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In vitro and in vivo evaluation of sustained release suspensions of ibuprofen

Paresh S. Dalal and Milind M. Narurkar

School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209 (U.S.A.)

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Summary

The objective of this study was to develop and evaluate suspensions of ibuprofen-loaded microspheres. The microspheres were prepared with cellulose acetate butyrate (CAB) and cellulose propionate (low molecular weight) (CPL) polymers with 1:2 and 1:3 drug loadings, respectively. To ensure minimum drug release from the microspheres into the suspension, the pH was buffered at 3.5. 0.5% w/v methylcellulose served as the suspending agent. Redispersibility and content uniformity studies carried out during a period of 6 months produced no evidence of a non-dispersible sediment and allowed for a uniform dose withdrawal. The suspensions were able to produce sustained ibuprofen blood levels and decrease gastrointestinal mucosal injury as compared to the free ibuprofen suspension. The suspension of CAB microspheres sustained ibuprofen blood levels for the longest time and produced least gastrointestinal mucosal damage.

Introduction

A significant proportion of the population have difficulty in swallowing solid oral dosage forms (Rhodes, 1990). This problem becomes more acute for the administration of sustained action dosage forms due to the increase in volume of the delivery system. Formulating such systems as a suspension presents a novel means of circumventing the potential problems associated with the administration of such systems (Jones and Matthews, 1970).

Microencapsulation of a drug has been suggested to sustain drug release and to reduce or eliminate gastrointestinal tract irritation (Deasy, 1984). The incorporation of microspheres as a dispersed phase in a suspension has been proposed earlier (Bodmeier and Chen, 1989; Kawashima et al., 1989) since multiparticulate systems may spread out more uniformly in the gastrointestinal tract, thereby causing a reduction in local irritation when compared to a single-unit solid dosage form.

In this study, we report on the characterization of ibuprofen-loaded microspheres and describe

Correspondence: M.M. Narurkar, Division of Pharmaceutics and Medicinal Chemistry, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209, U.S.A.

the preparation and evaluation of suspensions containing these microspheres.

Materials and Methods

Materials

Ibuprofen (lot no. RD01) was kindly provided by Boots Pharmaceuticals, Inc., Shreveport, LA. Cellulose acetate butyrate (butyryl content 17%) (CAB) and cellulose propionate (number average molecular weight < 15 000) (CPL) were supplied by Aldrich Chemical Co., Inc., Milwaukee, WI. Methylcellulose (4000 cps) was obtained from Mallinckrodt, Paris, KY. All reagents used were of analytical or higher grades and were used as supplied.

Instrumentation

Spectroscopic analysis was performed on a Gilford Response spectrophotometer (Gilford Systems, Oberlin, OH). Scanning electron micrographs were obtained with a Philips SEM 505 (Philips, Eindhoven, The Netherlands). Differential scanning thermographs were obtained from a Perkin Elmer DSC-4 thermal data station computer interfaced with a 660 printer (Perkin-Elmer, Norwalk, CT). Dissolution testing of the microspheres was performed using a VanderKamp 600, six-spindle dissolution tester kept at a constant temperature (Vankel Industries, Inc., Chatham, NJ). Examination of mucosal injury was carried out under a Bausch and Lomb binocular magnifier fitted with an illumination probe (Bausch and Lomb, Rochester, NY). HPLC analysis was carried out on a Varian 5000 pump equipped with a Varian UV-50 variable-wavelength detector (Varian Instrument Co., Palo Alto, CA), a Valco C10W injector (Valco Instrument Co., Inc., Houston, TX) and a Water's 740 data module (Millipore Corp., Heber's, MD) using methanol : 0.05% phosphoric acid (69 : 31) as the mobile phase. The effluent from a C-18, 5 μm column (Supelco Chromatography, Bellefonte, PA) was monitored at 228 nm using butyl paraben as the internal standard.

