Vaginal Absorption of a Potent Luteinizing Hormone-Releasing Hormone Analog (Leuprolide) in Rats I: Absorption by Various Routes and Absorption Enhancement

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Abstract □ The absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) through different routes was evaluated by determining the ovulation-inducing activity in diestrous rats. Vaginal administration showed the greatest potency among nonparenteral routes and was followed successively by rectal, nasal, and oral administration. Mixed micellar solution with monoolein–bile acids improved the intestinal absorption of leuprolide, and nasal absorption was enhanced by adding sodium glycocholate, surfactin, or polyoxyethylene 9 lauryl ether, but these bioavailabilities were still insufficient. The vaginal absorption was enhanced by organic acids: citric, succinic, tartaric, and glycocholic; the absolute bioavailability increased to ~20%. The vaginal absorption from jellies, as practical dosage forms, yielded sufficient activity of leuprolide, but absorption was slightly reduced with highly polar polymers or with higher concentrations of polymers. It was concluded that vaginal administration of leuprolide can be a rational dosage method for a long-term antitumor therapy.

Keyphrases □ Leuprolide—vaginal absorption, potent luteinizing hormone-releasing hormone analog in rats, ovulation-inducing activity □ Absorption, vaginal—potent luteinizing hormone-releasing hormone analog (leuprolide) in rats, ovulation-inducing activity □ Hormones—potent luteinizing hormone-releasing hormone analog (leuprolide) in rats, vaginal absorption

Leuprolide (I), a luteinizing hormone-releasing hormone (II) analog synthesized previously (1), has a high gonadotropin-releasing activity (2) and ovulation-inducing potency (50–80 times greater than II) (3). Recently, this analog

(Pyro)Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH-CH_2CH_3

I

(Pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

II

at relatively large doses, was found to effect regression of hormone-dependent mammary tumors (4–6). In addition, it appears to have birth control potential, without toxic side-effects, in both sexes (7–11).

Peptides are generally absorbed poorly, subject to decomposition in the GI tract, and have short biological half lives after parenteral administration. To establish a convenient and reliable method for nonparenteral self-administration of II and the analogs for long-term therapy, numerous studies have been carried out on the pharmacological effects after oral (12–14), sublingual (15), nasal (15–23), rectal (24), and vaginal (12, 14, 24) administration.

In the present study, the absorption of leuprolide through nonparenteral administration routes for an antitumor therapy was assessed by determining its ovulation-inducing activity in rats. The absorption enhancement was attempted in oral, nasal, and vaginal routes, and a study was also conducted on an experimental dosage form designed for vaginal administration.

EXPERIMENTAL

Animals—Mature female Sprague-Dawley rats[1] aged 120–150 days and weighing 250–330 g were used. Animals exhibiting two or more consecutive 4-day estrous cycles on daily morning examination of vaginal smears were used in the diestrous stage.

Materials—Leuprolide acetate[2] was used after dehydration at 50° under vacuum for 5 hr. Sodium glycocholate[3], sodium taurocholate[3], and polyoxyethylene 9 lauryl ether[4] of commercial grade were used without further purification. The other chemicals were of reagent grade quality.

Ovulation-Inducing Activity by Different Routes—The absorption of leuprolide through various administration routes was estimated by ovulation-inducing activity as described previously (25). A 0.9% NaCl solution of the analog containing 0.1% bovine serum albumin[5], 20 U/ml of aprotinin[6], and 0.1 *N* HCl was used for intravenous, subcutaneous, nasal, and oral administrations. For rectal administration, the analog was dispersed in an oleaginous base[7] molded in a cylindrical shape (Φ5 × 8 mm). For vaginal administration, the analog was dissolved in 0.9% NaCl solution containing 0.1% bovine serum albumin and 20 U/ml of aprotinin or dispersed in an oleaginous base[7]. After the suppository was placed by a glass inserter, the orifice was closed with a surgical adhesive agent. Although the rectal and vaginal administration using an oleaginous base were carried out at a dose per rat, their dose and ED_{50} are shown by dose per kilogram using mean body weight.

Enhancement of Oral, Nasal, and Vaginal Absorption—For the oral administration, a mixed micellar solution with monoolein[4], sodium taurocholate, and sodium glycocholate was prepared according to a previous method (26). One percent sodium glycocholate, surfactin[8], or polyoxyethylene 9 lauryl ether was added to the analog solution for the nasal administration. For the vaginal administration, the following adjuvants were dissolved or dispersed as fine particles at a concentration of 10% (as a free acid), except lactic acid (2%), in the oleaginous base: citric acid, succinic acid, tartaric acid, lactic acid, ascorbic acid, gluconic acid, taurine, glycine, boric acid, dipotassium edetate, sodium citrate, sodium glycocholate, and sodium oleate.

