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TRANSDERMAL CONTRACEPTIVE DELIVERY SYSTEM: PRECLINICAL DEVELOPMENT AND CLINICAL ASSESSMENT

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ABSTRACT

An once-a-week transdermal (monophasic) contraceptive delivery system (TCDS), intended to be marketed in Asian countries, was developed at the Controlled Drug-Delivery Research Center, Rutgers, College of Pharmacy under the sponsorship of Sintong

Chemical Industrial Co. Ltd. in Taiwan. This TCDS was designed to simultaneously deliver a low dose combination of levonorgestrel (LNG) and 17ß-estradiol (E2) through the skin for the fertility regulation in female. In-vitro permeation study using human cadaver skin shows that 60.0 ± 9.42 mcg/day/10 cm^2 of LNG and 28.8 ± 4.96 mcg/day/10 cm^2 of E2 can be delivered from this system. The result of the one-week dermal toxicity study on 6 rabbits indicated the minimal potential of this TCDS to cause skin irritation. Histopathological examination revealed that this system causes only mild to moderate inflammation to the test animal which also showed no significant body weight change and sign of toxicity.

A phase I bioavailability-dose proportionality clinical study which consists of pre-treatment, treatment and post-treatment cycles was conducted on Fertile Chinese women. During the pre-treatment cycle, the 48 recruited subjects were given placebo patches to study the wearability (including skin irritation and adhesion tests). During the treatment cycle, each of the 8 subjects in Groups A, B and C received weekly application of 1, 2 or 3 pieces of 10 cm^2 TCDS patches, respectively, while each subject in Group D received one Chinese-made oral contraceptive pill (each pill contains 150 mcg of LNG and 35 mcg of ethynyl estradiol) per day as reference.

The TCDS patches were found to be very well accepted by the subjects as indicated by the results of the wearability study (PDII is less than 1.0 and mean survival rate of the patches = 99.5%). Residual assay of the used patches indicated that this TCDS has delivered LNG and E2 at the rate of about 5.0 mcg/cm^2.day and 4.0 mcg/cm^2.day, respectively, during the treatment cycle of

the study. Radioimmunoassay of serum samples revealed that therapeutically effective serum concentration of LNG was achieved. Serum profiles of progesterone, LH and FSH also indicated that ovulation inhibition occurred in the majority of the all 3 groups of subjects receiving TCDS patches. The post-treatment hormonal profiles also indicated that upon the termination of the administration of TCDS patches, the majority of the subjects return to their normal state of menstrual cycle. It was also reported that none of the subjects participated in this phase I study became pregnant.

Due to the success of this phase I study, a pilot phase II study has been initiated which involves more than 100 study subjects. Up to the first week of September, 1993, 112 subjects have completed 3 cycles of study with a total of 342 woman-month. During this pilot phase II study, the participated subjects were found to be sexually active and none of the subject has been reported to become pregnant so far.

INTRODUCTION

Methods of fertility regulation has long been used in the human history dated back to ancient Egypt and China. However, modern and efficient contraceptive methods did not become available until the introduction of intrauterine device and oral contraceptive pills in the 1960s. World population reached 4 and 5 billion people in 1975 and 1987, respectively, and is expected to exceed 6 billion people by the end of this century. The so called "low" projection of the United Nations is for 8.4 billion people by the year 2050, and the "high" projection is 12.4 billion for that date. Which of these

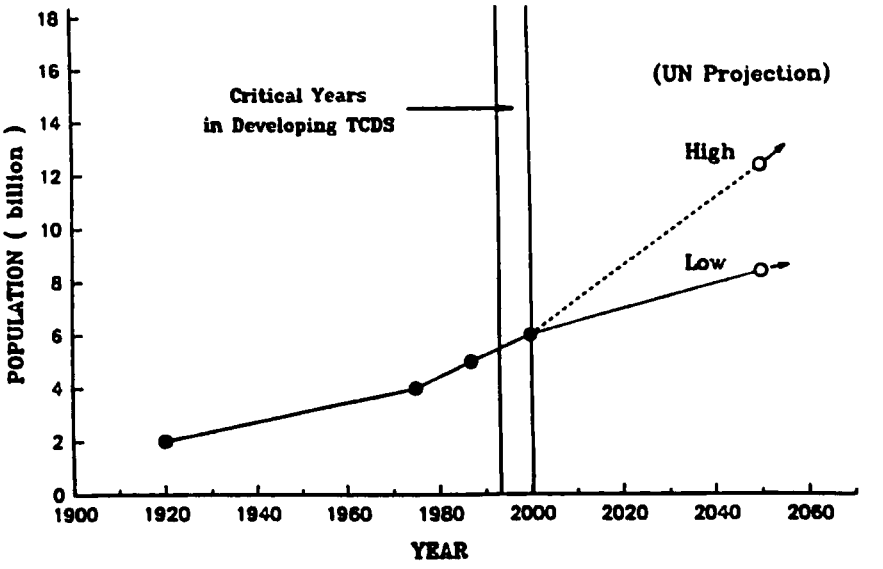

Figure 1
Projection of increse in world population by the United Nations helps to emphasize the need to develop new contraceptive methods in the nex few years.

two numbers is achieved depends largely upon the vigor with which birth control initiatives are pursued in the 1990s (Figure 1). Most of the expected population increase will come from developing nations in Africa and Asia. Take China, the world most populated nation whose birth rate has plummeted from 34 per thousand to 18 per thousand in the last 15 years, for example. China's population will still grow by 200 million in the next 15 years even if 70% or more of all Chinese couples now adopt the government's one-child family policy. Recent statistics (1) shows that only 63.1% of Chinese couples have complied with the one-child family policy and the rest of 36.9% of the family still produced two or even three children (Figure 2). To make the situation worse,

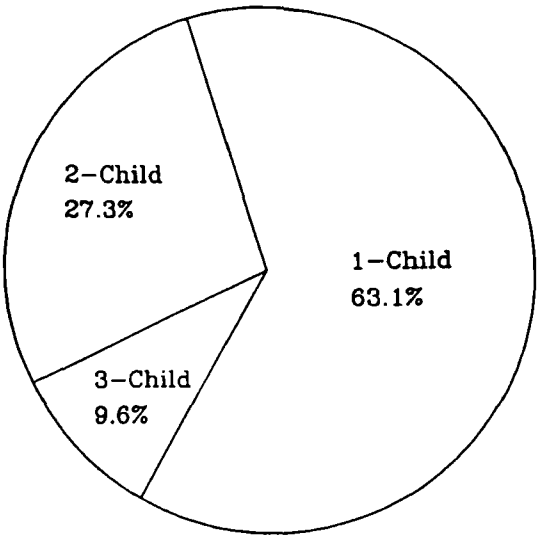

Figure 2
Percentage of Chinese couples that produce 1,2 or 3 children in 1992.

there has been a sharp decline in the percentage of married child-bearing age women using contraceptive methods in China between 1991 and 1992 (1). As the results, China's population is expected to grow by more than 20 million peoples this year (Figure 3).

