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Research Article

Transdermal Dual-Controlled Delivery of Contraceptive Drugs: Formulation Development, *in Vitro* and *in Vivo* Evaluations, and Clinical Performance

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Several transdermal contraceptive device (TCD) formulations were developed to provide a dual-controlled transdermal delivery of levonorgestrel (LN), a potent progestin, and 17β-estradiol (E_2), a natural estrogen. Using a sensitive HPLC method, the *in vitro* release and skin permeation profiles of LN and E_2 from various TCD formulations were simultaneously characterized in the hydrodynamically well-calibrated Valia–Chien skin permeation cells and both were found to follow zero-order kinetics. The rates of drug release and skin permeation were observed to vary significantly depending upon some formulation parameters. Six-month stability studies were performed on seven formulations at room and elevated temperatures (37 and 45°C), and two (Formulations 4 and 5) were found to be acceptable, based on drug recovery, release rate, and skin permeation rate data. Judging from the 6-month accelerated stability studies, it is projected these two formulations will have shelf-life of at least 2 years. As a result of development of an efficient manufacturing process, Formulation 4 was selected for further evaluation. One-week primary skin irritation evaluation in 6 rabbits indicated that Formulation 4 is nonirritating, and it was thus selected for Phase I clinical bioavailability/dose proportionality studies in 12 healthy female volunteers of child-bearing age. Results of pharmacokinetic and pharmacodynamic analyses demonstrated that it is capable of achieving and maintaining a steady-state serum level of LN throughout the 3-week treatment period by weekly applications of one or two TCD patches (10 or 20 cm^2). A dose proportionality was obtained in the serum drug levels, daily dose delivered, and contraception efficacy. An excellent correlation was obtained for the rates of transdermal delivery determined by the *in vitro* studies using human cadaver skin, the *in vivo* studies in rabbits, and the clinical studies in living subjects.

KEY WORDS: transdermal contraceptive devices; levonorgestrel/estradiol combination; formulation design; *in vitro/in vivo* clinical evaluations; stability testing; dermal irritation test.

INTRODUCTION

Various reversible contraceptive methods, such as oral contraceptive pills, intrauterine devices, condom, and diaphragm, have been widely used by many millions of women throughout the world. Among these methods, the oral contraceptive pills have enjoyed the most popularity as a result of their extremely high efficacy of fertility control and convenience of oral administration (1).

However, several major side effects have reportedly been associated with the administration of oral contraceptive. These side effects include thrombosis, myocardial infarction, and hypertension and have been largely attributed to the estrogen component in the pills (2–4). Use of the progestin-only preparations (minipills) have been found to decrease the side effects, but contraceptive efficacy is somewhat reduced and the menstrual cycle also becomes irregular. However, it has been reported that less incidence of irregular bleeding is observed if the progestin is administered at a more constant rate of delivery (5,6). Besides the side effects, the oral contraceptives also have the disadvantage of relying highly on patient compliance. The risk of pregnancy is known to increase with each pill missed (7).

An ideal and patient-acceptable contraceptive delivery system should provide the advantages of (i) a high efficacy of fertility control, (ii) minimum side effects, (iii) increased ease of administration, (iv) improved patient compliance, and (v) rapid termination of treatment, if needed. Transdermal controlled delivery of contraceptive drugs is expected to meet these criteria.

The interest in using the intact skin as the port of drug administration to the human body has been shown for several decades (8). The feasibility of transdermal drug delivery

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(TDD) for long-term systemic medication (24 hr or longer) has first been demonstrated by the development of a scopolamine-releasing TDD system for 72-hr prophylaxis of motion-induced sickness and then by the successful marketing of several nitroglycerin-releasing TDD systems for once-a-day medication of angina pectoris as well as very recently by the regulatory approval of a clonidine-releasing TDD system for weekly management of hypertension and of an estradiol-releasing TDD system for twice-a-week therapy of postmenopausal syndromes (9).

Over the years, several transdermal drug delivery devices have been developed at this Research Center, based on a patentable micro-drug-reservoir technique (10), to deliver systematically effective drugs at a controlled rate with enhanced skin permeability (11). The same controlled drug delivery technique has also been applied to the development of a transdermal contraceptive device to achieve a dual-controlled release of levonorgestrel, a potent synthetic progestin, and estradiol, a natural estrogen, continuously for a period of 7 days (12–15). This once-a-week transdermal contraceptive device will permit a female user to wear only one patch per week, beginning on Day 5 of the individual's cycle, for 3 consecutive weeks during each cycle (3 weeks "on" and 1 week "off") to achieve fertility regulation (16).

MATERIALS AND METHODS

Materials

All materials were obtained commercially and used as received. Release liner and backing laminate were obtained from 3M Co. (St. Paul, MN). Medical grade silicone elastomer (DC-382), silicone fluid (DC-360), and silicone adhesive (DC-355) were obtained from Dow Corning Corp. (Midland, MI). Levonorgestrel was obtained from Wyeth Laboratories (Philadelphia, PA) and 17β-estradiol was purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents such as polyethylene glycol 400 and acetonitrile (HPLC grade) were purchased from Fisher Scientific Co. (Springfield, NJ).

Formulation Development

The transdermal contraceptive device (TCD) was fabricated by first homogeneously dispersing the drug, along with a dispersing medium, as discrete microreservoirs in silicone medical-grade elastomer and then cross-linking the elastomer, between sheets of release liner and backing laminate, in a specially designed device maker. After curing, the device was removed and cut into patches of desired shapes and sizes using a specially designed stainless-steel die cutter.

During formulation development phase, a series of studies was performed to investigate various aspects of formulation design of TCD patches. The effect of variation in drug loading, composition of the microreservoir compartment and its density in the polymer matrix, adhesive coating, and incorporation of skin permeation enhancers on the release and skin permeation rate profiles of levonorgestrel and estradiol from the TCD patches was evaluated quantitatively.

In Vitro Drug Release and Skin Permeation Studies

The drug release and skin permeation rate profiles from the TCD patches were investigated in a hydrodynamically well-calibrated Valia–Chien skin permeation system (17), at 37°C, with (for permeation studies) or without (for release studies) a skin specimen which was freshly excised from the abdominal region of hairless mice (female, 5–7 weeks old) or human cadaver. Saline solution, which contained 40% (v/v) PEG 400 to maintain sink conditions throughout the experiment, was used as the elution medium. Samples were taken at regular time intervals from the elution medium and the concentrations of levonorgestrel and estradiol in each sample were assayed simultaneously by a sensitive HPLC method.