The concentration effect of citric acid and succinic acid was also examined at a range of 2–20% in the oleaginous base.

Leakage of Evan's Blue from the Vaginal Membrane—Under pentobarbital anesthesia, a dose of 1 ml/kg of 1% Evan's blue solution

[1] Clea Japan, Inc., Tokyo, Japan.
[2] Takeda Chemical Ind., Ltd., Osaka, Japan. The analog was synthesized in the Central Research Division.
[3] Tokyo Chemical Ind., Ltd., Tokyo, Japan.
[4] Nikko Chemical Ind., Ltd., Tokyo, Japan.
[5] Wako Pure Chemical Ind., Ltd., Osaka, Japan. Bovine serum albumin was added to prevent any loss by adsorption to the glass surface.
[6] Trasylol, Bayer A. G., Leverkusen-Bayerwerk, W. Germany. This peptidase inhibitor was added to inhibit a small quantity of peptidases derived from bovine serum albumin.
[7] WITEPSOL W35 (rectal) or WITEPSOL S55 (vaginal), Dynamit Nobel Aktiengesellshaft, W. Germany.
[8] A bacterial peptide lipid surfactant isolated from *Bacillus subtilis* (27).

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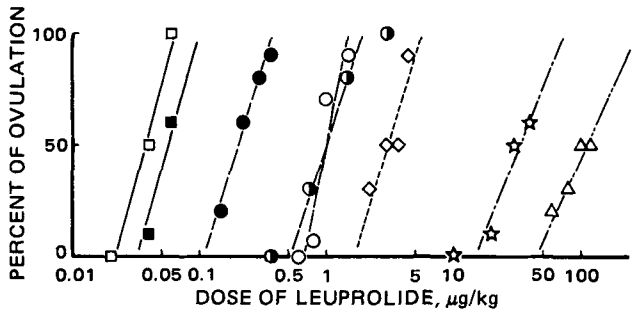

Figure 1—*Ovulation-inducing activity of leuprolide after intravenous, subcutaneous, oral, rectal, nasal, and vaginal administration to diestrous rats. Key: (□) intravenous; (■) subcutaneous; (○) vaginal in 0.9% NaCl; (◑) vaginal in oleaginous base; (●) vaginal with 10% citric acid; (◇) rectal; (☆) nasal; (△) oral.*

was injected intravenously 2 hr after the vaginal administration of the suppository containing 10% organic acids (citric, succinic, tartaric, and aspartic acids), sodium glycocholate, sodium citrate, calcium citrate, or dipotassium edetate. The rats were sacrificed by decapitation 30 min later and their vaginas were isolated and examined for the intensity of stain in the mucous membranes.

Vaginal Absorption from Jelly and Tablet—Methylcellulose[9] (2–7%), a mixture of xanthan gum[2] and locustbean gum[2] (2–3%), carrageenan[2] (5%), starch (7 and 10%), agar (10%), tragacanth gum[2] (4%), sodium carboxymethylcellulose[10] (5%), sodium polyacrylate[5] (5%), glycerogelatin[11], succinated gelatin[12] (61%), and polyethylene glycol 4000[13] (70%) were used as a jelly base. The concentration was adjusted to provide almost the same viscosity for each jelly. The jelly was prepared by dissolving the hydrophilic polymer in 5% citric acid solution (pH 3.5) containing various amounts of leuprolide. Each jelly was administered into the upper vagina by a glass inserter at a dose of 100 mg/rat.

Three kinds of tablets were prepared by a conventional method. The granules with the analog, citric acid, lactose, corn starch, and hydroxypropylcellulose[14] were formed by a wet method and blended with corn starch and magnesium stearate for compression into a cylindrically shaped tablet. The concentration of citric acid was 10% for tablet A, 5% for tablet B, and 10% as a sodium salt for tablet C. The pH value of the solution of tablet C dissolved in 3 ml of distilled water was 4.22.

The leakage of Evan's blue from the vaginal membrane was determined to compare the local reaction by methylcellulose jelly with 5% citric acid (pH 3.5), oleaginous suppository with 10% citric acid, and tablet A. Evan's blue was injected intravenously 30 min after vaginal administration of the suppositories.

RESULTS

Ovulation-Inducing Activity by Different Routes—The ovulation-inducing activities of leuprolide after intravenous, subcutaneous, vaginal, nasal, and oral administration to diestrous rats are shown in Fig. 1. The ED_{50}'s of the activity calculated by Finney's probit analysis (28) were 38 ng/kg by intravenous, 58 ng/kg by subcutaneous, 112 μg/kg by oral, 33.4 μg/kg by nasal, 3.12 μg/kg by rectal, and 1.0 μg/kg by vaginal administration. The activity of the analog by the oral route was 2950 times less than that by intravenous injection. Vaginal administration exhibited the greatest potency of all nonparenteral routes examined; the absolute bioavailability estimated by comparison with the potency of the intravenous injection was 3.8% in both the 0.9% NaCl solution and oleaginous suppository.

