

Development of Aqueous Based Formulation of Docetaxel: Safety and Pharmacokinetics in Patients with Advanced Solid Tumors

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Abstract

A well characterized Nanosomal Docetaxel Lipid Suspension (NDLS) formulation was developed without using any detergent or toxic organic solvents to avoid hypersensitivity reactions caused by the marketed Taxotere[®] product. The lyophilized NDLS formulation was easily resuspended in water and found to be physically and chemically stable for 48 hours. Physico-chemical characterization of NDLS confirmed a homogeneous formulation with an average particle size of less than 100 nm. Percent Docetaxel association with lipids in NDLS formulation was found to be greater than 95%. The in-vitro release assay showed a sustained release of 25% Docetaxel after 4 hours and 100% Docetaxel release after 42 hours of incubation. Sub-chronic toxicity in mice and rats showed comparable safety to Taxotere[®]. However, a pharmacokinetics study in rats revealed greater systemic availability of Docetaxel after administration of NDLS compared to Taxotere[®]. Further, a comparative safety and pharmacokinetic crossover study at 75 mg/m² of NDLS and Taxotere[®] in patients with advanced solid tumor also showed higher exposure of Docetaxel with NDLS formulation than patients treated with Taxotere[®] formulation.

Keywords: Docetaxel; Solid tumors; Drug delivery; Lipids; Pharmacokinetics; NDLS

Introduction

Taxanes are cytotoxic diterpenes used clinically to treat cancer patients. Among the taxanes, the use of Docetaxel is higher due to its enhanced efficacy in most types of cancers especially breast cancer [1] and non-small-cell lung cancer [2]. The enhanced efficacy of Docetaxel is due to its increased potency to stabilize the microtubular assembly and inhibit cell replication [3-5].

Docetaxel is prepared by semi-synthesis beginning with a precursor extracted from the renewable needle biomass of yew plants. It is highly lipophilic and practically insoluble in water. Due to its insolubility, the currently marketed Docetaxel (Taxotere[®]) is formulated in polysorbate 80 and ethanol. The use of ethanol and polysorbate 80 in Taxotere[®] formulation causes infusion related toxicities and hypersensitivity reactions in patients [3,6,7]. Thus, the patients are pre-medicated with antihistamines and corticosteroids to minimize such toxicities prior to the treatment. However, Taxotere[®] is still one of the most promising drug approved for the treatment of locally advanced metastatic breast, non-small cell lung, and ovarian cancer. Docetaxel in combination with other drugs is also used for several additional cancer types such as prostate, head and neck and gastric adenocarcinoma [8-10]. To avoid toxicities associated with the excipients such as polysorbate 80/ethanol, and to improve quality of patient's life, a well-characterized Nanosomal Docetaxel Lipid Suspension (NDLS) formulation was developed using Generally Recognized as Safe (GRAS) lipid excipients. The lipid based drug delivery system has been successfully used for various pharmaceuticals products to provide less toxic drug formulations that result in better quality of life for patients [11-13]. This may be due to the altered pharmacological distribution and minimal interaction with red blood cells (RBCs) [14].

One of the limitations of all lipid or liposome based delivery systems is the use of organic solvents to solubilize water insoluble drug and lipids. The organic solvents are removed using standard solvent removal methods to form a thin dry film before hydrating with aqueous medium to prepare lipid or liposome based preparations. However, the use of organic solvent and its removal process is quite cumbersome and expensive. Therefore, our laboratory is actively engaged in developing organic solvent-free lipid-based drug delivery systems [15,16].

NDLS was developed without the use of organic solvent or detergent at any step of the entire manufacturing process. Here we describe the preparation, physico-chemical characterization and preclinical studies of NDLS. In addition, a randomized crossover study is also described to assess safety and pharmacokinetics of NDLS and Taxotere[®] in advanced solid tumor patients.

Materials and Methods

Docetaxel was obtained from Scinopharm, Taiwan. Soyphosphatidylcholine was procured from Lipoid LLC (Newark, NJ, USA) and Sodium cholesteryl sulfate was obtained from Genzyme

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Pharmaceuticals (Cambridge, MA, USA). Taxotere® was procured from Aventis Pharma, S.A., France.

Preparation and Physico-chemical characterization of NDLS

Preparation of NDLS: Soy phosphatidylcholine and Sodium Cholesteryl Sulfate were mixed in sodium citrate buffer and subjected to high pressure homogenization using Emulsiflex C3 Homogenizer (Avestin, Inc. Ottawa, Canada). Docetaxel was added and the high pressure homogenization was continued to provide Docetaxel-lipid suspension with desired particle size. Sucrose solution was prepared and mixed vigorously with Docetaxel-lipid suspension before it was filtered through sterile 0.45 µm and 0.22 µm PVDF filters under aseptic conditions. The resulting suspension was filled in vials based on 20 mg Docetaxel per vial and lyophilized. The vials were then sealed using rubber stopper and flip-off aluminum seals. The lyophilized vial was reconstituted with 9 mL of sterile water for injection, to provide 10 mL of Docetaxel Lipid suspension for injection containing 2 mg/mL of Docetaxel. The reconstituted suspension was further diluted in 5% dextrose Injection. The lyophilized product was analyzed for drug content by prepacked Waters Sunfire column (3.5 µm particle size, 150 × 4.6 mm i.d.) attached with Agilent 1100/1200 Series HPLC system (Agilent technology, Palo Alto, CA) and a UV detector. The HPLC was run using a mixture of water-acetonitrile gradient with a flow rate of 1.2 mL/min at a wavelength of 232 nm. The Osmolality of the NDLS was measured in triplicate using Osmometer, Model M3250 (Advance Instrument Inc., Norwood, MA, USA). The Osmolality was found to be 239 mOsmol/kg and hence suitable for parenteral administration. The finished drug product was found to be endotoxin free.