The use of 40% PEG 400 in saline as the receptor solution was observed to prevent the bacterial overgrowth during the long-term skin permeation studies. Integrity of skin tissues was examined microscopically. No hydration of the stratum corneum occurred, in response to the long-term application of TCD patches, to an extent which affects the steady-state skin permeation profiles of both estradiol and levonorgestrel. Earlier studies with long-term permeation kinetics of estradiol in the same system demonstrated that freshly excised hairless mouse skin has maintained viable enzyme activities for the metabolism of estradiol and its esters (18,19).

The abdominal skin freshly excised from the hairless mouse (female, 5–7 weeks old) was used in the formulation development and stability studies. Because of its availability and easy control in its sex, age, and skin region as well as the possibility of removing the skin specimen just before an experiment is initiated, the results generated over the years indicated that reproducible data are constantly obtained, which permits one to evaluate the effect of variation in formulation parameters on skin permeation kinetics as well as to assess the changes in the physicochemical stability of TCD patches with a high degree of confidence. However, the skin permeation rates of both estradiol and levonorgestrel from the TCD patch formulations were always verified by conducting *in vitro* permeation studies using female human cadaver skin before a final formulation was selected for stability, dermatotoxicity, and clinical studies. The data accumulated to date suggested that the permeation rates of both estradiol and levonorgestrel across the female human cadaver skin are about one-third of that generated from the female hairless mouse skin.

Analytical Method

Assay of levonorgestrel and estradiol was performed using a HP 1090 HPLC system equipped with a HP 1040A diode-array detector and a HP 3392 integrator (Hewlett Packard, Mountainview, CA). An HP Hypersil ODS column [5 μm, 200 × 4.6 mm (i.d.); Hewlett Packard] was used as the analytical column and an acetonitrile–water (50:50) combination was used as the mobile phase at a flow rate of 1.5 ml/min. The analytical column was maintained at 37°C, the same temperature as for drug release and skin permeation studies, to minimize the potential drug precipitation in the column. With an injection volume of 5 μl and the detector wavelength first set at 210 nm and then switched to 245 nm

at the time of 4 min, the chromatographic peaks for estradiol and levonorgestrel were well resolved from each other at retention times of 3.18 and 5.30 min, respectively.

This set of HPLC conditions has a sensitivity of 0.1 μg/ml (at a peak/noise ratio of 5:1) with an injection-to-injection reproducibility of <1.0% variation and an inter-day variability of <3.0% variation. A linear peak height vs concentration relationship was established with a correlation coefficient of >0.999. The external standard established by a series of at least five reference concentrations was used to quantitate the amounts of estradiol and levonorgestrel in the samples.

Extraction Procedure

To determine the actual drug content in each 10-cm^2 TCD patch, the patches (including the drug reservoir polymer layer, backing laminate, and release liner, which was peeled off first) were each extracted with 40 ml of methanol three times for the duration of 2, 4, and 4 hr, respectively. The extracts for each individual patch were combined and assayed for estradiol and levonorgestrel by the HPLC method outlined above.

Near 100% recovery was achieved for both drugs by the first two extractions. Using the extraction procedure outlined above, reproducibility of drug recovery was constantly obtained with a coefficient of variation of less than 5% (n = 3). In view of its reproducibility, this extraction procedure was applied to recover the drug content in the TCD patches for Q.C. release, stability testing, and *in vivo*/clinical evaluation.

Stability Evaluation

The TCD patch formulations were fabricated, according to the fabrication procedure outlined above, and submitted to long-term (6-month) stability evaluation program under three storage conditions, i.e., 25, 37, and 45°C. TCD patches were individually sealed in paper/foil/polyethylene pouch (3 × 4 in.) and stored in the stability testing chambers (Gravity Convection Incubator, GCA Corp., Chicago, IL). Throughout the course of stability evaluation, triplicate samples each were taken, at each of the 16 sampling points, from each storage condition and evaluated for physical and chemical stability. Drug release and skin permeation rate profiles were used as the physical stability indicators. On the other hand, drug recovery assay, which was performed by using a solvent extraction procedure followed by HPLC assay of extracts (outlined above), was used as the chemical stability indicator. A 95% confidence interval, which was established using the mean value and standard deviation of each stability indicator generated from nine patch samples at week "0," was used to determine the stability of each patch formulation being tested.

Dermatotoxicity Testing

A 7-day primary skin irritation testing (modified Draize Test) was carried out in two groups of New Zealand White rabbits (females, nulliparous and not pregnant) (Group I, three rabbits as the negative controls; Group II, six rabbits that each received two placebo and two medicated patches simultaneously). The dosage of two placebo and two medicated patches applied to each animal was selected in order to delineate possible adverse reactions evoked using the maximal number of patches possible that could be applied. Also, the clinical protocol called for application of two patches to each human subject included in Group B of the Phase I Clinical Study. During the in-life phase (prior to sacrifice), the animals were observed for toxic signs and body weights were recorded weekly. After 1 week, the patches were removed and the skin application sites were graded for the occurrence of erythema and edema. The patches were also assayed to determine residual drug content and the dosages of levonorgestrel and estradiol delivered were calculated. At sacrifice, the organs and tissues were examined for gross changes. Histopathological examination of the skin taken from the application site was also performed.

Phase I Clinical Studies

Phase I clinical bioavailability and dose proportionality studies were conducted in 12 healthy female volunteers for 8 weeks, consisting of a 4-week pretreatment period (one menstrual cycle) followed by a 4-week treatment period (one menstrual cycle). They were randomly divided into two groups of six subjects: one group (Group A) received one TCD patch per week, while the other group (Group B) received two TCD patches per week consecutively for 3 weeks, beginning on Day 5 of the cycle for each subject. In the fourth week, the medicated patches applied in the third week were removed and replaced with placebo (nonmedicated) patches. All the patches were applied on the lower abdominal region of each subject. Blood samples were taken at regular intervals, during pretreatment, treatment, and posttreatment periods for radioimmunoassay (RIA) of serum concentrations of levonorgestrel and progesterone. Because of the potential interference of endogenous estrogens in the healthy female volunteers, no RIA determination of total estrogen levels in the serum samples was performed. The patches removed at the end of each 7-day application were first subjected to solvent extraction and the amount of drug extracted from each patch was then assayed individually using the sensitive HPLC method outlined above.