Enhancement of Oral, Nasal, and Vaginal Absorption—The ovulation-inducing activity of leuprolide after oral administration in the mixed micellar solution with monoolein and bile acids is shown in Table I. The absorption was enhanced 1.6 times more than that of the 0.9% NaCl solution, but the ED_{50} was still 1870 times larger than that after intravenous injection.

Three surfactants employed as absorption promoters markedly increased the potency of the analog applied nasally by the same degree: the absolute bioavailabilities were 1.8–3.0% (Table II).

For the vaginal administration, the polybasic carboxylic acids (citric, succinic, and tartaric acids) and glycocholic acid increased almost 5 times the ovulation-inducing activity of the analog (Table III). Acidic amino acids (glutamic and aspartic acids), the carboxylic acids having hydroxyl groups (ascorbic and lactic acids), and dipotassium edetate exhibited weak promoting effects, whereas taurine, boric acid, oleic acid, caproic acid, sodium oleate, and polyoxyethylene 9 lauryl ether scarcely enhanced the activity of the analog and glycine reduced it. The promoting effect of the carboxylic acids was reduced by using sodium citrate and sodium glycocholate salts. The concentration effects of citric acid and succinic acid on the vaginal absorption of the analog are shown in Fig. 2. These acids increased the activity of the peptide in concentrations ≤10%, and the activity approached a maximum level of >10%.

Leakage of Evan's Blue on Vaginal Membrane—Staining of the vaginal mucous membrane by Evan's blue injected intravenously after vaginal administration of a suppository containing an additive was examined. The organic acids (citric, succinic, and tartaric acids) and dipotassium edetate, both of which enhanced the vaginal absorption of leuprolide, induced deep staining of vaginal membrane, whereas little staining was observed with sodium glycocholate, sodium citrate, and calcium citrate.

Vaginal Absorption from Jelly and Tablet—The ovulation-inducing activities of leuprolide after vaginal administration of various hydrophilic jellies are shown in Table IV. The activity was affected by the jelly material and its concentration. Polymers having a highly polar functional group (sodium carboxymethylcellulose, sodium polyacrylate, and gelatin) tended to reduce the activity of the analog, whereas polysaccharides with less polar functions (methylcellulose, xanthan gum, locustbean gum, carrageenan, and starch) showed higher activity. In both polymer types, the activity was reduced as the polymer concentration increased.

Tablets containing the analog with 5% citric acid and 10% sodium citrate exhibited one third of the activity of tablet A with 10% citric acid, which induced the highest ovulation effect (Table V).

Staining of the vaginal membrane by Evan's blue injected after vaginal administration of the jelly, oleaginous suppository, and tablet, which were expected to show similar pharmacological effects, was compared. The oleaginous suppository and tablet induced deep blue staining, but the jelly induced only faint or no staining.

DISCUSSION

Determination of the ovulation-inducing activity of leuprolide after administration by different routes revealed that the absorption was good by vaginal administration, which was followed successively by rectal, nasal, and oral administration. The absorption of the analog from 0.9% NaCl solution and oleaginous suppository applied vaginally was almost identical. Their absolute bioavailabilities estimated by the pharmacological effect were 3.8%.

The vaginal absorption of a drug depends on its release from the suppository and on its ability to pass through the vaginal membrane. For the penetration of a drug, a physical model has been proposed (29) comprising an aqueous diffusion layer in series with a membrane consisting of aqueous pores and lipoidal pathways. The considerable absorbability of the analog, a hydrophilic compound, may indicate that its main absorption is thorough a pore-like route, such as intercellular channels, rather than through partition to the membrane cell.

Attempts were made to enhance absorption through the oral, nasal, and vaginal routes. Mixed micelles with lipid–bile salts are known to enhance the intestinal absorption of poorly absorbable polar drugs such as insulin (26), aminoglycosides (30), and heparin (31). The mixed micellar solution with bile salts and monoolein also improved the absorption of leuprolide, but its absolute bioavailability was 0.05%.

The bioavailability following nasal administration was 0.11% from the aqueous solution and was increased to 2–3% when sodium glycocholate, surfactin, or polyoxyethylene 9 lauryl ether was added. The absorption-enhancing effect with the promoters is in agreement with the effect observed previously with insulin (32).

