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In vitro and in vivo study of poly(ethylene glycol) conjugated ketoprofen to extend the duration of action

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Abstract

Ketoprofen–polyethylene glycol (PEG) conjugates (KPEG) were prepared and their potential as a prolonged release system was investigated. Three KPEG conjugates were synthesized from ketoprofen and methoxy PEG with three different molecular weights by esterification in the presence of DCC. The KPEG conjugates were characterized by FT-IR and ^{1}H NMR spectroscopy. The rate of hydrolysis profile showed a specific acid–base catalysis pattern with a minimum at pH 4–5. The pharmacokinetic study after the intravenous and intramuscular administration of KPEG750 showed that the plasma levels of KP increased slowly and reached a maximum concentration at later time. The AUC of KPEG750 was higher than that after administering an equivalent dose of ketoprofen except 40 mg/kg dose of intramuscular administration. The tail-flick experiment and paw edema test after intramuscular administration showed that KPEG750 had extended analgesic and anti-inflammatory effects compared with ketoprofen. These results suggest that KPEG could be a promising NSAID prodrug with an extended pharmacological effect owing to delayed-release of parent drug.

Keywords: Ketoprofen; Poly(ethylene glycol); Conjugation; Analgesic; Anti-inflammatory

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug with well-known anti-inflammatory, antipyretic and analgesic properties. It is a propionic acid derivative with low water solubility (Chen et al., 2006). It is most commonly administered orally and is rapidly absorbed to reach its maximal plasma concentration within 1–2 h. However, its short biological half-life of 2 h (Insel, 1996) means frequent doses are needed to maintain the therapeutic efficacy for an extended time.

The conjugation of a biologically active compound with a polymer is one of the many methods for altering and controlling the pharmacokinetics, biodistribution, and often toxicity of various drugs (Duncan and Kopeček, 1984; Fuertges and Abuchowski, 1990; Duncan et al., 1992; Nichifor et al., 1996; Cavallaro et al., 2004a,b). The preparation of a drug–polymer conjugate has been reported extensively in many reviews (Goddard, 1991; Nucci et al., 1991; Pizzo, 1991). Recently, for oral delivery of ketoprofen, polymeric prodrug with high water solubility was prepared using pH sensitive polymer containing ketoprofen as pendent group (Wang and Chang, 1999). Increase in water solubility was also achieved by the preparation of a conjugate with dextran. This ketoprofen-dextran ester prodrug improved ketoprofen solubility and dissolution and reduced its ulcerative side effects (Larsen et al., 1991). The conjugation with small molecules also has been employed to improve the pharmacokinetics and minimize the undesirable side effects of ketoprofen. For example, glycine methyl ester conjugate of ketoprofen was reported to have less ulcerogenicity with better anti-inflammatory/analgesic action than their parent drug when administered orally (Dhaneshwar and Chaturvedi, 1994). A conjugate with α-cyclodextrin was prepared by covalent bonding to one of the primary hydroxyl groups of α-cyclodextrin (Hirayama

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et al., 2002). This prodrug showed a typical delayed-release pattern after oral administration to rats, and plasma levels of KP increased after a lag time of about 3 h and reached a maximum concentration at about 9 h.

The covalent attachment of poly(ethylene glycol) (PEG), PEGylation, is a technique widely used to improve therapeutic value of drugs, mostly for protein/peptide drugs, with improved chemical/thermal stability, reduced immunogenicity, increased circulation half-life, and decreased toxicity (Guerra et al., 1998; Lee et al., 1999; Diwan and Park, 2001; Harris and Chess, 2003; Hinds and Kim, 2002). PEG is a water-soluble polymer that has been widely used in pharmaceutical preparations on account of its safety, hydrophilicity, biocompatibility, lack of antigenicity, and low toxicity (Pang, 1993; Ford, 1986). Pegylation was also utilized for the preparation of prodrug for small molecules, especially the antitumor drugs such as taxol, camptothecin and doxorubicin for conferring a passive targeting to solid tumors, by the enhanced permeability and retention effect (Veonese and Pasut, 2004; Maeda et al., 2000). These drugs have very low solubility and rapidly excreted from the body. By making prodrugs with PEG, the properties of PEG are generally conveyed to the conjugated drugs, and increased solubility, modification of pharmacokinetics and targeting have been described. For small molecular drugs, PEGylation also has been employed for improving the drug's pharmacokinetic profile by delaying the release of parent drug. The camptothecin-PEG prodrug showed some promising results (Rowinsky et al., 2003). Severe myelosuppression was consistently observed and patients tolerated repetitive treatment without severe side effects such as cystitis, nausea, vomiting, and diarrhea.

