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Effect of sodium chloride on the release, absorption and safety of diclofenac sodium delivered by poloxamer gel

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Abstract

Poloxamer solutions prepared with poloxamers and sodium chloride were previously reported to undergo a phase transition to bioadhesive gels at body temperature. For the development of a thermosensitive diclofenac sodium-loaded poloxamer gel, here we investigated the effect of sodium chloride on the release, safety and rectal absorption in rats of diclofenac sodium delivered by the poloxamer gels. P 188 delayed the release rates of diclofenac sodium from poloxamer gels. However, sodium chloride showed no significant effect on the release rates of diclofenac sodium from poloxamer gels. Release mechanism analysis showed the release of diclofenac sodium was proportional to the time. The initial plasma concentrations of diclofenac sodium in the rectal formulation [diclofenac sodium/poloxamer 407 (P 407)/poloxamer 188 (P 188)/sodium chloride (2.5/15/17/0.8%)] were significantly higher compared with those in semi-solid suppository. Furthermore, it gave significantly faster T_{max} of diclofenac sodium than did semi-solid suppository, indicating that the diclofenac sodium from poloxamer gel could be absorbed faster than that from semi-solid one in rats. It did not cause any morphological damage to the rectal tissues. These results suggested that poloxamer gel with sodium chloride could be a more effective and safe rectal delivery system of diclofenac sodium.

Keywords: Poloxamer gel; Diclofenac sodium; Sodium chloride; Release; Pharmacokinetics

1. Introduction

Ideal suppository would be easy to administer with good patient compliance and remain at the administered sites avoiding the first-pass effect in the liver and gastrointestinal tracts. Conventional suppository is a semi-solid dosage form that melts or softens in the rectum. Such a conventional suppository can give a feeling of alien, discomfort and refusal to the patients, possibly lowering patient compliance. Furthermore, a suppository, which may reach the end of the colon, has a loss of drug at colonic level and may also allow the carried drugs to undergo the first-pass effect (Huang et al., 1987). In order to solve the problems of conventional semi-solid suppository, an attempt was recently made to develop a rectal dosage form, thermosensitive poloxamer gel which was easy to administer to the anus, since it was a liquid form at room temperature and turned into a

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gel instantly at physiological temperature, and was also mucoadhesive to the rectal tissues without leakage after the dose (Choi et al., 1998; Miyazaki et al., 1998). Poloxamer [copolymer of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene)] solutions are known to exhibit the phenomenon of reverse thermal gelation; remaining as solutions at low temperatures and gelling upon increasing the temperature (Choi et al., 1998). In the development of thermosensitive poloxamer gel containing diclofenac sodium as a rectal dosage form, sodium chloride was used to control the gel strength and bioadhesive force of poloxamer gel. Yong et al. (2001) reported that the poloxamer gels composed of poloxamers and sodium chloride gelled at the physiological temperature without leakage after administration and remained mucoadhesive to the rectal tissues. However, the previous studies focused on modulating the physicochemical properties of poloxamer gels and there has been lack of information on the release and absorption of drugs from the rectal poloxamer gels. Thus, in this study, the release and pharmacokinetic profiles of diclofenac sodium from the poloxamer gels were investigated. Furthermore, the rectal tissue irritation of the poloxamer gel was evaluated.

2. Materials and methods

2.1. Materials

Diclofenac sodium and poloxamers (P 407, P 188) were supplied from SK chemical (Suwon, South Korea) and BF Goodrich (Breesville, OH, USA), respectively. Sodium chloride was of USP grade. Semipermeable membrane tube (Spectra membrane tubing No. 1) was from Spectrum Medical Industries Inc. (Los Angeles, CA, USA). Acetonitrile and methanol were from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of diclofenac sodium-loaded poloxamer gel

The poloxamer gel was prepared as previously described by Yong et al. (2001). In brief, various components such as sodium chloride and diclofenac sodium were dispersed or dissolved in distilled water at room temperature and the solution was cooled down to 4 °C. Poloxamer 407 and poloxamer 188 were then slowly added to the solution with continuous agitation.

2.3. Release test

Each poloxamer gel (5 g) containing 125 mg of diclofenac sodium was inserted into a semipermeable membrane tube. Both sides of the tube were tied up with a thread to prevent leakage. The semipermeable membrane tube was then placed in a dissolution tester (Shinseang Instrument Co., Korea). Release test was performed at 36.5 °C using the paddle method at 100 rpm with 500 ml phosphate buffer (pH 6.8) as a release medium. At predetermined interval, 5 ml of the medium was sampled and filtered. The filtrate was analyzed by UV-Vis variable wavelength detector (Philips, Model PU8730) at 250 nm (Anderson and Conradi, 1985; Chen-chow and Frank, 1981). The released rates of drug from various poloxamer gels were compared for statistical significance by the one-way analysis of variance (ANOVA). The statistical significance of means among different formulations was then compared by multiple range method of least significant difference.

