Macromolecular Research, Vol. 12, No. 1, pp 71-77 (2004)

Drug-Release Behavior of Polymeric Prodrugs of Ibuprofen with PEG and Its Derivatives as Polymeric Carriers

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Received Aug. 14, 2003; Revised Nov. 13, 2003

Abstract: We have synthesized various types of poly(ethylene glycol) (PEG)-ibuprofen conjugates by nucleophilic substitution of bromo-terminated PEG with ibuprofen-Cs salt. The conversion of the terminal hydroxyl groups to bromo-termini was quantitative, as was the drug conjugation process, which suggests that the present synthetic method is very useful for the preparation of PEG-based prodrugs from pharmaceuticals having carboxyl functionalities. The drug-release behavior of the prodrugs was examined in both phosphate buffer (PBS, pH 7.4) and rat plasma. From the drug-release behavior in PBS, we determined that each prodrug has high storage stability. The drug-release rate was observed to be much faster in rat plasma than in buffer solution as a result of the acceleration effect provided by enzymes present in the plasma. The drug-release rate in rat plasma depends on the degree of molecular aggregation of the prodrugs, which can be changed effectively by the nature of their spacer groups or by the use of Pluronic as the polymer carrier.

Keywords: nucleophilic substitution, drug-release, rat plasma, prodrug, polymeric carrier.

Introduction

Until now, drug delivery system (DDS) has been developed to improve the therapeutic availabilities of many pharmaceuticals. One of the effective methods is the use of polymeric prodrugs. Since polymeric substances are more easily incorporated into tumor cells than into normal cells, the prodrugs are particularly effective for the therapeutic treatment of cancer. This drug specificity is originated from the enhanced permeability and retention effect (EPR effect)[1] of tissues. "Polymeric prodrug" is strictly differentiated from "Polymer prodrug" as follows.[2]

1) Polymer prodrugs: polymeric substances that have a physiological activity. 2) polymeric prodrugs: low molecular pharmaceuticals immobilized on polymeric carriers. Polymeric prodrugs have various advantages in terms of elongation of circulation life, slow release, and targeting of drug. They liberate the conjugated pharmaceuticals when absorbed in tissues, organs, and blood vessels to enhance their bioavailability. Polymeric prodrugs are usually prepared by the covalent conjugation of the pharmaceuticals with appropriate polymer carriers. Therefore, the properties of the polymer carriers are very important. The requirements for the polymer carriers are as follows[3]; 1) water-solubility, 2) low toxicity and low immunogenicity, 3) low accumulation in organs and tissues, 4) stability within an appropriate time after administration, 5) appropriate molecular size, and 6) functional groups and spacers to be combined with drugs.

Poly(vinyl alcohol),[4,5] dextran,[6-10] poly(ethylene glycol) (PEG),[11-13] poly(malic acid)[14,15] and poly(L-lysine)[16,17] are typical polymer carriers that satisfy the requirements. Among them, PEG is the most useful polymer because of its good water-solubility, body reservation[18-21] and biological safety. However, PEG has only two functional groups on the terminals, and the amount of the pharmaceuticals attached to PEG is quite limited. Moreover, with increasing molecular weight of PEG, the concentration of terminal hydroxyl groups becomes lower, so that the terminal reaction with the pharmaceuticals is more difficult. Taking these demerits into consideration, PEG can be used as a polymeric carrier for the pharmaceuticals with high potency which a small quantity of drug should be administered for the therapeutic treatment.

In this study, the author reports on a novel method to directly conjugate unstable pharmaceuticals on PEG terminals that are functionalized with bromide. The conjugation reaction proceeds by the nucleophilic substitution of the bromide. If the drug contains carboxylate groups, the bromide is readily replaced by the drug under mild reaction conditions. Here, 4-isobutylphenyl-2-propanoic acid (Ibuprofen) is employed as a carboxyl-containing drug, which is well

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known as an anti-inflammatory drug and widely used in therapy. Various PEG-ibuprofen conjugates having different spacer groups are synthesized from PEGs with different molecular weight, and for which the drug-releasing behavior is evaluated.