Nowadays, the majority of Chinese couples still rely on invasive, nonreversible types of birth control methods such as tubal ligation, vasoligation and IUD. Only 3.8% of contraceptive users are using modern contraceptive pills for their fertility regulation (1) and (Figure 4). Oral contraceptive pills, although highly efficient, do require daily ingestion of regimen. If one pill is missed, the risk of getting pregnant is increased (2). Transdermal route of administration offers the advantage, as compared to oral route, of being able to deliver the

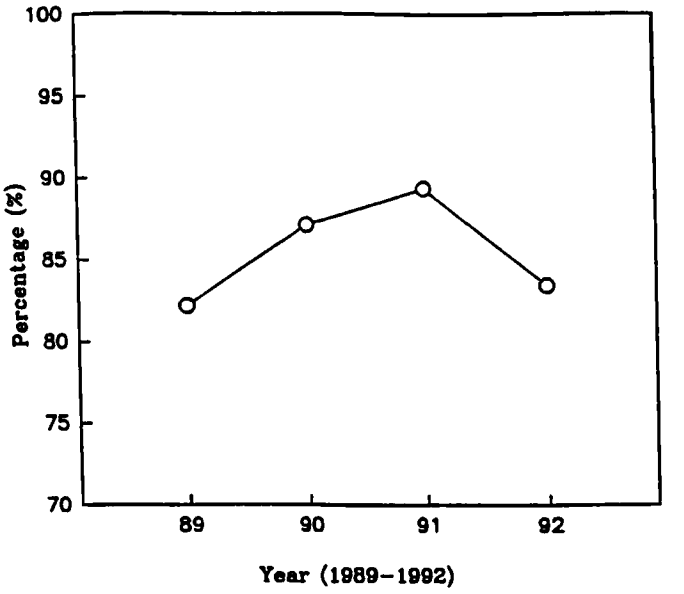

Figure 3
Changes of percentage of married Chinese women using contraceptive method.

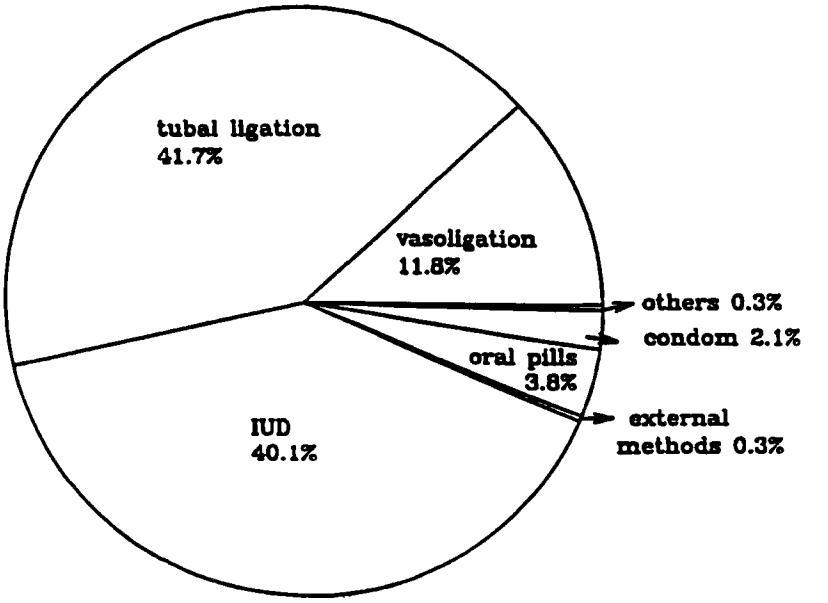

Figure 4
Percentage of various contraceptive methods used by the Chinese couples in 1992.

drug directly into the systemic circulation therefore can bypass the hepatic first pass metabolism. Because of this advantage, lower dose of drug is required by the transdermal route of administration to achieve the desired therapeutic level of drug in the blood. Since lower dose of drug is required, transdermal route of administration is expected to cause less undesirable side effects. An once-a-week transdermal contraceptive delivery system (TCDS) which can simultaneously deliver a low dose combination of progestin and estrogen would offer a viable and convenient way for fertility regulation in women. It is therefore the objectives of this project to develop an effective, safe, noninvasive and reversible contraceptive method which can be conveniently administered or terminated by the women users for fertility regulation in China.

Combined regimen of progestin and estrogen has long been used in the oral contraceptive pills. These combo pills has the advantage over the minipills (contain only progestin) of more effective and less incidence of irregular bleeding (3,4). Figure 5 shows the chemical structure of active ingredients chosen in the development of this TCDS. Levonorgestrel is a very potent synthetic progestin whose effects on endometrium growth and the amount and the viscosity of cervical fluid during the menstrual cycle are believed to contribute to the main efficacy of a contraceptive regimen. 17ß-estradiol, a natural estrogen whose anti-progestational effect can balance the effect of progestin and contribute to both higher efficacy and less side effect. Transdermal delivery of 17ß-estradiol has been demonstrated by the Estraderm-TTS (Ciba Geigy) as a hormonal replacement regimen for the twice-a-week therapy of postmenopausal syndromes (5). There is no transdermal product on the