The vaginal absorption of leuprolide was markedly facilitated by the polybasic carboxylic acids, and slightly increased with the hydroxy carboxylic acids and acidic amino acids. All of the carboxylic acids that

[9] METOLOSE (90SH4000, 90SH8000, 90SH15000, and 90SH30000), Shinetsu Chemicals Co., Tokyo, Japan. Four types of methylcellulose were used. In SH type, 4–12% of the methoxy groups was substituted by the hydroxypropoxy groups to raise the thermal gelation temperature. The former numbers represent the thermal gelation temperature and the latter the molecular weight.

[10] 7MF type, Hercules Inc., Wilmington, Del.

[11] Prepared as described in "Remington's Pharmaceutical Sciences," 15th (1975), Mack Publishing Co., p. 1545.

[12] Nitta Gelatin Co., Ltd., Tokyo, Japan.

[13] Sanyo Kasei Ind., Ltd., Kyoto, Japan.

[14] L type, Nippon Soda Co., Ltd., Tokyo, Japan.

Table I—Ovulation-Inducing Activities of Leuprolide after Oral Administration to Diestrous Rats [a]

Preparation	Dose of Leuprolide, μg/kg							ED_{50}, μg/kg
	10	20	40	60	80	100	120	
NaCl solution, 0.9%	—	—	—	2/10	3/10	5/10	5/10	112 (81–155)[b]
Mixed micellar solution[c]	0/5	2/10	2/10	4/9	5/10	7/10	—	71 (46–190)

[a] Number of rats with induced ovulation per number of rats examined. [b] Fiducial limits (95%). [c] The mixed micellar solution of leuprolide was prepared with 45 m*M* of monoolein, 20 m*M* of sodium taurocholate, and 20 m*M* of sodium glycocholate by sonication (Ref. 26).

Table II—Ovulation-Inducing Activity of Leuprolide after Nasal Administration with Surfactants to Diestrous Rats [a]

Additives, 1%	Dose of Leuprolide, μg/kg										ED_{50}, μg/kg
	0.8	1	2	3	4	5	10	20	30	40	
None	—	—	0/5	—	0/5	—	0/5	1/10	5/10	6/10	33.4 (25.7–69.8)[b]
Sodium glycocholate	0/10	3/10	9/10	—	—	5/5	5/5	—	—	—	1.28 (1.05–1.70)[c]
Surfactin	0/10	2/10	4/10	10/10	—	—	—	—	—	—	1.77 (1.38–2.35)[c]
Polyoxyethylene 9 lauryl ether	—	0/10	6/10	7/10	—	—	—	—	—	—	2.08 (1.52–2.93)[c]

[a] Number of rats with induced ovulation per number of rats examined. [b] Fiducial limits (95%). [c] Significant ($p < 0.05$).

Table III—Ovulation-Inducing Activity of Leuprolide after Vaginal Administration with Additives to Diestrous Rats [a]

Additives (10%)	Dose of Leuprolide, ng/rat										ED_{50}, ng/rat	Relative potency
	20	40	60	80	100	150	200	400	600	800		
None	—	—	—	—	0/10	—	3/10	8/10	9/10	10/10	270 (194–353)[b]	1
Citric acid	—	2/10	6/10	8/10	9/10	—	10/10	10/10	5/5	5/5	56 (38–69)	4.9[c]
Succinic acid	0/10	4/10	6/10	8/10	9/10	—	—	—	—	—	50 (37–63)	5.4[c]
Tartaric acid	—	—	1/10	5/10	8/10	5/5	—	—	—	—	82 (69–97)	3.3[c]
Glycocholic acid	1/10	5/10	5/10	8/10	9/10	—	—	—	—	—	47 (32–62)	5.6[c]
Ascorbic acid	—	—	3/10	2/10	4/10	7/10	—	—	—	—	113 (80–161)	2.4[c]
Lactic acid, 2%	—	—	2/10	0/10	3/10	8/10	—	—	—	—	117 (95–184)	2.3[c]
Aspartic acid	—	—	—	2/10	4/10	—	9/10	9/10	10/10	5/5	122 (79–167)	2.1[c]
Glutamic acid	—	—	—	—	0/10	—	7/10	10/10	10/10	5/5	177 (133–243)	1.6
Dipotassium edetate	—	—	0/10	3/10	5/10	8/10	—	—	—	—	104 (87–134)	2.6[c]
Taurine	—	0/5	—	2/10	3/10	—	5/10	8/10	—	—	182 (112–373)	1.5
Glycine	—	—	—	—	0/10	—	0/5	1/10	3/10	6/10	755 (570–5904)	0.4[c]
Boric acid	—	—	—	—	1/10	3/15	5/10	—	—	—	200 (153–261)	1.3
Caproic acid	—	—	—	—	1/10	2/10	5/10	8/10	9/10	—	341 (249–484)	0.8
Oleic acid	—	—	—	0/5	1/10	—	3/10	4/10	7/10	9/10	358 (244–541)	0.7
Polyoxyethylene 9 lauryl ether	—	—	—	—	0/10	1/10	6/10	7/10	9/10	—	254 (193–348)	1.1
Sodium glycocholate	—	—	3/15	6/15	2/15	—	10/15	—	—	—	151 (107–666)	1.9[c]
Sodium oleate	—	—	—	—	0/10	—	1/10	8/10	8/10	10/10	323 (237–461)	0.8
Sodium citrate	—	—	—	0/10	3/10	—	5/10	6/10	8/10	9/10	245 (160–365)	1.0

