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Sustained release characteristics and pharmacokinetic parameters of ketoprofen suppositories using chitosan[1]

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Abstract

The use of chitosan granules as a means of achieving sustained release of ketoprofen (KP) in suppository form was examined. The suppositories were prepared using different molecular weight polyethylene glycol in various portions. In contrast with the rapid release of conventional suppository form, sustained release form chitosan granules were observed. Furthermore, the release rate could be controlled by changing the mixing ratio of drug and chitosan. The potential of a suppository which has chitosan granules of KP was compared with a conventional form in rabbits. When a conventional suppository was administered rectally, the plasma concentration reached the maximum level in 1 h. The sustained release form also reached the maximum level in the same time but, produced a sustained plateau of the drug. This is due to the slow rate of release and a longer residence time in the rectum. However, the sustained release formulation reduces the rectal bioavailability by nearly 40% in comparison with the conventional formulation.

Keywords: Ketoprofen; Sustained release suppository; Chitosan; In vitro release; Rectal administration; Rabbit; Pharmacokinetics

1. Introduction

Chitosan (1,4)-2-amine-2-deoxy-β-D-glucan) is a natural polyamino saccharide prepared from chitin by *N*-deacetylation with alkali (Machida and Nagai, 1989). In pharmaceutical applications, it has been used as a direct compression diluent (Sawayanagi et al., 1982a; Nagai et al., 1984), a new drug carrier for sustained release preparations or a floating oral drug delivery system (Inouye et al., 1989; Miyazaki et al., 1988) as well as an agent for the enhancement of the dissolution properties of some less soluble drugs (Nagai et al., 1984; Sawayanagi et al., 1982b).

Ketroprofen (KP) is an analgesic and non-steroidal anti-inflammatory (NSAI) drug usually

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employed in the therapy of rheumatic disorder. It is rapidly eliminated from blood after dosing (plasma-half life 1–3 h) (Reynolds et al., 1989), and to maintain the therapeutic plasma levels the drug must be administered at least twice a day. In the usual oral administration of NSAI drugs, the tablets and capsules have led to peptic ulceration and anorexia (Thomas and Kantor, 1986).

Rectal administration of NSAI drug would be an alternative dosage route for patients with peptic ulcers and children. Sustained release suppositories are preferable to conventional suppositories because they reduce the frequency of drug administration.

The purpose of the study was to prepare the sustained release suppositories of KP using a gel matrix of chitosan. Also, the release behaviour and bioavaibility of the suppositories were investigated in rabbits after rectal administration of two samples.

2. Materials and methods

2.1. Materials

Ketoprofen was supplied by Drifen Pharm. (Turkey), PEGs from Merck, Chitosan from Dainichiseica Color and Chemicals (Japan), other reagents used were of analytical and HPLC grade.

2.2. Preparation of suppositories

Conventional suppositories (F1: F2: F3: F4) were prepared by fusion method at 48°C.

Sustained release suppositories F1-CH-11: F2-CH-11: F3-CH-11: F4-CH-11: F1-CH-12: F2-CH-12: F3-CH-12: F4-CH-12.

In order to obtain the formulations first, the chitosan granules of KP were prepared. Then they are mixed with the PEG bases. In this way, 12 different formulations were obtained.

2.3. Preparation of chitosan granules

Chitosan granules were prepared by the following procedures: KP (100 mg) was dissolved in 5 ml of methanol at 60°C and the calculated amount of chitosan was added to the drug solution. After evaporation of the solvent at 60°C, the residue was dissolved in 5 ml of acetic acid (10% w/v). The gelatinous chitosan-drug mixture was allowed to stand at room temperature for 1 h and than sucked into a glass syringe, and extruded on to a glass plate. After drying overnight at room temperature, the chitosan gel cord was cut into pieces and dried for an additional 8 h at 80°C in vacuum. Then the pieces were ground and sieved. The final chitosan granules used in the formulations were approximately 500 mm in sieve size.

