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In vitro and in vivo comparison of two diclofenac sodium sustained release oral formulations

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Abstract

The aim of this study was to investigate the effect of formulation on the pharmacokinetics of diclofenac in two sustained release formulations (formulation A and Voltaren SR®) after oral delivery. The dissolution of diclofenac from sustained release formulation was pH-dependent. While drug released from both formulations increased with increased pH, the release kinetics of these two formulations was different. The pharmacokinetic study was conducted in 12 healthy subjects administered with multiple doses of 100 mg of diclofenac in a crossover design. There was a significant difference in area under the plasma concentration–time curve [AUC(0–24)] and C_{max} observed. The formulation with a reduced diffusion exponent with increased kinetic constant results in increased absorption of diclofenac in vivo. This study demonstrated the impact of release mechanism of the formulation on the absorption in vivo.

Keywords: Diclofenac; Pharmacokinetic; Sustained release; Dissolution

1. Introduction

The release of drug from a sustained release formulation is controlled by various factors through different mechanisms such as diffusion, erosion, or osmosis. Several mathematical models have been proposed to describe the release profiles of drugs from various systems (Higuchi, 1963; Bamba et al., 1979; Peppas et al., 1980), and studies have also been performed to evaluate the effect of polymer ratio and particle sizes on the release of drug from formulations (Velasco et al., 1999; Liu et al., 1995). However, little studies were conducted to understand the relationship between the release mechanism and the in vivo performance of diclofenac. Therefore, it is relevant to study the effect of formulation on the release of diclofenac in vitro and its performance in vivo.

Diclofenac, a phenylacetic acid derivative, is a nonsteroidal anti-inflammatory analgesic with potent cyclooxygenase inhibition activity (Ku et al., 1985; Menasse et al., 1978). This drug is commonly used for pain control and the treatment of rheumatic diseases (Brogen et al., 1980).

Diclofenac is well absorbed after oral administration with extensive hepatic metabolism (Hasan et al., 1991; Fowler et al., 1983). This compound exhibits a terminal half-life of 1–2 h, volume of distribution of 0.17 l/kg, and 99% protein binding (Willis et al., 1979; Benson et al., 1985; Chan et al., 1987). In addition, diclofenac enters the synovial fluid (Wallis and

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Simkin, 1983). The effect of food on the absorption of diclofenac was reported (Terhaag et al., 1991). However, a study by Riad et al. (1995) showed that a more consistent absorption pattern with a single peak was observed for sustained formulation under fed condition. Controlled release formulations of diclofenac have been widely studied via different release matrices. Relationship between the in vitro release kinetics from different matrix and their in vivo performance is thus an important issue to evaluate different formulations of diclofenac sodium.

In the current study, an experimental diclofenac sustained released formulation was chosen while Voltaren SR® was chosen as the reference. A multiple-dose pharmacokinetic study was performed to evaluate the effect of formulation on the in vivo performance of diclofenac under fed state.

2. Materials and methods

2.1. Materials

The controlled release formulation of 100 mg diclofenac was an experimental formulation (formulation A, a multiple unit formulation or capsules with coated minipellets) and Voltaren SR® (100 mg sustained release diclofenac) (Norvatis, Basel, Switzerland) was chosen as a reference. The internal standard, nimesulide, was obtained from Lotus Medical Supply, Inc. (Taiwan, ROC). NaH_2PO_4 was obtained from E. Merck (Darmstadt, Germany). *n*-Hexane and 85% phosphoric acid were purchased from Fisher Scientific Co. (NJ, USA). All chemicals were either analytical or HPLC grade and deionized water was used.

2.2. Dissolution study

Dissolution studies were performed using a rotating paddle apparatus (USP apparatus II) (Pharma Test, type PTW SIII, Hainburg, Germany) at a stirring rate of 100 rpm at 37 °C. The dissolution media included phosphate buffers at different pHs (pH 2.5, 4.5, 6.5), 0.1N HCl and water. Samples were taken at different time intervals, and the concentrations of diclofenac sodium were measured at UV 277 nm. In order to compare dissolution profiles of diclofenac, the similarity factor (f_2) was calculated as following (Moore and Flanner, 1996):

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} W_t (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (1)$$

where W_t is the weighting factor at time t, R_t is the percent dissolved from Voltaren SR® at time t, and T_t is the percent dissolved from formulation A at time t.