Physico-chemical characterization

Particle size measurement: The particle size measurement was carried out using Nicomp Model 380/ZLS&S Potential/Sub-Micron Particle Sizer (Particle Sizing Systems, New Port Richly, FL, USA). The measurements were carried out at 23°C at a scattering angle of 90°.

Morphology: NDLS was characterized for its morphology by freeze-fracture electron microscopy. The samples were quenched using sandwich technique and liquid nitrogen-cooled propane. The cryo-fixed samples were stored in liquid nitrogen for less than 2 hours before processing. The fracturing process was carried out in JEOL JED-9000 freeze-etching equipment and the exposed fracture planes were shadowed with platinum for 30 sec in an angle of 25-35 degree and with carbon for 35 sec (2 kV/60-70 mA, 1×10^{-5} Torr). The replica produced was cleaned with concentrated, fuming HNO₃ for 24 hours followed by repeating agitation with fresh chloroform/methanol (1:1 by vol.) at least 5 times. The clean replica was examined at JEOL 100 CX TEM/Microanalytical laboratories, Inc., CA, USA and at JEOL 1230 TEM/Stanford University with a digital camera.

Zeta potential: The zeta potential of NDLS was measured using Nicomp 380 ZLS Particle Sizing System. The zeta potential was measured at 23°C at 14.1 degree scattering angle, 18.9 degree external fiber angle, and 632.8 nm laser wavelength. One mL of test sample was diluted with 9 mL of water for injection.

Percent drug association: Association of Docetaxel with the lipids was determined using size exclusion chromatography. Briefly, 300 µL of reconstituted Docetaxel-lipid suspension (2 mg/mL) was loaded on Sephadex™ G-25M PD-10 column equilibrated with 0.9% sodium chloride solution. The column was then eluted with 0.9% sodium chloride solution and small fractions (~300 µL) were collected. Fractions containing the lipids were pooled and volume was measured.

For control, 300 µL of reconstituted NDLS (2 mg/mL) was diluted to the measured volume with 0.9% sodium chloride solution. The eluted pooled fractions and control were analyzed by HPLC for the drug content.

In vitro drug release

The *in vitro* release of Docetaxel from NDLS was measured using Electro Lab TDT-08L Dissolution Test Apparatus (USP Type-II (Paddle) maintained at 37°C. Dialysis membrane used was of 110 kD cut-off. Hydroxyl propyl cellulose (HPC) solution was prepared by dissolving 50.0 mg of HPC in 1 L of water and pH of solution was adjusted to 4.5 with ortho phosphoric acid. A mixture of 900 volumes of HPC solution, pH 4.5 and 100 volumes of ethanol was used as dissolution media. This dissolution medium was used to enhance the *in vitro* release of the Docetaxel from NDLS. The sample was prepared by reconstituting NDLS vial with 9 mL of water to provide 2 mg/mL Docetaxel concentration. Reconstituted suspension (3.0 mL) was taken into a 20 mL volumetric flask and diluted to volume with 5% dextrose solution to yield 0.3 mg Docetaxel concentration per mL. The dissolution bowls were filled with the dissolution medium. The dialysis membrane was cut into equal 7 cm pieces. One end was closed tightly and 0.5 mL of the sample was placed into the membrane from other end close it with universal closure such that no sample will come outside. The universal closure to the paddle was attached with the help of thread and apparatus was immediately started. At the end of each specified time point 5 mL of the sample medium was withdrawn and replaced with the same volume with fresh dissolution medium equilibrated at 37°C. Docetaxel concentration in withdrawn sample at each specified time points were analyzed by HPLC.

Preclinical studies

Toxicity: A multiple-dose study was conducted in healthy Swiss Albino mice and Sprague Dawley rats (Indian Institute of Toxicology, Pune, India) of both sexes to test the toxicity of NDLS. Twenty four male and twenty four female mice and rats were divided into four groups of 6 animals in each sex. The animals were acclimated for 7 days prior to the initiation of dosing. The animals were housed 6 each, of the same sex in polycarbonate cages provided with bedding of husk. The temperature was maintained between 20 to 24°C and relative humidity between 30 to 70%; 12 hours each of dark and light cycle was maintained. The preclinical studies were conducted in accordance with the Schedule Y of the Drugs and Cosmetic Act (IInd Amendment) Rules, 2005 and regulations of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA, Indian Institute of Toxicology, Pune, India, Registration No. 15/1999/CPSEA).