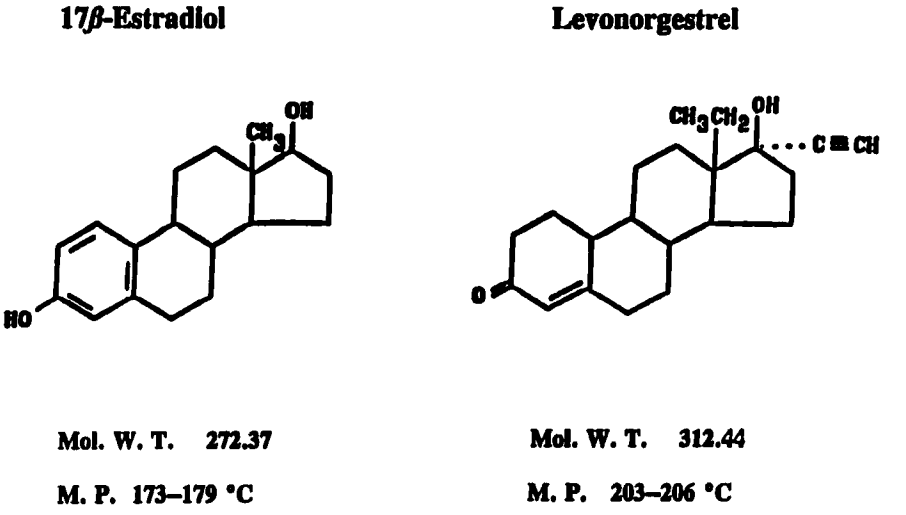

Figure 5
Chemical structure of estrogen (17β-estradiol) and progestin (levonorgestrel) used in the TCDS patch formulation.

market that can deliver levonorgestrel for either contraceptive or hormonal replacement uses yet. However, a levonorgestrel subdermal implant, Norplant, has been developed by the Population Council/New York and is now being marketed by Wyeth-Ayerst as a contraceptive device (6). Norplant is designed to continuously deliver levonorgestrel from 6 silastic capsules for up to 5 years of fertility regulation in women. The delivery rate of levonorgestrel from Norplant implants has been reported to be 60-80 mcg/day during the first 18 months and around 30 mcg/day thereafter (7). Serum concentration of levonorgestrel was found to be about 400 pg/ml at the beginning and gradually decreased to about 200 pg/ml (8). With the near 100% contraceptive efficacy achieved by the Norplant implants, it is reasonable to assume that if the

TCDS can deliver levonorgestrel and 17ß-estradiol at the rate of 5 mcg/cm².day and 2.5 mcg/cm².day, respectively, it should be able to attain the serum level high enough to achieve the same contraceptive efficacy that has been achieved by Norplant. It was estimated, based on the results of a phase I clinical study conducted on a similar TCDS ,that to achieve near 100% ovulation inhibition, it is necessary to deliver about 80 mcg/day of LNG which will produce serum level of 275 mcg/ml (9). Since inhibiting ovulation is only one of the three major mechanisms for achieving contraceptive efficacy (the other two mechanisms are changes in endometrium including its thickness and increase in cervical mucus viscosity), it is not necessary to achieve 100% ovulation inhibition in order to achieve 100% contraceptive efficacy. Norplant is believed to have achieved only about 50% of ovulation inhibition but still can yield near 100% contraceptive efficacy (10).

EXPERIMENTAL

Fabrication of TCDS Patches

Patches of TCDS for all the studies were made according to the following fabrication procedures:

1. In a glass container, disperse estradiol (made by Schering AG and purchased in the U.S.) and levonorgestrel (made by a Chinese pharmaceutical manufacturing company) crystals in the enhancer.

2. Add adhesive polymer (Duro Tak, by Chemical Starch and Chemical Co., Bridge water,NJ) to the LNG,E2/enhancer.

3. The mixture is mixed by using magnetic stirring bar in the glass container with air-tight cap. Mix until all the estradiol crystals are dissolved and a homogeneous solution is obtained.

4. Coat this formulation solution onto a piece of backing laminate (Scotch Pak 1109, by 3M Co. MN) at 650 microns wet thickness using a table top coater/dryer (Werner Mathis, Model LTSV/LTH).

5. Dry the coating at 60 °C for 15 minutes using the same table top coater/dryer.

6. Cover the dried coating with a piece of release liner (Scotch Pak 1022, 3M Co. MN).

7. Cut the product into 10 cm^2 patches by using a steel rule die cutter.

8. Weigh and give an identification number to each patches manufactured as part of quality control.

In Vitro Skin Permeation Study

To confirm the desired skin permeation flux of both LNG and E2 have been achieved by the developed formulation, the patches manufactured were subjected to the in vitro permeation study using human cadaver skin. The samples taken from the receptor compartment of the diffusion cell (Valia-Chien side-by-side skin permeation cell system; Crown Glass Co. NJ) were analyzed by high performance liquid chromatograph (Hewlett Packard 1090 HPLC System). The in vitro skin permeation profiles of each drug can then be established and the permeation flux of each drug can be assessed from the steady state of the permeation profiles.

Table 1

Draize scale used for scoring skin reactions in the primary skin irritation test.

Reaction	Value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

Dermal Toxicity Test

To investigate the potential of the developed TCDS patch formulation to cause skin irritation, an one-week dermal toxicity test was conducted (contracted out to Toxikon Corp. Woburn, MA). The test consists of an one-week primary skin irritation study on six rabbits followed by histopathological examination on each patch application site. Both medicated and placebo patches (10 cm^2) of this TCDS formulation were tested on either intact or abraded skin and a primary dermal irritation index (PDII) was given to each patch application site at 24 and 72 hours following the 7 days application period

based on the Draize Scale Scoring method (Table 1). The maximal possible PDII is 8.0 which is the sum of the total possible erythema and edema score (4.0 each).

Each animal were weighed for body weight change and observed for any sign of intoxication. Histopathological examination was immediately carried out after the irritation study by conducting microscopic examination of the dermal tissue removed from the patch application sites.

Phase I Clinical Study

With the collaboration of National Research Institute for Family Planning (NRIFP) in Beijing/China, a WHO Collaborating Center for Research in Human Reproduction, a phase I bioavailability-dose proportionality clinical study was conducted on this TCDS using fertile Chinese women. In this study, healthy female subjects of child-bearing age were randomly divided into 4 groups in a 4-way parallel study design (Table 2). The study consists of three menstrual cycles which are sequentially arranged as pre-treatment, treatment and post-treatment (or recovery) cycles. During the pre-treatment cycle, the 48 recruited subjects were given placebo patches to study the wearability (including skin irritation and adhesion tests) while they were being screened against the inclusion/exclusion criteria to be admitted in this clinical study. During the treatment cycle, each of the 8 subjects in Group A, B and C received weekly application of 1, 2 or 3 pieces of 10 cm^2 TCDS patches, respectively, while each subject in Group D received one Chinese-made oral contraceptive pill (each pill contains 150 micrograms of LNG and 35 micrograms of ethynyl estradiol) per day as reference.

Table 2

Study design of the phase I clinical study on TCDS patch formulation in fertile Chinese women.