2.4. Preparation of sustained release suppositories

First of all, the displacement value of the chitosan granules were calculated in the bases. Then they were mixed with the bases. The melt mass poured into the steel molds and allowed to solidify at room temperature. After solidification, the formed suppositories were removed from the mold, wrapped with aluminium foil and stored in a desiccatore in the refrigerator at +4°C. The content of KP in suppositories was 100 mg. The composition of the formulations are listed in Table 1.

2.5. In vitro release from suppositories

The release test were carried out according to the method reported previously (Ermiş and Tarimci, 1995). The test solution used was 300 ml of pH 7.2 phosphate buffer at 37°C. Each determination was carried out in triplicate. The data obtained for sustained release suppositories were evaluated kinetically using a computer program written for this purpose (Ağabeyoğlu, 1984).

2.6. Animal studies

White male rabbits weighing from 2.0 to 2.5 kg were randomly divided into two groups of four animals each. They were fasted for 15 h prior to the experiments but were allowed free access to water. The first group was administered conventional suppositories (F1), containing 100 mg of drug while the second group was administered sustained release suppositories (F1-CH-12) con-

Table 1
Code and constituents of suppositories

Codes	Substances (mg)								
	KP	PEG-400	PEG-1000	PEG-1540	PEG-2000	PEG-4000	PE-6000	Water	Chitosan
F1	0.100	—	2.184	—	—	0.0446	—	—	—
F2	0.100	0.116	2.149	—	—	0.0462	1.056	—	—
F3	0.100	—	—	0.939	—	—	—	0.352	—
F4	0.100	—	—	—	2.292	—	—	—	—
F1-CH-11	0.100	—	2.178	—	—	0.0444	—	—	0.100
F2-CH-11	0.100	0.110	2.047	—	—	0.0440	—	-	0.100
F3-CH-11	0.100	—	—	0.887	—	—	0.997	0.333	0.100
F4-CH-11	0.100	—	—	—	2.246	—	—	—	0.100
F1-CH-12	0.100	—	2.091	—	—	0.0427	—	—	0.200
F2-CH-12	0.100	0.105	1.955	—	—	0.0421	—	—	0.200
F3-CH-12	0.100	—	—	0.852	—	—	0.958	0.319	0.200
F4-CH-12	0.100	—	—	—	2.133	—	—	—	0.200

taining the same amount of drug. Blood samples (4 ml) were withdrawn from the ear vein at regular intervals after rectal administration of suppository. The suppositories were applied 15 min after removal the refrigerator. The plasma samples were frozen and stored at −4°C until assay.

2.7. Determination of KP in plasma

The high-performance liquid chromatographic method reported by Upton et al. (1980) was applied with a slight modification as follows: 1 ml plasma was mixed with 40 μl naproxen solution as internal standard (concentration 6.25 μl/ml 0.01 pH 6 phosphate buffer). Then the pH of the each sample was adjusted by addition of 0.5 ml of 1.0 M phosphate buffer at pH 2.0. After 10 ml of diethyl ether was added, tubes were vortexed for 1 min and centrifuged at about 2000 rpm for 3 min. The upper organic phase was transferred by Pasteur pipette to another glass tube and evaporated to dryness at 40°C under a stream of dry nitrogen. The residue was dissolved in 100 μl of mobile phase (0.05 M pH 7 phosphate buffer: acetonitril (90:10)) and eluent was injected into the HPLC column (Waters Associate μ Bondapack C_{18}) and monitored at 263 nm.

2.8. Pharmacokinetic data analysis

The data were processed by the ESTRIP striping program to determine the pharmacokinetic model of KP (Brown and Manno, 1978). The pharmacokinetic parameters taken from the pro-

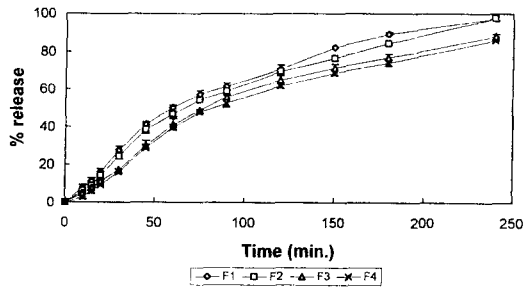

Fig. 1. The release profiles of KP from conventional suppositories.