A sub-chronic 28 day intravenous toxicity study was conducted by administrating NDLS daily for five days to Swiss Albino mice at 5 mg/kg, 10 mg/kg and 15 mg/kg and to Sprague Dawley rats at 0.312 mg/kg, 0.625 mg/kg and 1.25 mg/kg. The doses were freshly prepared every day for 5 days. The control animals were administered with 5% dextrose only.

Animals were observed daily for mortality and clinical signs. The weight of each animal was recorded on day 0 and weekly intervals throughout the course of the study. All animals were sacrificed on day 29 by using CO₂ asphyxiation technique. Necropsy of all the animals was carried out and the weights of liver, kidney, adrenals, spleen, brain, heart, lungs, testes/ovaries, and epididymis/uterus were recorded. Following tissue samples of organs from control and treated animals were examined for histopathological findings: Adrenals, aorta, brain,

colon, coagulation glands, duodenum, epididymis, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, esophagus, ovaries, prostate, skeletal muscle, skin, mammary gland, spleen, seminal vesicles, stomach, testes, thymus, thyroid, urinary bladder, uterus.

Pharmacokinetics: The study was undertaken to determine the concentration levels of Docetaxel after administration of NDLS or Taxotere® (Intravenous Injection of 5 mg/kg) in twelve male Sprague-Dawley rats. A total of seven blood samples were collected from each animal. The blood samples were withdrawn at pre-dose (0 h) and at 0.25, 1, 3, 6, 12 and 24 hours post-dose administration. LC-MS/MS system and data acquisition system MassLynx software Version 4.1 was used for the quantitative determination of Docetaxel in rat plasma. Scientific Data Management System, software Version 7.1.0.27 SR-1 (NuGenesis Technologies Corporation (Waters), USA) was used to review the chromatographic data. The pharmacokinetic parameters were calculated from the drug concentration-time profile by noncompartmental model using WinNonlin Professional Software Version 5.0.1 (Pharsight Corporation, USA) for NDLS and Taxotere®.

Clinical study

Histopathologically or cytologically confirmed patients with following primary tumors (breast, head and neck, lung, melanoma, or prostate) were enrolled in the study where Docetaxel was a viable treatment option. The patients were within 18 to 65 years of age with Body Mass Index (BMI) of atleast 17, and having life expectancy of atleast 3 months were screened. Female patients, who were not pregnant or nursing, having negative pregnancy test at screening and using effective form of birth control during and for 1 month after study participation were enrolled. The mean age for enrolled patients was 49.0 ± 9.1 years and the mean body mass index (BMI) was 22.2 ± 4.8 kg/m². The racial make-up of the study was 100% Asian. Patients who satisfied all inclusion criterion and none of the exclusion criteria were enrolled in the trial.

A total of 49 patients were screened where 17 patients failed the inclusion criteria. Thus, 32 adult advanced solid tumor patients were enrolled and treated. Twenty patients (62.5%) were males and 12 patients (37.5%) were females. Before enrollment in the study, subjects were fully informed of the aim, methods, expected study duration, anticipated risks and possible discomfort and afterwards, written informed consent was obtained.

Treatment and pharmacokinetic assessments

The study was planned to be an open label, balanced, randomized, two treatments, two-sequence, crossover, multi-centric study for comparison of pharmacokinetic profile and safety of two formulations containing Docetaxel for injection in advanced solid tumor patients. A total of 32 adult advanced solid tumor patients were enrolled and dosed in the study, out of them 28 patients completed the study. The screening phase was 11 days prior to the scheduled dosing day. All patients were pre-medicated prior to NDLS or Taxotere® administration in order to rule out any effect of pre-medication on pharmacokinetics of docetaxel. The Study medication was administered to the left upper limb of the patient at a dose of 75 mg/m² intravenously, over 1 hour, which is a standard Taxotere® dose prescribed for cancer patients.

The dosing schedules were as follows: Period I: Patients were dosed with Docetaxel Injection (either test, NDLS or reference, Taxotere®) on the first day of the first chemotherapy cycle (day 1) of the study as per the randomization schedule. Period II: Patients were crossed over to either test or reference drug (patients on test product to be crossed

over to reference product and vice-versa) on the first day of the next chemotherapy cycle (day 22) as per the randomization schedule. The time of administration of dose on day 1 was the reference time for the period 02 dosing. However a deviation of up to 30 minutes was allowed. Patients were admitted to the study site on day 0 and day 21, at least 11 hours prior to dosing on day 1 and day 22, respectively. Total 22 plasma samples (01 pre-dose and 21 post dose samples) were collected in each period for pharmacokinetic assessments. Following administration of investigational drugs, plasma samples for pharmacokinetic assessments were collected. Patients underwent a Study Completion evaluation (day 39 ± 3) and were discharged from the study site at the discretion of the investigator. The plasma samples obtained from these twenty eight patients were analyzed to obtain concentrations for Docetaxel. Pharmacokinetic parameters for both the test and reference formulations were estimated from the plasma drug concentrations of Docetaxel. Parametric 90% CI for the ratio of geometric least squares mean of the calculated pharmacokinetic parameters, C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of the two formulations were computed for Docetaxel.

