J. Pharm. Pharmacol. 1993, 45: 947–950
Received February 2, 1993

Use of Enzymatic Activity for Design of Orally Administered Enteric Dosing Forms

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Abstract—Liquid and semi-solid enteric dosage forms were prepared by entrapping drug with an appropriate partition coefficient in a lipid base vehicle which would then be released by the action of intestinal enzymes. Lipid ester derivatives such as glyceryl monocaprylate and polysorbate 80 were used as vehicles. These vehicles readily dissolved the poorly water-soluble compounds used in the study, itazigrel, indomethacin and the dye, sudan II, and were digested by lipase and esterase, releasing the test drugs with time profiles similar to those observed in dissolution studies. The vehicles released little or only a small amount of the drugs into aqueous medium in the absence of an appropriate enzyme. The enzyme-sensitive enteric vehicles when containing sudan II did not release the dye in the stomach of rats after oral administration, but released significant amounts of the dye in the small intestine.

The use of enzymes in drug design studies is well documented (Sinkula 1975; Kahns & Bundgaard 1991; Sinko 1992). However, enzymes may also be useful tools for optimizing dosing form design. To achieve the objective of devising formulations which dissolve and release drugs in the intestine (enteric formulations), it is desirable to have dosing forms which are sensitive to various intestinal enzymatic factors other than pH. As we previously reported (Nishihata et al 1986; Yoshitomi et al 1987), digestion of triglyceride by lipase occurs rapidly in the intestinal tract. Thus, the use of a lipase-sensitive oral dosage form would appear to be a good strategy for an enteric dosage form. In this study, we considered two key factors: the target enzyme, and the formulation type. We selected lipase and esterase as the target intestinal enzymes. The enzyme-sensitive materials used as diluents in the formulations included glyceride and other ester compounds that are degradable by lipase and esterase.

Partition theory (Park & Ripple 1977) was used to design a vehicle that would retain drugs until resident in the small intestine. Simply stated, the theory implies that a drug dissolved in an enzyme-sensitive vehicle, remains in that vehicle until the vehicle is degraded by the appropriate enzyme. When such a dosage form encounters an appropriate enzyme, the vehicle will be degraded and the drug rapidly released. This type of formulation is very effective for drugs which are poorly water-soluble. Dissolution of the drug in intestinal fluid would follow rapidly when the vehicle was degraded by an enzyme.

In the present study, we attempted to select an appropriate enteric formulation vehicle by investigating the solubilities of poorly water-soluble drugs in various vehicles, the partition coefficient of the drugs between the aqueous phase and vehicle, and the biodegradation of the vehicles by lipase and esterase.

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Materials and Methods

Materials

Glyceryl monocaprylate (GMC), glyceryl monostearate (GMS), propyleneglycol dicaprylate (PDC), and polysorbate 80 (Tween 80) were obtained from Nikko Chemicals (Tokyo, Japan) and were used as ester compounds. Indomethacin (Wako Pure Chemicals, Osaka, Japan), sudan II (Wako Pure Chemicals) and itazigrel (The Upjohn Company, Michigan, USA) were used as models of poorly water-soluble compounds. Lipase was obtained from porcine pancreas (EC 3.1.1.3., Type II, Sigma, St Louis, MO, USA) and contained 220 units of lipase (mg protein) $^{-1}$. Esterase was from porcine liver (EC 3.1.1.1., Sigma) and was suspended in 3·2 M $(NH_4)_2SO_4$ (11 mg protein mL^{-1}), with 230 units of esterase activity (mg protein)$^{-1}$. Other reagents used were of analytical grade.

Solubility of test materials in liquid vehicles

The solubility of the drugs and dye was determined in each vehicle at 25 ± 2°C. Approximately 500 mg of each drug was added to 1 g of each liquid vehicle and was shaken at 25 ± 2°C for 48 h. After centrifugation at 3000 rev min^{-1} for 10 min, the supernatant was collected. The concentration of the drug in the vehicle after dilution with ethanol was determined by HPLC as described below. The concentration of sudan II in vehicle after dilution with ethanol was determined by spectrophotometry (λ 540 nm). The assay limitation for sudan II was 10 $\mu g\ mL^{-1}$ in either water or ethanol.