[a] Number of rats with induced ovulation per number of rats examined. [b] Fiducial limits (95%). [c] Significant ($p < 0.05$).

showed an absorption promoting effect possess a chelating ability. The absolute bioavailability of the analog after vaginal administration increased to ~20% on the average by adding these polybasic carboxylic acids. The absorption was poorly enhanced with surfactants such as sodium glycocholate, sodium oleate, and polyoxyethylene 9 lauryl ether, which are known to enhance greatly the rectal and nasal absorption of hydrophilic drugs, and was reduced with glycine. The difference between the enhancement effects of surfactants on vaginal and rectal absorption may be attributed to structural differences of the membranes, *i.e.*, the vaginal epithelium, consisting of stratified squamous cells, may resist cleavage or desquamation of epithelial cells by the surfactants. Glycine seems to possess a stabilizing activity on the epithelium that results in the reduced vaginal absorption of salicylate[15].

An intimate relation was observed between the absorption-enhancing effect of leuprolide and the leakage from the vaginal membrane of Evan's blue injected intravenously after treatment with the promoters. Distinct staining of the vaginal epithelial membrane was elicited after vaginal administration of carboxylic acids with chelating ability, but not of calcium citrate. This indicates that the blood–vaginal epithelium barrier has been loosened by the organic acids and that the chelating ability may contribute to the absorption enhancement by expanding the channel bore through reversible electrical uncoupling with calcium ions as demonstrated in gap junctions (33), or by cleaving the intercellular tight junction through uptake of the calcium ions of binding proteins. Sodium citrate, which did not promote absorption, had no effect on the leakage of Evan's blue. The decrease of membrane permeability of the acid and/or of interaction between membrane and acid by dissociation, as a consequence of pH elevation, may reduce the effects.

[15] To be published.

Citric acid and succinic acid showed a similar concentration *versus* effect relation, *i.e.*, the activity increased with concentration to attain a maximum activity >10%. The exudation of body fluid out of the vaginal mucous membrane because of the hypertonicity of the acids may be one of the factors causing saturation of the promoting effect.

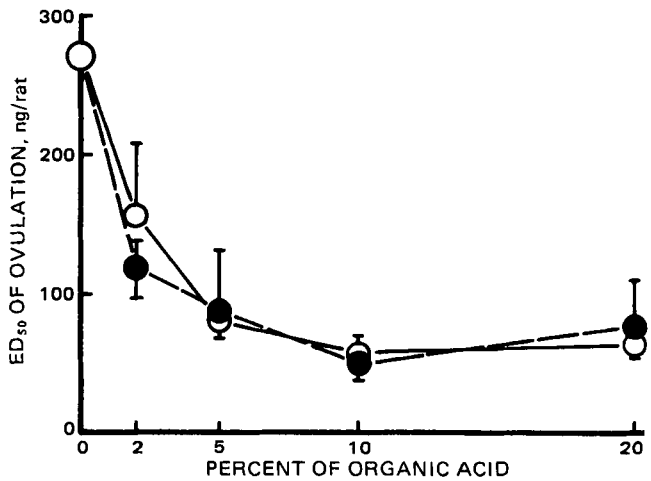

Figure 2—*Ovulation-inducing activity of leuprolide after vaginal administration with citric acid and succinic acid at the different concentrations to diestrous rats. The analog was administered in an oleaginous base containing the acid dispersed as fine powders. Bars represent 95% fiducial limits. Key: (○) citric acid; (●) succinic acid.*

Table IV—Ovulation-Inducing Activity of Leuprolide after Vaginal Administration in Various Kinds of Hydrophilic Jelly to Diestrous Rats [a]

