as the protons, a–e, was consistent with that obtained from the UV absorption at PD moiety (246 nm).CS-GP was evaluated from the in vitro release analysis [60], comparison of effectiveness and pharmacokinetic studies [61,62].

In vitro characteristics of CS-GP

The stability characteristics of the intermediate compound GP are essential to predict and utilize of the polymer conjugates with GP. GP was dissolved in a mixture of methanol and aqueous buffer (1: 3, v/v) and incubated at 37°C under horizontal shaking at 60 rpm. At appropriate time points, aliquot samples (50 μL) were withdrawn. Both GP and PD were analyzed by HPLC in order to examine the stability of GP.The stability of the ester of GP depended on pH. The decomposition rate of GP was promoted with the increase in pH (Figure 4-A1). The PD release was increased with the degradation of GP. However, at pH 8, the regeneration of PD was smaller than expected from the degraded GP (Figure 4-A2); GP degraded quickly at pH 8, but PD appeared to a slight extent, indicating abnormality in mass balance. In HPLC analyses at pH 8, other peaks except GP and PD were observed, suggesting that the glycine moiety appeared to promote the decomposition of GP at the parts other than the ester bond, though the detailed mechanism was unknown. This phenomenon reflected the kinetic parameter h_2 at pH 8. These results suggested that GP could be utilized as an intermediate for macromolecular prodrugs. The conjugate between CS and GP, named CS-GP, was examined for the release characteristics under the conditions similar to those in GP. The release patterns were obtained pH-dependently as shown in Figure (4B).

The stabilities of GP and CS-GP were analyzed using the pseudo-first order kinetics (Figure 5). The conversion rate constants were calculated by fitting the calculated profile to the observed one. Since GP is combined with CS via an amide bond, the glycine is not hydrolyzed in the present incubation conditions. These features were observed from HPLC analysis; no GP was observed in the incubation studies. From the results, PD was released gradually at the physiological pH (pH 7.4), and more slowly at the acidic conditions. After CS-GP has been targeted to RA joints through the EPR effect, the accumulated conjugate is exposed to arthritic joint pH conditions. The synovial pH in humans with RA was reported by Goldie and Nachemson the synovial fluid depends on the diseased states, ranging from 6.0 – 7.3. It is suggested that CS-GP should act in the prolonged manner due to the slow drug release of PD after it has been localized to the RA joints. Although the enzymes in the biological fluids could influence the release rate of PD, such effects were investigated

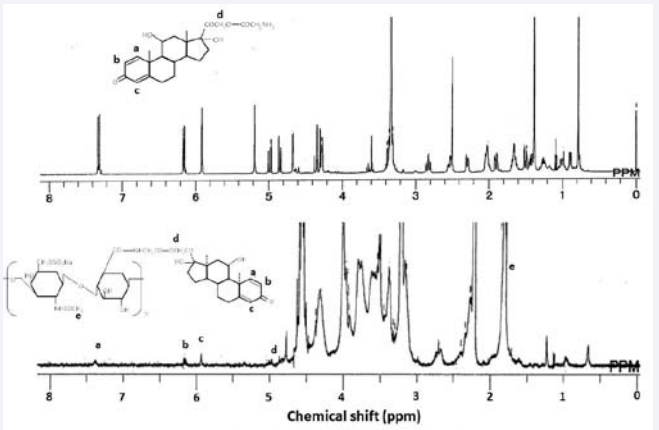

Figure 3. ^{1}H-NMR spectra of GP(A) AND CS-GP(B).

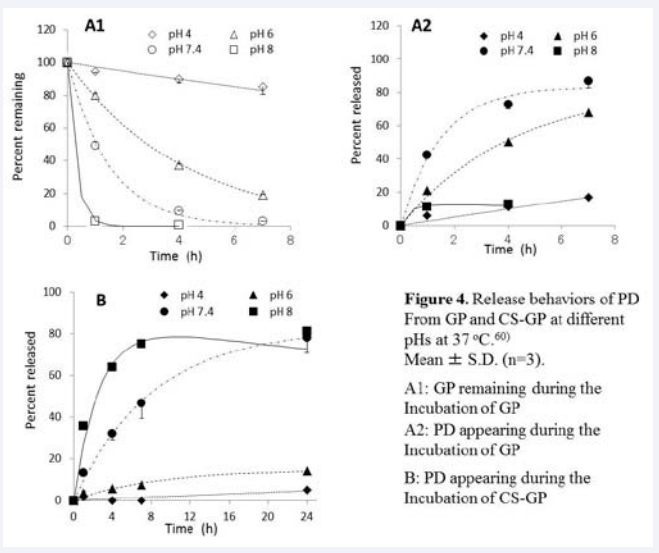

Figure 4. Release behaviors of PD From GP and CS-GP at different pHs at 37 °C.[60] Mean ± S.D. (n=3).