Methods

Preparation and characterization of microspheres

The microspheres were prepared by the emulsion-solvent evaporation process (Kondo, 1979; Chang et al., 1986) with methylene chloride as the organic solvent. The microspheres were washed twice with 30 ml aliquots of distilled water and vacuum dried at ambient temperature before screening through a nest of six sieves (nos 35, 45, 60, 80, 120, and 170) and stored in a desiccator. Accurately weighed samples of the microspheres were lysed in methylene chloride by sonication for 10 min and analyzed spectrophotometrically at 228 nm to determine the efficiency of microencapsulation. Microsphere samples (4–7 mg) were scanned under nitrogen atmosphere at 10°C/min in the range of 20–120°C followed by cooling at 320°C/min to obtain thermograms. The surface topography of the microspheres was examined using scanning electron microscopy. The in vitro release profiles of the drug from the microspheres were examined spectrophotometrically at 226 nm using a USP XXII type II dissolution apparatus at 100 rpm in a dissolution medium consisting of 900 ml of phosphate buffer with 0.05% Tween 80, kept at a constant temperature of 37°C.

Preparation of suspensions

CAB and CPL microspheres (no. 60) with a drug loading of 1 : 2 and 1 : 3, respectively, were selected for incorporation into suspensions as they exhibited a desirable balance of in vitro initial and sustained drug release as shown in Fig. 1. Based on results of preliminary data, 0.5% w/v methylcellulose (4000 cps) was chosen as the suspending agent for the microsphered suspensions as well as free ibuprofen suspension.

Effect of pH on the release of ibuprofen within the suspension

Triplicate samples of suspensions were prepared containing 2% w/v of the drug in 20 ml of citrate buffer (pH 3.4, 3.6, 3.8, and 4.0) and stored in closed vials at 25°C and ambient humidity conditions for 30 days. The release of ibupro-

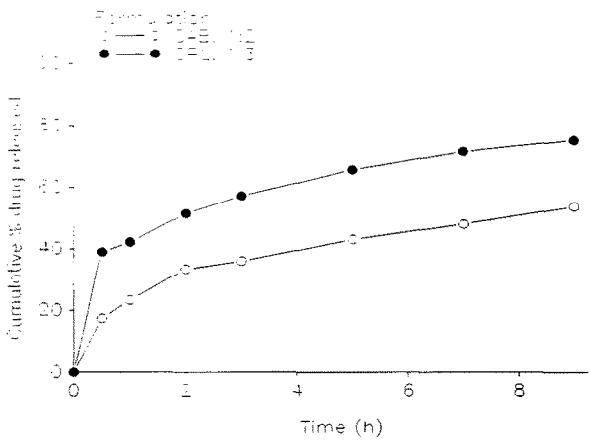

Fig. 1. Release profiles of the microsphere formulations selected for incorporation into a suspension.

fen within these suspensions at each pH was monitored spectrophotometrically at 226 nm.

The suspensions were buffered at pH 3.5 for the remaining studies to ensure minimum drug release within the suspension, while avoiding extreme acidity.

Redispersibility and content uniformity studies

The apparatus consisted of a controlled motor capable of rotating a 100 ml measuring cylinder at 20 rpm (Matthews and Rhodes, 1968; Jones and Matthews, 1970). Triplicate 100 ml samples of 2% w/v suspensions, were filled into 100 ml stoppered measuring cylinders and allowed to sediment for storage periods of 5, 10, 15, 30, 90, and 180 days. At the end of the storage period, the cylinders were placed onto the redispersibility apparatus and the number of revolutions necessary to restore homogeneity was recorded.

Upon restoration of homogeneity, an aliquot of 0.5 ml was withdrawn from the cylinders for content uniformity studies. The aliquot was dried under vacuum and the residual microspheres lysed with methylene chloride prior to spectrophotometric analysis at 228 nm.