Experimental

Materials. PEGs having number average molecular weights (M_n) of 7.5 and 20.0 kg/mol were purchased from Wako Pure Chemical Industry (Osaka, Japan). Their molecular weight distribution indexes in weight (M_w)/number (M_n) average molecular weight were 1.07 and 1.06, respectively. Ibuprofen of pharmaceutical grade was purchased from Wako Pure Chemical Industry. Cesium carbonate (Cs_2CO_3), 1,5-dibromopentane, 1,10-dibromodecane, ethanol, sodium hydride (NaH), magnesium chloride hexahydrate, and trifluoroacetic anhydride were of reagent grade and used as received. α,α'-Dibromo-*p*-xylene was purchased from Tokyo Chemical Industry (Tokyo, Japan) and were purified by distillation. Pyrene was purified by recrystallization from ethanol. Distilled water was further purified by filtration using a Milipore Milli-Q system. Pluronic F-108™ [PN: a triblock copolymer poly(oxyethylene)-*block*-poly(oxypropylene)-*block*-poly(oxythylene) with an oxyethylene/oxypropylene unit ratio of 20/80)] having a M_n of 15.5 kg/mol was supplied by Asahi Denka Kogyo (Tokyo, Japan). Its M_w/M_n was 1.06. Rat plasma was supplied by Ono Pharmaceutical Industry (Osaka, Japan).

Measurements. ^{1}H-NMR spectra were recorded in $CDCl_3$ and D_2O with tetramethylsilane (TMS) and 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as the internal standards, respectively, on a Brucker ARX 500 spectrometer (Japan) operated at 500 MHz. Gel permeation chromatography (GPC) was performed on an analyzer composed of a Shimadzu LC-10AS pump (Japan), a Shimadzu RID-10A refractive index detector (Japan), a Shimadzu CTO-10A column oven (35 °C), and a Shimadzu C-R4A chromatopac data processor. A Toso TSK gel G4500PW$_{XL}$ column was used together with a guard column, and the eluent was 0.02 M phosphate buffer (PBS, pH = 7.4) containing 5% acetonitrile. The molecular weight was calibrated with the PEG standards. High performance liquid chromatography (HPLC) was performed on an analyzer composed of a Shimadzu LC-10AS pump, a Shimadzu SPD-10AUV-VIS detector (264 nm), a Shimadzu CTO-10A column oven (35 °C), and a Shimadzu C-R4A chromatopac data processor. A Nacalai PG10C18-500 reversed-phase column was used, and the eluent was 0.02 M PBS (pH = 5.0) containing 26 vol% acetonitrile.

Syntheses of Bromo-Terminated PEG. A typical example is as follows: a two grams of PEG7.5K (0.34 mmol) was dissolved in a small volume of benzene and subjected to the lyophilization to remove water. The lyophilized PEG was re-dissolved in 20 mL toluene, and a 81 mg of (3.4 mmol) NaH suspended in 10 mL toluene was added to it at 0 °C under nitrogen. Then, the system was stirred at 60 °C for 1 hr to complete the terminal alkoxide formation of PEG and cooled again to 0 °C. To this system was added 7.7 g of 1,5-dibromopentane. After the addition, the mixture was stirred at 60 °C for 90 hrs. The resultant solution was filtered to remove the inorganic by-products, and the filtrate was reprecipitated into a large exess of diethyl ether to isolate the PEG products. The precipitates were filtered and dried under vacuum (**1**, 71%).

Bis(5-bromopentyl)-PEG20K (**2**), bis(10-bromodecanyl)-PEG7.5K (**3**), PEG20K (**4**), bis(10-bromodecanyl)-PN (**5**), and bis(*p*-bromomethyl benzyl)-PEG20K (**6**) were also prepared likewise (2: 87%, 3: 82%, 4: 83%, 5: 80%, 6: 81%). Their ^{1}H-NMR spectra are summarized in Table I and II, indicating that the terminal conversion was almost quantitative in each case.