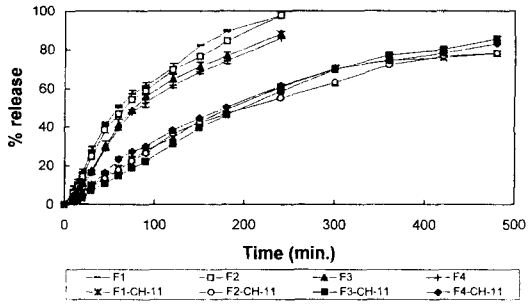

Fig. 2. In vitro diffusion rate profiles of KP suppositories prepared with chitosan granules (1:1 ratio).

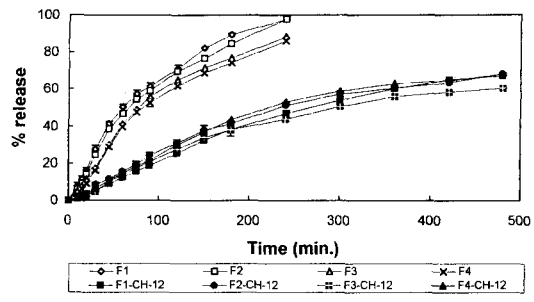

Fig. 3. In vitro diffusion rate profiles of KP suppositories prepared with chitosan granules (1:2 ratio).

gram were then employed as the inputs of MARQUART Non Linear Regression Program which yields optimised parameters (Nicholls and Peck, 1981).

3. Result and discussion

3.1. Release of KP from suppositories in vitro

The diffusion profiles of KP from the conventional suppositories are as shown in Fig. 1. It is seen from this figure that the drug is completely released over a period of 4–5.5 h. Since the membrane control method was used in which the drug diffuses from the constant area of the membrane, such low values of release rate have been obtained for conventional suppositories of KP. Similar results were obtained in the studies employing Muranishi method as reported in (Nakajima et al., 1987, 1990).

The release profiles of the sustained release suppositories which contain chitosan granules in different ratios are given in Figs. 2 and 3. As can be observed from these figures, nearly 78% of the drug releases in 8 h time from F1-CH-11 and F2-CH-11 coded samples which contain KP:CH (1:1) ratio granules. This value is approximately 85% for F3-CH-11 and F4-CH-11 coded samples. In the series of suppositories which contain KP:CH (1:2) ratio granules, the F3-CH-12 formulation yields 60% released amount of drug by diffusion in 8 h. This is the lowest rate among all results.

The remaining three formulations of the series do not show significant differences.

The kinetic assessment of the release data are given in Table 2. By calculating the determination coefficients it was found out that the best fits were obtained with the first order reaction and $Q \rightarrow \sqrt{t}$ kinetics. However, the best fit was observed to be the first order reaction kinetic when the evaluation of the same results were carried out according to SWSD (sum of the weighted square deviations) criterion. The reason is that drug releases by diffusion process through the gel layer and in proceeding hours the granules continue to swell in dissolution medium. This has the effect of slowing down the diffusion of the drug through the gel. The rate limiting step is the thickness of the gel layer.

3.2. The evaluation of invivo results

When the diffusion results of the formulations prepared with chitosan in two different ratios are compared, there was no significant difference between different formulations. This is because the selectivity of Muranishi Method is limited. For that reason, the drug release results of the samples obtained with USP XXII basket method, were assessed and the slowest release obtained with F1-CH-12 formulation (Ermiş and Tarimci, 1992). This formulation is also found suitable regarding its other physical properties. Because of this; F1-CH-12 conventional form of F1 formulations having no chitosan granules were used in-vivo experiments. The blood concentration-time profiles are given in Fig. 4. For determining the pharmacokinetic model of KP, the data of the mean blood concentrations belonging to both formulations were applied to ESTRIP stripping program.