In this work, ketoprofen was conjugated with PEG of various molecular weights (M_w = 750, 2000, and 5000) and its hydrolysis kinetics were examined in buffer solutions at different pHs. We also studied the potential of these prodrugs as a prodrug with slow release kinetics and prolonged pharmacological effect, which would reduce dosing schedule and improve compliance and quality of life in chronic patients.

2. Materials and methods

2.1. Materials

Ketoprofen and 4-dimethylaminopyridine (DMAP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). *N*,*N*-Dicyclohexylcarbodiimide (DCC) was purchased from Acros Organics (Geel, Belgium). Methoxy poly(ethylene glycol) with a molecular weight of 750, 2000 or 5000 was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation and characterization of the ketoprofen-conjugated PEG

2.2.1. Preparation of the ketoprofen-conjugated PEG (KPEG)

Methoxy poly(ethylene glycol) (1 mmol) and DCC (2 mmol) were dissolved in methylene chloride followed by the addition of ketoprofen (2 mmol) and DMAP (0.3 mmol). The mixture was stirred, and a white precipitate of dicyclohexylurea (DCU) was formed after 15–30 min. The reaction was monitored by thin layer chromatography (TLC). After approximately 6–8 h, the precipitate was filtered and the filtrate was evaporated to dryness. The resulting residue was dissolved in acetone, filtered to further remove any DCU, and evaporated again. Column chromatography was used to separate the KPEG from the reaction mixture. In order to remove the DMAP, a methanol/methylene chloride (5/95):hexane/ethyl acetate (1/5) mixture at a ratio of 15:1 was used as the mobile phase in the case of PEG with a molecular weight of 750, and at a 20:1 ratio of methanol/methylene chloride (9/91):hexane/ethyl acetate (1/2) in the case of PEG with a molecular weight of 2000 and 5000. Methanol/ethyl acetate (1/3) was used as a mobile phase to separate the KPEG from free ketoprofen. The purified products were dried under vacuum.

2.2.2. Spectroscopic study

The infrared absorption spectra of the ketoprofen, PEG and KPEG were obtained using a FT-IR spectrophotometer (FT/IR-430, Jasco, Tokyo, Japan). The samples were pressed into a potassium bromide pellet before obtaining their IR absorption spectra. The ^{1}H NMR spectra were obtained on a Bruker 300 spectrometer (Bruker Optics Inc., Bilerica, MA, USA).

2.2.3. Hydrolysis of KPEGs

The rate of KPEG hydrolysis was examined in an aqueous buffer solution at different pHs. The buffers used were 0.1 M phosphate buffer solutions with pHs of 1–10. The buffers contained 2% sodium azide to inhibit bacterial growth. Sample solutions of the KPEGs were prepared by dissolving the appropriate amount of KPEGs in phosphate buffers. All the sample solutions were kept in an eppendorf tube in an incubator at a constant temperature of 37 ± 0.5 °C. The samples were taken at predetermined times and analyzed immediately for the remaining KPEG as well as the free ketoprofen using high performance liquid chromatography (HPLC). The HPLC system (Shimadzu Scientific Instruments, Kyoto, Japan) consisted of an UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A). The wavelength of the UV detector was set to 258 nm. A reverse phase column (KR100-10018, 4.6 mm × 250 mm, Bohus, Sweden) was used for the analysis. The mobile phase used was 0.1 M ammonium phosphate buffer/acetonitrile (42:58) and the flow rate was 1.6 ml/min.

2.3. Pharmacokinetic study

2.3.1. Animal study

All the animal studies were carried out in accordance with the institutional guidelines as well as the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences. Male Sprague–Dawley rats weighing 300–350 g were obtained from Samtako Bio Co., Ltd. (Osan, Korea) and were given free access to normal standard chow diet (Jae II Chow, Korea) and tap water. The animals were acclimatized to these facilities for at least 1 week before the experiment. The rats were fasted overnight prior

to the experiments but were allowed water *ad libitum*. One day before the study, the external jugular vein and carotid artery were cannulated for sampling and drug administration, respectively. The animals were housed in plastic metabolic cages. A dose of ketoprofen (10 mg/kg or 30 mg/kg) or KPEG750 (40 mg/kg or 120 mg/kg, which contains 10 mg/kg or 30 mg/kg of ketoprofen, respectively) was administered to each animal either intravenously or intramuscularly. After administering the drugs, blood samples (0.25 ml) were collected at predetermined intervals over a 32 h period. All samples were stored at −20 °C until they were analyzed. The concentrations of free ketoprofen in the plasma were determined by HPLC after extraction from the plasma.