Syntheses of PEG-Ibuprofen Conjugates. Ibuprofen (8.0×10^{-2} mmol) and cesium carbonate (0.24 mmol) were dissolved in 1 mL of a water/ethanol (1/9 in vol.) mixture and stirred at 0 °C for 1 hr. The solution was then evaporated under vacuum to obtain the ibuprofen-Cs salt as the residue. It was mixed with a bromo-terminated PEG (2.0×10^{-2} mmol) in a small volume of benzene and lyophilized. The mixture was subsequently dissolved in 1 mL acetonitrile and stirred at 40 °C for 10 hrs. After the reaction being over, the reaction system was filtered to remove the excess ibuprofen-Cs salt, and the filtrate was poured into 50 mL *t*-butanol containing 5 g magnesium chloride hexahydrate. The resultant suspension was stirred at 30 °C for 3 hrs. This procedure was effective for removing the residual ibuprofen-Cs salt by adsorption. The suspension was finally evaporated under vacuum, and the residue was subjected to extraction with a dichloromethane/water system to recover the product in dichloromethane. The dichloromethane extract was dried over sodium sulfate, fitered, and evaporated under vacuum.

Table I. Chemical Shift Values of Bromo-Terminated PEGs in $CDCl_3$

Proton	Coupling	PEG Derivatives (ppm) 1	2	6
$BrCH_2CH_2\mathit{CH_2}CH_2CH_2O$-	m	1.51	1.51	
$BrCH_2CH_2CH_2\mathit{CH_2}CH_2O$-	m	1.63	1.63	
$BrCH_2\mathit{CH_2}CH_2CH_2CH_2O$-	m	1.89	1.89	
$BrCH_2CH_2CH_2CH_2\mathit{CH_2}O$-	t	3.42	3.42	
$\mathit{CH_2CH_2}O$ for PEG	m	3.67	3.67	3.68
$Br\mathit{CH_2}C_6H_4CH_2O$-	s			4.50
$BrCH_2C_6H_4\mathit{CH_2}O$-	s			4.58
$BrCH_2\mathit{C_6H_4}CH_2O$-	d			7.38

Table II. Chemical Shift Values of Bromo-Terminated PEGs and PN in $CDCl_3$

Proton	Coupling	PEG and PN Derivatives (ppm)		
		3	4	5
$CH_2CH(CH_3)O$ for PN 1.15	d			1.15
$BrCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.30	1.30	1.30
$BrCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.43	1.43	1.43
$BrCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.57	1.57	1.57
$BrCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.85	1.85	1.85
-$CH_2CH(CH_3)O$ for PN	m			3.40
$BrCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	t	3.41	3.41	3.41
-$CH_2CH(CH_3)O$ for PN	m			3.54
CH_2CH_2O- for PEG	m	3.66	3.67	3.64

The residue was purified by reprecipitation using a benzene/diethyl ether (solvent/precipitant) system. The finally obtained precipitates were filtered and dried under vacuum. The ibuprofen-PEG conjugates derived from 1, 2, 3, 4, and 6 by this method are named as prodrugs III (7, 0.085 g 53%), IV (8, 0.23 g, 56%), V (9, 0.073 g, 45%), VI (10, 0.20 g, 58%), and VII (11, 0.20 g, 49%), respectively. The ibuprofen-PN conjugate derived from 5 is named as prodrug PN (12, 0.15 g, 45%). Their ^{1}H-NMR spectra are summarized in Table III and IV from which the percent introduction of ibuprofen was known to be almost quantitative in each case.

Fluorescence Measurements. A stock solution of pyrene was prepared by dissolving a freshly recrystallized pyrene in ethanol in a concentration of 1.0×10^{-4} M. Each prodrug was dissolved in a distilled water to have a concentration of 0.1 or 0.6 wt%. 10, 40 and 100 μL portions or the stock solution of pyrene were charged into three separate reaction vials and dried up under a reduced pressure. Then, a 1 mL volume of the prodrug solution was charged into each of the vials and incubated at 37 °C for 24 hrs. The concentrations of pyrene in the prodrug solutions in the vials were 1.0, 4.0 and 10 μM, respectively. Each solution in the vial was then subjected to the fluorescence measurement using a Simadzu RF-5300PC spectrofluoro photometer (Japan). The fluorescence emission spectra of pyrene were recoded in a wavelength range of 350~600 nm at an excitation wavelength of 338 nm. The intensities of the first emission band I_1 (at 374 nm) and the third band I_3 (at 385 nm) were determined. Their ratio (I_1/I_3) was used as a measure of the polarity of the microenvironment where the hydrophobic pyrene molecule was located.