A1: GP remaining during the Incubation of GP

A2: PD appearing during the Incubation of GP

B: PD appearing during the Incubation of CS-GP

pH	Rate constant (h^{-1})		
	h_0	h_1	h_2
4	0.0	0.0263	0.0
6	0.0	0.200	0.0400
7.4	0.000967	0.559	0.108
8	0.00979	0.429	2.98

pH	Rate constant (h^{-1})		
	k_0	k_1	k_2
4	0.0	0.00195	0.0
6	0.0	0.0175	0.102
7.4	0.0	0.102	0.0222
8	0.00724	0.328	0.0596

Figure 5. Conversion rate constants at different pH at 37 °C based on pseudo-first order kinetics.[60]

in the conditions of CS-GP solution with rat plasma (23 %, v/v), which indicated that the addition of plasma was affected the release rate only to a small extent.

In vivo evaluation of CS-GP efficacy

The therapeutic effects of CS-GP were investigated using RA animal models. Namely, CS-GP was investigated for effectiveness by comparison with PD alone, CS alone and the mixture of PD and CS usingr at models with adjuvant-induced arthritis was made by the known method [63]. The in vivo experiment procedure was performed as in **Figure (6)**; heat-killed M. tuberculosis M37Ra was suspended in liquid paraffin at 5 mg/mL. The suspension (adjuvant) (100 μL) was injected intracutaneously into the pad of the right hind paw of each Lewis rat. The volume of the hind paws reached a plateau around 14 and 15 days after adjuvant injection. PD solution (2.5 mg/mL) was made using 50% (w/v) PEG400 saline as a solvent. CS-GP was dissolved in saline at 2.5 mg PD equiv/mL. CS was dissolved in saline at the same concentration as that of CS-GP. The mixture of CS and PD in saline contained the same concentrations of PD and CS as those in CS-GP, respectively. The preparations were injected intravenously via the jugular vein at 2.5 mg PD equiv./kg 14 and 15 days after injection of the adjuvant, that is, the total dose = 2.5 × 2 mg PD equiv./kg. As to PD, the treatment was additionally conducted in a similar manner at twice the dose (total dose = 5 × 2 mg/kg). CS alone was administered similarly with the same volume of CS–GP preparation. No treatment was done for the control group. On appropriate days after adjuvant injection, the rats were weighed and the volume of each hind paw was measured by immersing it in water and reading the buoyancy.

PD (2.5 mg/kg), CS-GP (2.5 mg PD equiv./kg), CS, PD (2.5 mg/kg)/CS and PD (5 mg/kg) were expressed as PD (2.5), CS-GP (2.5), CS, PD (2.5)/CS and PD (5) (Figure 7), and the control (no treatment) was expressed as control. For each group, the body weight changed in a similar pattern. Even after injection on the 14th and15th days, a decrease in body weight was hardly observed in each preparation, indicating that all the preparations had low toxicity under the present dosing conditions.

The therapeutic effect of each preparation was examined from suppression of the swelling of the hind paws. The inflammation extent was evaluated from swelling ratio of paw volume to the initial one. As to a right hind paw, CS-GP exhibited a significant reduction of paw volume against the control for a long period, while other preparations did not. PD alone, CS alone and the PD/CS mixture tended to decrease swelling but the significant effect was obtained only at some time points; PD (2.5 mg/kg) exhibited a significant reduction against the control on the 15th day ($p < 0.05$), and the swelling was significantly lower in PD (5 mg/kg) than the control on the 15th, 17th–19th day ($p < 0.05$). CS is known to exhibit anti-inflammatory actions on adjuvant arthritis in rats, although the effect appears to be low, particularly in therapeutic use [64-68]. In the present experiment, the tendency to suppressswelling was observed with CS, but the effect was not significant except for on the 19th day. The mixture of CS and PD was also not significantly effective against swelling; CS and PD appeared not to act synergistically.The suppressive effect of each preparation on the swelling of the left hind paw was observed to a less extent. Only CS-GP suppressed the swelling of the left hind paw significantly against the control ($p < 0.05$ on the 17th and 18th day). Although PD alone, CS alone and the PD/CS mixture tended to decrease the swelling of the left hind paw as compared with the control, none of them was significantly effective.