Jelly base [b]	Dose of Leuprolide, ng/rat										ED_{50}, ng/rat
	30	40	50	60	70	80	100	120	150	200	
Methylcellulose, 3%, [d]	—	—	2/9	—	3/9	—	6/9	—	—	—	81.8 (54.2–123) [c]
Methylcellulose, 5% [d]	—	—	1/10	2/10	3/10	8/10	9/10	—	—	—	75.0 (67.1–86.4)
Methylcellulose, 7% [d]	—	—	—	—	0/5	2/10	7/10	—	5/7	—	103.0 (82.7–153)
Methylcellulose, 5% [e]	—	—	1/10	—	4/10	—	5/10	—	—	—	93.6 (70.1–125)
Methylcellulose, 3% [f]	—	—	2/10	—	5/10	—	9/10	—	—		67.4 (54.8–83.2)
Methylcellulose, 2% [g]	—	—	0/10	—	3/10	—	8/16	—	—	—	105.0 (82.2–133)
Mixed gum, 2% [h]	—	—	3/5	—	—	—	—	—	—	—	44.0
Mixed gum, 3% [h]	1/10	3/10	7/10	8/10	—	—	9/9	—	—	—	45.4 (38.2–53.6)
Carrageenan, 5%	—	—	2/5	—	—	5/5	5/10	—	9/10	—	55.0
Starch, 7%	—	—	1/5	—	2/5	—	—	—	—	—	65.0
Starch, 10%	—	—	1/10	—	—	—	5/5	—	—	—	69.0
Agar, 10%	—	—	0/5	—	—	—	4/5	—	—	—	74.0
Tragacanth gum, 4%	—	—	—	—	0/5	—	—	—	—	—	—
Sodium carboxymethylcellulose, 5%	—	—	—	—	—	—	1/7	—	5/5	—	105.0
Sodium polyacrylate, 5%	—	—	—	—	—	—	1/5	—	2/5	—	160.0
Glycerogelatin, 44.5%	—	—	—	—	—	0/10	2/10	—	4/15	6/9	179.0 (145–349)
Succinated gelatin, 61.2%	—	—	—	—	—	—	—	0/5	—	—	—
Polyethylene glycol 4000, 70%	—	—	—	—	—	—	0/5	—	—	—	—

[a] Number of rats with induced ovulation per number of rats examined. [b] Each jelly was prepared in 5% citric acid solution (pH 3.5). [c] Fiducial limits (95%), ED_{50} without fiducial limits was estimated by using the adequate slope of the dose–response curve. [d] 90SH4000. [e] 90SH8000. [f] 90SH15000. [g] 90SH30000. [h] Xanthan gum and locustbean gum were mixed at the same weight ratio.

Table V—Ovulation-Inducing Activity of Leuprolide after Vaginal Administration in Tablet to Diestrous Rats [a]

Tablet [b]	Dose of Leuprolide, ng/rat									ED_{50}, ng/rat
	10	15	25	50	60	75	100	150	200	
A	0/10	1/10	8/15	5/10	7/10	10/10	8/10	—	—	34.6 (25.1–46.7) [c]
B	—	—	—	—	—	0/5	6/10	—	—	—
C	—	—	—	1/5	—	4/10	4/10	5/10	8/10	114.3 (57.4–256.6)

[a] Number of rats with induced ovulation per number of rats examined. [b] Each tablet consists of leuprolide, citric acid, lactose, corn starch, hydroxypropylcellulose, and magnesium stearate. Citric acid was dispersed as fine powders at the concentration of 10% for tablet A, 5% for tablet B, and 10% as a sodium salt for tablet C. [c] Fiducial limits (95%).

The absorbability of leuprolide from jellies or tablets containing citric acid was examined to determine a practical dosage form. Jellies with a highly polar polymer such as carboxymethylcellulose and polyacrylate reduced the absorption of the analog, whereas those with a less polar polysaccharide elicited sufficient absorption. Since the absorption was reduced by all polymers at a high concentration, the interaction of the analog with the jelly materials appeared to cause the suppression. The tablet containing 10% citric acid allowed considerable absorption of the analog and gave a smaller ED_{50} than the oleaginous suppository.

The local reaction of these suppositories, examined by the leakage of Evan's blue from the vaginal mucosa after a 1-hr treatment, revealed that the tablet and oleaginous suppository exhibited deep staining, while the jelly exhibited only faint staining, or none. This difference may be explained by the fact that citric acid, dispersed as fine particles in the tablet and the oleaginous suppository, is dissolved in exuded body fluids at close to the saturated concentration, resulting in an irritation to the vaginal mucosa. Discernible changes of the mucous membrane, such as destruction or exfoliation of vaginal tract surface cells of rats, were rarely observed by scanning electromicroscopy after 10 consecutive days of administration of jelly containing 5% citric acid[15]. These results indicate that the vaginal jelly will be the most suitable practical dosage form because of the activity with lower concentrations of citric acid, less local reaction, and easy handling due to a high water solubility.