Drug-Releasing Study in PBS. Each of the prodrugs was dissolved in a 0.1 M PBS (pH=7.4) in a concentration on 0.1 mg/mL. The solution was then incubated in a bath thermostated at 37 °C, and a small portion of it was taken for the HPLC analysis at intervals from 0 to 400 hrs.

Drug-Relesaing Study in Rat Plasma. Each of the prodrugs was dissolved in a 0.1 M PBS and mixed with nine times the volume of rat plasma to have an ibuprofen concentration of 0.1 mg/mL. A 50 μL portion of this plasma solution was taken out and poured into a 450 μL of ethanol to hydrolytic enzymes in rat plasma involved. The mixture was then centrifuged to precipitate the protein involved and filtered. The filtrate was dried up in a centrifugal evaporator (speed-vac) under vacuum and re-dissolved in an eluent of HPLC for the analysis mentioned above.

Table III. Chemical Shift Values of Prodrugs III, IV and VII in $CDCl_3$

Proton	Coupling	Prodrugs (ppm)		
		III(7)	IV(8)	VII(11)
$(CH_3)_2CH$- for ibuprofen	d	0.89	0.89	0.90
-$COOCH_2CH_2CH_2CH_2CH_2O$-	m	1.31	1.31	
CH_3CH for ibuprofen	d	1.48	1.48	1.50
-$COOCH_2CH_2CH_2CH_2CH_2O$-	m	1.56	1.56	
-$COOCH_2CH_2CH_2CH_2CH_2O$-	m	1.59	1.59	
$(CH_3)_2CH$- for ibuprofen	m	1.85	1.85	1.85
-$CH_2C_6H_4$- for ibuprofen	d	2.44	2.44	2.45
-$COOCH_2CH_2CH_2CH_2CH_2O$-	t	3.40	3.40	
-CH_2CH_2O- for PEG	m	3.64	3.64	3.66
-$COOCH_2CH_2CH_2CH_2CH_2O$-	t	4.05	4.05	
-$COOCH_2C_6H_4CH_2O$-	s			4.54
-C_6H_4- for ibuprofen	d	7.10	7.10	7.10
-$COOCH_2C_6H_4CH_2O$-	d			7.38

Results and Discussion

Drug-Releasing Behavior in PBS. Drug-releasing behavior of the synthesized ibuprofen-conjugated prodrugs

Table IV. Chemical Shift Values of Prodrugs V, VI and PN in $CDCl_3$

Proton	Coupling	Prodrugs (ppm)		
		(9)	(10)	PN(12)
$(CH_3)_2CH$- for ibuprofen	d	0.89	0.89	0.89
$CH_2CH(CH_3)O$ for PN	d			1.14
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.29	1.29	1.26
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.43	1.43	1.43
CH_3CH for ibuprofen	d	1.48	1.48	1.48
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.56	1.56	1.56
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	m	1.59	1.59	1.59
$(CH_3)_2CH$- for ibuprofen	m	1.86	1.86	1.86
-$CH_2C_6H_4$- for ibuprofen	d	2.44	2.44	2.44
-$CH_2CH(CH_3)O$- for PN	m			3.40
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	t	3.40	3.40	
-$CH_2CH(CH_3)O$- for PN	m			3.54
-CH_2CH_2O- for PEG	m	3.66	3.64	3.64
-$COOCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2O$-	t	4.04	4.04	4.05
-C_6H_4- for ibuprofen	d	7.10	7.10	7.10

was studied in PBS at 37 °C.