In vivo bioavailability studies

Twenty-four male Sprague-Dawley rats (180–205 g) were used. The rats were fasted for a 12 h period prior to the administration of the suspensions. The three formulations subjected to testing were (i) free ibuprofen suspension, (ii) suspension of CAB microspheres, and (iii) suspension of CPL microspheres. A formulation was administered to the rat at a dose of 30 mg/kg, using a gavage needle and washed down with two aliquots of 0.2 ml each of distilled water. Food was withdrawn during the first 4 h of the experiment. Water was available ad libitum throughout the experiment. 150 μl of the blood was withdrawn from the tail vein into a microsyringe prerinsed with heparin solution and stored in heparinized vacutainers (Omaye et al., 1987). Blood sampling was performed at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 15.0, 21.0, and 24.0 h post administration and the samples were frozen until analysis. Six determinations were made at each time interval for each formulation.

The samples were prepared for HPLC analysis using a slight modification of the method proposed by Lalande (Lalande et al., 1986) as follows: 300 μl of methanol spiked with the internal standard, butyl paraben, was added to thawed blood samples to precipitate the proteins. The samples were centrifuged at 4000 rpm for 20 min and the supernatant was separated. A drop of 85% phosphoric acid was added to the supernatant and the mixture was stirred on a vortex mixer. The samples were filtered through a 0.45 μm nylon filter and injected onto the HPLC.

The blood ibuprofen concentration-time curves were plotted for each individual formulation and the area under the curve $(AUC)_{0-\infty}$ was determined by the trapezoidal method.

In vivo ulcerogenicity studies

Twenty-four male Sprague-Dawley rats weighing 180–200 g were fasted overnight. The rats were administered doses of either 46, 100, or 150 mg/kg of ibuprofen or microsphered ibuprofen suspensions, using an oral gavage needle. This was followed by two 0.2 ml washings with distilled water. The control rats were administered an equal volume of dispersion medium and followed by two 0.2 ml aliquots of distilled water. The doses were repeated daily for four days. At 24 h following the last dose, the rats were euthanized in a carbon dioxide chamber. 0.5% solution of Evans blue was injected (10 ml/kg) into the tail

vein of the rats 30 min prior to killing (Cioli et al., 1980). Six determinations were made at each dose, for each formulation. The stomach was dissected out of the body along with the first 5 cm of the intestine. The stomach was filled with saline and the contents of the stomach were emptied. The stomach and the intestine was excised open along the greater curvature and gently wiped clean with a swab dipped in saline. The mucosal damage was examined under a binocular magnifier. The severity of mucosal damage was assessed by a scheme which is a slight modification of one reported earlier (Cioli et al., 1979):

Observation	Score
no lesions	0.0
punctiform lesions (lesions less than 1 mm)	0.5
five or more punctiform lesions	1.0
one to five small ulcers (1–2 mm)	2.0
more than five small ulcers or one large ulcer (greater than 2 mm)	3.0
more than one large ulcer	4.0

Based on the severity of the mucosal damage, the specimen was assigned an ordinal score as per the scoring scheme. For example, a specimen with five punctiform lesions and two small ulcers was assigned a score of 2.0. All specimens including the controls showed the presence of red spots and these were not accounted for in the observation scheme. However, the control specimens did not exhibit the formation of lesions or ulcers and accordingly the controls had a score of 0. The scores were averaged and the mean score was plotted as the severity index for the dose administered.

Results and Discussion

Characterization of microspheres

A higher rate and extent of drug release but poor microencapsulation efficiency was found to be associated with higher drug loadings. SEM micrographs showed the topography of the microspheres to assume a smoother surface, with a decrease in drug loading, indicating the combined effect of excessive drug precipitating within the microspheres and some adhesion of free ibuprofen crystals to the microsphere surface, at higher drug loadings. Surface adhesion of ibuprofen was found to be higher for CPL microspheres as compared to CAB microspheres, at equal drug loadings. DSC thermograms indicated that ibuprofen was probably present as discrete crystals within the polymer matrix and the drug had no apparent solubility in the polymer (Bodmeier and McGinity, 1987). The release of drug from the microspheres was best described by the equations proposed by Higuchi and Baker-Lonsdale for the release of the drug from a spherical matrix.