RESULTS AND DISCUSSION

Formulation Development and *in Vitro* Evaluations

The microscopic structure of the micro-drug-reservoir composition in the transdermal contraceptive device (TCD) is shown microphotographically in Fig. 1. *In vitro* drug release kinetics studies indicated that levonorgestrel and estradiol release from the TCD patches at a zeroth-order kinetic profile, with levonorgestrel releasing at a mean rate which is slightly higher than that of estradiol (0.82 vs 0.72 μg/cm^2/hr) (Fig. 2). The *in vitro* skin permeation studies also demonstrated that both levonorgestrel and estradiol permeate through the human cadaver skin at zeroth-order kinetic profile, in which levonorgestrel permeates through the skin at a mean rate (0.12 μg/cm^2/hr) which is slightly lower than that of estradiol (0.16 μg/cm^2/hr) (Fig. 3). But the difference in the skin permeation rates between levonorgestrel and estradiol is not statistically significant. The data also showed that the skin permeation of levonorgestrel requires a time lag

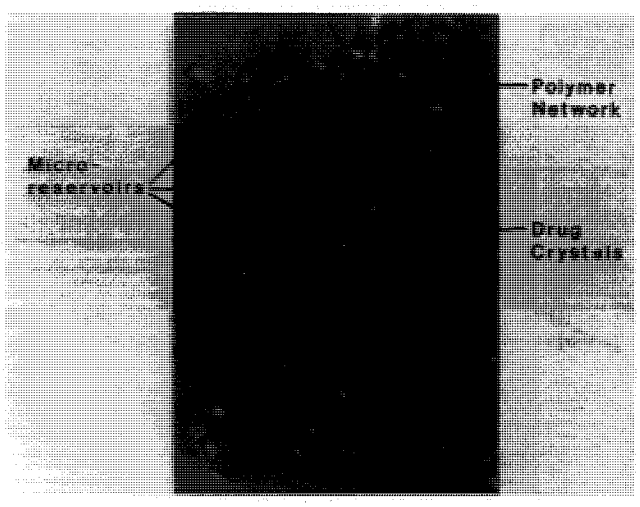

Fig. 1. Photographic illustration of the transdermal contraceptive device showing the microstructure under the microscope.

of 21.1 (±2.9) hr to reach the steady-state permeation flux, while estradiol takes a shorter lag time of 8.9 (±1.4) hr. The difference in the lag time between these two drugs is statistically significant ($P > 0.05$).

Results from a series of formulation development studies demonstrated that the rates of release and skin permeation of levonorgestrel and estradiol are dependent upon a number of system parameters which can be summarized as follows.

(1) Effect of Drug Loading. While increasing the loading dose of estradiol from 2.5 to 10% and that of levonorgestrel from 1 to 15% in the microreservoir, the release rates of estradiol and levonorgestrel from TCD patches were observed to increase linearly; the skin permeation rate for levonorgestrel was enhanced initially and then leveled off at 5% loading dose, while no enhancement was noted for estradiol.

(2) Effect of Microreservoir Composition. While increasing the volume fraction of PEG 400, a solubilizer for both levonorgestrel and estradiol, in the microreservoir, the drug release rate from the TCD patches was observed to increase linearly for estradiol but not for levonorgestrel. However, the skin permeation rates of levonorgestrel and estradiol were both seen to increase initially and then leveled off as the microreservoir contained more than 15–20% (v/v) of PEG 400.

(3) Effect of Density of Microreservoir. While increasing the density (weight fraction) of the microreservoir compartment in the polymer matrix, the release rate of the drug from the TCD patches was noted to increase linearly for levonorgestrel; but for estradiol, it increased linearly at the beginning and then reached a maximum level at the density of 12.5% (w/w). The skin permeation rate was also observed to increase initially and then reached the maximum rate at 5% (w/w) for estradiol and 9% (w/w) for levonorgestrel.

(4) Effect of Adhesive Coating. Coating a layer of silicone-based pressure-sensitive adhesive polymer on the drug-releasing surface of TCD patches was observed to reduce the drug release rate by 24% for estradiol and by 26% for levonorgestrel, but the skin permeation rate was decreased by more than twofold for levonorgestrel, while only slightly for estradiol.

(5) Effect of Skin Permeation Enhancers. Incorporation of skin permeation enhancers, such as isopropyl myristate (IPM), in the adhesive polymer was noted not only to overcome the diffusional resistance of adhesive polymer, but also to increase the skin permeation rate by twofold for estradiol and more than threefold for levonorgestrel. The extent of enhancement in the skin permeability of estradiol and levonorgestrel was found to be dependent upon the concentration of IPM incorporated into the adhesive polymer.

Based on the information generated above during the formulation development phase, seven TCD patch formulations were developed (Table I). These formulations were found to be capable of achieving a dual-controlled transder-

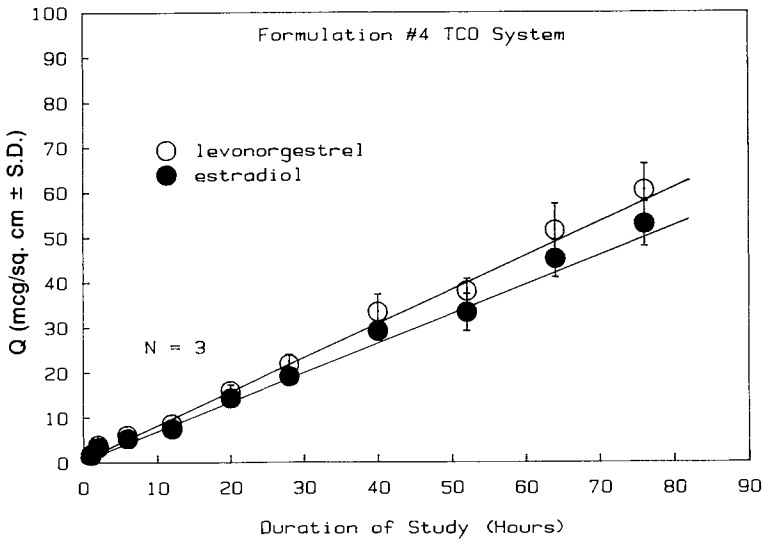

Fig. 2. Comparative zeroth-order release profiles of levonorgestrel and estradiol delivered by the transdermal contraceptive devices (Formulation 4) developed.