Figure 1 shows a typical HPLC curve of the buffer solution of prodrug VII after incubation for 290 hrs. In this curve, a peak due to ibuprofen is shown around 30 min in retension time. The amount of the released drug was calculated from the area of this peak relative to that of the corresponding peak observed for the sample after alkaline hydrolysis (control). Figure 2 shows the drug-releasing profiles. Each prodrug showed a very slow drug release. Only prodrug VII, having a benzyl ester bonding, showed a relatively fast releasing rate. In prodrugs III and IV having a pentamethylene spacer, more than 5% of the conjugated ibuprofen was released after 400 hrs, while in prodrugs V and VI having a decamethylene spacer, no ibuprofen was released. In prodrug PN, less than 1% of the conjugated ibuprofen was released for 400 hrs. This difference in releasing rate is reasonably attributed to the different microenvironments of the conjugated ibuprofen that are originated from the molecular aggregation of the prodrugs. It was indicated in the previous section that each prodrug aggregates in water to form a hydrophobic pocket where the conjugated ibuprofen is located. Since the I_1/I_3 ratios in the aqueous solutions of prodrugs III and IV are close to the ratio in water, their ibuprofen and the spacer moieties do not form the stable hydrophobic pocket and are exposed to hydrophilic environment. Contrarilly, in the prodrugs V, VI, and PN, showing much lower I_1/I_3 ratios, the ibuprofen moieties are in the stable hydrophobic pocket and isolated from the hydrophobic environment. This difference in aggregation nature is related with the different drug-releasing rate. Since PN has a less hydrophilic poly(oxypropylene) segment, the hydrophobic pocket formed in prodrug

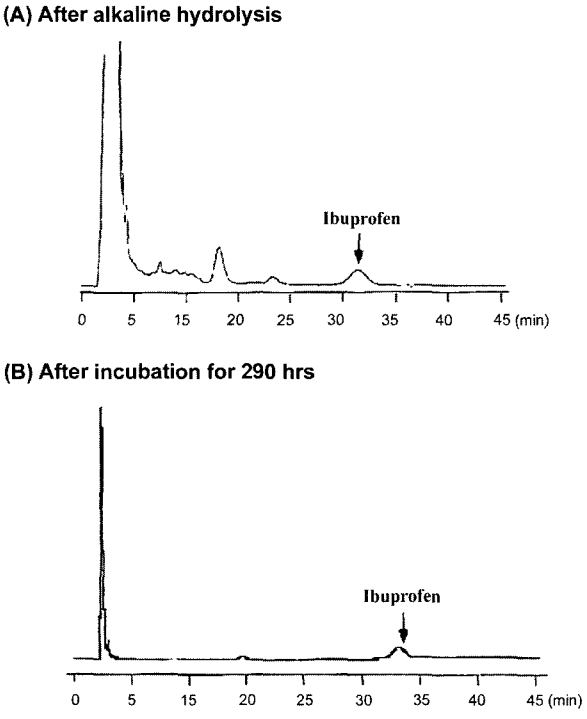


Figure 1. A typical HPLC curve of the buffer solution of prodrug VII after (A) hydrolysis (control) and (B) incubation in phosphate buffer for 290 hrs.

PN is more stable and less susceptible to the hydrolysis of the drug moiety in comparison with the PEG-based prodrugs. In prodrug VII, ibuprofen is connected through a more hydrolysable benzyl ester linkage, and the drug-releasing rate is the fastest among the prodrugs. The formation of the hydrophobic pocket is also supposed to be in lesser ratio, judging from its I_1/I_3 ratio. This slow drug-releasing nature in PBS supports the high stability to chemical hydrolysis of the prodrugs. Therefore, they can be stored for long time without hydrolysis occurring. The drug-releasing profiles shown in Figure 2 could be interpreted by the simple first order kinetic analysis assuming the first-order rate constant as k_1. Scheme I shows the drug-releasing mechanism of the prodrugs. Table V summarizes the k_1 value and the half-life time ($t_{1/2}$) of the release for each prodrug. In values for prodrugs I and II which were reported before are also involved. In prodrug I, k_2 for the second hydrolysis step is responsible for the final release of drug, and is shown in Table V. The k_1 values of prodrugs of II-IV, VII, and PN are in the order of 10^{-4} to 10^{-5} h^{-1} while those of prodrugs of V and VI are too slow to be determined.

