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Pulmonary Absorption of Liposomal Levonorgestrel

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ABSTRACT

The purpose of these studies was to achieve desired bioavailability after pulmonary administration of Levonorgestrel (LN) and to provide prolonged effective concentration of the drug in plasma and to reduce reported side effects of orally administered drug. The plain drug suspension, physical mixture (plain drug with liposomal constituents), and drug-encapsulated liposomes containing 10 µg of drug were instilled intratracheally in rats. Similarly, 10-µg drug suspension (LO) was administered orally. The blood samples were withdrawn at specific time intervals and were subjected to LN analysis by spectrofluorimetric technique. The plasma drug concentration data of both the treatments were plotted, and pharmacokinetics data were calculated and compared with that of oral administration. Percentage relative bioavailability (F*) of 97.6%, 98.6%, and 109.9% were observed after pulmonary administration of plain drug formulation (LP1), physical mixture (plain drug along with constituents of liposomes [LP2]), and liposomal (LP3) formulations of the drug, respectively. Following oral administration, C_{max} of 14.4 ± 0.6 ng/mL was observed at 2.1 ± 0.2 hours followed by subtherapeutic concentration beyond 30 ± 0.2 hours, while after pulmonary administration of LP1, LP2, and LP3 formulations, C_{max} of 4.4 ± 0.4 ng/mL, 4.2 ± 0.5 ng/mL, and 4.4 ± 0.6 ng/mL were observed at 6.0 ± 0.2 hours, 7.0 ± 0.2 hours, and 6.8 ± 0.2 hours, respectively, followed by maintenance of effective plasma drug concentration up to 60 ± 2 hours. These studies demonstrate superiority of pulmonary drug delivery with regards to maintenance of effective therapeutic concentration of the LN in the plasma over a period of 6 to 60 hours. Hence, the pulmonary delivery is expected to reduce frequency of dosing and systemic side effects associated with oral administration of LN.

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INTRODUCTION

It is well known that contraceptive needs change during a couple's reproductive life because of changing cultural, religious, and reproductive needs. In addition, many couples do not use modern methods for the fear of side effects. To meet the unmet needs of couples, a wider range of fertility regulation methods should be made available. Research is going on worldwide for the development of improved versions of existing technologies as well as the development of new methods.

An important feature of all oral contraceptive drugs is the interference with production and action of endogenously synthesized steroid hormones.[1] Indeed, changes in oestradiol metabolism after administration of exogenous hormones have been reported.[2] As the majority of contraceptives are administered by mouth, the hepatic first-pass effect may result in induction of hepatic enzymes, including liver microsomal cytochromes P450.[3] Therefore, an important side effect of oral contraceptive drugs is the interference with potency and duration of other medications such as anticoagulants,[4] antibiotics, or anticonvulsant drugs. In addition, orally administered steroids interfere to different degrees with hepatic protein synthesis of procoagulatory and fibrinolytic proteins.[5] Interference with liver function also explains why some oral contraceptive users develop a fatty liver as a consequence of long-term treatment. It is also likely that factors originating from or due to hepatic metabolism of exogenous steroids play a role in hypertension and dyslipidaemia, side effects frequently observed with oral contraceptive treatment.

Levonorgestrel (LN) has been used for many years both alone (in low doses) in the progestogen only pill (POP) and in combination with estrogen in combined oral contraception (COC) preparations. There have been many fewer studies on the safety of long-term use of the POP than of the COC, but the existing data are largely reassuring.[6]

New materials and new technologies have stimulated pharmaceutical researchers to identify and use alternatives to the classical oral and injectable routes. The pulmonary route is

being used for the effective delivery of drugs into the systemic circulation. For a long time, the lung has been used for the administration of drugs for the treatment of local conditions. However, more recently, spurred on by the advent of novel delivery devices, there is a growing interest in the use of the lung for the systemic delivery of challenging molecules, such as peptides and proteins, as well as analgesic agents and even vaccines.[7] The larger surface area of the lung is well known, although, interestingly, the permeability of the lung tissue in itself is not that different from other mucosal surfaces; it is the large area that provides for the rapid absorption. Liposomes are phospholipid vesicles composed of lipid bilayers enclosing one or more aqueous compartments. Liposomes provide an efficient delivery system because they are biocompatible, biodegradable, and relatively nontoxic.[8] As a drug delivery system, liposomes can significantly alter the pharmacokinetics and pharmacodynamics of entrapped drugs, for example, by enhancing drug uptake, delaying rapid drug clearance, and reducing drug toxicity.[9-11]

In the present study an attempt was made to develop formulations for pulmonary administration of LN and to establish pharmacokinetic parameters and comparable relative pulmonary bioavailability to oral route. It was an objective to enhance and maintain effective therapeutic concentrations of the drug for a prolonged period of time in the development of a pharmaceutically rational pulmonary drug-delivery system for maximizing the therapeutic index, reducing the dose/frequency of dosing and systemic side-effects, and thereby reducing the cost of therapy.

