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RESEARCH PAPER

Relative Hypoglycemia of Rectal Insulin Suppositories Containing Deoxycholic Acid, Sodium Taurocholate, Polycarbophil, and Their Combinations in Diabetic Rabbits

E. A. Hosny*

Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

ABSTRACT

In this study, insulin suppositories containing 50 U insulin incorporated with 50 mg of deoxycholic acid, sodium taurocholate, or both were placed in the rectum of alloxan-induced hyperglycemic rabbits. A large decrease in plasma glucose concentrations was observed, and the relative hypoglycemias were calculated to be 38.0%, 34.9%, and 44.4%, respectively, compared with insulin subcutaneous (s.c.) injection (40 U). Insulin suppositories containing 50 mg polycarbophil alone or mixed with 50 mg deoxycholic acid produced relative hypoglycemia of 43.1% and 42.2%, respectively. The most pronounced effect was observed with the addition of polycarbophil to the suppository formulation containing a combination of deoxycholic acid and sodium taurocholate, which produced a 56% relative hypoglycemia compared with subcutaneous injection. These suppository formulations could be very promising alternatives to the current insulin injections, being roughly half as efficacious as subcutaneous injection.

* To whom correspondence should be addressed. P.O. Box 2457, Riyadh 11451, Saudi Arabia. Telephone: (966)-1-4677504. Fax: (966)-1-467638. E-mail: Ehosny@ksu.edu.sa

INTRODUCTION

Peptides and protein drugs like insulin usually have a large molecular size, show poor permeability across the intestinal epithelium, are chemically unstable, and degrade rapidly in the gastrointestinal tract (GIT). These unique properties have delayed the development of appropriate nonparenteral dosage forms for peptides and proteins as needle-free alternatives. The nonparenteral routes (such as nasal, buccal, rectal, ocular, vaginal, and even oral) have shown promise (1–3). In coordination with enzyme inhibitors and/or absorption enhancers, these drugs can be delivered into the blood circulation, and sufficient bioavailability can be obtained.

The rectal route for delivery of peptide and protein molecules offers several advantages over some of the other common routes (4,5). Rectal absorption is independent of gastrointestinal transit time, gastric emptying rate, and presence of food. It is likely that the presence of degrading enzymes in the gut wall decreases from the jejunum to the colon and the rectum. One of the suggested advantages of rectal administration is the possibility of avoiding, to some extent, the hepatic first-pass metabolism (6). In the case of insulin, rectal delivery may have physiological advantages over parenteral administration since the rectal anatomy allows some of the drug to be absorbed into the portal circulation, thereby passing through the liver and being utilized there. The mucus membrane in the rectum is thicker and more vascular than that in the colon, and its mobility is also greater (7,8). Rectal drug absorption is primarily via simple diffusion through lipid membrane, is consistent with the pH partition theory (9), and is influenced by several physiological factors (5) and other factors related to the drug and dosage form, including drug solubility, pK_a, surface properties, particle size, partition coefficient, and concentration.

The rectal route has been used extensively for delivery of insulin (10–12). All these studies suggest the use of various surfactants that can enhance insulin absorption, but the relative bioavailabiltiy in most of these cases was rahter low. Other studies used, beside penetration enhancers, protease inhibitors to increase insulin bioavailability (13).

Bile acids and salts are sorption promoters that appear to increase significantly the rate of uptake and absorption of high molecular weight polar drugs like insulin. They enhance GIT transmembrane movements of endogenous and exogenous lipids (14), as well as transmembrane or paracellular movements of several polar molecules (15). Bile salts may act by solubilization of insulin in mixed bile salt micelles and by forming reverse micelles within the nasal membrane (16). Bile acids and salts augmented the protease inhibiting effects of aprotinin and soybean trypsin, thus enhancing insulin absorption (17). When used alone, they were less effective than the protease inhibitors in promoting oral insulin absorption.

The incorporation of a mucoadhesive in rectal formulations can serve three different, but related, functions. First, mucoadhesive attaches to mucin and/or epithelial layer and localizes the rectal suppository in the rectum, delaying its upward migration. Second, this localization of the rectal formulation allows more intimate contact of the drug with the absorbing epithelium and increases the bioavailability of the medicament. Third, close contact of the formulation with the mucosal layer promotes action of adjuvants that are present in the formulation and possibly reduces the amount of adjuvant needed for modification of tissue permeability. Thus, irritation of these adjuvants to the mucosal layer may be reduced. Mucoadhesives have been used for rectal delivery of drugs (18–22).