2.4. Pharmacokinetic study

2.4.1. In vivo experiments

Male Sprague–Dawley rats weighing 250 ± 20 g were fasted for 24–36 h prior to the experiments but allowed free access to water. Twelve rats were divided into two groups. The rats in each group were administered with conventional suppository [diclofenac sodium/PEG 4000 (2.5/97.5%)] and rectal poloxamer gel [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)], respectively.

2.4.2. Administration and blood-collecting

Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube was inserted into the right femoral artery of the rat. The poloxamer gel (1.5 g/kg equivalent to diclofenac sodium 37.5 mg/kg) was administered into the rectum 4 cm above the anus through a stomach sondle needle

fitted on a glass syringe. The conventional suppository was administered with a dose of 1.5 g/kg (equivalent to diclofenac sodium 37.5 mg/kg) into the rectum 4 cm above the anus (Miyazaki et al., 1987). The entrance of the anus was then blocked with a cyanoacrylate adhesive, since the preparations might be leaked out from the anus during the pharmacokinetic experiment, leading to not obtaining accurate pharmacokinetic data. Half milliliter of blood was collected from the right femoral artery at various intervals and centrifuged at 3000 rpm for 10 min using a centrifuge 5415C (Eppendorf, USA) (Choi et al., 1998; Schneeweis and Muller-Goymann, 1997).

2.4.3. Blood sample analysis

Plasma (0.1 ml) was mixed with 0.4 ml of acetonitrile solution containing flufenamic acid (0.5 μg/ml), as an internal standard. It was then centrifuged at 3000 rpm for 10 min to precipitate the proteins. The supernatant layer (0.4 ml) was evaporated under N_2 (g). The residue was reconstituted in 50 μl of ethanol. Then, the resulting solution was analyzed by HPLC (Hitachi, Model L-7100) equipped with an Inertsil ODS-3 C_{18} column (GL science, 0.5 μm, 15 cm × 0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of acetonitrile and phosphate buffer (pH 6.8) (4:6, volume ratio). The eluent was monitored at 280 nm with a flow rate of 1.0 ml/min (Garcia et al., 1998; Idkaidek et al., 1998; Nakanishi et al., 1994).

2.5. Safety of rectal tissues

Male Sprague–Dawley rats weighing 250 ± 20 g were fasted for 24–36 h prior to the experiments but allowed free access to water. The rectal poloxamer gel [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)] was administered at 1.5 g/kg into the rectum 4 cm above the anus through the stomach sondle needle. At 4 h after administration, the rectum was isolated, rinsed with a saline solution, fixed in 10% neutral carbonate-buffered formaldehyde, embedded in paraffin using an embedding center and cut into slices. The slices were stained with hematoxylin–eosin (Miyazaki et al., 1998) and observed under a light microscope (Leitz; Laborlux 12 Pols, Germany).

3. Results and discussion

3.1. Release of diclofenac sodium from poloxamer gel

To test whether P 188 or sodium chloride affects the release rates of diclofenac sodium from the poloxamer gels, we performed the release studies on the formulations composed of constant amount of P 407 (15%) and variable amounts of P 188 (15–20%) and sodium chloride (0–0.8%).

The release of diclofenac sodium was variously affected by P 188 and sodium chloride (Choi et al., 1998). The poloxamer gel containing 15% P 188 had significantly higher release rates than any other poloxamer gels tested. However, they gradually retarded the release rate as the concentration of P 188 increased from 17 to 20 % (Fig. 1A). Moreover, sodium chloride showed no significant effect on the release rates of diclofenac sodium from poloxamer gels, though it delayed them (Fig. 1B).