MATERIALS AND METHODS

LN was obtained from German Remedies Pvt Limited, Mumbai, India. Phosphatidylcholine (PC) (type-E 80) was a gift sample from Lipoid Gmbh, Ludwigshafen, Germany. Cholesterol (CHOL) was purchased from S. D. Fine Chemicals, Mumbai, India. All other reagents and chemicals used were of analytical grade unless otherwise specified.

Preparation of Levonorgestrel Formulations

Levonorgestrel Suspension (LP1)

One hundred milligrams of drug was weighed accurately and transferred to a 10-mL volumetric flask. Water was added and volume was made up to the mark using distilled water, and the resulting suspension was sonicated.

Levonorgestrel Physical Mixture (LP2)

One hundred milligrams of drug along with liposomal constituents, namely, egg PC and CHOL, were weighed accurately and transferred to a 10-mL volumetric flask. Water was added and volume was made up to the mark with distilled water, and the resulting suspension was sonicated.

Levonorgestrel Liposomes (LP3)

Liposomes of LN were prepared by reverse phase evaporation method (REV method)[12] with Drug:PC:CHOL molar ratio of 1:4:1 and dissolved in diethyl ether (organic phase) in a glass tube (Quick fit neck B-24, Durga Scientific Pvt Ltd, Vadodara, India). Distilled water containing 100mmol/L sucrose was injected rapidly into lipid solution through a 23-gauge hypodermic needle from a 5-mL syringe. The tube was closed with a glass stopper and vortexed for 5 minutes. The tube was then attached directly to a rotary evaporator to dry the contents at 40°C under vacuum (20" of Hg) until a gel was formed. Vacuum was released and the tube was removed from the evaporator and subjected to vigorous mechanical agitation on vortex mixture for 5 minutes. When the gel collapsed to fluid, it was again fitted to rotary flash evaporator for the removal of organic solvent. A cycle of 10 minutes drying and 5 minutes vortexing was again repeated twice. Final liposomal suspension was subjected to complete removal of last traces of organic solvent in a rotary flash evaporator under vacuum (20" of Hg) for 15 minutes. The liposomal dispersion so formed was subjected to ultrasonic downsizing in an ice bath for 30 minutes. Resulting suspension was frozen at -40°C for 2 hours and then thawed at room temperature for 15 minutes to get optimum percent drug entrapment (PDE) (ie, 98.3%). The dispersion was analyzed for PDE in liposomes and in the preparation of liposomal dry powder inhaler (LDPI); the liposomal dispersion was diluted with sufficient hydrating medium to obtain a lipid:sugar ratio of 1:1. An equivalent proportion of sorbolac, calculated to have a final strength of 10 μg entrapped drug (in purified liposomal dispersion) per 20 mg formulation, was dispersed into the liposomal dispersion. The paste so formed was frozen at -40°C overnight and dried under negative displacement pressure (model DW1 0–60E; Heto Drywinner, Birkerod, Denmark) for 24 hours. The porous cake thus formed was sized successively through no. 120 and no. 240 sieves.[13] Capsules (size "2") were filled with individually weighed powder (20 mg) containing 10 μg LN and packed under nitrogen atmosphere in high-density polyethylene (HDPE) bottles containing silica bags as desiccant. The bottle with desiccant was sealed with polyvinyl chloride-coated aluminum foils and stored in a refrigerator until further use. A fraction of the powder was rehydrated with triple-distilled water with gentle, occasional agitation. The rehydrated liposomal dispersion was separated from the leaked drug by centrifugation and analyzed for PDE and percentage free drug in the LDPI formulation.

Table 1. Analytical Profile of Levonorgestrel Formulations*

	LN Suspension (LP1)	LN Physical Mixture (LP2)	LN Liposomes Before Dehydration	LN Liposomes (LP3) After Rehydration
Drug Entrapped in Liposomes (%)	–	–	98.30 ± 0.21	98.17 ± 0.18
Size (μm) D [4,3]	2.7 ± 0.01	4.8 ± 0.02	2.5 ± 0.01	2.5 ± 0.01
Span (polydispersibility)	3.26 ± 0.01	16.20 ± 0.01	1.55 ± 0.01	1.97 ± 0.01

* D [4,3] indicates volume mean diameter; and LN, Levonorgestrel. Data are the mean ± SEM (n = 3).