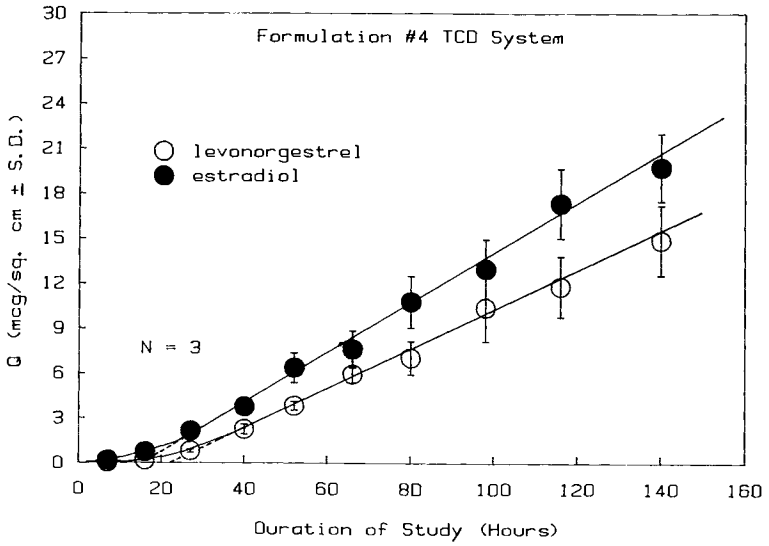

Fig. 3. Comparative zeroth-order permeation profiles of levonorgestrel and estradiol across the human cadaver skin following the delivery by the transdermal contraceptive devices (Formulation 4) developed.

mal delivery of estradiol and levonorgestrel at permeation rates that meet the target daily dosage rate (25–50 μg/day for estradiol and 5–50 μg/day for levonorgestrel with a patch size of 10 cm^2) as specified by NICHD/NIH. The long-term (6-month) physical and chemical stabilities of these formulations were also evaluated at three storage conditions. The results obtained with Formulation 4 are shown in Figs. 4–6. Among these TCD patch formulations studied, two (Formulations 4 and 5) have demonstrated excellent physical and chemical stabilities, with a projected shelf-life of at least 2 years. As a result of development of an efficient manufacturing process, Formulation 4 was selected for dermatotoxicity studies in rabbits.

Table I. Human Cadaver Skin Permeation Profiles of Estradiol and Levonorgestrel Delivered by Various Transdermal Contraceptive Device Patch Formulation

Formulation	Permeation rate (μg/cm^2/hr ± SD)[a]	Lag time (hr ± SD)[a]
	Estradiol	
1	0.12 (±0.03)	16.2 (±2.2)
2	0.16 (±0.03)	17.8 (±3.1)
3	0.49 (±0.10)	17.3 (±3.9)
4	0.16 (±0.03)	8.9 (±1.4)
5	0.14 (±0.03)	9.8 (±1.7)
6	1.18 (±0.24)	3.2 (±0.5)
7	0.68 (±0.14)	1.1 (±0.1)
	Levonorgestrel	
1	0.07 (±0.02)	33.7 (±3.9)
2	0.12 (±0.02)	34.5 (±5.1)
3	0.28 (±0.05)	31.8 (±4.4)
4	0.12 (±0.03)	21.1 (±2.9)
5	0.14 (±0.03)	24.4 (±3.2)
6	0.09 (±0.02)	5.2 (±0.6)
7	0.09 (±0.02)	3.7 (±0.5)

[a] Mean ± standard deviation (n = 3).

Dermatotoxicity Studies

To explore any skin irritation potential of the TCD patches developed and also to demonstrate their safety as required for regulatory submission before the initiation of a Phase I clinical bioavailability–dose proportionality study in human volunteers, a 1-week primary skin irritation study was initiated in six New Zealand female rabbits (with another three rabbits serving as the controls).

Placebo (nonmedicated) and medicated patches were prepared under cGMP conditions. After Q.C. release, each of the six rabbits in the treatment group received two placebo and two medicated patches on its shaved dorsal skin for 1 week. The results indicated that over the 1-week treatment period, a total of 439.6 (±59.2) μg of levonorgestrel and 560.0 (±28.2) μg of estradiol has been released to each rabbit

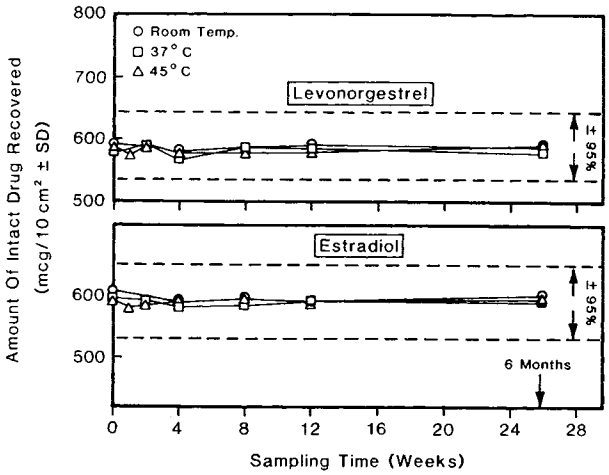

Fig. 4. Long-term (26-week) chemical stability profiles of levonorgestrel and estradiol, using the drug recovery data as the indicator, in the transdermal contraceptive devices under three storage conditions.

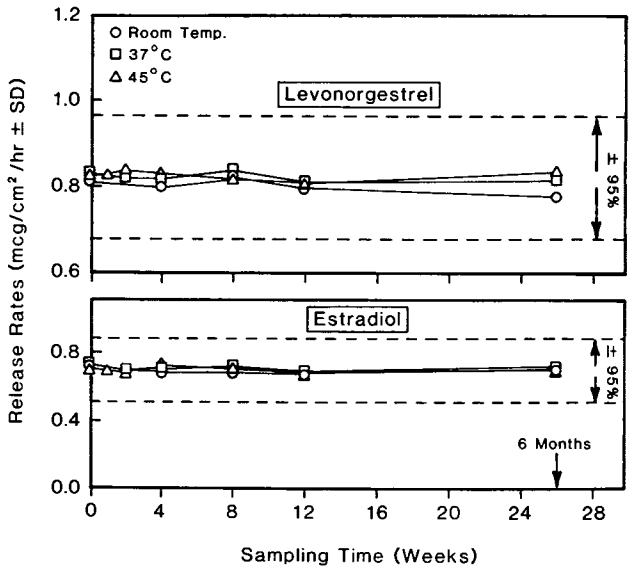

Fig. 5. Long-term (26-week) physical stability profiles of levonorgestrel and estradiol, using the drug release kinetics data as the indicator, in the transdermal contraceptive devices under three storage conditions.