The release kinetics of diclofenac from formulation was evaluated by the exponential Eq. (2) (Korsmeyer et al., 1983) for $Q_t/Q_\infty < 0.8$:

$$\frac{Q_t}{Q_\infty} = kt^n \quad (2)$$

where Q_t is the amount of drug released at time t, Q_∞ is the total amount of drug released, k is the kinetic constant, and n is the diffusional exponent.

2.3. Subjects

Twelve healthy male subjects aged between 20 and 40 (body weight of 58–78 kg) were recruited with informed consent. The study protocol was approved by the IRB of National Cheng Kung University Medical Center. All subjects were initially admitted to the Chi-Mei Foundation Hospital, Tainan, Taiwan for a 1-day check up of the health status as determined by medical history, physical examination and laboratory tests. Laboratory tests performed included complete blood analysis, plasma electrolytes, urine and liver function tests, urinalysis, and assay for hepatitis B antigen and HIV antibody. All subjects met the inclusion and exclusion criteria.

2.4. Study design

This study was performed in a two-way crossover design with a washout period of 1 week between two phases. No other medications were taken during the study period. The subjects were randomly divided into two groups. Subjects 1, 3, 5, 7, 9, and 11 were given 100 mg daily of formulation A followed by Voltaren SR® daily at the same dose 1 week later, and vice versa for subjects 2, 4, 6, 8, 10, and 12.

On study days, each subject arrived at the medical center at 08:00 h following an overnight fasting. A

standard meal (250 ml milk and one hamburger) was finished in 15 min followed by administration of diclofenac with 250 ml water for 7 days. No food was allowed until 4 h after dosing. Approximately 10 ml of blood samples were collected into heparinized tubes before each dosing and at 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 8, 10, 12 and 24 h after dosing on the seventh day. Subsequently, samples were centrifuged at 3000 rpm for 15 min and plasma was stored at −70 °C until analysis.

2.5. Sample analysis

The plasma concentration of diclofenac was determined by a modified high-performance liquid chromatographic (HPLC) assay (Chan et al., 1982). To 1 ml of plasma was added 3 ml of diethyl ether and 3 ml of *n*-hexane, followed by overtaxing and centrifugation at 300 rpm for 5 min. Subsequently, 100 μl internal standard (2 μg/ml of nimesulide) was added to the lower layer, followed by acidification with 1 ml of 0.83 M phosphoric acid and 4 ml of mixture of *n*-hexane:isopropyl alcohol (90:10 (v/v)). The mixture was vortexed, and was centrifuged at 3000 rpm for 5 min. The upper liquid layer was evaporated under N_2 gas. The residue was reconstituted with 200 μl of mobile phase and 150 μl was injected for HPLC analysis. HPLC system consisted of a pump (Waters, Model 510, Milford, MA, USA), an automatic injector (Waters, WISP Model 710), a reverse phase C8 column (Microsorb-MV, 4.6 mm × 250 mm, Rainin Instrument Co., Woburn, MA, USA), a UV detector (Waters, Model 486) at 295 nm and an integrator (Hewlett-Packard, Model 3392A, Avondale, PA, USA). The mobile phase included 49% methanol, 10% acetonitrile, and 41% 40 mM NaH_2PO_4 at a flow rate of 1 ml/min. The logarithmic peak height ratio of diclofenac to internal standard (nimesulide) versus logarithmic concentration was fitted by linear regression. The concentrations of plasma diclofenac were interpolated from the calibration curve.