Pharmacokinetic evaluation of CS-GP

First, CS-GP was examined for plasma concentration and body distribution after i.v. injection at 2.5 mg PD eq./kg using normal rats. The distributed PD and CS-GP were determined according to the extraction method [61]. Namely, free PD in plasma or tissue homogenate was extracted using the mixture of *t*-tributylmethyl ether and pentane (3:2, v/v) after the addition of saturated NaCl

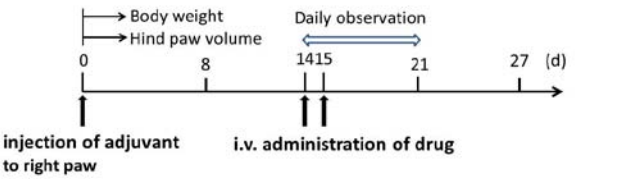

Figure 6. Animal experiment schedules using rats with adjuvant-induced arthritis.[61] Animals were divided into 5 groups as follows: **CS-GP-L** (2.5 mg PD eq./kg), **CS** (60 mg/kg), **PD** (2.5 mg/kg), **CS/PD** (60 mg/kg, 2.5 mg/kg), **Control** (Non-treated).

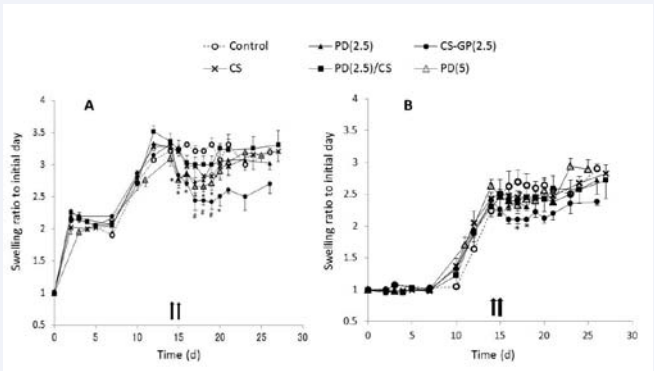

Figure 7. Effect of each substance on joint swelling of right (A) and left (B) hind paws in rats with adjuvant-induced arthritis.[61] Mean ± S.E. (n=3). * $p < 0.05$, # $p < 0.01$ vs. control (Dunnett's test).

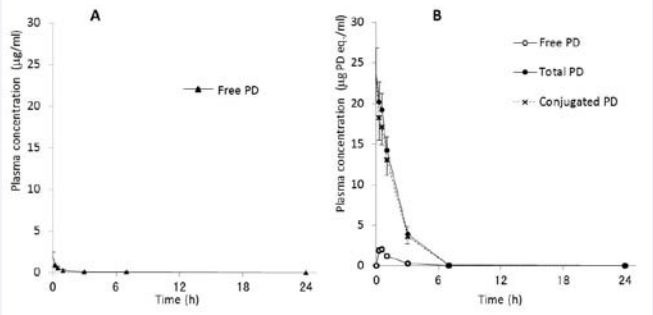

Figure 8. Plasma concentration-time profiles after i.v. injection of PD alone (A) and CS-GP (B) in normal rats.[61] Mean ± S.E., (n=3).

aqueous solution and phosphoric acid.Then, PD concentration was analyzed by HPLC.Total (conjugated + free) PD concentration was determined similarly after the alkaline hydrolysis of the carboxy ester of CS-GP. The drug recovery ratios were good in the determinations of both free and total PDs. Conjugated PD was calculated by the subtraction of free PD from the total PD. After i.v. injection of PD alone, the plasma concentration declined rapidly until 1 h, and then, it was eliminated slowly (Figure 8-A). As to CS-GP, both free and total (free + conjugated) PD concentrations were pursued after i.v. administration at 2.5 mg PD eq./kg. The conjugated PD concentration is shown in the broken line (Figure 8-B). The total PD concentrations were 20.2, 19.2, 14.2 and 3.9 μg/ml at 0.25, 0.5, 1 and 3 h, respectively, after the injection, which were approximately 10 times larger than the free PD concentrations, respectively. CS-GP enhanced the systemic retention of PD extensively, which was due to the much higher localization of CS-GP in the blood circulation. Although CS-GP did not prolong the systemic retention of PD so much, the area under the plasma concentration curve (AUC) value increased to a great extent. The high localization of CS-GP in the systemic circulation was expected to facilitate the accumulation of the drug at the inflammatory site based on the EPR effect [69,70]. As CS is not so retentive in the blood circulation, CS-GP was considered not to maintain the plasma PD level so long.