2.3.2. Extraction procedure

Fifty microliter of a 200 μg/ml solution of internal standard (butyl-4-aminobenzoate) in ethyl acetate was added to 100 μl of the plasma sample. The sample was then acidified by adding 0.1 ml of 1.0 M phosphate buffer at pH 3.0 and extracted with 550 μl ethyl acetate for 15 min using a vortex mixer. The sample tube was centrifuged at 10,000 rpm for 3 min. Five hundred microliter of the supernatant was transferred to an eppendorf tube and evaporated to dryness at 45 °C under a vacuum using a Speed Vac (Labconco, Kansas, USA). The extracted residue was reconstituted with 60 μl of a 0.1 M phosphate buffer at pH 7.4 and the solution was injected into the HPLC system.

2.3.3. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using the BA Calc 2002 program (Lee et al., 2000). The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal method. The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were read directly from the plasma concentration–time data.

2.3.4. Tail-flick test

The analgesic effect of ketoprofen and KPEG750 after intramuscular administration was estimated using the Tail-flick method (7360 Tail-flick, Ugo Basile, Varese, Italy) (D'Amour and Smith, 1941). Before injecting the drug, the base-line (control) tail-flick latencies were measured for each animal. The intensity of the heat stimulus was adjusted to yield a base-line latency of approximately 3 s, and a 10 s cut-off was used to prevent tissue damage (Dewey and Harris, 1971). The base-line was measured 30 min before administering the drug. After administering the drug, the test latency was measured as a function of time. The data was transformed to the percentage of maximum possible effect (%MPE) (Thornton et al., 1997; Preechagoon et al., 1998), which was calculated from the tail-flick latencies using the following equation: %MPE = [(test latency − control latency)/(10 − control latency)] × 100.

2.3.5. Carrageenan-induced paw edema

The anti-inflammatory effect after intramuscular administration was measured by Carrageenan-induced paw edema in rats weighing 150 ± 10 g. Edema was induced on the right hind foot of the rat by a subplantar injection of 0.1 ml/rat of 1.0% carrageenan (type IV, Sigma) dissolved in saline (Winter et al., 1962; Bonina et al., 1996). Each group of rats ($n = 7$) was treated with the vehicle, ketoprofen, or KPEG750 by an intramuscular injection 30 min before the carrageenan injection. The swelling volume of the paw was measured before (time 0) and at 0.5, 1, 2, 3, 4, 24 and 28 h after the carrageenan injection. The degree of paw edema was determined by measuring the hind paw volume with a Plethysmometer (Ugo Basile, Varese, Italy). The percentage increase in the paw volume was calculated from the normal paw volume.

2.3.6. Statistical analysis

The data is expressed as a mean ± S.E.M., except for the data from the pharmacokinetic studies, which are expressed as a mean ± S.D. The Student's *t*-test was used to compare the data. A p value <0.01 was considered significant.

3. Results and discussion

3.1. Preparation of KPEG

In general, the conjugation chemistry for non-protein drugs faces fewer problems because of the reduced number of functional groups present on a small molecule, the absence of conformational constrains, and the easier purification and characterization steps for the drug–polymer conjugates (Veonese and Pasut, 2004). In this work, KPEG was synthesized by the esterification of the carboxyl group of ketoprofen with the hydroxyl group of PEG at room temperature, as shown in Fig. 1. The esterification was performed using DCC as the coupling agent and DMAP as a catalyst (Zacchigna et al., 2004). The conjugation between ketoprofen and PEG was confirmed by FT-IR and ^{1}H NMR. The position of peak on IR spectrum for KPEG2000 was assigned as follows; IR (pellet): 2887, 1734, 1660, 1467, 1112 cm^{-1}. When conjugated, the hydroxyl groups of ketoprofen and PEG are expected to disappear due to the esterification process. The carbonyl peak of ketoprofen appearing at 1697 cm^{-1} due to intermolecular hydrogen bonding will be shifted to a higher frequency after conjugation with PEG (Chun et al., 2002). In addition, larger and sharper aliphatic stretching band of C–H will appear, due to the increased number of C–H bonds by PEG. As shown in Fig. 2, there was no hydroxyl peak in the KPEG2000 spectrum and the carbonyl peak shifted to 1734 cm^{-1}. Also, the larger and sharper C–H stretching band appeared at 2887 cm^{-1}. Fig. 3 shows the chemical shift of the KPEG2000 measured by ^{1}H NMR. The result confirmed the conjugation, and each peak was appropriately assigned to KPEG as follows; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.41$–7.78 (m, 9H), 4.15–4.32 (m, 2H), 3.85–3.93 (m, 1H), 3.53–3.73 (m, 189H–193H), 3.38 (m, 3H), 1.55 (d, 3H, $J = 7.1$ Hz). At room temperature, the KPEG prepared using PEG with a molecular weight of 750 (KPEG750) was oily, and the KPEG prepared using PEG with molecular weight of 2000 (KPEG2000) and 5000 (KPEG5000) were solid. All the conjugates were soluble in water and organic solvents such as ethanol, chloroform and dichloromethane. The position of each peak in