2.6. Data analysis

Area under the plasma concentration–time curve [AUC(0–24)] on the seventh day was calculated by the trapezoidal method. C_{max} was the highest observed concentration and T_{max} was the time within which C_{max} occurred. Mean residence time (MRT) was calculated as AUMC/AUC, where AUMC is the area under the moment versus time curve. Fluctuation at plateau was estimated as the ratio of $(C_{ss,max}-C_{ss,min}):C_{ss,min}$. Differences between the pharmacokinetic parameters (AUC and C_{max}) were tested for statistical difference by ANOVA, and the difference in T_{max} was tested by nonparamatric analysis.

3. Results

3.1. In vitro dissolution studies

In vitro dissolution tests were performed using five different pHs of medium. Based on the Guidance of SUPAC-MR, the rotation speed of 100 rpm was chosen in this study due to the limited dissolution in acidic solutions. At least 90% of diclofenac was released after 17 h in either water or pH 6.8 buffer. However, steady state was reached after 8–10 h with only 8.5, 2.3, and 2% of diclofenac released from formulation A in medium of pH 4.5, 2.5, and 0.1N HCl, respectively (Fig. 1). The similarity factor (f_2) was calculated to compare the dissolution profiles (Table 1). Fig. 2 exhibits the release kinetics of both formulations as a function of time ($\log t$). The kinetic constant (k) and the diffusional exponent (n) of both formulations were shown in Table 2, and significant differences were observed in both parameters ($P < 0.05$).

3.2. Pharmacokinetic study

The plasma concentrations of diclofenac reached steady state after the first dose. The plasma concentrations of diclofenac before dosing were in the range of 34–46 ng/ml (Fig. 3). A multiple-peak behavior in the plasma concentration–time profiles was observed when Voltaren SR® was administered, but not when formulation A was administered (Fig. 3). Table 3 lists

Table 1
Comparison of the dissolution profiles of formulation A and Voltaren SR®

	0.1N HCl	pH 2.5	pH 4.5	pH 6.8	Water
f_2[a]	94.1	96.1	77.5	57.9	59.9

[a] Similarity factor.

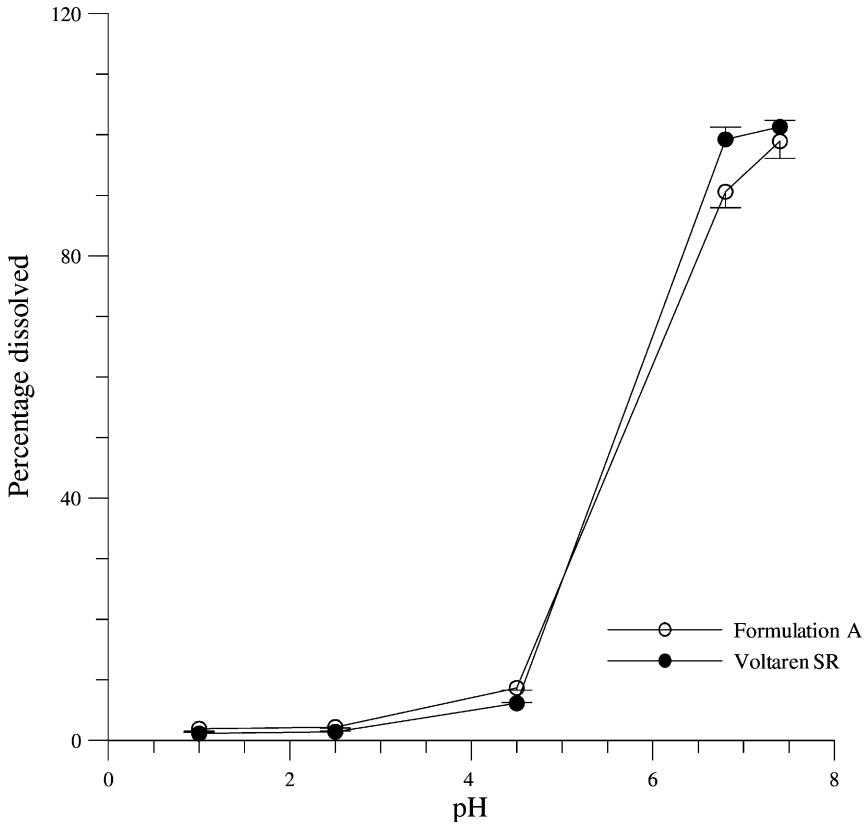

Fig. 1. Effect of pH on the dissolution of diclofenac. (○) Formulation A; (●) Voltaren SR®. Data represent the mean ± S.E. of six experiments.