Drug concentration measurements

Collection of blood samples for pharmacokinetic measurements: 21 blood samples each of 03 mL and 1 pre-dose sample of 05 mL were collected from each patient in each period during the study. For Period I or Period II, blood samples were collected at 0.167, 0.333, 0.50, 0.667, 0.833, 1.00, (during infusion); 1.333, 1.667, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 36.00, 48 and 72 hours post-dose.

Bioanalytical method for docetaxel

The plasma samples of Patients were analyzed using a validated LC-MS/MS method for Docetaxel at the Bioanalytical facility of Lambda Therapeutic Research Ltd., Ahmedabad, India. Calibration curves using an 8-point calibration curve standards for Docetaxel, with concentrations ranging from 10.832 ng/mL to 4995.343 ng/mL, were used to determine the concentrations of Docetaxel in the samples of various Patients.

Safety assessments

Adverse events were assessed every cycle for the duration of the trial and graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 4.02. Data on serious adverse events (SAEs) were collected throughout the study. Medical history, demography, Physical examination and vitals, Body measurement, ECOG, Hepatic screening, β -HCG test (Serum), Hematology biochemistry and urine analysis, CT scan, Bone Scan, ECHO and ECG was carried out as a part of safety and efficacy evaluations.

Statistical analysis

Descriptive statistics was computed and reported for all pharmacokinetic parameters of Docetaxel. ANOVA, two one-sided tests for bioequivalence, power and ratio analysis for un-transformed and in-transformed pharmacokinetic parameters C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were computed for Docetaxel. ANOVA model included terms for Centre, Sequence, Sequence by Centre, Patient (Within Sequence by Centre), Treatment, Treatment by Centre and Period (Within Centre). The Sequence and Centre effects were tested using Patient within Sequence by Centre effects as the error term. The 90% parametric confidence intervals were calculated for the un-transformed and in-transformed pharmacokinetic parameters, C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of the Docetaxel. All statistical analysis for Docetaxel was performed using PROC GLM of SAS® Release 9.1.3 (SAS Institute Inc., USA).

Conduct of the clinical study

Written informed consent was obtained from all patients before enrollment. The clinical study was initiated as per the protocol after the approval from Independent Ethics Committee or Institutional Review Board. In addition, the Study was conducted as per International Conference on Harmonization Good Clinical Practice based on the basic principles of Good Laboratory Practice, Indian Council of Medical Research Guidelines for Biomedical Research on Human subjects, and Declaration of Helsinki (Seoul 2008) on the rights of research participants.

Results and Discussion

Physico-chemical characterization of NDLS

A well characterized organic solvent and polysorbate 80 free formulation of Docetaxel was developed using lipids which are Generally Recognized as Safe by the US Food and Drug Administration. The size and distribution of NDLS was measured using the dynamic light scattering method. The particle size of NDLS was found to be <100 nm after reconstitution with water for injection (Figure 1). Electron microscopy revealed a homogenous population of small unilamellar vesicles having average size less than 100 nm (Figure 2). The surface charge was assessed by the measurement of zeta potential, which is a

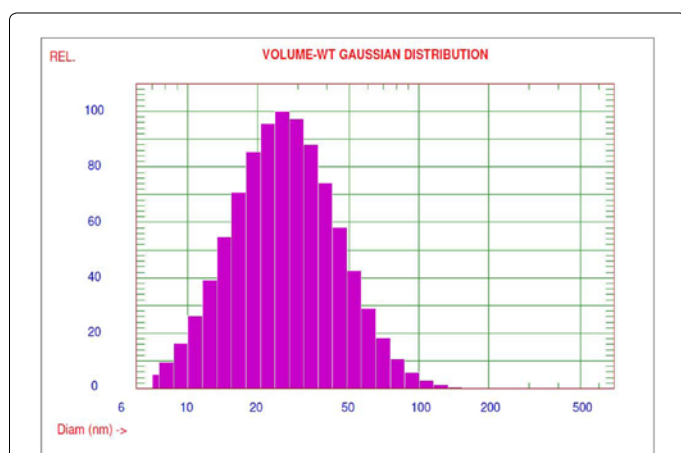


Figure 1: Particle size distribution of Nanosomal Docetaxel Lipid Suspension: mean particle size, 30 nm, distribution (<%) D25 18.4 nm, D50 26.2 nm, D75 37.5 nm, D90 52.0 nm, and D99 91.0 nm. The measurements were carried out at 23°C at a scattering angle of 90°.

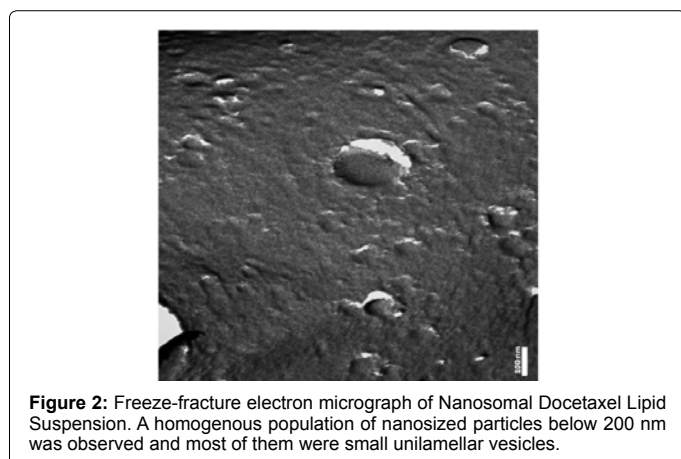


Figure 2: Freeze-fracture electron micrograph of Nanosomal Docetaxel Lipid Suspension. A homogenous population of nanosized particles below 200 nm was observed and most of them were small unilamellar vesicles.