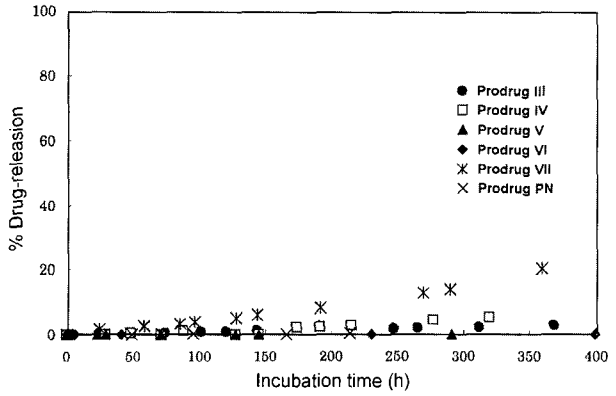
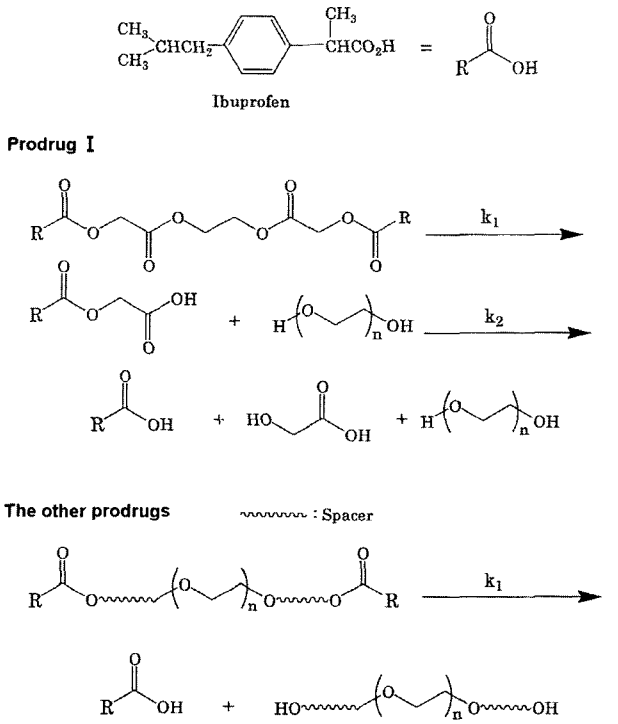


Figure 2. Drug-releasing profiles of various ibuprofen-conjugated prodrugs incubated in PBS (pH = 7.4) at 37 °C.

Drug-Releasing Behavior in Rat Plasma. In order to verify the therapeutic applicability of the prodrugs, their drug-releasing behavior was examined in rat plasma. Figure 3 shows their drug-releasing profiles in rat plasma. For all the prodrugs the drug-releasing rate became much faster in



Scheme I. Drug releasing reactions of the ibuprofen-conjugated prodrugs and the constants defined.

Table V. Rate Constants k_1 and Half-Life Times ($t_{1/2}$) of Drug Releasing for Various Ibuprofen-Conjugated Prodrugs in PBS (pH = 7.4) and Rat Plasma

Prodrug Type	in PBS		in Rat Plasma	
	k_1 (h^{-1})	$t_{1/2}$	k_1 (h^{-1})	$t_{1/2}$
I	$k_1 = 3.2 \times 10^{-2}$	21 h [a]	k_1 = over 100	25 sec [a]
	$k_2 = 5.3 \times 10^{-4}$	1.3×10^3 h [b]	$k_2 = 2.9 \times 10^{-2}$	24 h [b]
II	5.0×10^{-4}	1.4×10^3 h	2.5	17 min
III	8.0×10^{-5}	8.7×10^3 h	5.4	7.7 min
IV	2.0×10^{-4}	3.5×10^3 h	4.2	10 min
V	N.D. [d]	N.D.	18 (k_1' [c] = 0.33)	2.4 min ($t_{1/2}$' = 2.1 h)
VI	N.D.	N.D.	9.5 (k_1' [c] = 0.34)	4.4 min ($t_{1/2}$' = 2.1 h)
VII	5.8×10^{-4}	1.2×10^3 h	8.1	5.2 min
PN	1.3×10^{-5}	5.5×10^4 h	0.86	49 min

The concentration: 0.1 mg/mL realtive to the amount of ibuprofen.
[a] First-stage. [b] Second-stage. [c] k_1': The rate constant at the plateau releasing stage. [d] N.D.: Not determined.