the pharmacokinetic parameters of diclofenac in both formulations. A significant difference in AUC(0–24) was observed with 90% confidence interval (CI) of the difference (formulation A−Voltaren SR®) of the mean in the range of 0.61–0.81. The relative bioavailability of formulation A to Voltaren SR® is 71%, and the peak concentrations (C_{max}) of these two formulations were also significantly different, while the 90% CI of the difference of the logarithmic mean was in the range of 0.47–0.56. However, no significant difference was observed in the time to peak concentration (T_{max}), and MRT between these two formulations,

Table 2
The release kinetics of formulation A and Voltaren SR® under different conditions

		Formulation A	Voltaren SR®
Water	n[a]	0.75 ± 0.06	0.54 ± 0.01*
	k (h^{-n})[b]	0.23 ± 0.01	0.27 ± 0.01*
pH 6.8	n	0.81 ± 0.02	0.64 ± 0.02*
	k (h^{-n})	0.14 ± 0.01	0.19 ± 0.01*

Data represent the mean ± S.E. of six experiments.
[a] Diffusional exponent.
[b] Kinetic constant.
* $P < 0.05$.

Table 3
Pharmacokinetic parameters of formulation A, and Voltaren SR®

Parameters	Formulation A	Voltaren SR®
AUC(0–24) (μg h/ml)	2.43 ± 0.15	3.43 ± 0.26*
C_{max} (μg/ml)	0.45 ± 0.04	0.96 ± 0.14*
T_{max} (h)	4.33 ± 0.33	4.88 ± 0.53
MRT (h)	8.35 ± 0.29	7.74 ± 0.48
Fluctuation	24.79 ± 6.95	118.4 ± 30.0

Data represent the mean ± S.E. of six experiments.
* $P < 0.05$.