treated. The data can be translated to suggest that a daily dosage of 28.6 (±3.8) μg/kg/day for levonorgestrel and 36.4 (±1.8) μg/kg/day for estradiol has been administered to each rabbit on body-weight basis. With these daily dosages delivered, the in-life studies demonstrated that compared to the controls, both the medicated and the placebo patches have been well tolerated, without the occurrence of any significant dermatotoxicity (with a irritation score of 0.6 for medicated patch, as compared to 0.3 for placebo patch, while a grade of 1.0 denotes the observation of a very slight irritation). All rabbits completing the 1-week treatment were observed in good condition, with no significant loss in body weight. Microscopic examination of skin excised from the patch application sites did not show compound-related changes. A trace to slight increase in the thickness of medicated epidermis layers (acanthosis) occurred to a similar degree in placebo and medicated patch sites; this is a nonspecific effect of patch application, probably due to retention of moisture. Trace to slight leukocytic infiltration was observed in skin excised from nonmedicated controls and from both placebo and medicated patch application sites; this is attributed to skin preparation (clipping and shaving).

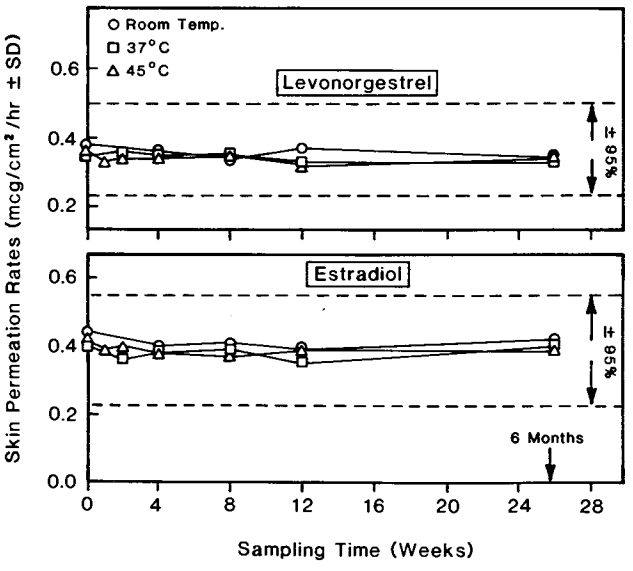

Fig. 6. Long-term (26-week) physical stability profiles of levonorgestrel and estradiol, using the skin permeation kinetics data as the indicator, in the transdermal contraceptive devices under three storage conditions.

The expected maximum clinical doses to be delivered in Phase I clinical bioavailability and dose proportionality studies in human subjects are 57.6 μg/day (or 1.15 μg/kg/day) of levonorgestrel and 76.8 μg/day (or 1.54 μg/kg/day) of estradiol (which were calculated from the *in vitro* human cadaver skin permeation data in Table I, using Formulation 4 with a patch size of 20 (or 2 × 10) cm^2 and 50 kg as the body weight). This expected clinical dose is at least 20 times less than the dosages (28.6 μg/kg/day for levonorgestrel and 36.4 μg/kg/day for estradiol) delivered *in vivo* to each rabbit during the 1-week skin primary irritation study. Based on the results, the toxicologist and clinician both concluded that the TCD patch (Formulation 4) developed is reasonably safe for Phase I clinical bioavailability–dose proportionality studies.

Phase I Clinical Bioavailability Studies

Upon completion of the 1-month pretreatment cycle, the subjects were immediately admitted to the 1-month treat-

Table II. Transdermal Controlled Delivery of Levonorgestrel from a TCD[a] System in the Healthy Female Volunteers

Subject code	Amount delivered (μg/week) Week 1	Week 2	Week 3	$\bar{x}$(±SD)[b] (n = 3)
Group A (one patch)				
KP	235.3	136.8	211.8	194.6 (51.4)
RA	228.6	211.4	198.6	212.9 (15.1)
DK	186.6	194.6	183.6	188.3 (5.7)
MM	235.4	91.3	205.4	177.4 (76.0)
RD	242.0	186.2	202.4	210.2 (28.7)
GL	196.1	186.4	141.3	174.6 (29.2)
$\bar{x}$(±SD)[c]	220.7	167.8	190.5	
(n = 6)	(±23.3)	(±44.9)	(±25.9)	
Group B (two patches)				
IP	418.5	425.0	388.9	410.8 (19.2)
IR	372.1	388.7	381.4	380.7 (8.3)
MG	373.2	337.7	396.6	369.2 (29.7)
IC	421.9	408.5	408.0	412.8 (7.9)
KG	443.4	440.2	389.5	424.4 (30.2)
KB	412.4	430.7	435.8	426.3 (12.3)
$\bar{x}$(±SD)[c]	406.9	405.1	400.0	
(n = 6)	(±28.5)	(±37.7)	(±19.7)	

[a] Transdermal contraceptive delivery.
[b] Mean (±standard deviation) for the weekly levonorgestrel dose delivered to the same subject in 3 consecutive weeks.
[c] Mean (±standard deviation) for the weekly levonorgestrel dose delivered to different subjects in the same week.

Table III. Transdermal Controlled Delivery of Estradiol from a TCD[a] System in the Healthy Female Volunteers

Subject code	Amount delivered (μg/week) Week 1	Week 2	Week 3	$\bar{x}$(±SD)[b] (n = 3)
Group A (one patch)				
KP	291.6	246.8	275.6	271.3 (22.7)
RA	255.8	298.9	279.4	278.0 (21.6)
DK	248.8	273.7	281.4	270.0 (17.0)
MM	303.1	258.8	296.8	286.2 (24.0)
RD	321.6	261.8	293.7	292.4 (29.9)
GL	251.7	323.6	299.8	291.7 (36.6)
$\bar{x}$(±SD)[c]	278.8	277.3	287.8	
(n = 6)	(±30.8)	(±28.8)	(±10.2)	
Group B (two patches)				
IP	529.4	513.4	585.6	542.8 (37.9)
IR	646.4	610.4	600.2	619.0 (24.3)
MG	543.2	617.8	623.1	594.7 (44.7)
IC	583.0	647.6	630.8	620.5 (33.5)
KG	545.2	577.6	645.0	589.3 (50.9)
KB	631.8	584.1	639.0	617.6 (46.1)
$\bar{x}$(±SD)[c]	579.8	591.8	620.6	
(n = 6)	(±49.5)	(±45.9)	(±23.2)	

[a] Transdermal contraceptive delivery.
[b] Mean (±standard deviation) for the weekly estradiol dose delivered to the same subject in 3 consecutive weeks.
[c] Mean (±standard deviation) for the weekly estradiol dose delivered to different subjects in the same week.