The objective of the present work was to study the effect of rectally administered insulin in the presence of deoxycholic acid, sodium taurocholated, polycarbophil, and their combinations on the plasma glucose levels of overnight food-deproved hyperglycemic rabbits. The hypoglycemia of these formulations was calculated and compared to that produced by subcutaneous (s.c.) injection of insulin suspension.

MATERIALS AND METHODS

Materials

Crystalline insulin 23 U/mg, deoxycholic acid, and taurocholic acid sodium salt hydrate (sodium taurocholate) were purchased from Fluka Chemicals (AG, CH-9470 Buchs, Switzerland). Alloxan monohydrate was obtained from Winlab (Middlesex, UK). Polyethylene glycol 4000 (PEG 4000) came from BDH Chemicals Limited (Poole, England). Polycarbophil was kindly provided by Lee Laboratories (Petersburg, VA). Glucose GOD-PAP came from Randox Laboratories Limited (Antrim, UK).

Methods

Induction of Hyperglycemia

Male white rabbits of crossed strains of Chinchilla and Branco, France, weighing 3.86 $\pm$ 0.37 kg ($X \pm$ SD), bred in the Experimental Animal Care Center (College of

Pharmacy, King Saud University, Riyadh, Saudi Arabia), were deprived of food overnight and rendered hyperglycemic with a single intravenous injection of 5% solution of alloxan monohydrate in normal saline (60 mg/kg). This study was approved by the College of Pharmacy, King Saud University.

Preparation of Insulin Suppositories

PEG 4000 was selected as a base for the formulation of insulin suppositories. Each suppository was formulated to contain 50 U of insulin with 50 mg deoxycholic acid, 50 mg sodium taurocholate, or 50 mg of each. Polycarbophil (50 mg) was added to insulin suppositories and to those containing either deoxycholic acid or both deoxycholic acid and sodium taurocholate. The displacement values of these additives were determined in PEG base. The suppositories were prepared by the fusion method, in which PEG base was melted at 70°C in a water bath. Deoxycholic acid and/or sodium taurocholate were added subsequently and triturated. Insulin was added and triturated in the melted mass after it was allowed to cool. Finally, polycarbophil was added (to certain formulations), the molten mass was triturated, poured into a 1-g mold, and cooled. The suppositories were kept at 4°C until used on the next day.

Rectal Administration

Each cage-restrained hyperglycemic rabbit food deprived for 16 hr before the experiments received one suppository containing 50 U of insulin with 50 mg deoxycholic acid, 50 mg sodium taurocholate, or both. All suppositories were with or without 50 mg of polycarbophil 30/40 mesh particles.

Subcutaneous Injection of Insulin

Each food-deprived, cage-restrained hyperglycemic rabbit received a 2 ml (40 U/ml) subcutaneous injection of insulin suspended in sterile normal saline (20 U/ml).

Blood Sampling

Blood samples (0.5 ml) were taken into heparinized tubes before the experiments and every 1 hr after rectal administration of the suppositories for 7 consecutive hours by inserting an Abbocath-T cannula (20 G $\times$ 14 inches, i.d. 0.8 $\times$ 32 mm; Abbott, Ireland) in the central ear artery of the rabbits. The tubes were centrifuged immediately at 4000 rpm for 5 min using a Beckman centrifuge (Model J-6B, Palo Alto, CA). The plasma was then aspirated and stored at -20°C pending analysis at the end of the sampling time.

Plasma Glucose Measurement

The plasma glucose levels were estimated using the glucose-oxidase method (23). Plasma (10 μl) was added to 1 ml glucose reagent (Glucose GOD-PAP, Randox Laboratories Ltd.). After vortexing for 10 sec, the tubes were incubated for 25 min at room temperature. The absorbances of the standard and plasma glucose samples were measured within 60 min against the reagent blank at 500 nm using a Spectronic 21D Spectrophotometer (Milton Roy, Rochester, NY). The plasma glucose concentration was calculated as milligrams per deciliter.