The drug distribution was investigated for several organs at 24 h after i.v. injection. The distribution profiles were obtained as described in Figure (9). For PD alone, PD was detected in only kidney and liver. The kidney concentration was lower than 0.4 μg/g, and the concentration was less in the liver. As to CS-GP, free and total concentrations were examined. PD and CS-PD were detected in only liver and lung. The total PD concentrations in liver and lung were 2.2 and 0.2μg/g, respectively, which were larger than the PD concentrations observed in PD alone. In the liver, conjugated PD was observed more than free PD. However, the drug distribution extent was very low in each organ; even in liver, being a largest tissue, the total distributed amount for CS-GP was 20.3μg PD eq. (less than 5 % of dose) per rat. Probably, these low organ distributions were due to the biological features of CS, which is metabolized and excreted moderately. These properties of non-accumulation in organs suggested that CS should be a suitable carrier of the drug delivery system.

In addition, the pharmacokinetic studies were performed by the i.v. injection of CS-GP using rats with adjuvant-induced arthritis, and also, the targeting potential of CS-GP to the inflammatory joint were investigated using the diseased rats. These animal experiments were conducted according to the schedules shown in Figure (10). As to the pharmacokinetic experiment, 14 d after adjuvant injection, the rats were administered with CS-GP or PD solution at 2.5 mg PD eq./kg/mL via the jugular vein (Figure 10-A). Blood sampling (each, 0.3 mL) was performed immediately before dosing and 0.25 h, 0.5 h, 1 h, 3 h and 7 h after drug administration.For CS-GP, the plasma concentration of free PD and that of total (free + conjugated) PD were measured.After PD alone and CS-GP were intravenously administered to rats with adjuvant-induced arthritis, the plasma levels were investigated from 0.25–7 h. The conjugated PD concentration was calculated by subtraction of the free PD level from the total level. For both administrations, the results were basically similar to those in normal rats (Figures 8 and 11). For PD alone, the plasma concentration was eliminated in a bi-phasic manner, in which rapid elimination at 0–0.5 h and slow decline at 0.5–7 h were observed. For CS-GP, the total concentration was eliminated in a mono-exponential manner from 0.25 h to 7 h. At 0.25–3 h after administration, the total concentration was more than 10 times greater in CS-GP than in PD alone; a significant difference was observed from 0 h to 3 h in comparison of PD alone with both total and conjugated PDs (p <0.05 or 0.01). The concentration of free PD was much less than the total concentration from 0 h to 7 h after administration of CS-GP, and not so different from that given by PD alone. CS-GP was considered to be concentrated well in the systemic circulation. Thus, it could be proposed that the high distribution of CS-GP in the systemic circulation should promote the localization of PD at the inflamed tissue due to the enhanced permeability and retention (EPR) effect [16,69,70].

Biodisposition of CS-GP to inflammatory joints

The targeting abilities of CS-GP were examined in the schedules shown in Figure (10-B), which was performed separately from the pharmacokinetic studies. The drug concentrations in the inflammatory joints were examined at 1, 7 and 24 h after i.v. injections of PD alone and CS-GP at 2.5 mg PD eq./kg to the diseased rats. For CS-GP, the tissue concentration of free PD and that of total (free + conjugated) PD were measured; the distribution of conjugated PD was calculated by subtracting the free PD amount from the total PD amount. The determination methods of the joint concentrations of free and total PDs were similar to those in the above studies of plasma or tissue drug concentration [61,62]. The drug distribution

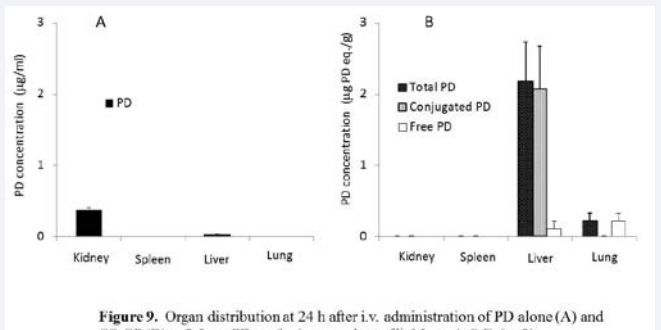

Figure 9. Organ distribution at 24 h after i.v. administration of PD alone (A) and CS-GP (B) at 2.5 mg PD eq./kg in normal rats.[61] Mean ± S.E. (n=3).