Partition study

The partition coefficient of the drugs or dye was determined at 37°C. One gram of oleaginous vehicle (GMC or PDC) containing 2·5 or 1·25 mg drug was placed in an aqueous phosphate buffer, pH 6.8 or 0·01 M HCl pH 1·2, and shaken at 100 rev min^{-1} for 6 h. After centrifugation at 3000 rev min^{-1} for 10 min, the aqueous and oleaginous phases were collected and the concentration (% w/w) of drug or dye was determined by HPLC or spectrophotometry, respectively.

The partition coefficient (P) was determined using the following equation:

$$P = [Drug]_o/[Drug]_w \quad (1)$$

where $[Drug]_w$ was the drug concentration in the aqueous phase and $[Drug]_o$ was the drug concentration in the oleaginous phase. The partition coefficient (P) of the drug to Tween 80 was determined by measuring the increased solubility of drug in the presence of Tween 80 in micellar conditions, using the following equation:

$$P = [S_o - S_w/W_o]/(S_w/W_w) \quad (2)$$

where S_w and S_o represent the solubility in the buffer and in the buffer containing 1% Tween 80, respectively. W_w and W_o represent the composition ratio of buffer and Tween 80 in the medium.

Degradation of lipid vehicles

The medium for the degradation study consisted of lipase (4 μg mL^{-1}) or esterase (10 μg mL^{-1}) and sodium taurocholic acid (24 mM) in 0·05 M phosphate buffer (pH 6·8). Five hundred milligrams of the oleaginous vehicle was immersed in 100 mL of the medium with shaking (50 rev min^{-1}) at 37°C. Subsequently, 500 μL of the medium was collected at 0, 15, 30 and 60 min to determine the concentration of free fatty acid, which was used as an indicator for the degradation of the oleaginous ester vehicle. Free fatty acid was assayed using a NEFAC-Test Wako assay kit (Wako Pure Chemicals). The percent degradation of the lipid was calculated as:

$$\frac{\text{(amount of free fatty acid in medium) (vehicle wt)}}{\text{(calculated fatty acid content)/(mol. wt)}} \times 100$$

In-vitro release of test materials from vehicle

The formulations used in this study are described in Table 1. They were prepared by dissolving drug or dye in vehicle at room temperature (21°C), except for GMC/GMS-Ind which was prepared by dissolving indomethacin in the vehicle at 60°C and then was solidified at room temperature. The in-vitro release of drug from vehicle was determined using the procedure previously described. Two hundred and fifty milligrams of the oleaginous formulations (2·5 g of the formulation for GMC-Sud and Tween 80-Sud) were gently placed in the medium at 37°C without shaking and 1 mL of the aqueous phase was collected at 15, 30, and 60 min to determine the concentration of drug or dye in the medium.

In-vivo release study

The in-vivo release of sudan II from the oleaginous formulated vehicles (Table 1) was determined by naked-eye observation of the staining of the gastrointestinal mucosa of rats after oral administration. At 1·5 h after oral administration of 500 mg of each formulation (containing 12·5 mg sudan II), male Sprague-Dawley rats, 200–250 g, were anaesthetized with pentobarbitone (30 μg mL^{-1}, i.p.), the stomach and all the intestines were removed and the mucosal side of the gatrointestinal tract was rinsed gently with 50 mL saline. The degree of staining of the mucosa of the stomach and small intestine was determined by naked-eye observation.

Table 1. Formulation codes and compositions of oleaginous vehicles used in this study.

Formulation code	Drug (g)	Vehicle (g)
GMC-Ita	Itazigrel, 2·5	GMC, 247·5
GMC-Ind	Indomethacin, 2.5	GMC, 247·5
GMC-Sud	Sudan II, 2·5	GMC, 97·5
Tween 80-Ita	Itazigrel, 2·5	Tween 80, 247·5
Tween 80-Ind	Indomethacin, 2·5	Tween 80, 247·5
Tween 80-Sud	Sudan II, 2·5	Tween 80, 97·5
GMC/GMS-Ind	Indomethacin, 2·5	GMC/GMS (1:1), 247·5
Suspension-Sud	Sudan II, 2·5	0·5% sodium CMC, 97·5
Beeswax-Sud	Sudan II, 2·5	Beeswax, 97·5