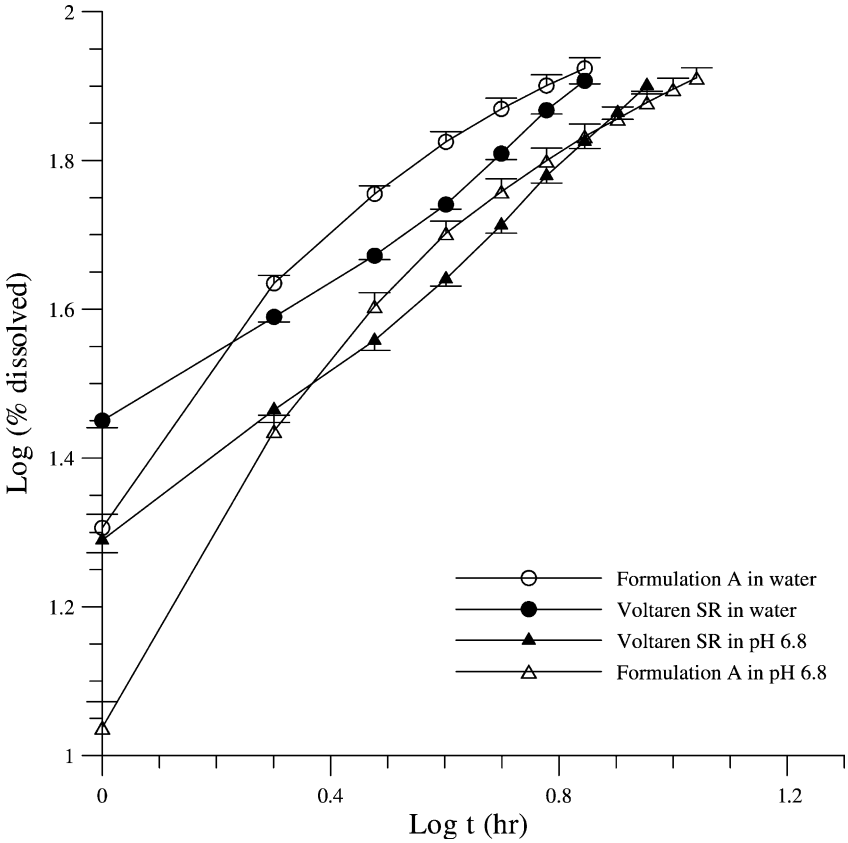

Fig. 2. The in vitro release profiles of formulation A and Voltaren SR®.

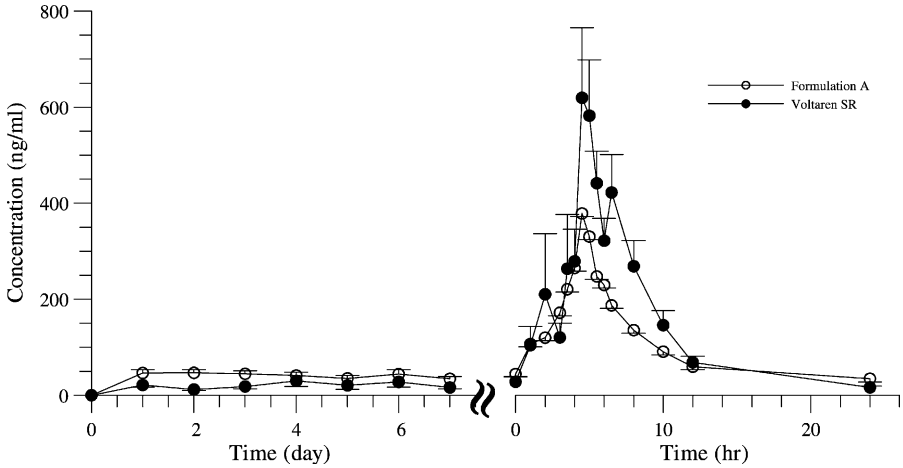

Fig. 3. The plasma concentration–time profiles of diclofenac in 12 healthy subjects administered diclofenac sustained release formulation orally. (○) Formulation A; (●) Voltaren SR®. Each point represents the mean ± S.E.

and approximately fivefold reduction in fluctuation was observed when formulation A was administered.

4. Discussion

The results from in vitro dissolution tests indicate that the dissolution of diclofenac was pH-dependent; less than 10% of drug was released at acidic pH. Diclofenac is a weak acid with $pK_a = 4.0$ and $\log P(n\text{-octanol/water}) = 4.4$. Increased pH could improve the dissolution of diclofenac from both formulations. The dissolution profiles were similar when the f_2 value is between 50 and 100 (Moore and Flanner, 1996). In the current study, the f_2 values were in the range of 77–96 in acidic pH. This suggests that the release of diclofenac from both formulations were similar at lower pH since diclofenac is practically insoluble in acidic solution. However, the dissolution of diclofenac from formulation A was slower in the initial 2 h, followed by a higher release rate in the next 7 h at higher pH (pH 6.8 and water). It is therefore interesting to evaluate the release kinetics of diclofenac from both formulations.