CYCLE	CLINICAL ACTIVITIES	ASSAY AND MEASUREMENTS
1. Pre-treatment (21+7 days)	a. recruit 48 women b. admit 32 subjects c. randomly divide the subjects into 4 groups of 8 subjects d. initiate wearability test on groups A,B & C with placebo patches e. hormonal base lines establishment on groups A,B,C & D subjects	Basal body temp. E2,P,LH & FSH Hematologic determinations Clinical Chemistry Urinalyses Skin irritation test Adhesion test
2. Treatment (21+7 days)	a. conduct a 4-way parallel bioavailability-dose proportionality study: Group A : 1 X 10 cm^2 patch/week Group B : 2 X 10 cm^2 patch/week Group C : 3 X 10 cm^2 patch/week Group D : 1 tablet/day	LNG, E2, P, LH & FSH Hematologic determinations Clinical chemistry Urinalyses Recording adverse reactions
3. Post-treatment (21 days)	a. recovery of normal menstrul cycle b. drug recovery study on the used patches to determine the amount of drug delivered.	LNG, E2, P, LH & FSH Hematologic determinations Clinical chemistry Urinalyses Recording adverse reactions

Blood samples obtained from these three cycles of studies were assayed by radioimmunoassay (RIA) methods for their serum concentration of LNG, E2, progesterone (P), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Ultrasonic measurement of follicle size and endometrium thickness measurement were also performed during the mid-treatment cycle. Bioavailability of LNG will be assessed by the serum LNG profile of each subject group. Suppression of post-ovulatory progesterone peak and mid-cycle surges of LH and FSH will provide the hormonal indications of ovulation inhibition. Contraceptive efficacy of the TCDS in the study was assessed according to the results of hormonal indications of ovulation inhibition, follicle size and endometrium changes.

Pilot Phase II Clinical Study

Upon the completion of the phase I clinical study, a pilot phase II clinical study on this TCDS patch formulation was immediately conducted at NRIFP. The purposes for conducting this pilot phase II study are to investigate the multiple-cycle bioavailability of LNG and to further establish the contraceptive efficacy involving more subjects. A total of 118 fertile Chinese women of child-bearing age with history of successful conception and delivery of child were admitted in this study. The study is divided into two parts and all the subjects participated in both parts of this pilot phase II study were given two 10 cm^2 of TCDS patches. 35 subjects were included in the part A to participate in a 3-cycle long bioavailability study. Serum concentrations of LNG, E2, P, FSH and LH will be assayed by the same RIA methods used in the phase I study. Another 83 subjects were admitted in the part B for a 6-month study of

contraceptive efficacy and adverse effect on this TCDS. Serum concentration of P, FSH and LH will also be assayed to provide hormonal indications of ovulation inhibition for assessing the contraceptive efficacy. Ultrasonic measurement of follicle size and observation on endometrium changes will also be performed to provide additional means of assessing the contraceptive efficacy.

RESULTS AND DISCUSSION

Fabrication of TCDS Patches

The TCDS patches fabricated are very thin (total thickness is about 400 microns) and have very flexible embodiment. These two features are essential to the survival of the patches under the week long real-life wearing situation. Thick and rigid patches tends to fall off easier than the patches with thinner and more flexible embodiment. The drug-containing matrix is made up of a monolithic layer of polyacrylate adhesive polymer which has been cured during the drying process. The transparent release liner of the patches was found very easy to peel and the surface of the adhesive matrix is tacky but can be easily separated from the finger tips during the application of the patch. The overall elegance and the ease of application of these TCDS patches are considered important factors to the acceptability of the patches to the potential women users.

In Vitro Skin Permeation Study

Figure 6 shows the in vitro skin permeation profiles of both estradiol and levonorgestrel using human cadaver

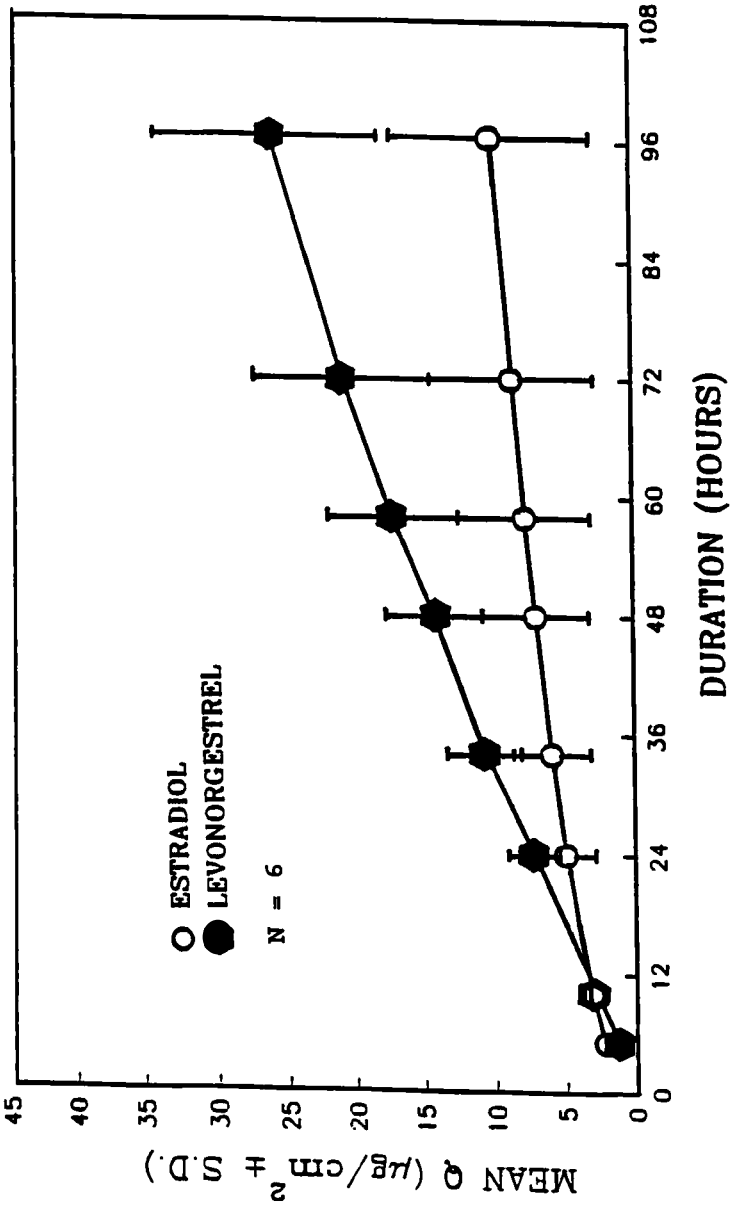

Figure 6

In vitro human cadaver skin permeation profiles of estradiol and levonorgestrel delivered form the TCDS patch formulation.