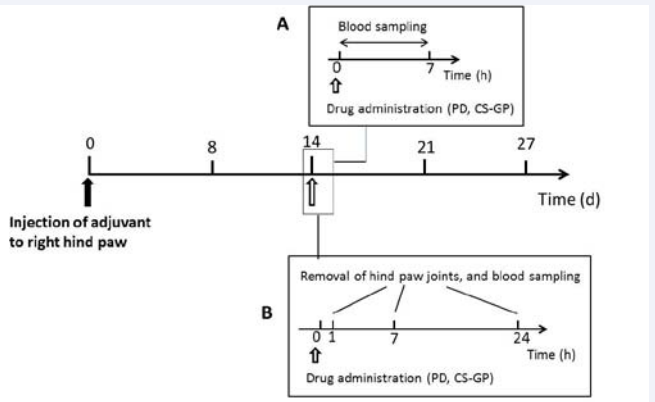

Figure 10. Animal experiment schedules for pharmacokinetics (A) and drug distribution in the inflammatory joints (B) after i.v. injection at 2.5 mg PD eq./kg to rats with adjuvant-induced arthritis.[62]

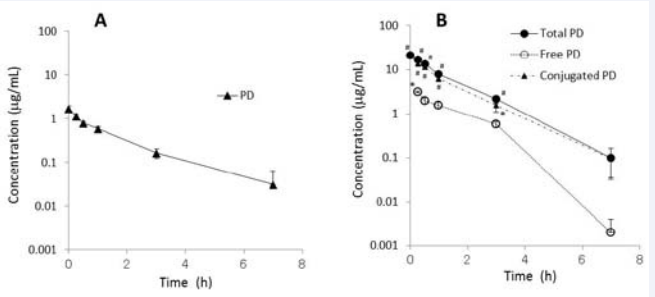

Figure 11. Plasma concentration-time profiles after i.v. injection of PD alone (A) and CS-GP (B) at the dose of 2.5 mg PD eq./kg in rats with adjuvant-induced arthritis.[62] Mean ± S.E. (n=4). * $p < 0.05$, # $p < 0.01$ vs. PD alone (Dunnett's test).

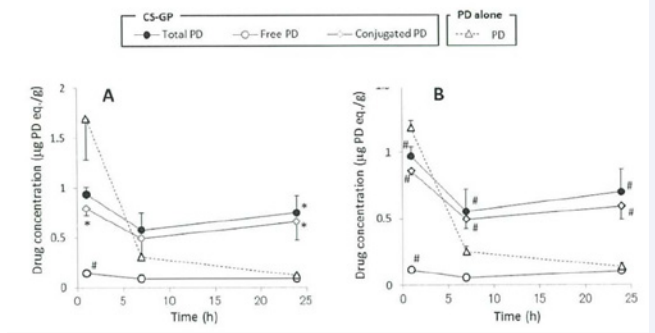

Figure 12: Drug distribution I the right (A) and left (B) hind paws after i.v. injection of PD alone and CS-GP at the doses of 2.5mg PD eg./kg to rats with adjuvant-induced arthritis.
Mean± S.E. (n=3 for 1 and 7h, 4hr for 24h). *p< 0.05, # p< 0.01 vs. PD alone (Dunnett's test)