Table V. *In Vivo–In Vitro* Correlation in Transdermal Dual-Controlled Delivery of Contraceptive Drugs

Experimental conditions	Transdermal permeation rate ($\bar{x}$ ± SD μg/10 cm²/day) Levonorgestrel	Estradiol
(A) *In vitro* studies		
Human cadaver skin (n = 3)	28.8 (±7.2)	38.4 (±7.2)
(B) *In vivo* studies		
Ovary-intact rabbits (n = 12)	31.4 (±4.2)	40.0 (±2.0)
(C) Clinical studies		
Group A females (n = 18)	27.6 (±2.3)	40.2 (±3.4)
Group B females (n = 36)	28.9 (±1.7)	43.1 (±3.6)

ment cycle and each subject received either one or two TCD patches per week consecutively for 3 weeks, beginning on Day 5 of the cycle for each subject. In the fourth week, the medicated patches were removed and replaced with same size placebo (nonmedicated) patches. All the patches were fabricated under cGMP conditions and, after Q.C. release, were applied on the lower abdominal region of each subject.

The patches removed from each subject at the end of 7-day treatment were assayed for residual drug content using the solvent extraction drug recovery procedure and the sensitive HPLC method outlined earlier. The assay data on the amount of levonorgestrel and estradiol delivered from each of the medicated patches during each weekly application on the subjects in Groups A and B are summarized, respectively, in Tables II and III. The results indicate that the doses of estradiol and levonorgestrel delivered weekly vary from one subject to another, but this intersubject variability is not statistically significant ($P > 0.05$). The weekly doses of estradiol and levonorgestrel also vary from one week to an-

Table IV. Clinical Pharmacokinetics of Levonorgestrel Delivered from TCD Patches[a]

Subject code	Total dose delivered (μg)[b]	Daily dose (μg/day ± SD)	AUC (pg · hr/ml)[c]	AUC/dose (hr/ml × 10^6)
Group A (one patch)				
KP	583.9	27.8 (7.3)	37,350	64.0
RA	638.6	30.4 (2.2)	45,603	71.4
DK	564.8	26.9 (0.8)	30,896	54.7
MM	532.1	25.3 (10.9)	72,105	135.5
RD	630.6	30.0 (4.1)	70,057	111.1
GL	523.8	24.9 (4.2)	56,707	108.3
Mean (±SD)[d]	579.0 (48.3)	27.6 (2.3)	52,120 (17,048)	90.8 (32.0)*
Group B (two patches)				
IP	1,232.4	58.7 (2.7)	118,922	96.5
IR	1,138.6	54.4 (1.2)	60,334	53.0
MG	1,108.5	52.7 (4.2)	79,961	72.1
IC	1,238.4	59.0 (1.1)	94,373	76.2
KG	1,274.1	60.6 (4.3)	105,303	82.6
KB	1,278.6	60.9 (1.8)	27,629	21.6
Mean (±SD)[d]	1,211.8 (71.4)	57.7 (3.4)	81,087 (33,118)	67.0 (26.4)*

[a] Formulation 4 transdermal contraceptive device (TCD) (10 cm²/patch).
[b] Total amount of levonorgestrel dose delivered to individual subject over three weekly applications.
[c] Area under plasma levonorgestrel concentration–time profile in 0–648 hr.
[d] Mean (±standard deviation) of various values obtained for six subjects in either Group A or Group B.
* The difference is not statistically significant (P = 0.1894).

other, but the interweek variability for all the subjects also is not statistically significant ($P > 0.05$).

Analysis of the data in Table II shows the attainment of dose proportionality for transdermal delivery of levonorgestrel: the six subjects in Group B (each received two TCD patches weekly) have been administered a mean weekly levonorgestrel dose (404.03 ± 27.93 μg/wk), which is twice the dose (192.99 ± 38.06 μg/wk) delivered weekly to each of the six subjects in Group A (each received one patch weekly). The same dose proportionality is also observed for estradiol: 597.42 (±42.51) vs 281.27 (±24.00) μg/wk (Table III). The results also point out that no statistical difference between groups can be assessed in the mean weekly doses of estradiol and levonorgestrel delivered that were normalized by applied dose in each 10-cm^2 patch.

The daily doses of levonorgestrel delivered transdermally to each of the 12 subjects during the three weekly topical applications of TCD patches were also calculated and are summarized in Table IV. The results indicate that, on average, a daily levonorgestrel dose of 27.6 (±2.3) and 57.7 (±3.4) μg/day, respectively, has been delivered to each subject in Group A and Group B given one or two 10-cm^2 patches, respectively.

Additionally, the mean daily dosage rate of levonorgestrel delivered from each 10-cm^2 patch (27.6–28.9 μg/10 cm^2/day) to each of the 12 subjects during the three weekly treatments is in fairly good agreement with the projected daily dosage rate (28.8 μg/10 cm^2/day) determined from the *in vitro* skin permeation studies using human cadaver skin as well as the *in vivo* daily dosage rate (31.4 μg/10 cm^2/day) delivered to the rabbits during the week-long skin primary irritation studies (Table V). Good agreement was also achieved among the *in vitro, in vivo,* and clinical data for estradiol.

The serum data obtained were analyzed pharmacokinetically in Figs. 7 and 8. The results indicate that by weekly application of transdermal contraceptive devices (one patch for Group A and two patches for Group B), a fairly constant serum level of levonorgestrel was achieved for the first 2 weeks of the treatment [84.7 (±8.3) pg/ml for Group A and 133.7 (±13.1) pg/ml for Group B], while in the third week the serum levels were observed to increase by approximately 30–40% [122.6 (±21.0) pg/ml for Group A and 177.4 (±28.3) pg/ml for Group B]. The observed higher serum levels achieved in the third week of the treatment cannot be related to the weekly dosages of levonorgestrel delivered, since the levonorgestrel doses delivered in the third week show no significant difference from the first or second week (Table II). Based on the existing data available to date, no suitable explanation could be provided for this unexpected phenomenon. Further studies are planned to explore the possibility that either the skin tissue has become saturated during the first 2 weeks of the treatment or the sex hormone-binding globulins have been saturated and/or its concentration has been altered by levonorgestrel (20). But in any case, the serum levels of levonorgestrel attained in all 3 weeks also show some proportionality as that demonstrated by the weekly doses delivered to the subjects in Groups A and B (Tables II and III).