Vaginal administration of leuprolide resulted in a high ovulation-inducing activity, which was enhanced by addition of the carboxylic acids: citric, succinic, tartaric, and glycocholic. It is suggested that vaginal administration of leuprolide in humans can be a rational dosage method especially from the standpoint of self-administration for long-term antitumor therapy.

REFERENCES

(1) M. Fujino, T. Fukuda, S. Shinagawa, S. Kobayashi, I. Yamazaki, R. Nakayama, J. H. Seely, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **60**, 406 (1974).

(2) J. A. Vilchez-Martinez, D. H. Coy, A. Arimura, E. J. Coy, Y. Hirotsu, and A. V. Schally, *ibid.*, **59**, 1226 (1974).

(3) R. H. Rippel, E. S. Johnson, W. F. White, M. Fujino, T. Fukuda, and S. Kobayashi, *Proc. Soc. Exp. Biol. Med.*, **148**, 1193 (1975).

(4) E. S. Johnson, J. H. Seely, W. F. White, and E. R. DeSombre, *Science*, **194**, 329 (1976).

(5) E. R. DeSombre, E. S. Johnson, and W. F. White, *Cancer Res.*, **36**, 3830 (1976).

(6) A. Danguy, N. Legros, J. A. Heuson-Stiennon, J. L. Pasteels, G. Atassi, and J. C. Heuson, *Eur. J. Cancer*, **13**, 1089 (1977).

(7) U. K. Banik and M. L. Givner, *J. Reprod. Fertil.*, **44**, 87 (1975).

(8) A. Corbin, C. W. Beattie, J. Yardley, and T. J. Foell, *Endocr. Res. Commun.*, **3**, 359 (1976).

(9) D. Gonzalez-Barcena, D. H. Coy, A. J. Kastin, K. Nikolics, and A. V. Schally, *Lancet*, **ii**, 997 (1977).

(10) S. J. Nillius, C. Bergquist, and L. Wide, *Contraception*, **17**, 537 (1978).

(11) D. Heber and R. S. Swerdloff, *Science*, **209**, 936 (1980).

(12) A. De La Cruz, K. G. De La Cruz, A. Arimura, D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, and A. V. Schally, *Fertil. Steril.*, **26**, 894 (1975).

(13) D. Gonzalez-Barcena, A. J. Kastin, M. C. Miller, D. S. Schalch, D. H. Coy, A. V. Schally, and A. Escalante-Herrera, *Lancet*, **ii**, 1126 (1975).

(14) N. Nishi, A. Arimura, D. H. Coy, J. A. Vilchez-Martinez, and A. V. Schally, *Proc. Soc. Exp. Biol. Med.*, **148**, 1009 (1975).

(15) S. Jeppsson, S. Kullander, G. Rannevik, and J. Thorell, *Br. Med. J.*, **4**, 231 (1973).

(16) H. G. Solbach and W. Wiegelmann, *Lancet*, **i**, 1259 (1973).

(17) H. G. Dahlén, E. Keller, and H. P. G. Schneider, *Horm. Metab. Res.*, **6**, 510 (1974).

(18) J. P. Bourguignon, H. G. Burger, and P. Franchimont, *Clin. Endocrinol.*, **3**, 437 (1974).

(19) G. Fink, G. Gennser, P. Liedholm, J. Thorell, and J. Mulder, *J. Endocrinol.*, **63**, 351 (1974).

(20) D. Gonzalez-Barcena, A. J. Kastin, D. S. Schalch, D. H. Coy, and A. V. Schally, *Fertil. Steril.*, **27**, 1246 (1976).

(21) G. Potashnik, N. Ben-Adereth, B. Lunenfeld, and C. Rofe, *ibid.*, **28**, 650 (1977).

(22) G. Potashnik, R. Homburg, A. Eshkol, V. Insler, and B. Lunenfeld, *ibid.*, **29**, 148 (1978).

(23) R. F. Lambe, I. Werner-Zodrow, A. Darragh, and M. Mall-Häfeli, *Lancet*, **ii**, 801 (1979).
(24) M. Saito, T. Kumasaki, Y. Yaoi, N. Nishi, A. Arimura, D. H. Coy, and A. V. Schally, *Fertil. Steril.*, **28**, 240 (1977).
(25) I. Yamazaki, H. Nakagawa, K. Yoshida, and R. Nakayama, *Jpn. J. Fertil. Steril.*, **22**, 136 (1977).
(26) R. H. Engel and M. J. Fahrenbach, *Proc. Soc. Exp. Biol. Med.*, **129**, 772 (1968).
(27) K. Arima, A. Kakinuma, and G. Tamura, *Biochem. Biophys. Res. Commun.*, **31**, 488 (1968).
(28) D. J. Finney, "Probit Analysis," Cambridge University Press, 1952.
(29) S. Hwang, E. Owada, T. Yotsuyanagi, L. Suhardja, N. F. H. Ho, G. L. Flynn, and W. I. Higuchi, *J. Pharm. Sci.*, **65**, 1574 (1976).
(30) S. Muranishi, N. Muranushi, and H. Sezaki, *Int. J. Pharm.*, **2**, 101 (1979).
(31) Y. Tokunaga, S. Muranishi, and H. Sezaki, *J. Pharmacobio. Dyn.*, **1**, 28 (1978).
(32) S. Hirai, T. Yashiki, and H. Mima, *Int. J. Pharm.*, **9**, 165 (1981).
(33) C. Peracchia and A. F. Dulhunty, *J. Cell Biol.*, **70**, 419 (1976).