Since limited amount (<10%) of diclofenac was dissolved from both formulations in acidic solutions, the release kinetics from both formulations in water and pH 6.8 buffer were evaluated. In the exponential equation, the kinetic constant (k) incorporates the overall solute diffusion coefficient and geometric characteristics of the system (Korsomeyer et al., 1983). The kinetic constants of diclofenac in water were 0.23 ± 0.01, and 0.27 ± 0.01 from formulation A and Voltaren SR®, respectively. The diffusion exponents of diclofenac from formulation A and Voltaren SR® in water were calculated as 0.75 ± 0.06 and 0.54 ± 0.01, respectively, when the dissolution profiles were fitted into the exponential equation. Significant differences were observed in both parameters. This indicated that the release kinetics of diclofenac from both formulations were different. One explanation is that these two formulations are of different types, while Voltaren SR® is a matrix type sustained release formulation, and formulation A is a multiple unit formulation. Voltaren SR® exhibits a larger kinetic constant but with a slower release rate in comparison to formulation A. Therefore, a larger amount of diclofenac dissolved from formulation A was observed between 2 and 7 h. The release of diclofenac from Voltaren SR® was square root of time dependent, implying that the dissolution is controlled by diffusion, a typical release mechanism in matrix type formulation. On the other hand, formulation A is a multiple unit sustained release formulation. The value of diffusion exponent from formulation A is greater than 0.75, suggesting a time-dependent non-Fickian diffusion. In addition, the increased diffusion exponents with decreased kinetic constants were observed as pH of dissolution medium increased in both formulations.

The concentration of diclofenac reached steady state after the first dose without drug accumulation for both formulations. Significant differences in the pharmacokinetic parameters (AUC, C_{max} after log transformation) between formulation A and Voltaren SR® were observed. The relative bioavailability of formulation A to Voltaren SR® is 71%. However, the time to peak concentration (T_{max}) and MRT were equivalent between these two diclofenac formulations. This suggested that the rate of absorption was similar, while the extent of absorption between these two sustained formulations differed. In addition, a fivefold reduction of fluctuation in plasma concentration was observed in subjects taken formulation A.

A study by Riad et al. (1995) demonstrated that a multiple-peak behavior occurred in fasted condition, whereas a single peak at 5–6 h after dosing under fed state when diclofenac sustained release tablets were administered. In our study, the multiple-peak behavior was observed in 6 of 12 and 2 of 12 subjects when Voltaren SR® and formulation A were administered, respectively, under fed state. An early peak occurred at the initial 1–2 h followed by a second peak at approximately 5 h. Diclofenac exists in unionized form in acidic condition such as gastric fluid. In fed state, the pH in the stomach increased accompanied with an increase in the ionized fraction of diclofenac. Moreover, food intake could also prolong gastric emptying time with an increased gastric residence time of the drug. As a result, little absorption occurred during the early phase, followed by major absorption at 5 h after dosing when lunch was provided and at the time when drug entered the small intestine where pH favors dissolution and absorption of diclofenac. This indicates that absorption of diclofenac in the gastrointestinal tract is rate-limited by dissolution. One of the possibilities for the discrepancy in the peak behavior between

these two studies could be the difference in meal composition, which might affect gastric emptying time and dissolution of diclofenac in the gastrointestinal tract.

In subjects taken Voltaren SR®, the higher diclofenac concentrations during the first 2 h could be due to its higher dissolution rate. The results from in vitro dissolution study showed that approximately 90% of diclofenac was released from formulation A at the steady state after 17 h, while 99% from Voltaren SR® in pH 6.8 buffer. About 80% of drug was released between 8 and 9 h from Voltaren SR®, although it occurred between 8 and 14 h from formulation A. This might reflect in the plasma concentrations, in which higher diclofenac concentrations were observed during the early 12 h when Voltaren SR® was administered.

The current study investigated the impact of sustained release formulation on the pharmacokinetics of diclofenac. The absorption of diclofenac in the gastrointestinal tract is dissolution rate-limited. For dissolution rate-limited drug, a lower diffusion exponent suggests slower release with extensive absorption, resulting in increased absorption in vivo. Therefore, the release kinetics of the formulation plays an important role in the in vivo absorption of diclofenac. Formulation A, a multiple unit sustained release formulation resulted in a reduced AUC and C_{max}. However, this formulation caused less fluctuation in plasma concentration as compared to Voltaren SR®, a matrix type of sustained release formulation. The formulation with a reduced diffusion exponent and increased kinetic constant could result in increased absorption of diclofenac in vivo. Further studies will be conducted to evaluate the degree of influence of these two parameters on the absorption of drugs.

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