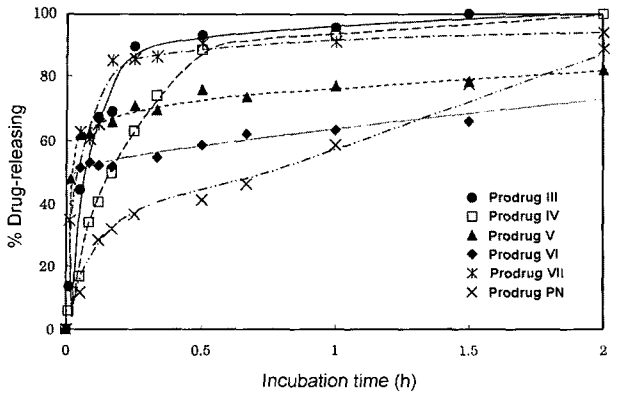


Figure 3. Drug-releasing profiles of various ibuprofen-conjugated prodrugs incubated in rat plasma at 37 °C.

rat plasma than in buffer solution because of the action or the hydrolysis by enzymes contained in rat plasma such as esterases. The releasing profiles could also be interpreted by the first-order kinetics similarly to those in buffer. The k_1 and $t_{1/2}$ values are summarized in Table V.

The change in the drug-releasing rate in rat plasma also depended on the spacer groups of the prodrugs. In prodrugs III and IV having a pentamethylene spacer, the ibuprofen release reached about 95% in 1 hr after the incubation. Their half-life times were 7.7 and 10 min, respectively. In prodrugs V and VI having a decamethylene spacer, 50~60% of the ibuprofen was released in 10 min after the incubation. This releasing feature is so-called "burst-releasing" whose rate is even faster than that of prodrugs III and IV. However, the drug-release reached a plateau after this "burst-releasing" and extremely slowed down until having a drug-release of about 80% in 2 hrs after the incubation. In the first-order kinetics, the rates in the "burst-releasing" and plateau releasing stages are separately analyzed by assuming the respective rate constants as k_1 and k_1'. In prodrug PN with a decamethylene spacer, the initial "burst-releasing" was successfully retarded, and the sustainable drug-release was allowed to continue constantly for a period of 2 hrs after the incubation ($t_{1/2}$ = 49 min). With this profile, a possible side effect by the "burst-releasing" may be escaped to establish an ideal slow drug-release. The drug-releasing rate of prodrug VII having a *p*-oxymethylbenzyl spacer was much faster ($t_{1/2}$ = 5.2 min) than that of the other prodrugs. Here, the benzyl ester linkage is more highly susceptible to the enzymatic hydrolysis.

Especially, prodrugs V, VI, and PN aggregate strongly through the hydrophobic bond of the spacer groups and ibuprofen moieties. It is considered, however, that interaction of the prodrugs and enzymes in blood plasma may dissociate the weak bonds between the spacers and ibuprofen to incur the burst-release. These easily dissociated bonds are those in the external domain of the aggregates are slowly hydrolyzed to give the slow-release. Prodrug PN showed the slowest drug-releasing rate of all the prodrugs because it aggregates more strongly than the other prodrugs. The drug-releasing rate of prodrug VII is faster than that of prodrugs III and IV in spite of its stronger aggregation. The effect of molecular weight of the polymer carrier on the drug-releasing rate was not so significant. This fact also supports the conclusion that the drug-releasing rate in rat plasma depends on the molecular aggregation of prodrugs through their hydrophobic spacer groups.

Conclusions

Various types of PEG-ibuprofen conjugates were synthesized by the nucleophilic substitution of the bromo-terminated PEG with ibuprofen-Cs salt. PN was also used instead of PEG. The terminal conversion from the hydroxyl to the bromo-terminal was quantitative, and the drug conjugation proceeded in quantitative yield as well. It was therefore suggested that the present synthetic method is very useful for preparing the PEG-based prodrugs with pharmaceuticals having carboxy- functionality.

The drug-releasing behavior of the prodrugs was examined both in PBS (pH = 7.4) and rat plasma. Each prodrug was known to have high storage stability from the drug-releasing behavior in PBS. The drug-releasing rate was known to be much faster in rat plasma than in PBS owing to the acceleration by the enzymes involved in the plasma. The drug-releasing rate in rat plasma depended on the molecular aggregation of the prodrugs, which was effectively changed by their spacer groups and the use of PN as the polymeric carrier.

Acknowledgements. This work was supported by Korea Research Foundation Grant (KRF-2002-D00148).

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