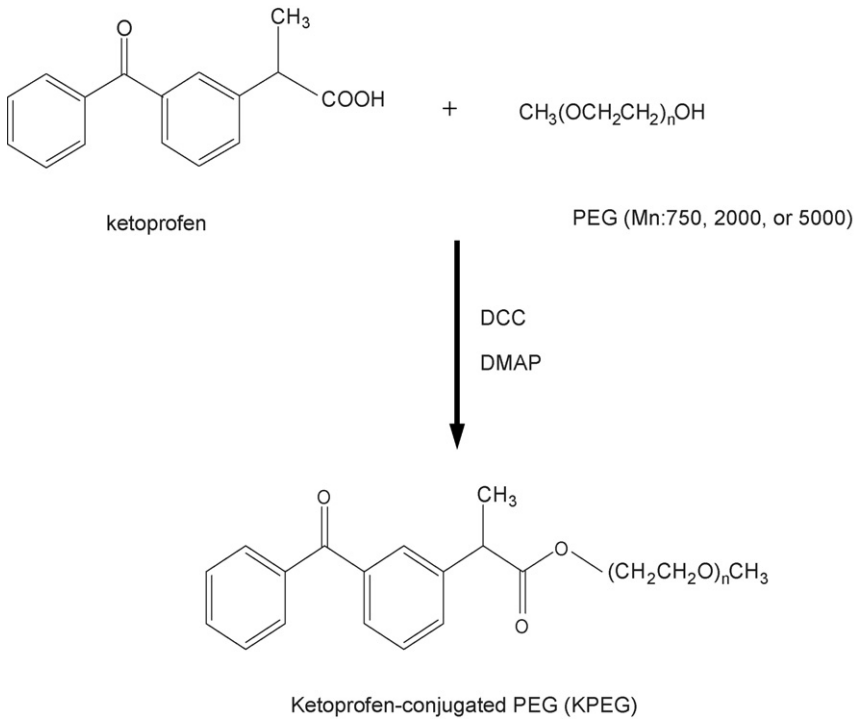

Fig. 1. Synthesis of the ketoprofen–PEG conjugate.

IR and NMR spectrum for KPEG750 & 5000 was as follows; KPEG750: IR (neat) 2872, 1732, 1659, 1450, and 1107 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.65–8.03$ (m, 9H), 4.38–4.52 (m, 2H), 4.02–4.09 (m, 1H), 3.74–3.92 (m, 64H–72H), 3.59 (s, 3H), 1.75 ppm (d, 3H, $J = 7.1$ Hz), KPEG5000: IR (pellet): 2887, 1734, 1660, 1467, and 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.41–7.78$ (m, 9H), 4.15–4.32 (m, 8H), 3.87 (m, 1H), 3.53–3.87 (m, 411H), 3.38 (m, 3H), 1.55 (d, 3H, $J = 7.1$ Hz).

3.2. Hydrolysis of KPEG

The appropriate rate of KPEG hydrolysis is important for it to be used as a prodrug. An extended release behavior cannot be expected if the hydrolysis rate is too fast. On the other hand, if the hydrolysis rate is too slow, most of the ketoprofen would remain in the conjugated form with PEG and the appropriate efficacy may not be obtained. Fig. 4 shows a plot of the rate of hydrolysis of the KPEGs as a function of pH in phosphate buffer solution. The hydrolysis rate constant was high at the low and high pHs, and showed a minimum at an intermediate pH (pH 4–6). As the pH was increased from 1 to 4, $\log k_{obs}$ decreased linearly and $\log k_{obs}$ increased linearly with increasing pH from 5 to 10, with a slope of approximately −1 and 1, respectively. The pH-rate profile resembled the typical specific acid–base catalysis, particularly in the basic pH region. In addition, hydrolysis rate increased with increasing length of PEG, indicating that the rate of hydrolysis can be modified by changing the molecular weight of PEG.