Characterization

Measurement of PDE of liposomes was carried out by separating unentrapped drug from liposomes by centrifugation at 2000 rpm for 15 minutes and analyzed according to the method described in *United States Pharmacopeia* 26-NF. Results obtained are recorded in Table 1.

The mean vesicle size of rehydrated liposomes was determined by a laser light scattering technique using Mastersizer (Malvern Instruments, London, UK). The particle size of the formulations was described by the volume mean diameter (*D* [4,3]). The polydispersity of the powder was expressed by the span. Span = $[D(v,90) - D(v,10)]/D(v,50)$, where $D(v,90)$, $D(v,10)$, and $D(v,50)$ are the equivalent volume diameters at 90%, 10%, and 50% cumulative volume, respectively. The results are given in Table 1.

Animal Studies

Six female Albino rats (250 ± 20 g) for each group were used in this study. All animals were housed in polypropylene cages with free access to palletized chow and tap water. The animals were exposed to alternate cycles of 12 hours light and darkness. Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India.

Methodology

Pulmonary Administration

The method of Enna and Schanker[14] for measurement of absorption rates of instilled compounds from the lungs of anesthetized rats was modified to allow measurements in conscious animals for periods of up to 72 hours after instillation. Animals were anesthetized using urethane intraperitoneally. Anesthetized animals were placed in supine position on a 45° slanted support, and a small middle incision was made over the trachea. The trachea was exposed by blunt dissection of the sternohyoideus muscle. A small hole was made in the trachea between the fifth and the sixth tracheal rings using a 20-gauge needle. A short (10- to 15-cm) length of PE50 tubing was inserted into the hole and advanced to the bifurcation of the trachea. Formulations of LN (0.1 mL) were slowly instilled over a 1-minute period using a 1-mL syringe attached to the PE50 tubing. Following instillation, the tubing was withdrawn and a small drop of cyanoacrylate adhesive was placed over the hole to seal the opening. The skin was clothed with 3-0 Dexon sutures. The animal was removed from anesthesia and allowed to recover under a heating lamp. After recovery, animals were housed in individual plastic cages with access to food and water for the remainder of the study.

Oral Administration

For oral administration, the drug was instilled through the mouth using a 28-gauge, long, blunt needle.

Blood was sampled from tail vein at 2, 4, 6, 12, 24, and 36 hours after oral administration and further at 48, 60, and 72 hours after pulmonary administration.

Analytical Methods

To determine intact LN in blood, a specific spectrofluorimetric method was used as described earlier.[15] Ten microliters of plasma samples were taken and extracted with 2 mL and 1 mL methylene chloride, twice. Methylene chloride was again extracted with 80% sulphuric acid in ethanol, and samples were analyzed at excitation wavelength of 460 nm and emission wavelength of 520 nm. Blood samples were collected and calibration curve of LN in blood was prepared by adding known quantity of drug (after dissolving in ethanol, volume not exceeding 1% of the blood volume) in blood, and the procedure was performed as described above.

Pharmacokinetics

The AUC (area under the blood LN concentration time curve) of both orally administered and intratracheally instilled LN formulations was calculated by the trapezoidal rule.[16] C_{max}, t_{max} and Half-lives of plasma drug disappearance $t_{1/2}$, were determined from plasma LN level-time curves. Data were compared using analysis of variance

Table 2. Pharmacokinetics of Different Formulations Following Oral and Pulmonary Administration of Levonorgestrel in Rats*

Formulation	Route	AUC (ng-h/mL)	F*	T_{max} (hours)	C_{max} (ng/mL)	$T_{1/2}$ (hours)
Plain Drug **(LO)**	Oral	261.41 ± 12.36	–	2.1 ± 0.2	14.4± 0.6	16.9 ± 0.2
Plain Drug **(LP1)**	Pulmonary	255.16 ± 9.87	97.6 ± 1.2	6.0 ± 0.2	4.4± 0.4	61.2 ± 0.2
Physical Mixture **(LP2)**	Pulmonary	257.63 ± 10.15	98.6 ± 1.4	7.0 ± 0.2	4.2± 0.5	61.4 ± 0.2
Liposomes **(LP3)**	Pulmonary	287.24 ± 11.29	109.9 ± 1.4	6.8 ± 0.2	4.4± 0.6	64.4 ± 0.2

*AUC indicates the area under the blood LN concentration time curve

(ANOVA), and the difference at $P > .05$ was considered significant.