Pharmacokinetic Calculations

The maximum reduction in plasma glucose concentration C_{max} and the time to reach this C_{max} (T_{max}) were obtained from the plasma glucose concentration-time curves (% of initial) for each rabbit. The area under percentage glucose reduction-time profile $AUC_{0 \rightarrow 7\,hr}$ and the area under the first moment curve $AUMC_{0 \rightarrow 7\,hr}$ were determined using the linear trapezoidal rule (% glucose reduction). The mean residence time (MRT) for glucose reduction was calculated using the following equation: MRT = AUMC/AUC. The hypoglycemia of insulin rectal formulations relative to that after subcutaneous injection was determined by comparing their AUCs, taking dose differences in consideration. All data are given as mean ± SE. There were at least 5 animals per group.

Statistical Analysis

Plasma glucose levels (1–7 hr after rectal or subcutaneous administration) were compared in each group with the respective initial values using repeated measures analysis of variance (ANOVA) followed by a Bonferroni multiple comparisons test. Differences between groups in C_{max}, T_{max}, AUC, and MRT were carried out by one-way ANOVA followed by a Tukey-Kramer multiple comparisons test. Statistical calculations were performed using the Graph Pad Instat Computer program (1990–1993; Graph Pad Software, V2.04, San Diego, CA).

RESULTS AND DISCUSSION

In this study, the effect of the presence of deoxycholic acid, sodium taurocholate, polycarbophil, and their com-

binations in insulin suppositories on the plasma glucose levels of hyperglycemic rabbits deprived of food overnight was investigated. Deoxycholic acid and sodium taurocholate were used in suppositories containing 50 U of insulin as they serve two purposes: to inhibit the mucosal proteolytic activity (24) and to promote absorption of insulin (25). The influence of the enhancers alone or in combination on the insulin effect on plasma glucose levels of hyperglycemic rabbits is shown in Table 1 and Fig. 1A.

Insulin suppositories containing deoxycholic acid (50 mg) produced a significant ($p < .01$) reduction in plasma glucose levels from initial fasting levels of 346 ± 46.2 mg/dl to 224 ± 42.2 mg/dl after 2 hr of rectal administration of the suppositories. This formulation produced a C_{max} (% maximum glucose concentration reduction) of 51%, a T_{max} of 3 ± 0.48 hr, and an $AUC_{0 \rightarrow 7\ hr}$ of 209 ± 17.2 mg%hr, thus resulting in 38% relative hypoglycemia when compared to a subcutaneous injection of insulin suspension (40 U), as shown in Table 2. Insulin suppositories containing sodium taurocholate (50 mg) resulted in a significant ($p < .001$) reduction in initial plasma glucose levels by the first hour, indicating the enhanced effect of sodium taurocholate on insulin absorption, as shown in Table 1 and Fig. 1A. This rapid reduction in plasma glucose levels (39%) was increased to 49% by the second hour. The $AUC_{0 \rightarrow 7\ hr}$ was 192 ± 19.6 mg%hr, which achieved 34.9% hypoglycemia relative to subcutaneous insulin injection (40 U). The C_{max}'s and the relative hypoglycemia obtained with these formulations were similar to those obtained by Ichikawa et al. (26) and Aungst, Rogers, and Shefter (27).

The concomitant addition of deoxycholic acid and sodium taurocholate (50 mg each) in one formulation caused insulin to produce a significant reduction ($p < .001$) in initial plasma glucose levels by the second hour. This actually carries the features of deoxycholic acid, which caused insulin to produce a late significant ($p < .01$) reduction in plasma glucose levels by the second hour and the prolonged significant ($p < .001$) reduction produced in the presence of sodium taurocholate, which lasts from the second to the fourth hour. The mixture of the two enhancers indicated a synergistic action on insulin effects in which the C_{max} reached (68.57% ± 6.14%) was insignificantly ($p > .05$) different from the C_{max} obtained after subcutaneous injection (as shown in Table 2). The T_{max} of 2.6 ± 0.25 hr was between that produced by the insulin formulation containing deoxycholic acid (3 ± 0.48 hr) and that produced by the suppositories containing sodium taurocholate (2.2 ± 0.49 hr). The $AUC_{0 \rightarrow 7\ hr}$ obtained for this formulation was 244 ± 29.5 mg%hr, which resulted

Table 1

Effect of Insulin (I) Given by Subcutaneous Injection (40 U) or Rectally in Suppository Form Containing 50 U Insulin Incorporated with Deoxycholic Acid (D), Sodium Taurocholate (T), Polycarbophil (P), and Their Combinations on Plasma Glucose Levels of Overnight Food-Deprived Alloxan-Induced Hyperglycemic Rabbits