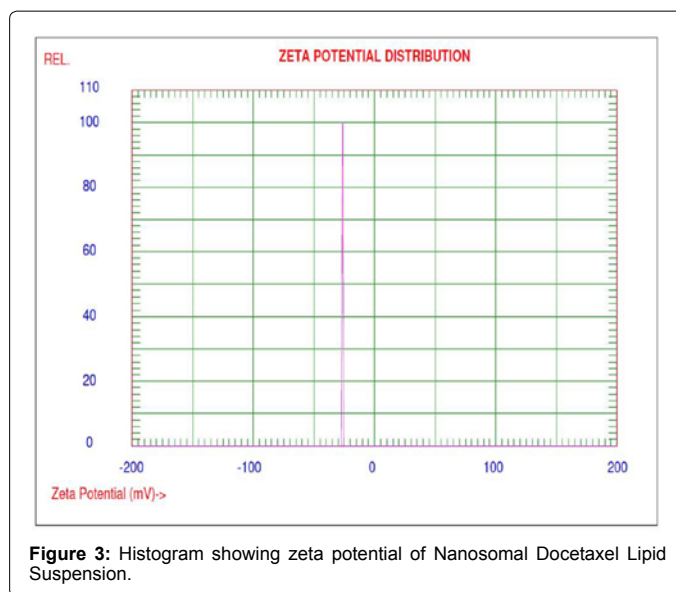


Figure 3: Histogram showing zeta potential of Nanosomal Docetaxel Lipid Suspension.

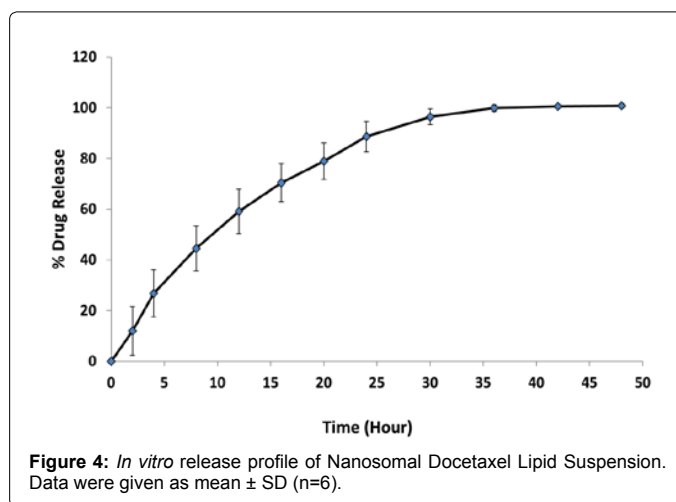


Figure 4: *In vitro* release profile of Nanosomal Docetaxel Lipid Suspension. Data were given as mean \pm SD (n=6).

measure of the magnitude of the electrostatic or charge repulsion or attraction between particles. The zeta potential of NDLS was found to be -26.37 mV (Figure 3).

Docetaxel, being a hydrophobic molecule is expected to be associated with hydrophobic fatty acid chains of soy phosphatidylcholine. Size exclusion chromatography experiment revealed that 95% of Docetaxel was associated with the lipids. *In vitro* release profile of NDLS in HPC dissolution medium showed 25% release of Docetaxel after 4 hours, 50% release after 10 hours and 100% release after 42 hours of incubation indicating sustain release of Docetaxel from NDLS (Figure 4).

The stability studies of NDLS were carried out in both accelerated and long term storage conditions according to ICH stability guidelines. The drug substance, Docetaxel, the lipid excipients Soyphosphatidylcholine, Sodium Cholesteryl Sulfate assay values, pH, mean particle size, and endotoxins levels were within the specifications after storage at 2-8°C and 25°C for 24 months. Further, the NDLS was physically and chemically stable for 48 hours after reconstitution and after dilution with 5% dextrose. The advantages of this aqueous-based NDLS formulation include the ease in preparation and use of lipids as

excipients that can circumvent the toxicities normally associated with detergents such as polysorbate 80. Treatment with this new formulation may deem it unnecessary to premedicate the patients.