Further analysis indicated that the mean serum concentrations of levonorgestrel in the initial treatment period increase gradually at zero-order kinetics with a rate constant of 1.99 pg/ml · hr for Group A and 1.93 pg/ml · hr for Group B (Fig. 9). The steady-state serum levels were attained within 30–36 hrs, which suggest the possibility of skin binding of levonorgestrel. Following removal of the TCD patch, the serum levonorgestrel declined by first-order kinetics (Fig. 10), with elimination rate constants of 0.0180 (±0.0031) hr^{-1} for Group A and 0.0132 (±0.0021) hr^{-1} for Group B. The difference in the elimination rate constants between Group A and Group B is not statistically significant ($P > 0.05$). These low rate constants of elimination are translated

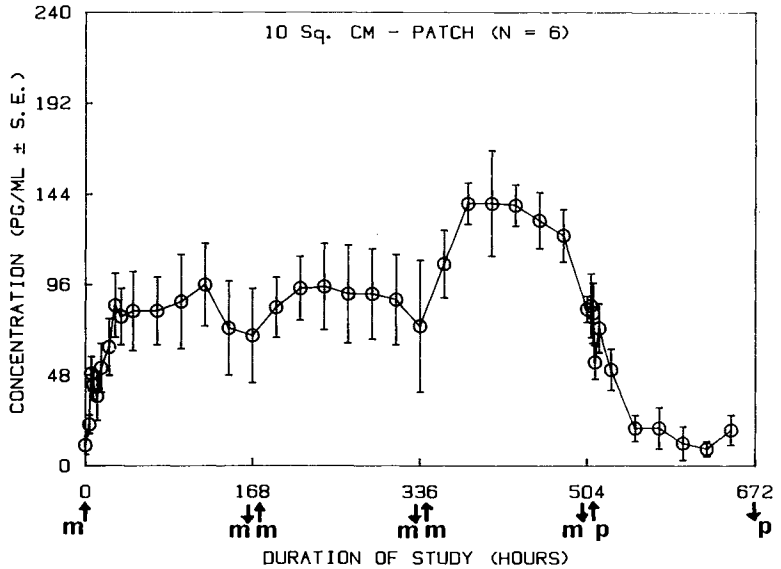

Fig. 7. Serum concentration profiles of levonorgestrel in the six healthy female volunteers in Group A. Each received one 10-cm^2 patch of transdermal contraceptive device per week, beginning on Day 5 of the cycle of each subject, consecutively for 3 weeks. In Week 4, the medicated patches were removed and replaced with placebo patches. (m: mediated patch; p: placebo patch; ↑: patch applied; ↓: patch removed).

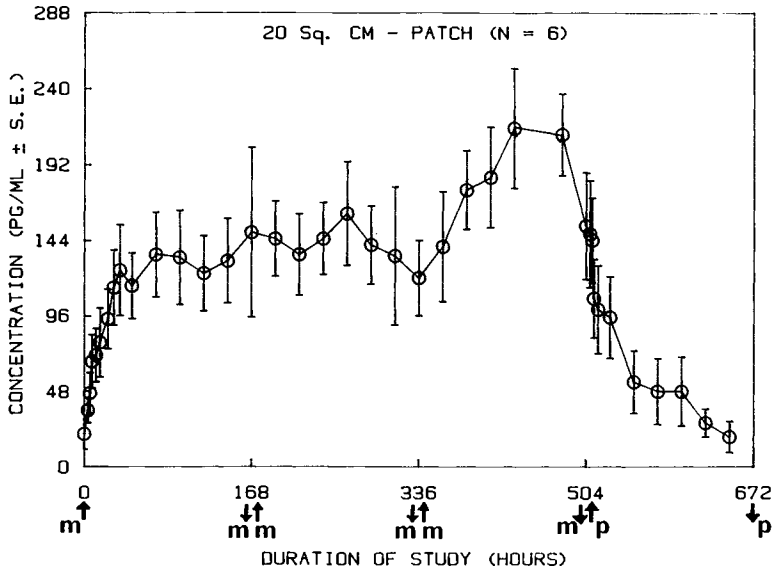

Fig. 8. Serum concentration profiles of levonorgestrel in the six healthy female volunteers in Group B. Each received two 10-cm^2 patches of transdermal contraceptive devices per week, beginning on Day 5 of the cycle of each subject, consecutively for 3 weeks. In Week 4, the medicated patches were removed and replaced with placebo patches. (m: medicated patch; p: placebo patch; ↑: patch applied; ↓: patch removed).

into a long elimination half-life for levonorgestrel [38.5 (±6.6) hr for Group A and 52.5 (±8.4) hr for Group B]. The values of elimination half-life obtained in this investigation are significantly greater than the 13.8 (±5.6) hr after i.v. administration, 11.9 (±1.7) hr after oral administration, and 15.6 (±6.4) hr after intravaginal administration of levonorgestrel (21). The observed long elimination half-life may suggest the existence of skin depot for levonorgestrel following transdermal delivery.

It is encouraging to note that the long time lag observed in the *in vitro* skin permeation studies for levonorgestrel [21.2 (±2.9) hr for Formulation 4; Table I] does not occur in the transdermal drug delivery to the living human body. The observed difference in lag time could be attributed to the difference in the skin used in the *in vitro* permeation kinetics studies and in the clinical pharmacokinetics studies. In the *in vitro* studies, the skin specimen (with a thickness of 200–250 μm) obtained from the left anterior thigh region of an adult female Caucasian cadaver was used, while in the clinical studies, the TCD patches were applied to the lower abdominal region of young female caucasian subjects. In the living human body, the drug molecules which are absorbed by the