	Plasma Glucose Levels (mg% ± SEM) (Number of Animals/Group)						
Time (hr)	I Subcutaneous (6)	I+D Suppository (5)	I+T Suppository (5)	I+DT Suppository (5)	I+P Suppository (5)	I+DP Suppository (5)	I+DTP Suppository (6)
0	259 ± 42.9	346 ± 46.2	219 ± 22.9	390 ± 18.8	330 ± 34.3	278 ± 39.0	407 ± 32.8
1	155 ± 52.4[a]	296 ± 49.9	136 ± 21.5[c]	336 ± 35.5	270 ± 44.4[b]	239 ± 37.7	333 ± 56.4
2	126 ± 44.5[c]	224 ± 42.2[b]	118 ± 25.7[c]	183 ± 45.7[c]	220 ± 34.1[c]	187 ± 34.1[a]	223 ± 59.7[c]
3	88 ± 39.7[c]	206 ± 30.5[b]	127 ± 24.2[c]	126 ± 23.9[c]	195 ± 28.5[c]	116 ± 36.9[c]	180 ± 50.3[c]
4	74 ± 26.9[c]	190 ± 26.0[c]	144 ± 20.1[c]	225 ± 35.6[c]	187 ± 26.8[c]	136 ± 28.5[c]	170 ± 32.0[c]
5	69 ± 16.1[c]	215 ± 26.9[b]	182 ± 17.8	262 ± 30.6[a]	192 ± 24.4[c]	149 ± 30.6[c]	172 ± 21.3[c]
6	57 ± 9.5[c]	243 ± 31.7[a]	197 ± 16.8	291 ± 29.1	205 ± 27.2[c]	191 ± 40.6[a]	206 ± 25.3[c]
7	60 ± 10.3[c]	267 ± 35.9	204 ± 17.4	321 ± 23.6	220 ± 31.4[c]	230 ± 30.1	201 ± 25.9[c]

Probablity compared with the respective initial value (0 hr) using repeated measures analysis of variance followed by Bonferroni multiple comparisons test.

[a] $p < .05$.

[b] $p < .01$.

[c] $p < .001$.

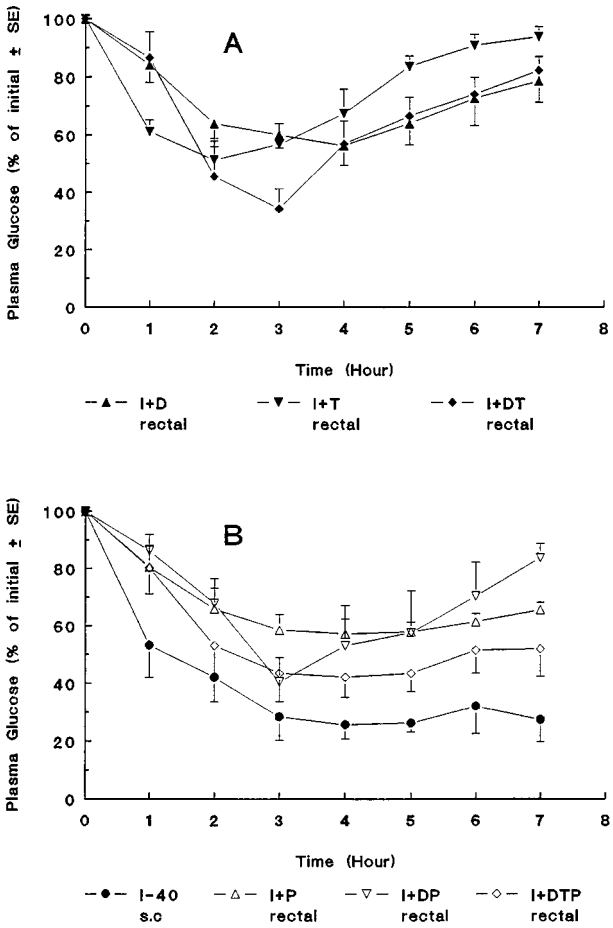

Figure 1. Effect of insulin (I) given rectally in a suppository form containing (A) insulin (50 U) plus 50 mg of either deoxycholic acid (I + D), sodium tauracholate (I + T), or their combinations (I + DT); (B) insulin subcutaneous injection (40 U, I-40) or rectally in a suppository form containing insulin (50 U) plus 50 mg polycarbophil (I + P) with 50 mg deoxycholic acid (I + DP) alone or with 50 mg sodium taurocholate (I + DTP) on plasma glucose levels (% of initial ± SEM) of overnight food-deprived, alloxan-induced hyperglycemic rabbits.

in relative hypoglycemia of 44.4% compared with subcutaneous injection (40 U).