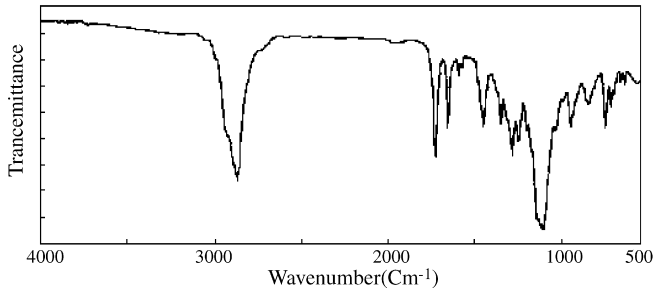

Fig. 2. FT-IR spectroscopy of KPEG2000.

3.3. Pharmacokinetic study

Based on the in vitro characteristics of KPEG, the pharmacokinetic profile of KPEG750 was compared with that of ketoprofen by measuring the ketoprofen concentration in plasma up to 32 h after the intravenous and intramuscular administration. Fig. 5 shows the ketoprofen concentration profiles in the plasma with time after the intravenous administration of ketoprofen (10 mg/kg and 30 mg/kg) and KPEG750 (40 mg/kg and 120 mg/kg, which contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively). The ketoprofen concentrations from KPEG in the plasma were generally higher than those of ketoprofen at both doses tested. The AUC of KPEG750 was approximately 1.5 times higher than that of ketoprofen, as shown in Table 1. Fig. 6 shows the ketoprofen concentration profiles in plasma with time after the intramuscular administration of 10 mg/kg and 30 mg/kg ketoprofen, and 40 mg/kg and 120 mg/kg KPEG750. At both doses tested, the T_{max} of KPEG750 in plasma was later than that of ketoprofen. The AUC after the administration of 120 mg/kg KPEG750 was similar to that of ketoprofen. However, the AUC was increased about 20% in case of 40 mg/kg of KPEG750