Preclinical toxicity and pharmacokinetics

Multiple dose toxicity studies were conducted with NDLS in mice and rats. Mice administered with NDLS at highest dose of 15 mg/kg consecutively for 5 days resulted in mortality of 2 male and 1 female mice during the study period of 28 days (Table 1a). In a separate study, the rats were administered with NDLS at 0.312 mg/kg, 0.625 mg/kg and 1.25 mg/kg consecutively for 5 days and were monitored for 28 days resulting in no mortality (Table 1b). Biochemical investigation revealed that normal biological and laboratory limits or the effect was not dose dependent, except elevated levels of alanine aminotransferase and alkaline phosphatase were recorded in the highest dose group of mice and rats. Histopathological examination did not reveal any significant findings in male or female animals. In the highest dose level only minimal to marked degenerative changes and/or atrophy of testes and moderate to markedly reduced spermatozoa in epididymis in male animals in both mice and rats were observed. Histopathological examination of ovaries in female mice (5 mg/kg and 10 mg/kg dose groups) or female rats (0.312 mg/kg and 0.625 mg/kg dose groups)

Group No.	Dose (mg/kg)		Mortality/Total	
	Male	Female	Male	Female
I	Control	Control	0/6	0/6
II	5	5	0/6	0/6
III	10	10	0/6	0/6
IV	15	15	2/6	1/6

Table 1a: Toxicity of NDLS in mice (n=6).

Group No.	Dose (mg/kg)		Mortality/Total	
	Male	Female	Male	Female
I	Control	Control	0/6	0/6
II	0.312	0.312	0/6	0/6
III	0.625	0.625	0/6	0/6
IV	1.25	1.25	0/6	0/6

Table 1b: Toxicity of NDLS in Rats (n=6).

revealed no abnormality attributable to the treatment. Mild follicular cyst and/or reduced number of corpora lutea was observed in ovaries in female animals from highest dose groups. It was noted that rats are more sensitive to NDLS compared to mice in rodents similar to Taxotere® [17].

The comparative Pharmacokinetics was evaluated after administration of a single 5 mg/kg intravenous dose of NDLS and Taxotere® in Sprague Dawley female rats. NDLS treated rats showed higher AUC_{0-t} (2128 vs. 916 ng h/ml), C_{max} (2459 vs. 310 ng/ml), T_{max} (0.375 vs. 0.25 h) and t_{1/2} (10 vs. 6.1 h) compared to Taxotere® treated rats (Table 2). It was apparent that the NDLS administration resulted in higher systemic bioavailability. There was no toxicity related to greater exposure of Docetaxel after NDLS administration that led to the investigation of pharmacokinetics in patients.

Clinical pharmacokinetics and safety

Evaluation of safety and pharmacokinetic comparison of intravenous infusion of NDLS and Taxotere® was conducted in advanced solid tumor patients (Figure 5). A total number of 172 Adverse Events (AEs) were observed. Among 172 AEs, 80 occurred before treatment and 92 occurred post treatment. The 92 post treatment AEs were reported by 24 of the 32 patients who were exposed to atleast one of the treatments. Out of these 92 post treatment AEs, 17 were judged as related to the study drug. The breakdown by treatment group is as follows: 44 AEs were reported by 72.41% (n=21) of the 29 patients who received Taxotere® treatment. 48 AEs were reported by 58.06% (n=18) of the 31 patients who received NDLS treatment. A total of 55 AEs in Period I, 05 AEs in Period II, 30 AEs in End study and 02 AEs in follow ups were reported during the study. The most frequently AEs

PK Parameters	Mean (±SD)	
	NDLS	Taxotere®
T _{max} (h)	0.375 (±0.306)	0.250 (±0.250)
C _{max} (ng/mL)	2458.89 (±1300.190)	309.745 (±49.091)
AUC _{0-t} (ng.h/mL)	2128.168 (±777.830)	916.259 (±234.152)
AUC _{0-inf} (ng.h/mL)	2251.664 (±672.955)	958.602 (±231.376)
t _{1/2} (h)	9.977 (±7.106)	6.126 (±1.566)

Table 2: Pharmacokinetics Analysis of Docetaxel after single dose administration of 5 mg/kg of NDLS or Taxotere® to rats.