These results show that deoxycholic acid and sodium taurocholate are effeiceint absorption enhancers for insulin form rectal suppositories, as shown by the hypoglycemic effect of these rectal formulations.

Bile acids and salts have been shown to be effective in promoting the absorption of insulin from the rat ileum (28), improving its conjunctival penetration in albino rabbits (15) and its nasal absorption in rats (29). Bile salts also enhanced the rectal permeability to insulin in albino rabbits (30). The mechanisms for the enhancement of insulin absorption from these routes show that the promoting effect of bile acids and salts was attributed not only to their direct effect on mucosa, but also to their inhibitory effect on proteolytic enzymes (15,28–32).

Bile acids and salts appear to inhibit the proteolytic activity by denaturing the enzyme and preventing the enzyme-substrate complex formed to undergo the necessary conformational changes that align the catalytic site on the protease with the susceptible bond of the substrate (33).

Tables 1 and 2 and Fig. 1B also show the effect on the plasma glucose level of alloxan-induced hyperglycemic rabbits of incorporating the bioadhesive polycarbophil (P) with insulin (I) alone (I + P), in the presence of deoxycholic acid (D) (I + DP), or in a mixture of deoxycholic acid and sodium taurocholate (T) (I + DTP) (Table 1), percentage of initial glucose level (Fig. 1B), and the pharmacokinetic parameters produced as a result of rectal administration of these formulations (Table 2). Insulin suppositories containing polycarbophil produced a rapid, significant ($p < .01$) decrease in plasma glucose level (19.4% glucose reduction) by the first hour, which increased to 41.5% ± 12.20% and 42.0% ± 7.7% by the third and fourth hours, respectively. This significant ($p < .001$) reduction in plasma glucose levels was maintained for the duration of the experiment. This resulted in late T_{max} (4.2 ± 0.2 hr), an MRT of 4.1 ± 0.13 hr, an $AUC_{0 \rightarrow 7\,hr}$ of 237 ± 32.6 mg%hr, and relative hypoglycemia of 43.1%. This effect of insulin on reducing the plasma glucose levels of the hyperglycemic rabbits is due to the ability of the multifunctional anionic charged macromolecule polycarbophil to make close contact between the rectal suppository and the site of absorption, which has a direct influence on the permeability of mucosal epithelium. It also exerts such effects as a locally high concentration within a confined area, thus reducing the luminal diffusion pathway of the insulin and has an effect on inhibiting the proteolytic enzymes (34). Because of its large molecular weight, polycarbophil itself is not absorbed and is not expected to exert any undesirable effects. It is also approved by the Food and Drug Administration for use in humans as an antidiarrheal and laxative product.

On incorporation of polycarbophil in a rectal suppository formulation containing deoxycholic acid, insulin produced a delayed significant ($p < .05$) reduction in plasma glucose levels. This reduction increased signifi-

Table 2

Mean (±SEM) Pharmacokinetic Parameters of Insulin (I) Following Subcutaneous Injection of Insulin Suspension (40 U) and Rectal Administration of Insulin Suppositories (50 U) Incorporated with Deoxycholic Acid (D), Sodium Taurocholate (T), Polycarbophil (P), and Their Combinations to Overnight Food-Deprived Alloxan-Induced Hyperglycemic Rabbits

	Isulin Formulation						
	I (s.c.)	I+D	I+T	I+DT	I+P	I+DP	I+DTP
C_{max} (% reduction)	82.49 ± 1.34	50.89 ± 4.02[a,b]	55.79 ± 4.08[b,c]	68.57 ± 6.14	44.35 ± 4.93[a,b]	64.09 ± 6.09[d,e]	67.94 ± 5.27[d,e]
T_{max} (hr)	4.67 ± 0.62	3.00 ± 0.48	2.20 ± 0.49[b,d]	2.60 ± 0.25[d,f]	4.20 ± 0.20	3.40 ± 0.25	4.80 ± 0.84
MRT (hr)	4.1 ± 0.14	3.7 ± 0.33	2.8 ± 0.23[b,c]	3.6 ± 0.28	4.1 ± 0.13[d,f]	3.7 ± 0.15	4.1 ± 0.27[c,f]
$AUC_{0 \rightarrow 7\ hr}$ (mg% hr)	440 ± 27.1	209 ± 17.2[a,b]	192 ± 19.6[a,b]	244 ± 29.5[b,c]	237 ± 32.6[b,c]	232 ± 60.4[b,c]	310 ± 40.1
RH		38.0	34.9	44.4	43.1	42.2	56.4

Probability calculated using one-way analysis of variance followed by Tukey-Kramer multiple comparisons test.