HPLC of indomethacin and itazigrel

Indomethacin was assayed by HPLC (Yaginuma et al 1981). The assay limit was 50 ng mL^{-1}. Itazigrel was also assayed by HPLC with the following assay conditions: a liquid chromatograph (Model LC-6A, Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF-535, Shimadzu) was used. The separation column was 4·6 mm i.d. by 15 cm long and contained a reverse-phase (LiChro-CART 125-4, RP 8-e endcapped, Merck, USA). The mobile phase was a mixture of 85 mL methanol and 15 mL distilled water. The flow rate was 0·8 mL min^{-1}. Itazigrel was detected by fluorescence (excitation 320 nm, emission 430 nm). The assay limit was 10 ng mL^{-1}.

Results and Discussion

Although the various drugs and dye tested had very poor aqueous solubility, their solubilities were much higher in GMC, PDC, and Tween 80 at 25°C (Table 2) and log P values were obtained for the three liquid oleaginous vehicles for itazigrel, indomethacin and sudan II (Table 3). These results indicated that the three test compounds were incorporated into the lipid vehicles even when the vehicles were suspended in an aqueous medium. The relatively small log P value for indomethacin at pH 6·8 compared with that at pH 1·2 was the result of ionization of indomethacin in the aqueous phase at pH 6·8.

The in-vitro degradation of lipid vehicles is shown in Fig. 1. Lipase degraded GMC rapidly and completely within 30 min, whereas esterase degraded GMC much more slowly. Lipase degraded PDC faster than esterase. In comparison, esterase degraded PDC faster than GMC and Tween 80 was degraded faster by esterase than by lipase.

Because GMS did not melt even at 50°C, it was only slightly degraded in the presence of either lipase (degradation

Table 2. Solubility of itazigrel and indomethacin in aqueous and oleaginous vehicles at 25±2°C.

Solvent	Itazigrel	Indomethacin	Sudan II
Aqueous solubility (mg g^{-1}):			
pH 1·2	0·00008±0·00001	0·0009±0·00005	<0·001
pH 6·8	0·00007±0·000003	0·5±0·02	<0·001
Oleaginous vehicle (mg kg^{-1}):			
GMC (mol. wt 246·2; fatty acid content 155·1)	69·2±5·8	17·8±1·3	52·5±6·9
PDC (mol. wt 364·4; fatty acid content 288·3)	154·8±9·6	4·9±0·5	—
Tween 80 (mol. wt 1459·2; fatty acid content 346·3)	177·0±11·2	67·2±3·8	—

Each value represents the mean±s.d. (n=3)

Table 3. Log P values of itazigrel, indomethacin and sudan II in an oleaginous and aqueous phase at 37°C.

Oleaginous phase	pH of aqueous phase	Itazigrel	Indomethacin	Sudan II
GMC	1·2	5·87 ± 0·21	5·62 ± 0·13	> 7
	6·8	5·92 ± 0·16	3·67 ± 0·092	> 7
PDC	1·2	> 7	> 7	—
Tween 80	1·2	5·62 ± 0·19	4·42 ± 0·13	—
	6·8	5·48 ± 0·10	3·23 ± 0·14	—

Each value represents the mean ± s.d. (n = 4).

of 8·1 ± 1·4% at 30 min) or esterase (2·4 ± 1·1% at 30 min). Although the mixture of GMS and GMC (ratio of 1 : 1) was solid at 37°C, the mixture was significantly degraded by lipase but not by esterase (Fig. 1D). None of the lipids tested produced more than 1·5% fatty acid in the medium in the absence of lipase or esterase. Among the vehicles tested, GMC and PDC had high sensitivity to lipase, and Tween 80 had higher sensitivity to esterase.

In the studies of release of drug or dye from the vehicles, 250 mg of the formulation vehicles for itazigrel and indomethacin and 2·5 g of the formulation vehicle for sudan II (Table 1) were placed in 100 mL aqueous medium to mimic conditions in the gastrointestinal tract. As anticipated from the partition coefficients, liquid vehicles such as GMC and Tween 80 did not release itazigrel and sudan II into the aqueous medium without use of an enzyme. When lipase or esterase was present in the medium at pH 6·8, itazigrel and sudan II were rapidly released from both vehicles (Table 4), and an apparent super-saturation of itazigrel in the aqueous medium (about 25 μg mL^{-1}) was observed. Because the release profile of drug or dye seemed to be almost identical to the enzyme-mediated vehicle degradation profile (Fig. 1, Table 4), it was concluded that drug release occurred as the result of vehicle degradation.