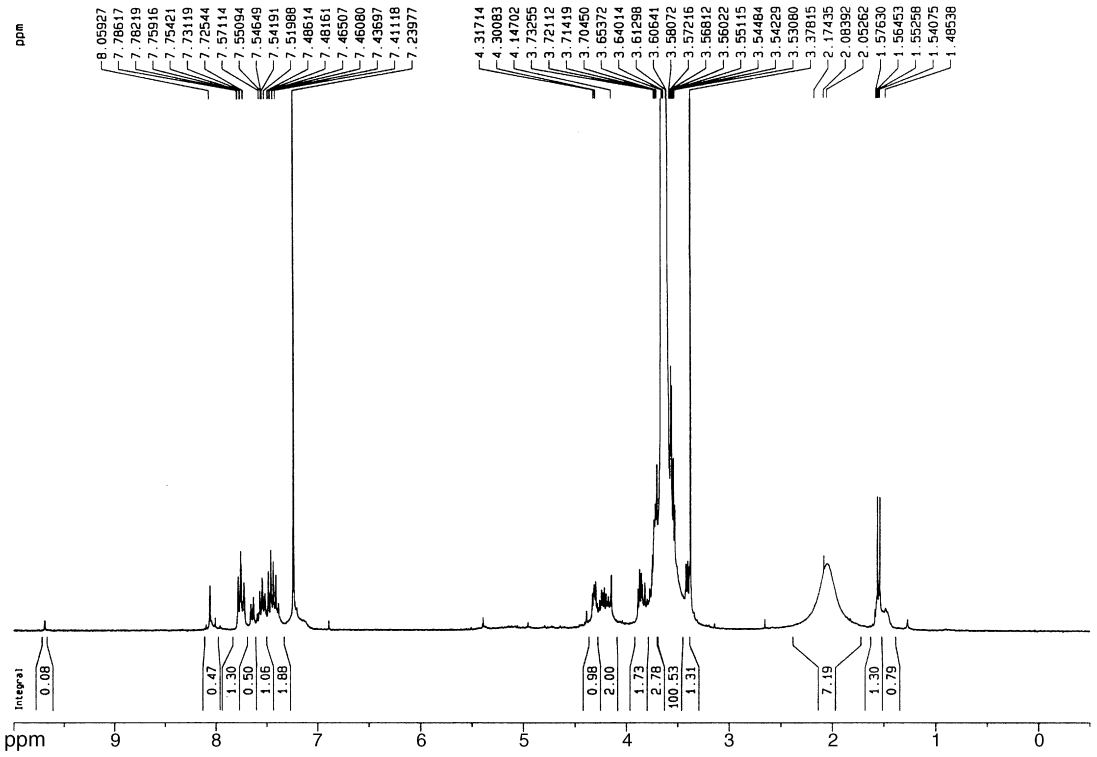

Fig. 3. ^{1}H NMR spectroscopy of KPEG2000.

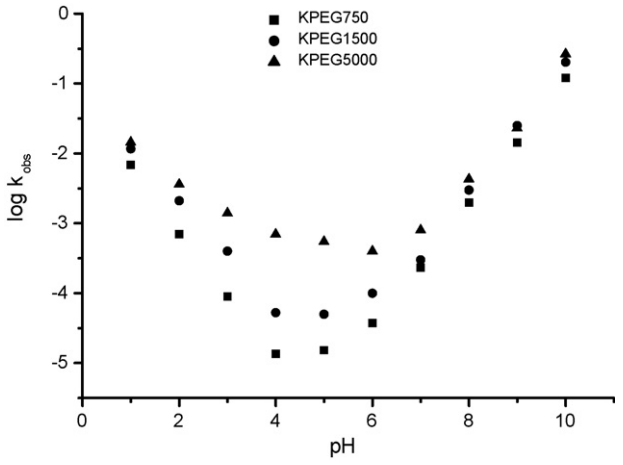

Fig. 4. Rate-pH profile for the hydrolysis of the KPEGs in phosphate buffer solution (pH 1–10).

Table 1
Pharmacokinetic parameters of ketoprofen after intravenous administration of KPEG750 and ketoprofen

Parameter[a]	Ketoprofen		KPEG750[b]	
	10 mg/kg	30 mg/kg	40 mg/kg	120 mg/kg
AUC (μg/(ml h))	72.4 ± 11.2	202.6 ± 44.8	103.5 ± 10.3	293.8 ± 31.2
C_{max} (μg/ml)	83.5 ± 4.8	92.3 ± 8.2	55.2 ± 5.2	70.6 ± 8.3
T_{max} (h)	–	–	0.25	0.25

[a] Parameters values expressed as mean ± S.D. ($n = 5$).
[b] KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively.

(Table 2). Although AUC after intravenous administration suggested that the efficacy may be improved by KPEG750, the results of Tables 1 and 2 were inconclusive in confirming the extended duration of action of KPEG750. However, it is highly probable that the conjugation of ketoprofen with PEG would maintain the efficacy of ketoprofen for an extended period due to slow hydrolysis. Therefore, it was decided to determine the effect of PEG conjugation on the duration of ketoprofen activity by measuring in vivo analgesic and anti-inflammatory effects.

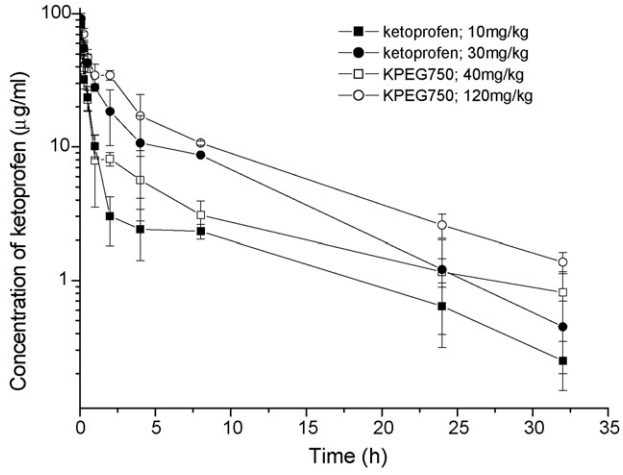

Fig. 5. Plasma concentration–time profile following the intravenous administration of KPEG750 and ketoprofen in rats. KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively. The values are reported as a mean ± S.D. ($n = 3$).