Effect of pH on the drug release within the suspension

The suspension of CAB microspheres had less than 5% release after a 30 day period with the release being pH independent. However, CPL microspheres showed a comparatively higher extent ($\approx 8\%$) of drug release from the microspheres, which was observed to be pH dependent. This may be due to the higher permeability of the cellulose propionate polymer matrix which allowed for drug solubilization by the permeating vehicle, thereby producing a pH-dependent drug release. In the case of CAB microspheres, the polymer successfully withheld most of the drug within its matrix and allowed a very small amount

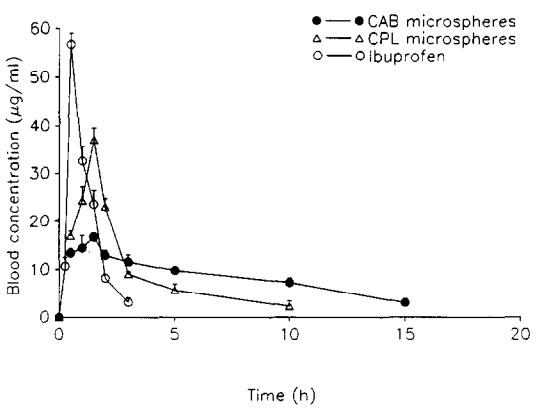

Fig. 2. Blood level profiles of ibuprofen after oral administration of free ibuprofen suspension, suspension of CAB 1:2 (no. 60) microspheres, and suspension of CPL 1:3 (no. 60), microspheres to Sprague-Dawley rats (dose = 30 mg/kg, $n = 6$).

TABLE 1

Redispersibility and content uniformity measurements for suspensions prepared using CAB 1:2, no. 60, and CPL 1:3, no. 60, microspheres

Storage period (days)	Redispersibility values (rpm) [a]		Content uniformity (%) [a]	
	CAB, 1:2	CPL, 1:3	CAB, 1:2	CPL, 1:3
0	9.67 ± 0.58	6.67 ± 0.58	–	–
5	6.33 ± 1.12	3.00 ± 0.00	86.93 ± 7.08	91.46 ± 8.84
10	5.67 ± 0.58	2.56 ± 1.76	96.55 ± 1.19	98.77 ± 1.54
15	5.67 ± 0.58	2.56 ± 1.76	92.78 ± 10.5	105.46 ± 0.96
30	5.67 ± 0.58	2.56 ± 1.76	98.72 ± 2.41	102.21 ± 4.17
90	5.33 ± 0.58	3.33 ± 0.58	93.67 ± 4.63	96.34 ± 3.67
180	5.67 ± 0.58	3.00 ± 0.00	97.36 ± 2.97	99.40 ± 5.43

[a] $n = 3$, mean ± S.D.

of vehicle to diffuse in and solubilize the drug. Thus, the effect of pH was insignificant. As stated earlier, increased surface adhesion of ibuprofen in the case of CPL microspheres was evident in the micrographs, which may also have been a contributing factor.

Redispersibility and content uniformity studies

The suspensions were readily redispersible and provided for a uniform dose withdrawal as shown in Table 1, suggesting that storage did not lead to the formation of aggregates.

In vivo bioavailability studies

The blood level profiles of ibuprofen after oral administration of the three formulations are shown in Fig. 2. It is evident that the microsphered suspensions were able to produce sustained ibuprofen blood levels as compared to the free ibuprofen suspension with the CAB microspheres sustaining levels for a much longer period than the CPL microspheres. The calculated $(AUC)_{0-\infty}$ values consistently increased with the sustaining effect of the formulation. This may be due to a more complete absorption of the drug resulting from a slow release from the microspheres and prolongation of drug residence time in the gastrointestinal tract.