RESULTS AND DISCUSSION

The data of drug plasma concentration are shown in Figure 1, and from the figure various pharmacokinetic parameters were calculated and recorded in Table 2. The AUC following oral and pulmonary administrations of formulations was found to be significantly different. However, no significant difference was observed in AUC after pulmonary administration of these formulations. The F* values after pulmonary administration were 97.6%, 109.88%, and 98.55% for LP1, LP2, and LP3 formulations, respectively. The T_{max} for these formulations were found to be 6.0, 6.8, and 7.0 hours for pulmonary administration with a C_{max} of 4.40, 4.42, and 4.20 ng/mL, respectively, followed by a plateau up to 48 hours, while for oral administration of LN suspension, T_{max} and C_{max} were 2.1 hours and 14.4 ng/mL, respectively. Following oral drug delivery, C_{max} of 14.4 ng/mL was followed by decline in plasma concentration with $t_{1/2}$ of 16.9 hours. In contrast, pulmonary delivery gave effective plasma drug concentration for the period of 56 to 60 hours with the zero-order release kinetics following C_{max} of 4.40, 4.42, and 4.20 ng/mL for LP1, LP2, and LP3 formulations, respectively.

The rate and extent of lung uptake depend on drug physicochemical properties such as degree of ionization and lipophilicity.[17-21] Pulmonary delivery of all 3 formulations resulted in similar pharmacokinetic behavior because of the similarity in lipophilicity and size of the drug and liposomes.

Solubilization and diffusion of the drug and drug from liposomes into alveolar fluid before absorption into systemic circulation through transcellular uptake may be responsible for prolonged and zero-order absorption of LN (up to 60 ± 2 hours). It has also been reported that liposomally encapsulated drug remains in the lung for a prolonged period of time.[22] Slow and prolonged absorption of the drug after pulmonary delivery significantly reduces C_{max} and is also expected to reduce dose-dependent progestronic side effects associated with orally administered LN.

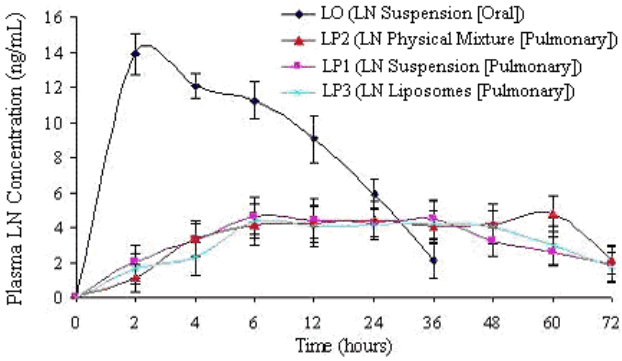

Figure 1. Plasma LN concentration (ng/mL) after oral and intratracheal instillation up to 72 hours.

Different portions of the bronchopulmonary tree possess different characteristics; it is possible that drug release from liposomes is affected by the distribution of formulation achieved during administration and later altered by mucociliary transport and other mechanisms. Animal studies to date have utilized instillation of liquid formulations in order to obtain accurate dosimetry.[23,24] Such results are dependent upon the spreading of the instilled dose within the lung and their interpretation may be complicated by the presence of components capable of affecting the spreading process. The distribution and absorption of inhaled aerosols in the lungs and airways are different from those of instilled liquids,[25-27] and it is possible that release kinetics of drug from instilled formulations in animals and from inhaled aerosols may be significantly different. In addition, the size and aerodynamic behavior of the powder through human airways may result in a significantly different distribution and rehydration of aerosolized liposomes compared with rodent test systems, which may affect observed release kinetics, duration, onset, and intensity of effect.

LN, an orally active progestronic derivative, is associated with various side effects possibly due to the initial very high plasma concentration (C_{max}) achieved, which is significantly higher than the therapeutic window of the drug (active therapeutic window, 4-6 ng/mL). Pulmonary dosage forms, however, give an extended release of the drug over a long period of time without resulting in initial higher plasma con-

centrations. This may further reduce the frequency of dose administration. While more work is needed to extrapolate these findings to better contraceptive efficacy by pulmonary route, the present study clearly indicates the important role of this route as an alternative to oral administration with regards to sustainability, and slow zero-order release kinetics may help in reduction of various side effects of oral contraceptives. The role of the formulations developed in this investigation can only be settled after pharmacodynamic studies and clinical investigations.

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