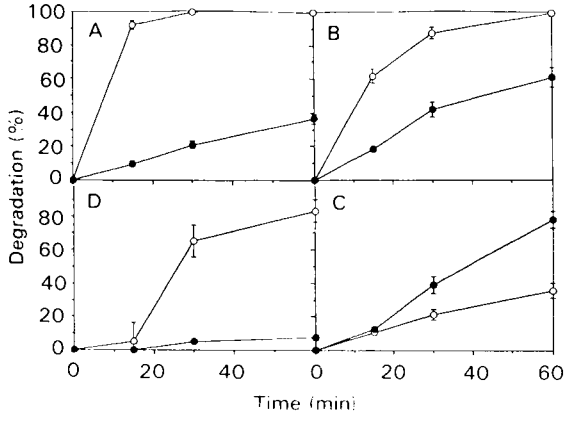

FIG. 1. Degradation of lipids in an aqueous medium in the presence of either lipase (○) or esterase (●). A, glyceryl monocaprylate (GMC); B, propyleneglycol dicaprylate (PDC); C, polysorbate 80 (Tween 80); and D, mixture of 50% GMC and 50% glyceryl monostearate (GMS) which is solid (semi-solid) at 37°C. The degradation was not observed in the absence of enzyme. The amount of free fatty acid was less than 1·5% in each lipid preparation, and the amount did not change during the experimental period. Each value represents the mean ± s.d. (n = 3).

A small amount of indomethacin was released into the aqueous medium from either GMC or Tween 80 at neutral pH (pH 6·8) without the presence of enzymes; whereas no drug was released into the acidic buffer. These results indicate that the release of indomethacin from vehicles is dependent on the partition coefficient. Indomethacin was rapidly released into the aqueous medium (pH 6·8) containing either lipase or esterase. To prevent the release of indomethacin at neutral pH without enzyme, the solidified formulation (GMC/GMS-Ind in Table 1) was examined. Without enzyme at neutral pH, smaller amounts of indomethacin were released into the medium from the solidified vehicles than from GMC alone. When lipase or esterase was added to the neutral medium, a much greater release of indomethacin from the solidified vehicle was observed (Table 4).

Using the information obtained in these in-vitro studies, an enzyme-sensitive enteric dosage formulation was designed to achieve optimal dissolution of drug in the intestine with little release of the drug in gastric juice (even at elevated pH). A dosage form for a poorly water-soluble drug that has a significantly high partition coefficient in oleaginous vehicles, such as itazigrel, could be easily accomplished by dissolving the drug in a liquid oleaginous vehicle. As illustrated in Table 4, itazigrel, which has a log P value greater than 5 in GMC or Tween 80, was not released from the formulated vehicle into the aqueous medium. However, itazigrel was released rapidly when lipase or esterase was present.

A small release of drug from the oleaginous vehicles may not be a significant problem in designing an enteric dosage form for indomethacin. However, to achieve an optimal enteric formulation, solidified vehicles which would decrease the degradation rate and prevent release of drug in the stomach were prepared by mixing the two vehicles. In designing such dosage forms, it was important to balance lipid degradation by enzymes and drug release without enzymes.

The in-vitro degradation studies and the in-vitro drug release studies with the Tween 80 formulations were performed without agitation of the medium to avoid the formation of a micellar system in the medium. Because no significant release of drug from the Tween 80 vehicle was observed in the absence of enzyme and a correlation between drug release and degradation of Tween 80 was observed in the presence of enzyme, the goal of avoiding the formation of a micellar system seems to have been achieved. If a micellar system had formed, the apparent release of drug should have been observed even in the absence of enzyme without

Table 4. In-vitro release of compounds from the formulated vehicles at 37°C.