profiles were obtained as shown in Figure (12).At 1 h after i.v. administration, PD alone exhibited a higher PD level than the total PD level given by CS-GP, but the drug concentration was eliminated fairly rapidly. At 7 h after i.v. administration, the total and conjugated PD concentrations by CS-GP were higher than the PD concentration by PD alone; a significant difference was observed for the left hind paw joint (p <0.01). At 24 h, the total and conjugated PD levels were significantly higher than that by PD alone (p <0.05 for the right hind paw joint, p <0.01 for the left hind paw joint). These total and conjugated PD levels tended to be rather higher at 24 h than at 7 h. This might be due to the following reasons; although the plasma level of CS-GP decreased to the fairly low level at 7 h after administration, CS-GP still remained in the systemic circulation to some extent. Therefore, CS-GP was considered to be possibly delivered to the inflammatory site even after 7 h. In addition, CS-GP seemed to be well-retained in the joint tissues once delivered there; CS-GP might interact with the joint tissue components, which was found from the preliminary studies in the mixing of CS-GP and the tissues. Also, as the release of PD from CS-GP was slow at the acidic joint pH, CS-GP was kept long in the conjugate form at the joint. These features were presumed to contribute to the high localization of CS-GP in the inflammatory joint at 24 h. After administration of CS-GP, free PD was observed at low but almost constant levels, which were 0.09–0.14 μg/mL for the right hind paw joint and 0.06–0.11 μg/mL for the left hind paw joint. At 24 h after i.v. administration, the free PD concentrations were similar for both CS-GP and PD alone. Since the pH of an inflammatory joint tends to be weakly acidic, the drug release was considered to be caused slowly. As CS-GP remained in inflammatory joints at much higher level at 24 h, the free PD level was expected to be retained longer because of the drug supply from the conjugate distributed in the inflammatory tissue. On the other hand, the drug was eliminated faster for PD alone. Reportedly, the effective concentration of PD for immunological suppression was shown to be several dozen – nearly one hundred ng/mL [71,72]. When considering the concentration of free PD in the inflamed joints based on this information, CS-GP appeared appeared to fulfill the PD effective levels, which obviously supported the relevance of CS-GP as a RA therapeutic system. CS-GP showed adequately the localization to inflammatory joint, prolonged retention there and sustained release of free PD, leading to higher efficacy of CS-GP than PD alone. In order to evaluate the passive targeting ability of CS-GP, the concentrations of the total (conjugated + free) PD distributed into the joint tissue at 24 h after i.v. administration of CS-GP were compared between AIA and healthy rats. The total PD concentration in the joint tissue was found to be higher in AIA rats than healthy rats (Figure 13). The total PD concentration of the left hind paw was significantly greater in AIA rats than in healthy rats (p < 0.05). As to the right hind paw, the mean total PD level was more than twice greater in AIA rats than in healthy rats, though the significant difference was not found (p> 0.05), which was probably due to the large variation of the data in AIA rats. These results suggested that CS-GP could give better localization of the drug in the arthritic tissue than in the normal one, which

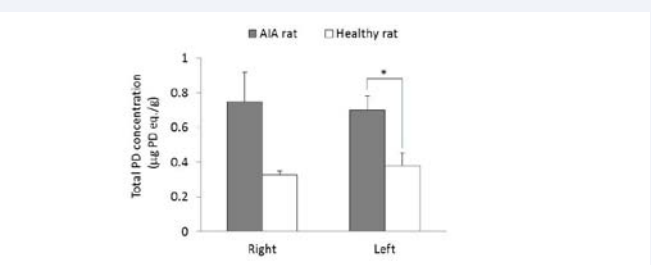

Figure 13. Total drug concentration in the right (A) and left (B) hind pawsat 24 h after i.v injection of CS-GP at the dose of 2.5 mg PD eq./kg to arthritic and healthy rats. [62]
Mean± S.E. (n=4 for AIA rats, 3 for healthy rats). * $p < 0.05$ (upaired t-test).

supported that CS-GP should exhibit a good passive targeting ability based on EPR effect.

CONCLUSION

There are many effective drugs in clinical usage, but some are restricted for dosage or dosing period due to several adverse effects. Drug targeting therapy is a technique in which a drug is delivered to target tissues and remaining for appropriate time periods to maximize the efficacy and minimize the side effect. This concept is true in the treatment of rheumatoid arthritis. Nano-carrier systems or macromolecular prodrugs are possibly useful delivery systems as a strategy to such arthritis. Considering the clinical approaches, the safety, biological fate and theoretical adequacy of the delivery systems are critical points. CS-GP can fulfill those conditions, in particular, pharmacokinetic studies and joint distributions demonstrated the adequate characteristics of retention and release of the drug; though the systemic retention is less than stealth liposomes or nano-micelles. Although the latter nano-carrier systems are reported to display much higher retention, they sometimes show long-term or unexpected tissue distributions. Considering these aspects, CS is a most simple carrier and well-known for biological features, which are the strong points of the CS-GP system. This approach is considered to be applicable for other anti-inflammatory or anti-rheumatoid drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) or disease-modifying anti-rheumatic drugs (DMARDs).In the present review, CS-GP was introduced as a strategy toward to the targeting system for the therapy of rheumatoid arthritis (RA).The resultant physicochemical characteristics, biological functions and pharmacokinetic features suggest the possible usefulness of CS-GP as an anti-arthritic delivery system.

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