Table 3

Draize scores of primary skin irritation test on placebo formulation of TCDS patches.

Animal #	01/15/93 24 h Intact Skin Erythema	Edema	Abraded Skin Erythema	Edema	01/17/93 72 h Intact Skin Erythema	Edema	Abraded Skin Erythema	Edema
27492	1	0	1	0	1	0	1	0
27493	1	0	1	0	1	0	1	0
27494	1	0	1	0	1	0	1	0
27495	1	0	1	0	1	0	1	0
27496	1	0	1	0	1	0	1	0
27497	1	0	1	0	1	0	1	0
Mean	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0

Note: Primary Dermal Irritation Index (PDII) = 1.00

skin from the thigh region of a young caucasian female. Both profiles indicated that the TCDS patches allow both drugs to be released and permeated through the skin at constant rates for up to 96 hours without significant permeation lag time. The slopes of the profiles at their steady state allow the in vitro permeation flux of the drugs to be estimated which are 6.0 ± 0.94 mcg/cm².day and 2.9 ± 0.50 mcg/cm².day, respectively, for levonorgestrel and estradiol. The skin permeation flux obtained along with the lack of skin permeation lag time suggested that this TCDS patch formulation will be able to deliver both drugs into the body if the patches are worn by the subjects during the clinical study.

Dermal Toxicity Test

The individual Draize scores at each patch application sites on each rabbits used in the primary

Table 4

Draize scores of primary skin irritation test on medicated formulation of TCDS patches.

Animal	01/15/93 24 h				01/17/93 72 h			
	Intact Skin		Abraded Skin		Intact Skin		Abraded Skin	
#	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
27492	1	0	1	0	1	0	1	0
27493	2	1	1	1	2	0	1	0
27494	2	2	2	2	2	0	2	0
27495	2	2	2	2	1	0	2	0
27496	1	1	1	1	1	0	1	0
27497	1	0	1	0	1	0	1	0
Mean	1.5	1.0	1.3	1.0	1.3	0.0	1.3	0.0

Note: Primary Dermal Irritation Index (PDII) = 1.85

skin irritation study of the test are shown in Table 3 and Table 4, respectively, for placebo and medicated TCDS patch formulation.

It is encouraging to note that the PDII scores are 1.0 and 1.85 for placebo and medicated patch formulation, respectively. On the scale of 8.0, both placebo and medicated patch formulations can be categorized as slightly irritating. The results of this one-week primary skin irritation study suggested that the developed TCDS patch formulation will be unlikely to cause severe skin irritation to the subjects during the clinical study. The outcome of the histopathological examination also revealed that both placebo and medicated formulations of TCDS caused only mild to moderate degree of inflammation to the test animal (Table 5). All the

Table 5

Scores of histopathological examination on the placebo and medicated TCDS patches in the dermal toxicity test.

Animal #	Intact Skin		Abraded Skin	
	Control	Test	Control	Test
27492	1+	2+	1+	2+
27493	2+	2+	2+	1+
27494	3+	2+	2+	3+
27495	1+	2+	1+	3+
27496	1+	3+	2+	2+
27497	1+	2+	2+	3+
Mean	1.5	2.2	1.7	2.3

1+ = Minimal to Mild
2+ = Mild to Moderate
3+ = Moderate
4+ = Severe

Table 6

Body weight change and signs of toxicity on the rabbits during the dermal toxicity test.

Animal #	Sex	Body Weight (Kg) 01/07/93 Day 0	01/17/93 Day 10	Weight Change	Signs of Toxicity*
27492	Female	2.51	2.53	0.02	none
27493	Female	2.52	2.53	0.01	none
27494	Female	2.57	2.60	0.03	none
27495	Female	2.55	2.56	0.01	none
27496	Female	2.50	2.52	0.02	none
27497	Female	2.56	2.58	0.02	none

* Summary of Observations (excluding skin irritation) Day 0 through Day 10.

Table 7
Primary dermal irritation index of the placebo TCDS patches obtained in the pre-treatment cycle of phase I clinical study on Chinese women.

Subject Group	Erythema	Edema	PDII
Group A (n=8)	0.10	0	0.10
Group B (n=7)	0.10	0	0.10
Group C (n=7)	0.42	0.12	0.53

test animal showed no significant body weight change and no signs of toxicity during the study (Table 6).

Based on the overall results of this dermal toxicity test, this TCDS patch formulation was considered dermatotoxically safe to be used on the live human subjects in the clinical study.

Phase I Clinical Study

The placebo TCDS patches were found very well tolerated by the women subjects in all three study groups as indicated by the low (less than 1.0 on the scale of 8.0) PDII values obtained during the pretreatment cycle (Table 7).

It is interesting to note that the PDII values obtained in the phase I clinical study on women subjects were much lower than the value (1.0 on the scale of 8.0)

Table 8

Mean patch survival rate of the placebo TCDS patch formulation during the pre-treatment cycle of the phase I clinical study on Chinese women.

Subject Group	Position	Total Number of Days / Maximum Number of Days	Patch Survival Rate
Group A	Right Abdomen	96/98	98.8%
	Left Abdomen	69/70	98.6%
Group B	Right Abdomen	168/168	100%
	Left Abdomen	126/126	100%
Group C	Right Abdomen	230/231	99.6%
	Left Abdomen	210/210	100%
		Mean Survival Rate =	99.5%

obtained in the dermal toxicity test on rabbits. It was also very encouraging to find that the placebo TCDS patches stayed very well (99.5% of mean survival rate) on the skin of all three groups of the women subjects in the real-life wearing situation during the pre-treatment cycle of the phase I clinical study (Table 8).

Residual drug assay from the used patches from the phase I clinical study allows the daily delivery rate of both drugs to be calculated. Figure 7 shows that the daily delivery rate of LNG is linearly proportional to the increase in dosage of transdermal regimen. However, this linear relationship between the daily delivery rate of E2 and dosage was established only for the dosages of the 1 and 2 pieces of 10 cm^2 TCDS patches and does not extent to the 3 pieces of 10 cm^2.