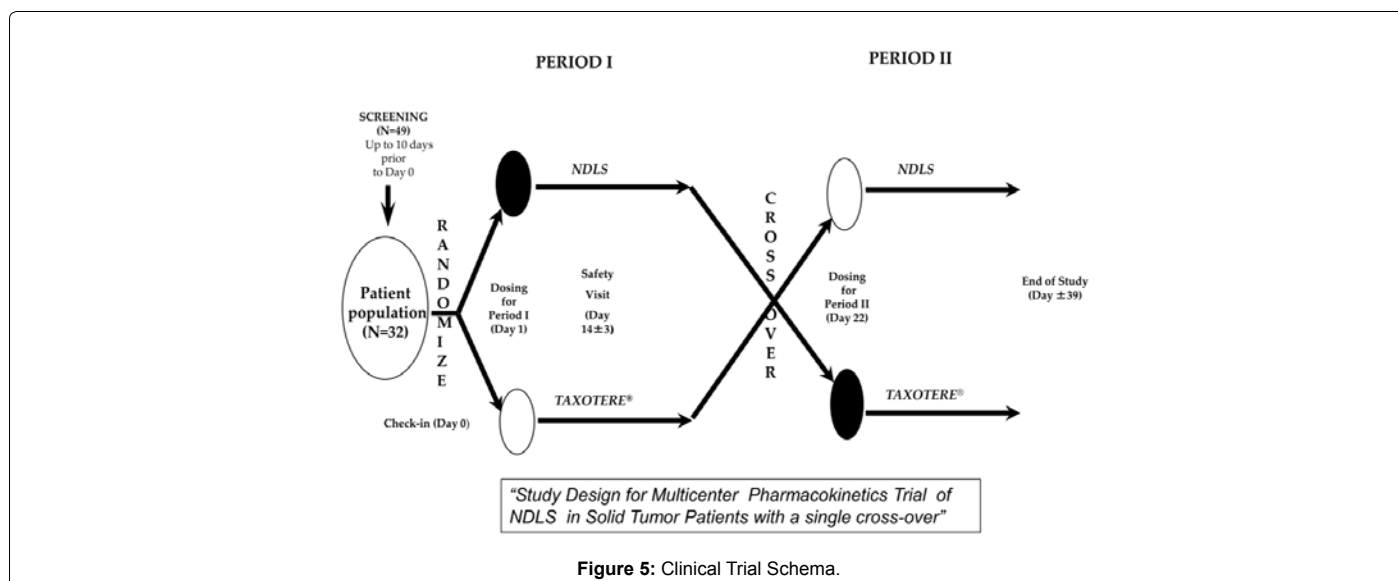


Figure 5: Clinical Trial Schema.

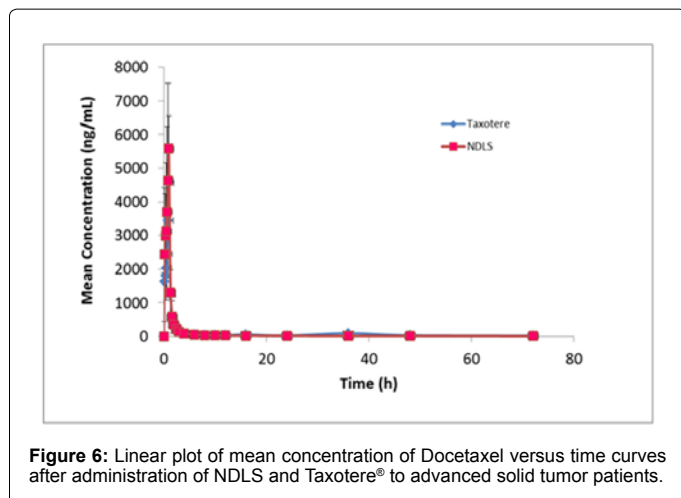


Figure 6: Linear plot of mean concentration of Docetaxel versus time curves after administration of NDLS and Taxotere® to advanced solid tumor patients.

Parameters (Units)	MEAN ± SD (un-transformed Data)	
	Taxotere	NDLS
T _{max} (h)	1.000	1.000
C _{max} (µg/mL)	5.03 ± 2.47	7.03 ± 230
AUC _{0-t} (µg.h/mL)	5.73 ± 4.50	6.01 ± 2.65
AUC _{0-∞} (µg.h/mL)	6.13 ± 4.56	6.41 ± 2.78
λ _z (1/h)	0.059-1.041	0.055 ± .042
T _{1/2} (h)	21.24 ± 26.1	21.28 ± 18.1
Vz (L/m ²)	434 ± 497	356 ± 239

T_{max} is represented in median value

Table 3: The Pharmacokinetic Parameters of Docetaxel for NDLS and Taxotere® (N=28 Patients).

among 44 AEs in Taxotere® group and 48 AEs in NDLS group were hypertriglyceridemia [2 events (4.55%) in case of Taxotere® product and 7 events (14.58%) in case of NDLS product], hyperglycemia [3 events (6.82%) in case of Taxotere® and 5 events (10.42%) in case of NDLS] and hypoalbuminemia [4 events (9.09%) in case of Taxotere® and 4 events (8.33%) in case of NDLS].

Figure 6 shows a plasma concentration–time curve of NDLS and Taxotere® at 75 mg/m². A marked inter-patient variability was observed for AUC and C_{max} and other PK parameters of NDLS and Taxotere®. Table 3 shows PK parameters of NDLS and Taxotere®. The ratio of NDLS and Taxotere® for C_{max} and AUC_{0-t} was about 149.3% (CI, 124.4–179.24) and 119.3% (CI, 98.05 -145.10%), respectively and the calculated 90% confidence interval for AUC and C_{max} did not fall within the bioequivalence range. The pharmacokinetic data such as AUC_{0-∞} obtained in this study were compared with other studies where Docetaxel was administered as 1 h intravenous infusion at 75 mg/m². Baker et al., [18] and ten Tije et al., [19] reported AUC_{0-∞} values (Mean ± SD, µg h/mL) of 5.75 ± 2.51 and 5.69 ± 2.27 respectively for Taxotere®. It is to be noted here that Docetaxel PK parameters such as AUC_{0-∞} from Taxotere® and NDLS observed in Indian patients were similar to black and white patients. Thus, the study indicates that Docetaxel pharmacokinetics is not significantly altered by ethnic and racial characteristics [20].