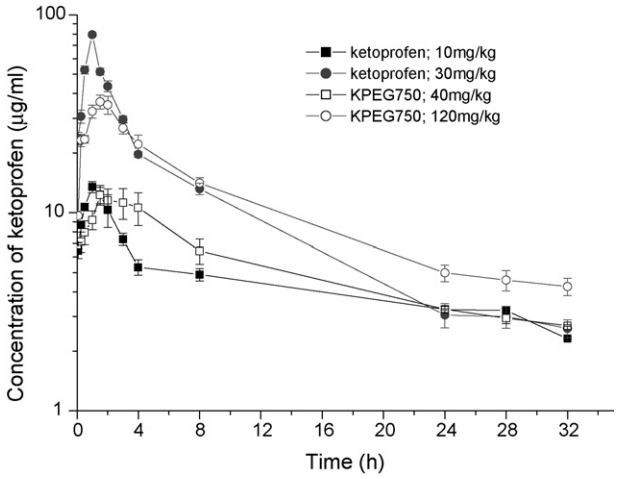

Fig. 6. Plasma concentration–time profile following the intramuscular injection of KPEG750 and ketoprofen to rats. KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively. The values are reported as a mean ± S.D. ($n = 3$, ketoprofen 10 mg/kg; $n = 5$, ketoprofen 30 mg/kg, KPEG750 40 mg/kg; $n = 6$, KPEG750 120 mg/kg).

Table 2
Pharmacokinetic parameters of ketoprofen after the intramuscular injection of KPEG750 and ketoprofen

Parameter[a]	Ketoprofen		KPEG750[b]	
	10 mg/kg	30 mg/kg	40 mg/kg	120 mg/kg
AUC (μg/(ml h))	145.7 ± 5.7	363.7 ± 5.4	175.2 ± 4.1	361.4 ± 8.3
C_{max} (μg/ml)	13.5 ± 0.9	79.3 ± 0.2	12.3 ± 0.1	36.4 ± 0.4
T_{max} (h)	1.0	1.0	1.5	1.5

[a] Parameters values expressed as mean ± S.D. ($n = 3$, ketoprofen 10 mg/kg; $n = 5$, ketoprofen 30 mg/kg, KPEG750 40 mg/kg; $n = 6$, KPEG750 120 mg/kg).
[b] KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively.

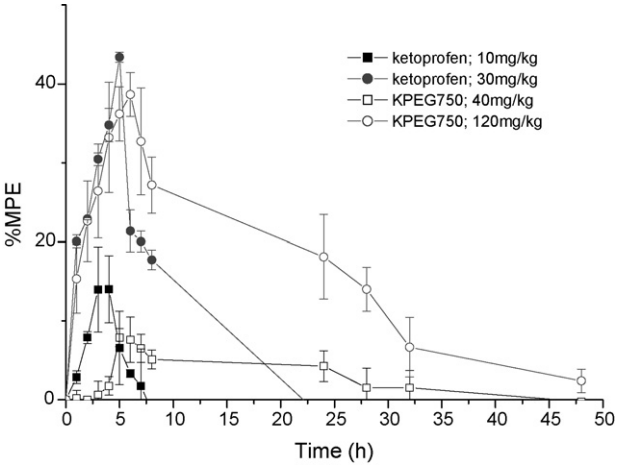

Fig. 7. %MPE of analgesic effect vs. time curve for KPEG750 and ketoprofen. The drugs were injected intramuscularly. KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively. The control data was omitted for clarity. The base-line latency was recorded 30 min before the intramuscular injection. The values are reported as a mean ± S.E.M. ($n = 6$).

Table 3
Comparison of the analgesic effect of KPEG750 and ketoprofen

Time[a] (h)	%MPE[b] ± S.E.M.			
	Ketoprofen		KPEG750	
	10 mg/kg	30 mg/kg	40 mg/kg	120 mg/kg
1	2.7 ± 0.9	20.1 ± 0.8***	0.2 ± 1.0	15.3 ± 4.3
2	7.9 ± 0.8**	22.9 ± 0.1***	−0.1 ± 0.8	22.6 ± 5.1*
3	14.0 ± 5.4	30.5 ± 0.8***	0.6 ± 1.7	26.4 ± 6.0*
4	14.0 ± 4.2	34.8 ± 2.1***	1.8 ± 1.2	33.2 ± 7.0*
5	6.6 ± 4.7	43.4 ± 0.6***	7.9 ± 1.1*	36.2 ± 3.4**
6	3.4 ± 0.5*	21.5 ± 2.7*	7.6 ± 2.9	38.7 ± 2.8**
7	1.7 ± 4.7	20.0 ± 1.3**	6.5 ± 1.8	32.7 ± 6.8*
8	−1.4 ± 1.8	17.7 ± 1.2**	5.1 ± 1.2*	27.2 ± 3.6*
24		−2.5 ± 1.9	4.3 ± 2.0	18.1 ± 5.3
28			1.5 ± 2.5	14.0 ± 2.8*
32			1.5 ± 2.2	6.7 ± 3.9
48			−0.2 ± 0.6	2.4 ± 1.5