MRT, mean residence time; $AUC_{0 \rightarrow 7\ hr}$, the area under percent glucose reduction time curve up to 7 hr; RH, relative hypolgycemia of rectal suppositories containing 50 U insulin as a percentage of insulin subcutaneous injection (40 U).

[a] $p < .001$.

[b] Compared to I.

[c] $p < .01$.

[d] $p < .05$.

[e] Compared to I+P.

[f] Compared to I+T.

cantly ($p < .001$) for 3 hr (3–5 hr sampling time) before it started to decrease by the sixth hour (Table 1 and Fig. 1B). The C_{max} was 64.9% ± 6.09%, which was insignificantly ($p > .05$) different from that of subcutaneous injection. The addition of polycarbophil increased the relative hypoglycemia from 38% to 42.2% (Table 2).

The effects of polycarbophil were much more pronounced on its addition to insulin suppositories containing a mixture of deoxycholic acid and sodium taurocholate. Insulin produced a significant ($p < .001$) reduction in plasma glucose levels by the second hour and lasted for the experimental time (7 hr), as shown in Table 1. It produced a C_{max} of 67.94% ± 27%, which is the same as that produced in the absence of polycarbophil. But, T_{max} is prolonged from 2.6 ± 0.25 hr to 4.8 ± 0.84 hr, and the MRT is prolonged from 3.6 ± 0.28 hr to 4.1 ± 0.27 hr. The $AUC_{0 \rightarrow 7\ hr}$ increased to 310 ± 40.1 mg%hr, which is insignificantly different ($p > .05$) from that produced after subcutaneous injection of 40 U insulin, thus producing 56.4% relative hypoglycemia (Table 2). These improvements may be due to the effect of sodium taurocholate in increasing the mucus secretion (35), which gives polycarbophil a better chance for mucoadhesion. This mucoadhesion increases contact time and permits localization, which are essential factors when modification of tissue permeability is important for delivery. Polycarbophil also inhibits the proteolytic enzymes in this area of localization. But, in the case of the formulation containing deoxycholic acid (I + D), the addition of polycarbophil did not produce such pronounced effects as the bile acid (36) affects the mucus viscosity as a result of chelation of Ca^{2+} and Mg^{2+} ions necessary to maintain the mucus layer structure (37). While the mucus thinning effect of deoxycholic acid when given alone may help absorption, it could hinder the mucoadhesion of polycarbophil.

In conclusion, the presence of deoxycholic acid, sodium taurocholate, or both appears to enhance markedly the relative hypoglycemia of insulin suppositories. In this study, the greatest relative hypoglycemia was obtained from the suppository formulation containing polycarbophil together with a mixture of deoxycholic acid and sodium taurocholate. These results show that the use of bioadhesives in the delivery of insulin, coupled with the use of penetration enhancers and protease inhibitors, is beginning to be realized as a way to improve insulin bioavailability greatly; in this study, a relative bioavailability of 56.4% was achieved relative to that of subcutaneous injection. So, rectal delivery may represent a practical alternative to the parenteral route of insulin administration.