To understand the release mechanisms of diclofenac sodium, we described the release rate using the following equations

$$\frac{M_t}{M} = kt^n \tag{1}$$

$$\log\left(\frac{M_t}{M}\right) = \log k + n\log(t) \tag{2}$$

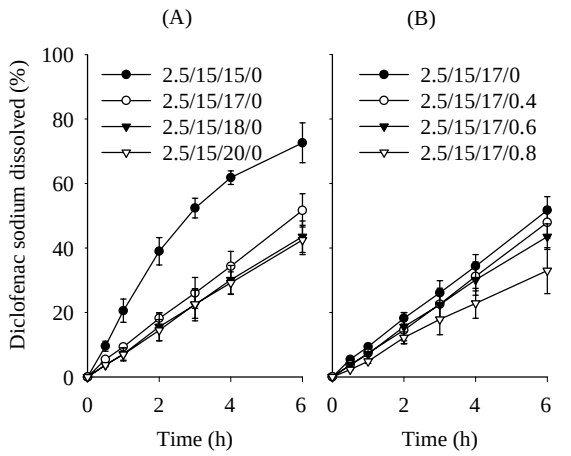

Fig. 1. Effect of poloxamer P 188 (A) and sodium chloride (B) on the release of diclofenac sodium. Poloxamer gels were composed of [diclofenac/P 407/P 188/sodium chloride]. Each value represents the mean ± S.E. ($n = 6$).

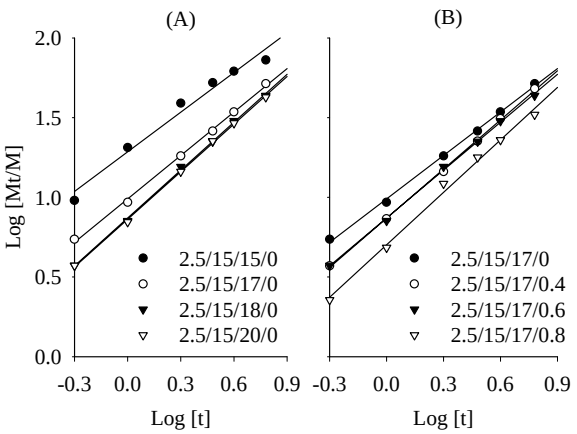

Fig. 2. Release kinetics of diclofenac sodium. Poloxamer gels were composed of [diclofenac/P 407/P 188/sodium chloride]. Logarithm of released fractions of diclofenac sodium was plotted against logarithm of time.

where M_t/M is the fraction of released drug at time t, k is a characteristic constant of the poloxamer gel and n is an indicative of release mechanism. As the k value becomes higher, the release occurs faster. The n value of 1 corresponds to zero-order release kinetics, $0.5 < n < 1$ means a non-Fickian release model and $n = 0.5$ indicates Fickian diffusion (Higuchi model) (Peppas, 1985). From the plot of $\log(M_t/M)$ versus $\log(t)$ (Fig. 2), kinetic parameters, n and k, were calculated. Table 1 shows that most of n values are close to 1, suggesting that the release rate of diclofenac sodium was independent of the time. It was reported that the poloxamer gels composed of acetaminophen, poloxamer and bioadhesive polymers (carbopol, polycarbophil) showed the n values of 0.5, indicating that the drug might be released from the suppositories by Fickian diffusion (Choi et al., 1998). As a possible mechanism by which sodium chloride containing poloxamer gels showed the zero-order release kinetics, it might be considered that, unlike carbopol and polycarbophil, sodium chloride was easily released with the drug from the poloxamer gels due to its very water-soluble property, resulting in no effect of release mechanism. The relatively parallel slopes of the plots (Fig. 2) indicated that the content of sodium chloride and P 188 might not affect the release mechanisms. The k values indicated that diclofenac sodium was more slowly released from poloxamer gels with higher concentration of P 188 and sodium chloride (Table 1). Among the poloxamer gels tested, the formulation [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)] had the smallest k value, indicating that the drug was most slowly released from this suppository. Such a slow release of diclofenac sodium was due to the higher gel strength of the formulation with 17% P 188 and 0.8% sodium chloride than other formulations. The higher gel strength means stronger viscosity at 36.5 °C and more compact structure of poloxamer molecules in formulations (Choi et al., 1998). It was reported that it had higher gel strength than other poloxamer gels (35.66 s versus 6.37–13.8 s) (Yong et al., 2001).

3.2. Pharmacokinetic study

The pharmacokinetic parameters of diclofenac sodium were determined after rectal administration

Table 1
Release kinetic parameters

Diclofenac sodium/P 407/P 188/sodium chloride (%)	Release exponent, n	Kinetic constant, k (%/h^n)	Correlation coefficient, r
2.5/15/15/0	0.931	1.285	0.976
2.5/15/17/0	0.959	0.920	0.981
2.5/15/18/0	1.002	0.870	0.977
2.5/15/20/0	0.994	0.862	0.979
2.5/15/17/0	0.959	0.920	0.981
2.5/15/17/0.4	1.008	0.868	0.989
2.5/15/17/0.6	1.001	0.863	0.989
2.5/15/17/0.8	1.057	0.702	0.984