The pharmacokinetic parameters obtained from ESTRIP results are given in Table 3. These results show that KP fits one compartment open model.

The two exponential equation describing one compartment open absorption model is as follows:

$$C = C_1 e_1^{-kt} + C_2 e_2^{-kt}$$

Table 2
The kinetic assessment of release data

Kinetics		F1-CH-11	F2-CH-11	F3-CH-11	F4-CH-11	F1-CH-12	F2-CH-12	F3-CH-12	F4-CH-12
0°	kr°	10.5	10.3	11.8	10.7	9.1	8.9	8.1	9.4
	r^2	0.932	0.945	0.968	0.923	0.961	0.929	0.931	0.934
	SSD	0.145	0.115	5.76×10^{-2}	0.182	4.77×10^{-2}	0.107	8.11×10^{-2}	9.21×10^{-2}
	SWSD	0.442	0.274	0.573	0.518	9.10×10^{-2}	0.171	0.171	0.167
1°	kr	0.209	0.202	0.257	0.230	0.150	0.149	0.125	0.161
	r^2	0.982	0.995	0.993	0.983	0.994	0.981	0.975	0.983
	SSD	7.94×10^{-3}	3.77×10^{-3}	6.08×10^{-2}	1.22×10^{-2}	4.44×10^{-3}	2.08×10^{-2}	2.16×10^{-2}	1.26×10^{-2}
	SWSD	2.36×10^{-2}	7.85×10^{-3}	0.102	3.03×10^{-2}	7.43×10^{-3}	3.32×10^{-2}	3.23×10^{-2}	2.28×10^{-2}
$Q\rightarrow\sqrt{t}$	kr°	0.567	0.532	0.569	0.622	0.358	0.403	0.322	0.417
	r^2	0.987	0.994	0.990	0.987	0.992	0.991	0.990	0.988
	SSD	8.27×10^{-2}	0.077	0.158	7.85×10^{-2}	8.79×10^{-2}	5.97×10^{-2}	5.46×10^{-2}	8.38×10^{-2}
	SWSD	0.132	0.118	0.279	0.124	0.125	7.99×10^{-2}	7.03×10^{-2}	0.117

kr°, Zero order release rate constant; kr, first order rate constant; k, the rate constant from the slope of the linear regression of cumulative amount release per unit area versus square root of time; SSD, sum of the square deviations; SWSD, sum of the weighted square deviations.

where C_1, C_2 are the coefficients of two exponential equations, k_1 is the elimination rate constant, k_2 is the absorption rate constant, and t is time.

As seen from Table 3, the determination coefficients obtained with this program are not high. For that reason; the pharmacokinetic data were decided as base values and for improving them they were applied to MARQUART non-linear regression program. The improved results are shown in Table 4. MARQUART non-linear regression program yields higher values for determination coefficients in comparison with those of ESTRIP program.

The parameters of the polyexponential equations for the formulations F1 and F1-CH-12 are also obtained by running the MARQUART program. The resulting equations obtained by this way are as given in Eqs. (1) and (2) respectively for F1 and F1-CH-12 formulations.

$$C = 11.040e^{-0.137t} - 11.065e^{4.410t} \quad (1)$$

$$C = 5.930e^{-0.126t} - 5.980e^{2980t} \quad (2)$$

When the absorption rate constant of the two formulations are compared it is observed that the absorption rate constant of formulation F1-CH-12 is relatively low; that is around 40%. From Table 2, the dissolution rate constant of the same formulation is observed to be smaller than the absolution rate constant mentioned above. These results prove that the absorption rate of the drug for the formulation F1-CH-12 is not dictated by the absorption rate of the drug itself but by the

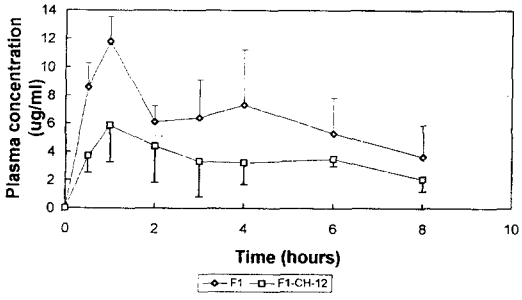

Fig. 4. Plasma levels of KP after rectal administration of suppositories in rabbits (Each point represents the mean + S.D. ($n = 4$).