	Percent of compound released (mean ± s.d.)					
	With enzyme (lipase or esterase) (min)			Without enzyme (min)		
Formulation code	15	30	60	15	30	60
Medium pH of 1·2						
GMC-Ita	—	—	—	< 1	< 1	< 1
GMC-Ind	—	—	—	< 1	< 1	< 1
GMC-Sud	—	—	—	< 1·6	< 1·6	< 1·6
GMC/GMS-Ind	—	—	—	< 1	< 1	< 1
Tween 80-Ita	—	—	—	< 1	< 1	< 1
Tween 80-Ind	—	—	—	< 1	< 1	< 1
Tween 80-Sud	—	—	—	< 1·6	< 1·6	< 1·6
Medium pH of 6·8						
With lipase						
GMC-Ita	78·6 ± 16·2	98·2 ± 2·1	101·4 ± 1·4	< 1	< 1	< 1
GMC-Ind	89·4 ± 7·2	99·8 ± 1·6	100·6 ± 1·2	8·1 ± 1·0	8·0 ± 0·9	8·1 ± 1·3
GMC-Sud	86·2 ± 4·1	94·9 ± 1·3	100·6 ± 1·5	< 1·6	< 1·6	< 1·6
GMC/GMS-Ind	28·9 ± 8·1	64·2 ± 9·0	89·6 ± 5·1	< 1	1·2 ± 0·2	2·0 ± 0·5
With esterase						
Tween 80-Ita	15·2 ± 2·8	28·5 ± 4·1	74·2 ± 6·6	< 1	< 1	< 1
Tween 80-Ind	19·9 ± 8·1	34·2 ± 9·0	86·6 ± 4·8	2·1 ± 0·2	3·5 ± 0·2	5·2 ± 4·1
Tween 80-Sud	11·8 ± 4·2	24·6 ± 3·6	71·2 ± 5·0	< 1·6	< 1·6	< 1·6

Each value represents the mean ± s.d. (n = 3).

degradation of Tween 80 because there was no separation of drug in aqueous medium from drug incorporated in the micelles.

On the other hand, after oral administration of the Tween 80 vehicle, a micellar system should be formed in the gastrointestinal fluid by agitation when the concentration of Tween 80 is more than the critical micellar concentration (CMC) of Tween 80 (0·06%). In an unpublished study in rats, a 1% aqueous micellar vehicle of Tween 80 containing itazigrel increased the rectal absorption of itazigrel significantly when coadministered with esterase compared with that without esterase; it was determined that the intrinsic esterase activity in the rat rectal compartment was negligible despite the high activity in the rat small intestine. Thus, it was possible to achieve an enzyme-sensitive enteric dosage form using a Tween 80 vehicle when a micellar system of Tween 80 is maintained in the small intestinal fluid after oral administration of Tween 80 vehicle.

The in-vivo evaluation of the release of sudan II from vehicle was conducted after oral administration of the formulation vehicle containing sudan II (Table 1) in rats. The oral administration of an aqueous suspension of sudan II (Suspension-Sud) resulted in a moderate staining of the mucosa of both the stomach and the small intestine (Table 5).

Table 5. Effect of vehicle on staining of rat gastrointestinal mucosa by sudan II (naked-eye observation) and on the disappearance of sudan II from gastrointestinal tract after oral administration.

	Stained status of lumen	
Formulation code	Gastric	Small intestine
Suspension-Sud	+ + (+ + +)	+ + (+ + +)
Beeswax-Sud	−	−
GMC-Sud	−	+ + +
Tween 80-Sud	−(+)	+ + +

The coding is as follows: + + + intensely stained, + + moderately stained, + faintly stained, − unstained. The parentheses indicate that the tissue from one of the five rats showed the indicated difference in staining. In all other cases, the tissue from the five rats was stained in the same manner.

No stain was observed after oral administration of the beeswax formulation (Beeswax-Sud) which did not melt at body temperature. When either the GMC-Sud or Tween 80-Sud formulation was administered orally to rats, no stain was observed in the gastric mucosa, but an intense stain was observed in the small intestine. These results indicate that the GMC vehicle and Tween 80 vehicles are stable in the gastric juice (no release of sudan II) and only release sudan II by enzymatic degradation of the vehicle in the small intestine. The in-vivo study as well as the in-vitro study indicated that formulations of GMC-Sud and Tween 80-Sud function as specific enteric formulations.

Acknowledgements

The authors thank Dr John E. Lund, Upjohn Labs, The Upjohn Company (Michigan, USA) for useful comments and technical assistance on the manuscript.

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