REFERENCES

1. M. Ishida, Y. Machida, N. Nambu, and D. T. Nagai, Chem. Pharm. Bull., 29, 810 (1981).
2. X. H. Zhou and A. L. Wan Po, Int. J. Pharm., 75, 116 (1991).
3. L. Illum and S. S. David, Clin. Pharmacokinet., 23, 30 (1992).
4. A. G. de Boer, F. Moolenaar, L. G. J. de Leede, and D. D. Breimer, Clin. Pharmacokinet., 7, 285 (1982).
5. A. G. de Boer, L. G. de Leede, and D. D. Breimer, Br. J. Anaesth., 56, 69 (1984).
6. A. Kamiya, H. Ogata, and H. L. Fung, J. Pharm. Sci., 71, 621 (1982).
7. R. Ger, Surg. Clin. North Am., 68, 1201 (1988).
8. M. E. Kraeling and W. A. Ritschel, Methods Find. Exp. Clin. Pharmacol., 14, 199 (1992).
9. S. Muranishi, Methods Find. Exp. Clin. Pharmacol., 6, 763 (1984).
10. W. A. Ritschel, G. B. Ritschel, B. E. Ritschel, and P. W. Lucker, Methods Find. Exp. Clin. Pharmacol., 10, 645 (1988).
11. E. J. Van-Hoogdalem, C. D. Heijligers-Feijin, J. C. Verhoef, A. G. de-Boer, and D. D. Dreimer, Pharm. Res., 7, 180 (1990).
12. E. A. Hosny, O. Al-Ahmady, H. El-Shattawy, A. Nabih, H. El-Damacy, S. Gamal, and N. El-Kabbany, Arzneim.-Forsch., 44, 611 (1994).
13. T. Nishihata, G. Liversidge, and T. J. Higuchi, Pharm. Pharmacol., 35, 616 (1983).
14. A. C. Moses, G. S. Gordon, M. C. Carey, and J. S. Flier, Diabetes, 32, 1040 (1983).
15. E. Hayakawa, D. S. Chien, Y. Inagaki, A. Yamamoto, and V. Lee, Pharm. Res., 9, 769 (1992).
16. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier, and M. C. Carey, Proc. Natl. Acad. Sci. USA, 82, 7419 (1985).
17. E. Ziv, O. Lior, and M. Kidron, Biochem. Pharmacol., 36, 1035 (1987).
18. K. Morimoto, E. Kamiya, T. Takeeda, Y. Nakamoto, and K. Morisaka, Int. J. Pharm., 14, 149 (1983).
19. E. A. Hosny and A. A. Al-Angary, Int. J. Pharm., 113, 209 (1995).
20. E. A. Hosny, E. M. Niazy, and A. S. El-Gorashi, Int. J. Pharm., 117, 147 (1995).
21. E. A. Hosny, E. M. Niazy, and M. M. El-Dardiri, Int. J. Pharm., 136, 37 (1996).
22. E. A. Hosny, S. S. Abdel-Hady, and K. E. H. El-Tahir, Int. J. Pharm., 142, 163 (1996).
23. P. Trinder, Ann. Clin. Biochem., 6, 24 (1969).
24. X. H. Zhou and A. L. Wan Po, Int. J. Pharm., 69, 29 (1991).
25. K. Takada, M. Yamamoto, H. Nakae, and S. Asada, Chem. Pharm. Bull., 28, 2806 (1980).
26. K. Ichikawa, I. Ohata, M. Mitomi, S. Kawamura, and H. J. Kawata, Pharm. Pharmacol., 32, 314 (1980).

27. B. J. Aungst, N. J. Rogers, and E. Shefter, J. Pharm. Pharmacol. Exp. Ther., 244, 23 (1988).
28. M. Kidron, H. Bar-On, E. M. Berry, and E. Ziv, Life Sci., 31, 2837 (1982).
29. S. Hirai, T. Yashiki, and H. Mima, Int. J. Pharm., 9, 173 (1981).
30. A. Yamamoto, E. Hayakawa, Y. Kato, A. Nishiura, and V. H. J. Lee, Pharmacol. Exp. Ther., 263, 25 (1992).
31. A. Yamamoto, E. Hayakawa, and V. H. Lee, Life Sci., 47, 2465 (1990).
32. S. J. Hersey and R. T. Jackson, J. Pharm. Sci., 76, 876 (1987).
33. E. Hayakawa and V. H. L. Lee, Pharm. Res., 9, 535 (1992).
34. H. L. LueBen, C. M. Lehr, C. O. Rentel, A. B. J. Noach, A. G. de Boer, J. C. Verhoef, and H. E. Junginger, J. Controlled Release, 29, 329 (1994).
35. F. G. J. Poelma, J. J. Tukker, and D. J. A. Crommelin, J. Pharm. Sci., 78, 285 (1989).
36. T. Murakami, Y. Sasaki, R. Yamajo, and N. Yata, Chem. Pharm. Bull., 32, 1948 (1984).
37. J. F. Forstner and G. G. Forstner, Biochim. Biophys. Acta, 386, 283 (1975).