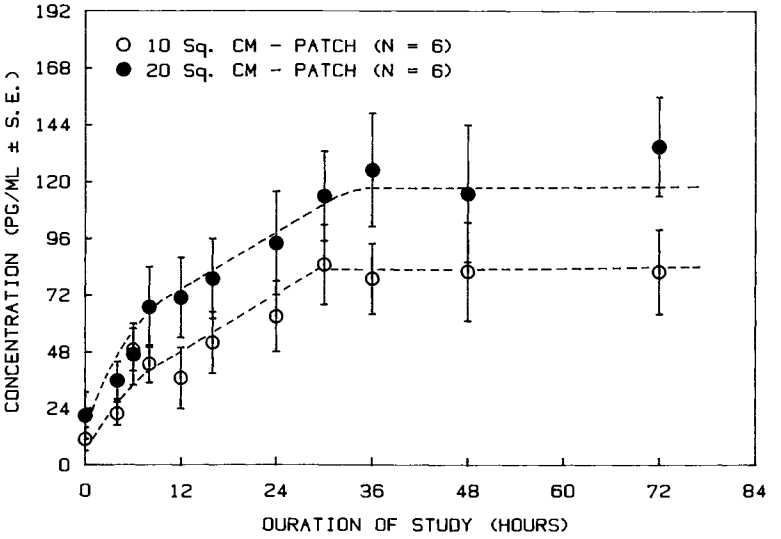

Fig. 9. Serum concentration profiles of levonorgestrel during the absorption phase of transdermal drug delivery in both Group A and Group B subjects. The zeroth-order rate of absorption is 1.99 and 1.93 pg/ml · hr, respectively, for subjects (n = 6 each) in Group A and Group B.

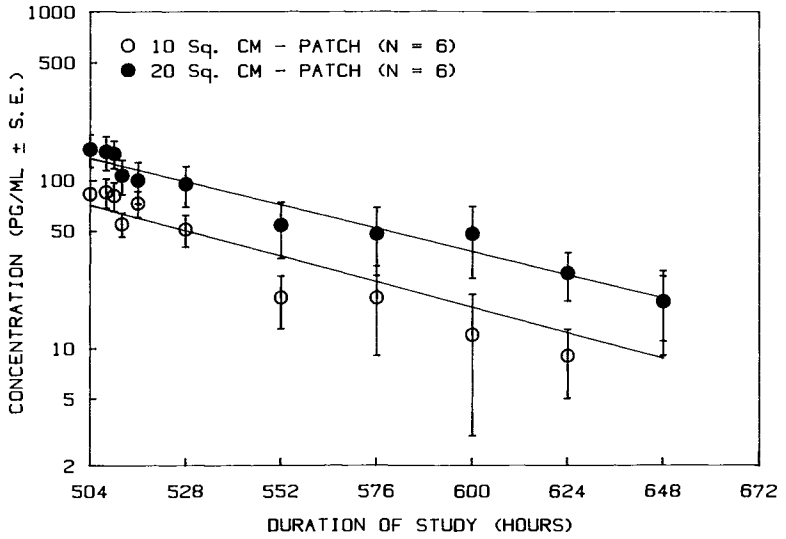

Fig. 10. Serum concentration profiles of levonorgestrel during the elimination phase of transdermal drug delivery in both Group A and Group B subjects. The first-order rate constant for elimination is 0.0180 (±0.0031) and 0.0132 (±0.0021) hr^{-1}, respectively, for subjects (n = 6 each) in Group A and Group B.

skin are expected to be taken up and transported to the systemic circulation by the rich microcirculation network at the dermal–epidermal junction. On the other hand, in the *in vitro* skin permeation studies, the drug molecules absorbed have to penetrate additional skin layer-dermis before reaching the receptor solution which has been maintained under sink conditions. And the lag time (t_l) is known to be directly proportional to the square of thickness of the skin barrier (h_s) (9) as follows:

$$t_l = \frac{h_s^2}{6D_s}$$

where D_s is the effective diffusivity of drug in the skin barrier.

By the trapezoidal rule method, we have calculated the AUCs for individual subjects and found they are 52,120 (±17,048) pg · hr/ml for Group A and 81,087 (±33,118) pg · hr/ml for Group B (Table IV). The mean AUC values also show dose proportionality as that demonstrated by the total dose of levonorgestrel delivered to each subject [579.0 (±48.3) and 1211.8 (±71.4) μg] over the three weekly applications of TCD patches. The AUC/dose was calculated to be 90.8 (±32.0) × 10^{-6} hr/ml for Group A and 67.0 (±26.4) × 10^{-6} hr/ml for Group B (Table IV). The difference in AUC/

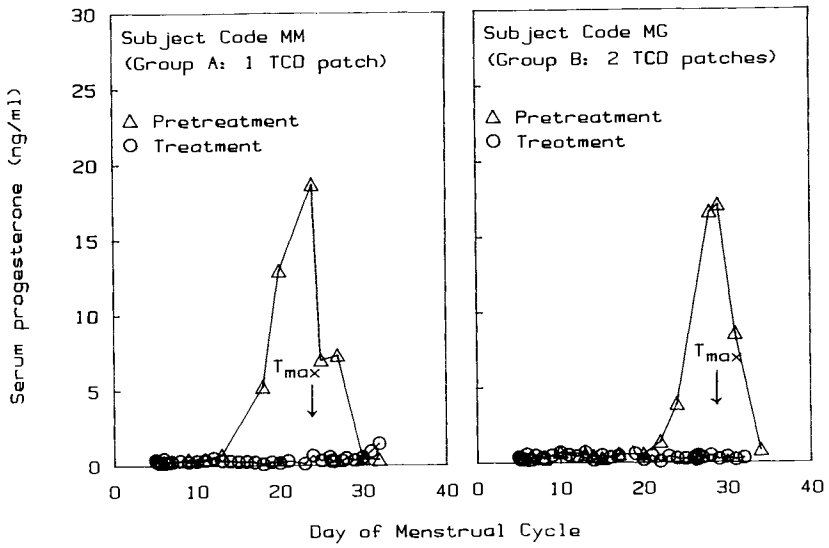

Fig. 11. Comparative serum concentration profiles of progesterone, during the pretreatment and treatment cycles, in two subjects, each as the representative for Group A and for Group B, respectively. The suppression of progesterone peak during the treatment cycle is an indication of effective fertility control.

dose values between Group A and Group B was found not statistically significant ($P > 0.05$).

By assaying the serum concentration of progesterone in the pretreatment and treatment cycles and comparing the elevated serum progesterone levels in Days 17–23 in each subject, one can assess the ovulatory status of individual subject and her response to the treatment of TCD patches. The entire abolition, as shown in Fig. 11, or substantial suppression of the progesterone peak will suggest the fertility regulation effectiveness of the TCD patches used. The results indicated that the weekly treatment with one 10-cm^2 patch has achieved the suppression of serum progesterone peaks in two of the five subjects treated (or 40%), while two 10-cm^2 patches have accomplished the suppression in four of the six subjects treated (or 67%). A dose-dependent effectiveness in the suppression of progesterone peak was observed.

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