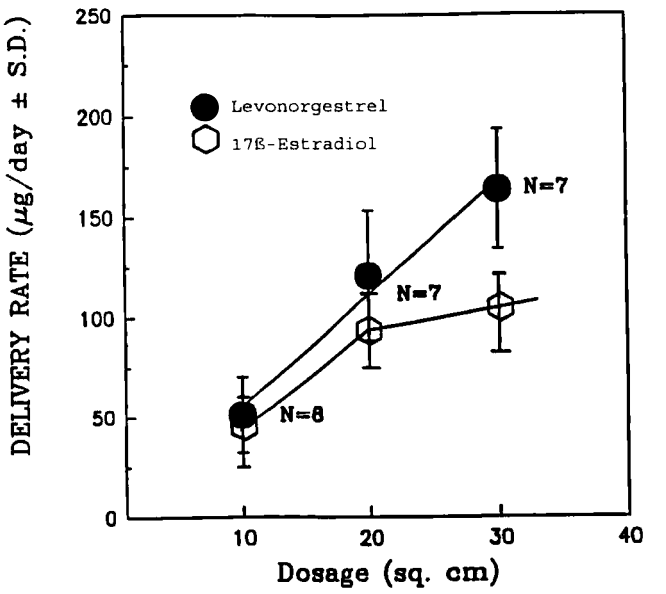

Figure 7
Relationship between daily drugs delivery rates and the dosage of TCDS patches used in the phase I clinical study on Chinese women.

Figure 8 shows the serum profiles of levonorgestrel resulted from weekly application of 1, 2 or 3 pieces of 10 cm^2 of TCDS patches by the subjects of Group A, B or C, respectively, in the phase I clinical study. The profiles also show that all three dosages of TCDS patches allowed the 275 pg/ml of target serum concentration of LNG to be reached two days after the first dose of the patches were applied. Subjects in Group B and C were found to have LNG serum concentration maintained above the target concentration through out the three weeks of patch application (except for the 20th day of the Group C). The serum LNG profiles obtained in this bioavailability study suggested that high percentage of ovulation inhibition could be achieved in both group B

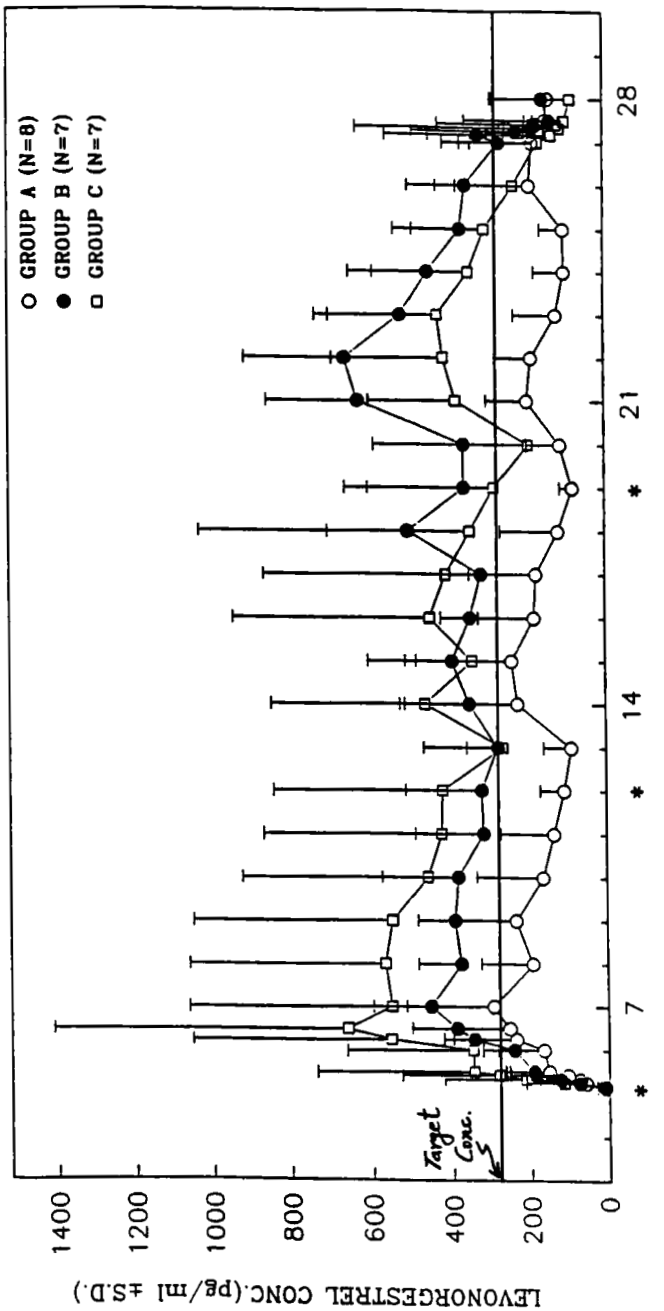

Figure 8

Serum levonorgestrel profiles obtained from the groups A, B and C subjects during the phase I clinical study on TCDS patch formulation.