Table 3
Pharmacokinetic parameters taken from the ESTRIP program

Codes	C_1	k_1	C_2	k_2	R^2	SSD
F1	10.6	0.128	−10.6	4.13	0.846	12.9
F1-CH-12	5.78	0.120	−5.78	2.42	0.868	2.73

C_1 and C_2, the coefficients of the second order equation; k_1, elimination rate constant; k_2, absorption rate constant; r^2, coefficient of determination; SSD, sum of square deviations.

Table 4
Pharmacokinetic parameters taken from the Marquart non-linear regression program

Codes	C_1	k_1	C_2	k_2	R^2	SSD
F1	11.04	0.137	−11.06	4.41	0.851	12.48
F1-CH-12	5.93	0.126	−5.98	2.90	0.885	2.38

C_1 and C_2, the coefficients of the second order equation; k_1, elimination rate constant; k_2, absorption rate constant; r^2, coefficient of determination; SSD, sum of square deviations.

dissolution rate of drug from the sustained release preparation.

Other pharmacokinetic parameters of the two formulations are calculated from Eqs. (1) and (2) as given in Table 5 t_{max} values for the formulations F1 and F1-CH-12 are found to be 0.813 and 1.131 h, respectively. The plasma concentration of KP rises to a maximum value in a short time.

t_{max} values presented here are also found to be in good agreement with those of a similar in vivo study reported in (Kamiya et al., 1983). In formulations F1 and F1-CH-12, t_{max} values are found to be very close to each other. This is because, in the first few hours of the total release period, the granules which contain chitosan are not swallowed efficiently enough to block the drug. Therefore, at the beginning, it is rapidly released. If the maximum value of drug concentration were reached in a longer time period then the t_{max} value would probably be larger than that of F1 formulation. When the peak plasma concentration values (C_{max}) are compared for the two formulations it is observed that the sustained release formulation F1-CH-12 gives a C_{max} value which is being 40% lower than that of conventional formulation F1.

Table 5
Pharmacokinetic parameters of ketoprofen after rectal administration in rabbits

Codes	C_{max} (μg/ml)	T_{max} (h)	AUC (μg/h/ml)	$t_{1/2}$ (h)
F1	9.57	0.813	80.59	5.06
F1-CH-12	5.01	1.131	45.00	5.50

C_{max}, peak plasma ketoprofen concentration; T_{max}, time when C max was obtained; AUC, area under the curve of plasma concentration 8 h after administration; $t_{1/2}$, half time of ketoprofen elimination in plasma.

When all these results are evaluated in combination with the plasma concentration curves in Fig. 4, F1-CH-12 formulation yields sustained release action for a period of 8 h as expected. However sustained release formulation reduces rectal bioavailability of KP by an amount of 40% in comparison with that of conventional formulation F1. This can be understood from Fig. 4 by making a comparison between the areas under plasma concentration-time curves of the two formulations.

In conclusion from the in vitro experiments, chitosan can be considered as a suitable matrix material for sustained release suppository formulation. However, in vivo results did not look promising. The reason is, the concentration of the polymer in a matrix type formulation of chitosan based suppositories was the determining factor in controlling dissolution and absorbsion rate of the drug from the rectal mucosa. Within a few hours right after the administration of suppository into the rectum, KP was probably encapsulated in the gel matrix which was formed by chitosan granules. Therefore, the absorbsion rate drops down. We think that a 1:1 (drug:chitosan) containing formulation might give better bioavailability.

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