*$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$, significantly different from the control ($n = 12$).
[a] Time after the intramuscular administration of test drug.
[b] The degree of analgesic effect was quantified by the percent maximum possible effect (%MPE), which was calculated from the tail-flick latencies.

3.4. Analgesic effect

The analgesic effect of ketoprofen and KPEG750 after intramuscular injection was evaluated using the Tail-flick method (Fig. 7 and Table 3). Although the peak analgesic effect of KPEG750 occurred later than ketoprofen, it exhibited a significantly extended analgesic effect compared with ketoprofen, which coincides with the plasma profile where the T_{max} of KPEG750 was later than ketoprofen. The analgesic effect lasted for approximately 6–7 h when the ketoprofen dose was 10 mg/kg. On the other hand, the analgesic effect of the 40 mg/kg of KPEG750, which is equivalent to 10 mg/kg of ketoprofen, lasted for almost 1 day. The analgesic effect lasted for a longer period as the dose was increased. When the ketoprofen dose

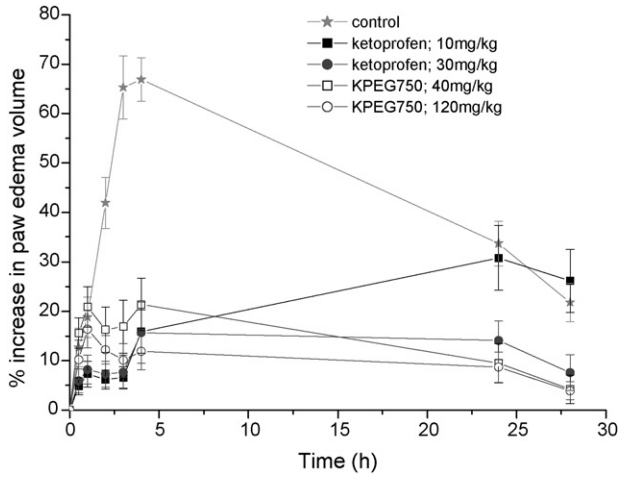

Fig. 8. Anti-inflammatory effect of KPEG750 on carrageenan-induced paw edema in rats. KPEG750 40 mg/kg and 120 mg/kg contain 10 mg/kg and 30 mg/kg of ketoprofen, respectively. The values are reported as a mean ± S.E.M. ($n = 7$).

was 30 mg/kg, the analgesic effect lasted for more than 8 h. When 120 mg/kg of KPEG750, which is equivalent to 30 mg/kg of ketoprofen, was administered, the analgesic effect lasted for more than 48 h.

3.5. Anti-inflammatory effect

Fig. 8 shows the effect of ketoprofen and KPEG750 on the volume of acute inflammatory paw edema in rats caused by carrageenan after intramuscular administration. The paw edema volume increased significantly within a few hours after the carrageenan injection. The results showed that both ketoprofen and KPEG750 could inhibit the inflammatory process in a dose-dependent manner. As was the case in the analgesic effect, ketoprofen showed a better efficacy in the initial phase and KPEG750 showed a better efficacy in the later phase. The anti-inflammatory effect lasted more than 24 h after the administration of 40 mg/kg and 120 mg/kg KPEG750 and 30 mg/kg ketoprofen.

4. Conclusion

KPEG prodrugs were prepared and their hydrolysis kinetics, pharmacokinetic behavior, analgesic effect and anti-inflammatory activity were investigated. The pharmacokinetic results were inconclusive in confirming extended duration of action of KPEG. The in vivo results after intravenous and intramuscular administration to rats showed that KPEG750 had a typical delayed action pattern and reached a maximum efficacy at later time. The effects of PEG conjugation were well reflected in the analgesic and anti-inflammatory effect tested using tail-flick and carrageenan-induced paw edema paw method. These results indicate that KPEG could be a promising NSAID prodrug with extended pharmacological effects by delayed release of parent drug.

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