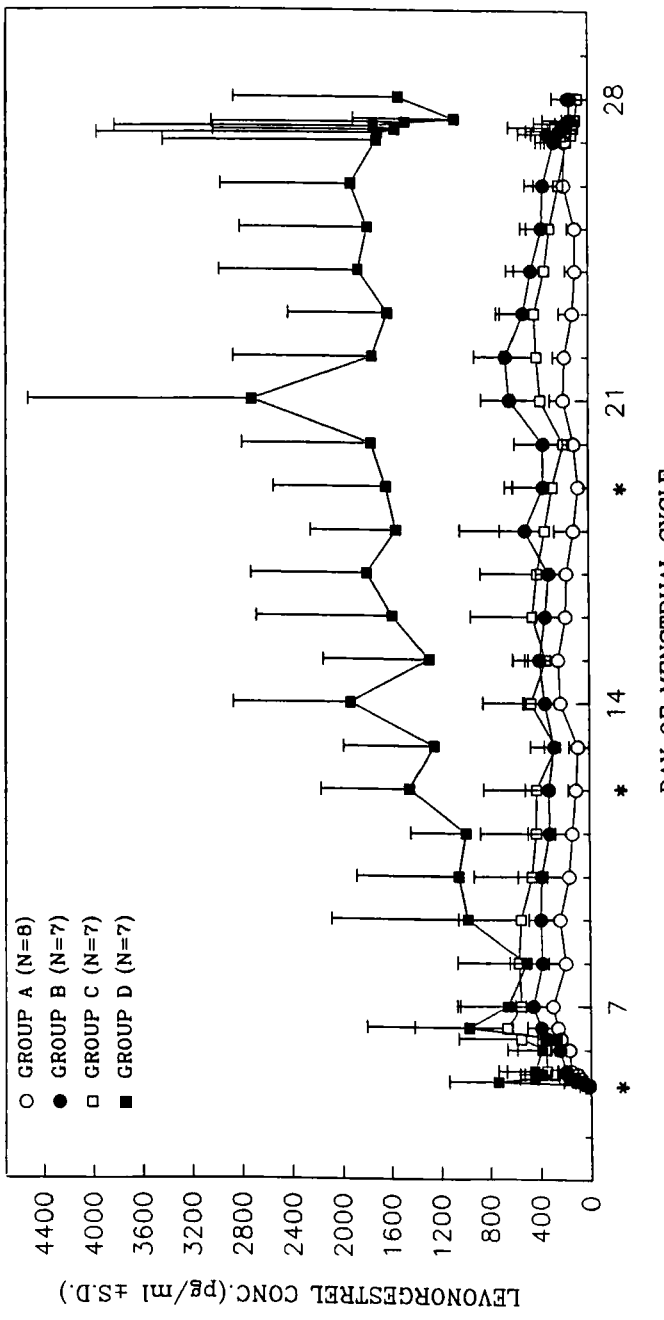

Figure 9

Serum levonorgestrel profiles obtained from the groups A, B and C subjects as compared to the profiles obtained from the group D (reference group) subjects during the phase I clinical study on TCDS patch formulation.

and group C subjects. Compared to the daily oral pill regimen taken by the group D subjects during the study, the TCDS patches seemed to produce more sustained and less fluctuated serum LNG profile (Figure 9). Additionally, daily ingestion of oral pill produced serum LNG that is considered unnecessarily high for achieving the desired near 100% contraceptive efficacy.

Serum profiles of progesterone, FSH and LH for each subjects participated in this clinical study were examined for their indication of ovulation inhibition. As shown, for example, in Figure 10, the subject (ID# B0018) had normal menstrual cycle during the pre-treatment cycle as indicated by the mid-cycle surges of serum FSH and LH and the post-ovulatory elevation of serum progesterone. After she took the TCDS patches (2x10 cm^2/week for 3 weeks), all three serum hormonal peaks were completely suppressed during the treatment cycle. Moreover, her menstrual cycle was found to return to normal after the termination of TCDS patch application as indicated by the reappearance of the mid-cycle surges of serum FSH and LH and the post-ovulatory rise of the serum progesterone.

Table 9 summarizes the results of examination of serum progesterone, FSH and LH profiles of each subject of groups A, B and C in this 3-cycle long phase I clinical study. It was found that 7 out of 8 subjects in group A, 6 out of 7 subjects in group and 7 out of 7 subjects in group C showed the occurrence of ovulation inhibition as indicated by the hormonal indicators. In group D, ovulation inhibition occurred in all 7 subjects participated in the study (Table 10). Subject ID# A0025 showed the occurrence of ovulation as indicated by the mid-cycle FSH and LH surges, however, the lack of

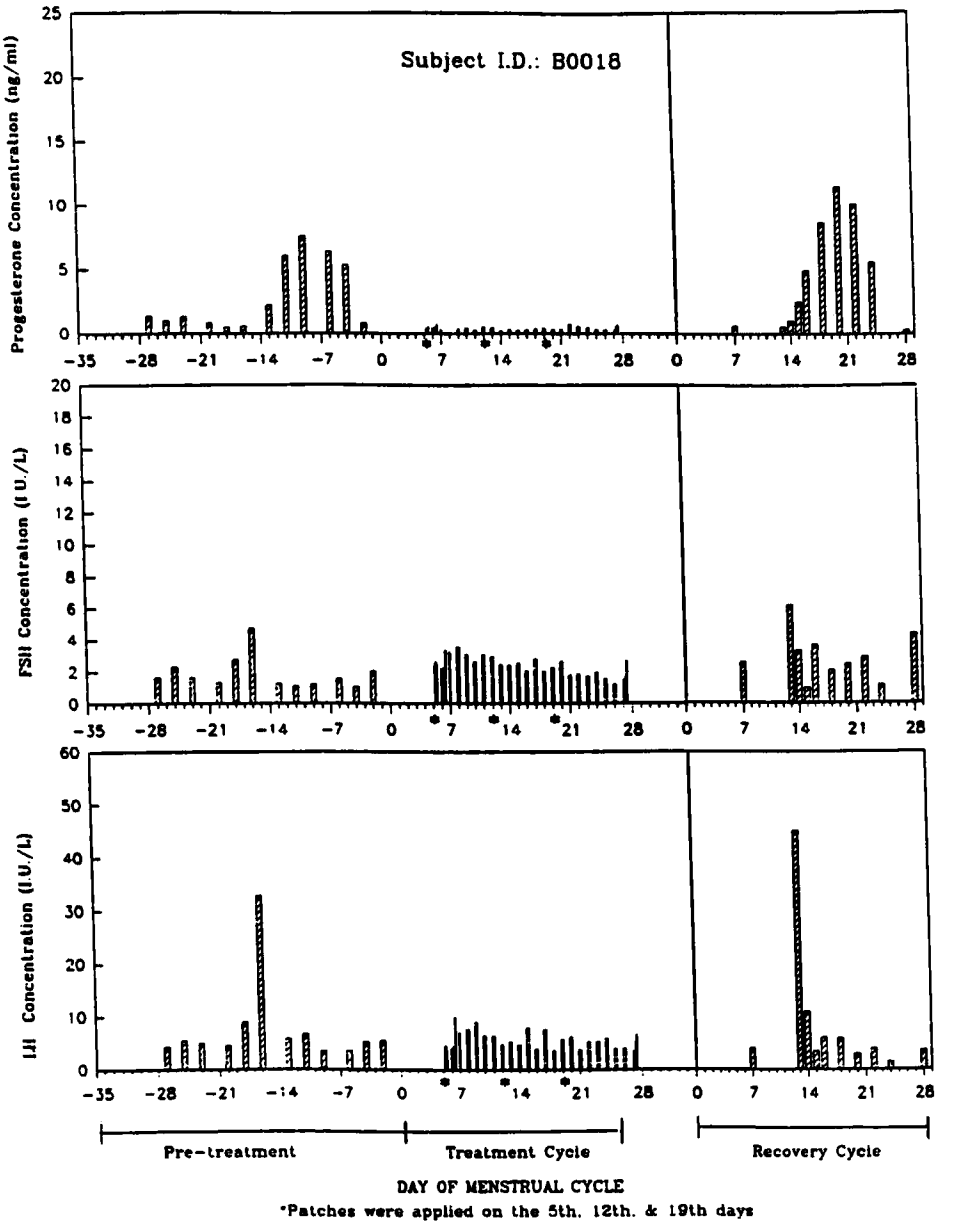

Figure 10
Serum profiles of progesterone, FSH and LH during the 3-cycle of phase I clinical study on a Chinese woman (subject ID#B0018) using TCDS patches in the phase I clinical study .