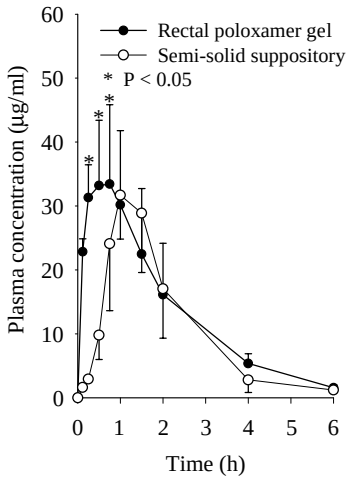

Fig. 3. Plasma concentration–time profiles of diclofenac sodium after rectal administration of rectal poloxamer gel and semi-solid suppository to rats. Rectal poloxamer gel and semi-solid suppository were composed of [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)] and [diclofenac sodium/PEG 4000 (2.5/97.5%)], respectively. Each value represents the mean ± S.D. ($n = 6$). $^{*}P < 0.05$ compared to semi-solid suppository.

of rectal poloxamer gel [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)] and conventional semi-solid suppository [diclofenac sodium/PEG 4000 (2.5/97.5%)]. This poloxamer gel with the suitable gelation temperature (33.1 ± 0.5 °C), gel strength (35.66 ± 1.23 s) and bioadhesive force ($61.8 \pm 7.5 \times 10^2$ kg m^{-1} s^{-2}/cm^2) was easy to administer to the anus and mucoadhesive to the rectal tissues without leakage after the dose (Yong et al., 2001).

Fig. 3 shows the change of mean plasma concentration of diclofenac sodium after rectal administration of diclofenac sodium in rats. The initial plasma concentrations of diclofenac sodium in poloxamer gel were higher compared with those in conventional suppository. In particular, in poloxamer gel, from 7 to 30 min, the plasma concentrations of diclofenac sodium (22–34 μg/ml) were significantly higher than those in conventional suppository (1–10 μg/ml). However, from 1 h after the dose, the plasma concentrations of diclofenac sodium in poloxamer gel, were not significantly different from those in the conventional suppository. Our results indicated that the diclofenac sodium from poloxamer gel could be absorbed faster than that from conventional one in rats. The reason for this fast absorption might be dependent upon the dispensability (fluidity) and bioadhesive force (Yong et al., 2001). Conventional suppository was not bioadhesive, and gradually dissolved and dispersed. In contrast, poloxamer gel was spread easily in the rectum, gelled and attached on the rectal mucous membranes, since bioadhesive poloxamer gel was a fluid initially (Kim et al., 1998).

The pharmacokinetic parameters are shown in Table 2. Poloxamer gel gave significantly faster T_{max} of diclofenac sodium (0.50 ± 0.22 h) than did conventional suppository (0.94 ± 0.13 h) ($P < 0.05$). However, the AUC, C_{max}, K_{el} and $t_{1/2}$ values of diclofenac sodium from poloxamer gel were not significantly different from those from conventional suppository (80.55 ± 25.03 h μg/ml versus 63.78 ± 25.03 h μg/ml; 33.42 ± 9.90 μg/ml versus 31.70 + 7.33 μg/ml; 0.59 ± 0.13 h^{-1} versus 0.73 ± 0.24 h^{-1}; 1.18 ± 0.38 h versus 0.99 ± 0.32 h). Our results suggested that poloxamer gel with sodium chloride would be useful to deliver diclofenac sodium in a pattern that allows fast absorption in the initial phase.

3.3. Safety

The safety test of poloxamer gel [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)] was performed by observing any irritation of poloxamer gel on the rectal tissues. Morphological assessment was performed by observing any irritation of poloxamer gel on the rectal tissues followed by evaluating the observation rate of the three types of gland changes in the rectal epithelium (Reid et al., 1987). Poloxamer gel showed the similar observation rates of normal gland to control (71.4 ± 22.3 versus

Table 2
Pharmacokinetic parameters of diclofenac sodium delivered by semi-solid suppository or rectal poloxamer gel

Parameters	Semi-solid suppository	Rectal poloxamer gel
AUC (h μg/ml)	63.78 ± 20.08	80.55 ± 25.03
MRT (h)	1.76 ± 0.61	1.66 ± 0.53
T_{max} (h)	0.94 ± 0.13	0.50 ± 0.22*
C_{max} (μg/ml)	31.70 ± 7.33	33.42 ± 9.90
K_{el} (h^{-1})	0.73 ± 0.24	0.59 ± 0.13
$t_{1/2}$ (h)	0.95 ± 0.32	1.18 ± 0.38

Each value represents the mean ± S.E. ($n = 6$).
* $P < 0.05$ compared with semi-solid suppository.