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Effect of Moisture and Crushing Strength on Tablet Friability and *In Vitro* Dissolution

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Abstract □ The friability and dissolution of a formulation of compressed tablets were studied by varying the granulation moisture and tablet crushing strength. A general quadratic response surface model was used to analyze the data. The response surface contour plots of tablet friability consisted of a series of ellipsoidal curves. The optimum friability corresponding to a granulation moisture content and a tablet crushing strength was a simple minimum. The *in vitro* dissolution contour plots showed a stationary ridge system. Along the ridge, a large number of combinations of tablet crushing strength and granulation moisture represented 100% drug dissolution. The contour overlays of friability and dissolution contour plots showed a region where both the friability and dissolution requirement could be met. The analysis of the data by means of multiple linear regression was helpful in understanding the role of granulation moisture and tablet crushing strength on tablet friability and *in vitro* dissolution.

Keyphrases □ Dissolution—*in vitro*, effect of moisture and crushing strength, tablet friability □ Crushing strength—effect on tablet friability and *in vitro* dissolution □ Friability—tablets, effect of moisture and crushing strength

Previous studies (1–3) from these laboratories discussed the interrelationships between moisture, crushing strength, and *in vitro* drug dissolution in compressed tablets. Another physical parameter of importance to the tablet formulators, especially in coating and packaging operations, is friability of compressed tablets. The friabilator[1] (4) provides falling as well as frictional abrasion to the tablet sample and is used to measure the resistance to abrasion or attrition of tablets. The loss of weight is measured after a fixed number of revolutions of a drum rotating at a controlled rate. In the development of tablet dosage forms, formulation factors are generally checked to reduce comparative loss in friability testing. Two types of friabilator[1,2] apparatuses were compared (5) using 10 tablet formulations differing in method of granulation or choice of binder. In all instances the percentage of weight loss was higher with friabilator A[1], the differences ranging from 6.2 to 39.7%, depending upon the formulation. After 25 years of use, it was concluded (6) that the weight loss of not more than 0.8% by friabilator A was valid for the control of most pharmaceutical tablets.

Although friability is generally considered important in the development of tablet formulations, factors affecting friability have not been fully explored. The present report describes a study of the interdependence of tablet friability and *in vitro* drug dissolution on granulation moisture content and tablet crushing strength. The data were analyzed using a general quadratic response surface model and the analysis suggested that rational specifications on the in-process variables such as the granulation moisture and initial tablet hardness could ensure proper control of the tablet friability and *in vitro* dissolution.

EXPERIMENTAL

Materials—The drug, ticlopidine hydrochloride[3], 5-(*o*-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[3,2-*c*]pyridine hydrochloride was at least 99.0% pure. The excipients used were microcrystalline cellulose[4] NF, povidone[5] USP, citric acid[6] USP, stearic acid powder[7] NF, corn starch[8] NF, and lactose[9] USP.

Granulation—The formulation used in this study contained 64.1% drug, 22.4% microcrystalline cellulose, 10% starch, 1% citric acid, 2% povidone, and 0.5% stearic acid. The drug and microcrystalline cellulose were mixed together in a small planetary mixer for 10 min. Povidone and citric acid were dissolved in water and the powder mixture was granulated with the binder solution. The wet granulation was mixed for 10 min and passed through a 1.4-mm aperture and dried in a forced-air oven at 60°

[1] Roche type friabilator A.
[2] Erweka Friability Apparatus B.

[3] Sanofi Research Co. Inc. New York, NY 10019.
[4] Avicel pH 101, FMC Corp., Philadelphia, PA 19103.
[5] GAF Corp. New York, NY 10020.
[6] Mallinckrodt, Inc., St. Louis, MO 63147.
[7] Emery Industries, Inc., Cincinnati, OH 45232.
[8] Staley Manufacturing Co., Decatur, IL 62525.
[9] Regular grade, Foremost Co., San Francisco, CA 94104.

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