Table 9

Summary of hormonal indications of ovulation inhibition for the groups A, B, and C subjects participated in the phase I clinical study on TCDS patch formulation.

Subj. ID#	Hormonal Indications of Ovulation Inhibition			
	P	LH	FSH	
A0001	+	+	+	
A0005	-	+	+	
A0009	+	+	+	
A0013	+	+	+	
A0017	+	+	+	
A0025*	+	-	-	
A0029	+	+	+	
A0033	+	+	+	
Group A : 7/8 subjects show ovulation inhibition.			Efficacy	8 out of 8 = 100%
B0002	+	+	+	
B0006	+	+	+	
B0010	+	+	+	
B0014*	-	+	+	
B0018	+	+	+	
B0022	+	+	+	
B0026	+	+	+	
Group B : 6/7 subjects show ovulation inhibition.			Efficacy	7 out of 7 = 100%
C0003	+	+	+	
C0007	+	+	+	
C0011	+	+	+	
C0019	+	+	+	
C0023	+	+	+	
C0027	+	+	+	
C0031	+	+	+	
Group C : 7/7 subjects show ovulation inhibition.			Efficacy	7 out of 7 = 100%

"+" indicate ovulation inhibition; "-" indicate no ovulation inhibition
Efficacy is assessed based on ovulation inhibition, follicle size and endometrium thickness.

progesterone surge suggested the abnormality of hormonal condition in the ovary. Subject ID# B0014 showed delayed maturation of ovum and might have resulted in unsuccessful follicular rupture to release the ovum. Supplemental evidence obtained from the observation of the endometrium changes further suggested the impossibility for those two subjects to get pregnant. As the results of these indications and evidence, contraceptive efficacy of 100% has been achieved by all three groups of the subjects that received TCDS patch regimen. Since all the subjects in group D showed the indications of ovulation inhibition, it can be concluded that 100% contraceptive efficacy has also been achieved by the ingestion of daily oral contraceptive pill. It is also noteworthy that successful contraception can be achieved not only by the mechanism of inhibiting ovulation along but also by the combination of the other two mechanism, namely increased cervical mucus viscosity and endometrium changes. Synthetic progestins, including levonorgestrel, taken via oral route or by subdermal implants has long been known to greatly increase the viscosity of cervical mucus during the mid-cycle (11 & 12) and therefore preventing the fertilization of ovum from happening. It is very encouraging to find that none of subjects participated in this phase I clinical study **has become pregnant during this 3 months long of study.**

In Vitro and In Vivo Correlation of Transdermal Delivery Rates of LNG and E2

Table 11 summarized the in vitro and in vivo correlation of daily levonorgestrel delivery rate for the developed TCDS patches.

Table 11

Summary of in vitro and in vivo correlation for the daily delivery rates of levonorgestrel and estradiol.

	Target	Daily Delivery Rate (micro gram/day ± S.D.) In Vitro Human Cadaver Skin (N = 12)	In Vivo Rabbit Skin Intact (N = 6)	In Vivo Rabbit Skin Abraded (N = 6)	Phase I Clinical Study on Chinese Women
Levonorgestrel	50	60.0 ± 9.42	64.0 ± 32.88	61.8 ± 39.22	51.0 ± 19.12 (Group A) 121.1 ± 32.24 (Group B) 163.7 ± 29.48 (Group C)
Estradiol	25	28.8 ± 4.96	37.0 ± 19.77	35.1 ± 26.70	41.2 ± 16.32 (Group A) 89.7 ± 28.40 (Group B) 110.4 ± 26.61 (Group C)

It was found that the in vitro human cadaver skin permeation rate of both LNG and E2 correlate very well with those of the in vivo delivery rate in rabbits during the dermal toxicity test. These two delivery rates were also found correlate rather well with the daily delivery rate obtained in the phase I clinical study on Chinese women. Additionally, in vivo delivery rate of LNG on the Chinese women during phase I clinical study was found very close to the target delivery rate originally at all three dosage regimen of this TCDS patch formulation. However, a higher than the targeted E2 delivery rate was observed for all three dosage regimen of TCDS patches. The excellent in vitro and in vivo correlation obtained for transdermal levonorgestrel delivery in this study has increased our confidence on the use of in vitro skin permeation cell system to be a good predictive tool for developing TCDS patch formulation.

Pilot Phase II Clinical Study

Up to the first week of September, 1993, 112 subjects have completed three cycles of study with a total of 342 woman-months in this pilot phase II clinical study. The woman subjects were found to have normal sexual activity during this study as indicated by their frequency of intercourse (3-7 times per month for group A subjects and 3-4 times per month for group B subjects). It is also very encouraging to learn that none of the subjects has become pregnant so far during this study. The effectiveness of this TCDS patch formulation in preventing pregnancy among those sexually active Chinese women participated in this study was very well demonstrated. This result seems to suggest that at the dosage of 2x10 cm^2/week, the developed TCDS patches formulation might be a more effective contraceptive regimen than the 6-capsule Norplant implants which was shown to cause 19 pregnancies during 21,589 woman-months of the study among 458 women users (10).

CONCLUSIONS

The results obtained from the preclinical testings and the clinical assessment of the TCDS patch formulation during the phase I and pilot phase II clinical studies allow us to conclude :

1) A monophasic-type once-a-week transdermal contraceptive delivery system which can simultaneously deliver a low-dose combination of levonorgestrel and 17ß-estradiol was developed.

2) The TCDS patches has very thin and flexible embodiment which allow the patches to adhere very well to the skin and causes very minimal degree of skin irritation in the real life wearing situation on Chinese women during the clinical study.

3) Therapeutically effective serum concentration of levonorgestrel was achieved which results in high percentage of ovulation inhibition in fertile Chinese women during the clinical study.

4) The developed TCDS patches, at the dosage of 2X10cm²/week, seemed to be very effective in preventing sexually active women from getting pregnant during both phase I and pilot Phase II clinical studies conducted in China.

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