The higher systemic availability of Docetaxel in the plasma compartment from NDLS may be related to altered tissue distribution of Docetaxel due to the presence of lipid in the formulation. The changes in tissue distribution can affect the concentration of Docetaxel at the

sites of action and hence may improve the overall efficacy. We have recently demonstrated that NDLS without pre-medication produced a greater therapeutic response when compared to Taxotere® [21].

Conclusion

The development of NDLS, a novel formulation of Docetaxel is described in this report. NDLS was well characterized and found to be stable, safe, and bioavailable. Taken together, NDLS may provide alternate treatment option with enhanced antitumor activity for cancer patients and may not necessitate pre-medication with corticosteroids prior to the treatment.

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References

- Valero V, Jones SE, Von Hoff DD, Booser DJ, Mennel RG, et al. (1998) A phase II study of docetaxel in patients with paclitaxel-resistant metastatic breast cancer. *J Clin Oncol* 16: 3362-3368.
- Edelman MJ, Gandara DR (1996) Promising new agents in the treatment of non-small cell lung cancer. *Cancer Chemother Pharmacol* 37: 385-393.
- ten Tije AJ, Verweij J, Loos WJ, Sparreboom A (2003) Pharmacological effects of formulation vehicles : implications for cancer chemotherapy. *Clin Pharmacokinet* 42: 665-685.
- Gradishar WJ (2008) Current data and ongoing trials with novel taxane formulations in metastatic breast cancer. *Community Oncology* 5: 2-7.
- Hennenfent KL, Govindan R (2006) Novel formulations of taxanes: a review. *Old wine in a new bottle? Ann Oncol* 17: 735-749.
- Masini E, Planchenault J, Pezziardi F, Gautier P, Gagnol JP (1985) Histamine-releasing properties of Polysorbate 80 in vitro and in vivo: correlation with its hypotensive action in the dog. *Agents Actions* 16: 470-477.
- Loos WJ, Baker SD, Verweij J, Boonstra JG, Sparreboom A (2003) Clinical pharmacokinetics of unbound docetaxel: role of polysorbate 80 and serum proteins. *Clin Pharmacol Ther* 74: 364-371.
- Valero V, Holmes FA, Walters RS, Theriault RL, Esparza L, et al. (1995) Phase II trial of docetaxel: a new, highly effective antineoplastic agent in the management of patients with anthracycline-resistant metastatic breast cancer. *J Clin Oncol* 13: 2886-2894.
- Pronk LC, Stoter G, Verweij J (1995) Docetaxel (Taxotere): single agent activity, development of combination treatment and reducing side-effects. *Cancer Treat Rev* 21: 463-478.
- Hortobagyi GN, Kris MG (2002) Expanding horizons: an update on the use of docetaxel in non-small cell lung, ovarian, and breast cancers. *Semin Oncol* 29: 1-3.
- Gates C, Pinney RJ (1993) Amphotericin B and its delivery by liposomal and lipid formulations. *J Clin Pharm Ther* 18: 147-153.
- Chrai S, Murari R, Ahmad I (2002) Liposomes (a Review) Part Two: Drug Delivery Systems. *BioPharm* 40-49.
- Gregoriadis G (1991) Overview of liposomes. *J Antimicrob Chemother* 28 Suppl B: 39-48.
- Ahmad I, Sarkar AK, Bachhawat BK (1990) Liposomal amphotericin-B as a therapeutic measure to control experimental aspergillosis in BALB/c mice. *Indian J Biochem Biophys* 27: 370-374.
- Sheikh S, Ali SM, Ahmad MU, Ahmad A, Mushtaq M, et al. (2010) Nanosomal Amphotericin B is an efficacious alternative to Ambisome for fungal therapy. *Int J Pharm* 397: 103-108.
- Ali SM, Ahmad A, Sheikh S, Ahmad MU, Rane RC, et al. (2010) Polyoxyl 60 hydrogenated castor oil free nanosomal formulation of immunosuppressant Tacrolimus: pharmacokinetics, safety, and tolerability in rodents and humans. *Int Immunopharmacol* 10: 325-330.

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17. Bissery MC, Nohynek G, Sanderink GJ, Lavelle F (1995) Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: Preclinical experience. *Anticancer Drugs* 6: 339-355.
 18. Baker SD, Li J, ten Tije AJ, Figg WD, Graveland W, et al. (2005) Relationship of systemic exposure to unbound docetaxel and neutropenia. *Clin Pharmacol Ther* 77: 43-53.
 19. ten Tije AJ, Verweij J, Carducci MA, Graveland W, Rogers T, et al. (2005) Prospective evaluation of the pharmacokinetics and toxicity profile of docetaxel in the elderly. *J Clin Oncol* 23: 1070-1077.
 20. Lewis LD, Miller AA, Rosner GL, Dowell JE, Valdivieso M, et al. (2007) A comparison of the pharmacokinetics and pharmacodynamics of docetaxel between African-American and Caucasian cancer patients: CALGB 9871. *Clin Cancer Res* 13: 3302-3311.
 21. Ahmad A, Sheikh S, Taran R, Srivastav SP, Prasad K, et al. (2014) Therapeutic efficacy of a novel nanosomal docetaxel lipid suspension compared with taxotere in locally advanced or metastatic breast cancer patients. *Clin Breast Cancer* 14: 177-181.