In vivo ulcerogenicity studies

Large ulcers in the gastric mucosa were observed following the administration of free

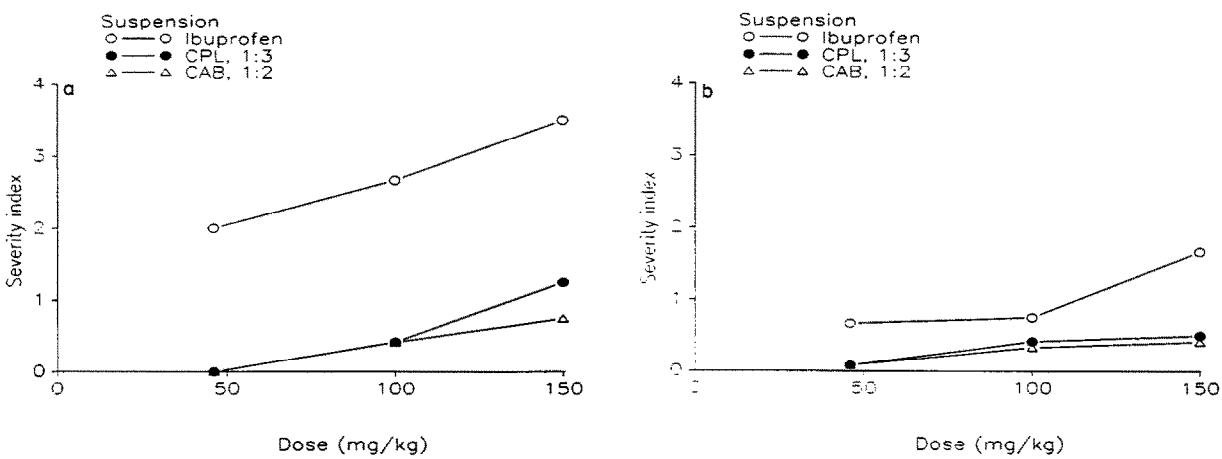

Fig. 3. Dose-severity index relationship in (a) stomach (b) duodenum, following chronic oral administration of suspensions to Sprague-Dawley rats ($n = 6$).

ibuprofen suspension at 100 and 150 mg/kg, but were notably absent at all doses of the microsphered suspensions. The overall severity of mucosal injury was most prominent for the ibuprofen suspension as compared to the microsphered suspensions, at all doses. The repression of the mucosal damage from the microsphered suspensions is linked to the entrapment of drug within the polymer matrix and the subsequent gradual drug release. Fig. 3a and b depicts the dependency of gastric and duodenal mucosal injury on the dose for all three suspensions. The small rise in the gastric severity index for the CPL suspension with an increase in the dose may be due to the presence of ibuprofen crystals adhering to the surface of the microspheres, which may have provided additional free drug crystals in the gastric environment, thereby contributing to the dose effect. The dose dependency is relatively smaller for CAB microspheres, since these showed a much lesser degree of free ibuprofen adhering to the surface, based on SEM micrographs. The damage to the mucosa was quite poignant in the gastric region as compared to the duodenal region. This may be related to the greater solubility of the drug at the duodenal pH which in turn would lessen the local contact of the drug crystals with the mucosal surface. It is also noted that the duodenal mucosal injury severity index increased sharply following the administration of 150 mg/kg of the free ibuprofen suspension. This effect may be due to the dose exceeding the solubility of the drug. It is postulated that at a dose of 150 mg/kg ibuprofen was predominantly present as insoluble crystals in the duodenal environment which augmented the mucosal injury.

Conclusions

Stable suspensions of ibuprofen-loaded microspheres could be formulated at pH 3.5 with 0.5% w/v methylcellulose (4000 cps) as the suspending agent. Prolonged storage did not adversely affect the integrity of the suspension and uniform dose withdrawal was possible upon storage up to 6 months. The suspensions prepared with CAB microspheres sustained ibuprofen blood levels for the longest period and produced minimum mucosal damage, amongst the formulations evaluated.

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