73.5 ± 31.6) (Table 3). The morphology of rectal tissues shown in Fig. 4 and Table 3 indicated that poloxamer gel with sodium chloride did not irritate or damage the rectal tissues. Previously, poloxamers, the non-ionic surfactants were reported to be inert, giving no damage to mucous membranes (Dumortier et al., 1991; Watanabe et al., 1993), whereas sodium chloride was shown to be able to irritate mucous membranes (Carstens et al., 1998). No irritation of liquid suppository containing sodium chloride might be explained by that the content (0.8%) of sodium chloride was lower than tissue-damaging threshold level.

Table 3
Observation rate of the three types of changes observed in the rectal epithelium at 4 h after the rectal administration of poloxamer gel

	Classification (%)			
	Normal	Type I	Type II	Type III
Control[a]	71.4 ± 22.3	18.3 ± 8.2	7.2 ± 2.6	3.2 ± 1.3
Poloxamer gel	73.5 ± 31.6	9.7 ± 5.7	11.3 ± 6.9	5.5 ± 3.2

Each value represents the mean ± S.D. ($n = 6$).
[a] Control means fresh rectal epithelium without drug administration.

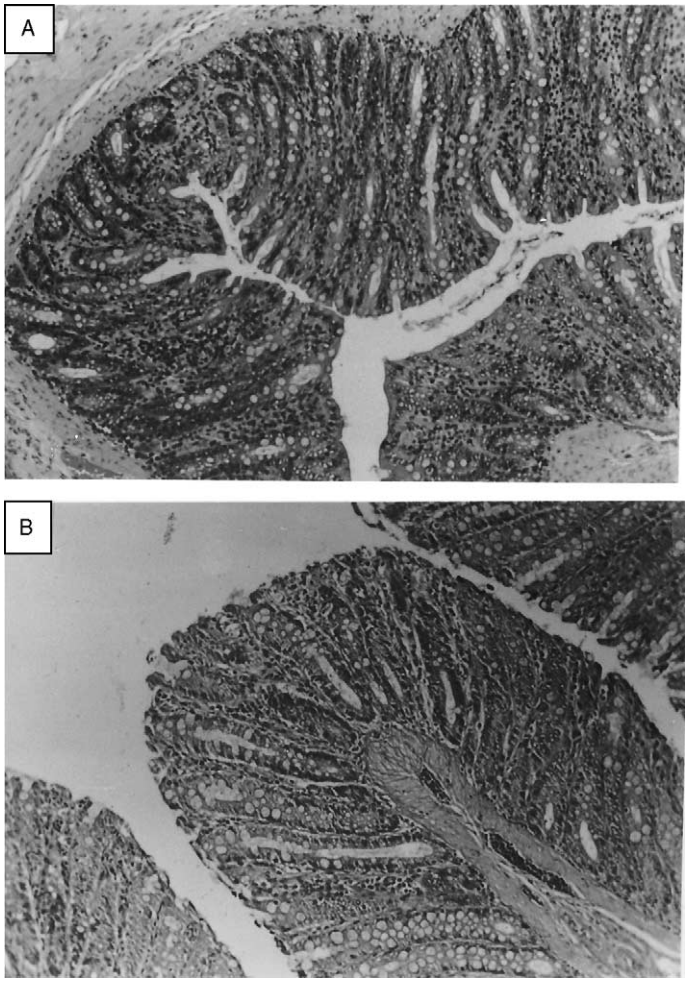

Fig. 4. Morphology of rectal mucosa of rats after rectal administration of diclofenac sodium-loaded poloxamer gel (250×). (A) Before administration and (B) 4 h after administration.

Taken together, it is concluded that the rectal poloxamer gel [diclofenac sodium/P 407/P 188/sodium chloride (2.5/15/17/0.8%)], could provide fast absorption without damaging the rectum. Furthermore, the desirable physicochemical properties such as thermosensitive property, suitable gel strength and bioadhesive force of the liquid type suppository, could alleviate the patients a feeling of alien, discomfort and refusal during application, increasing patient compliance (Yong et al., 2001). Thus, the thermosensitive poloxamer gel with sodium chloride could be a more effective and safe rectal delivery system of diclofenac sodium. The further study on design of poloxamer gel-filled vehicles to be conveniently administered in the human rectum will be performed.

Acknowledgements

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