Journal of Orthopaedics 35 (2023) 64–68

Contents lists available at ScienceDirect

Journal of Orthopaedics

journal homepage: www.elsevier.com/locate/jor

An open-label, randomized, crossover study to evaluate the bioavailability of nanoemulsion versus conventional fat-soluble formulation of cholecalciferol in healthy participants

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ARTICLE INFO

Keywords:
Area under curve
Nanotechnology
Vitamin D deficiency
Therapeutic equivalency
Biological availability
Lipid digestion
Absorption

ABSTRACT

Background: Nanoemulsion preparations of cholecalciferol available in the market claim to have better bioavailability than the conventional fat-soluble cholecalciferol. However, limited data are available in humans for such preparations. We, therefore, compared the relative bioavailability of two formulations of 60,000 IU cholecalciferol (nanoemulsion oral solution, water-miscible vitamin D3 [test] vs soft gelatin capsules [reference]) in healthy adult participants.

Methods: In this randomized, open-label, two sequence, single-dose, two-way crossover study (CTRI/2018/05/013839), Indian participants aged 18–45 years received single dose of nanoemulsion and capsule formulations, under fasting conditions. Blood samples collected over 120 h were assessed to determine cholecalciferol concentrations. Pharmacokinetic parameters (area under the concentration-time curve up to 120 h [AUC_{0-120h}], maximum observed drug concentration [C_{max}], time to reach maximum drug concentration [T_{max}], terminal half-life [$T_{½el}$], and terminal elimination rate constant [K_{el}]) were estimated using baseline corrected data and analyzed using analysis of variance.

Results: Among the 24 eligible participants, the relative bioavailability of nanoemulsion was significantly higher than the capsules by 36% ($p = 0.0001$) based on AUC_{0-120h}. Similarly, C_{max} of the nanoemulsion was significantly higher by 43% ($p = 0.0001$) than that of the capsules. The intra-participant variability for AUC_{0-120h} and C_{max} were 23.22% and 26.51%, respectively. The T_{max}, $T_{½el}$, and K_{el} were comparable for both the formulations. No adverse effects were noted with either of the two formulations.

Conclusions: Nanoemulsion oral solution of cholecalciferol showed a greater bioavailability compared with soft gelatin capsules, under fasting conditions, in healthy human participants.

1. Introduction

Vitamin D primarily maintains skeletal health through calcium-phosphate homeostasis.[1] Although 90% of vitamin D is endogenously synthesized in the skin by ultraviolet B radiation of the Sun,[2] its deficiency has become pandemic and remains a widely underdiagnosed and undertreated medical condition worldwide.[3–5] Vitamin D deficiency is prevalent in about 1 billion people, globally.[6] Despite being a tropical country, 40–99% of the general population of India is diagnosed with vitamin D deficiency.[5] Sub-clinical vitamin D deficiency prevails across all regions in India, both urban and rural areas, irrespective of socio-economic factors, gender, age, geographical regions, environmental conditions, or profession.[4,7,8] The primary reason being limited

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https://doi.org/10.1016/j.jor.2022.10.017
Received 19 October 2022; Accepted 31 October 2022
Available online 4 November 2022

Abbreviations

ANOVA	Analysis of variance
$AUC_{0–120h}$	Area under the concentration-time curve up to 120 h
BLQ	Below the limit of quantification
CI	Confidence intervals
C_{max}	Maximum observed drug concentration
CV	Coefficient of variation
GCP	Good Clinical Practices
GIT	Gastrointestinal tract
GLP	Good Laboratory Practices
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
K_{el}	Terminal elimination rate constant
LSM	Least squares mean
PK	Pharmacokinetic
SAS	Statistical Analysis System
SD	Standard deviation
T_{max}	Time to observe maximum drug concentration
$T_{½el}$	Terminal half-life;
25-[OH]D	25 hydroxy vitamin D3

sun-induced vitamin D synthesis due to factors, such as seasons, time of the day, air pollution, altitude, skin pigmentation, use of sunscreens, obesity, working predominantly indoors or in evening or night shifts, etc., and poor availability of vitamin D in Indian diets.[4,9–11] Sun exposure and Indian diets may not be the tenable measures for prevention of vitamin D deficiency in majority of the population.[7,12–14]

In view of the above mentioned factors and absence of mandatory food fortification with vitamin D, supplementation would play an important role in reducing the burden of vitamin D deficiency.[8] Majority of vitamin D formulations (99.9%) available in the Indian market are conventional fat-soluble preparations of vitamin D, which are available in the form of tablets, capsules, sachets, or liquid preparations.[8] Vitamin D is a non-polar lipid, having poor bioavailability owing to its low solubility in aqueous fluids of the gastrointestinal tract (GIT). Therefore, a reliable and robust drug delivery system is needed to improve the vitamin D status in the Indian population.[11] Nanotechnology-based nanoemulsion formulations of vitamin D3 claim to have better bioavailability as it disperses fatty molecules into aqueous micellar spheres enabling better absorption and bioavailability.[15–17]

In view of limited literature with regard to efficacy and bioavailability of recently introduced nanoemulsion preparations of vitamin D3, we undertook this open-label, crossover study to compare the bioavailability of [3]DePURA, an oral nanoemulsion formulation and [4]Uprise D3, a soft gelatin capsule formulation in healthy participants.

2. Material and methods

2.1. Study design and settings

This was a single-center, randomized, single-dose, open-label, balanced, two-way crossover study (CTRI/2018/05/013839) to assess the relative bioavailability of the study drugs in healthy Indian participants. The study consisted of two treatment periods, separated by a washout period of 11 days. Non-smoking adults, aged 18–45 years, with a body mass index between 18.5 kg/m^2 and 24.99 kg/m^2, and serum 25 hydroxy vitamin D3 (25-[OH]D) level between 10 ng/mL and 20 ng/mL, were enrolled for the study. Details of the inclusion and exclusion criteria are given in the Appendix. Participants were randomized according to the block randomization scheme produced by inVentiv/Syneos Health (Quebec, Canada) and were required to avoid direct exposure to sunlight during the period of blood sample collection. Participants with history of any systemic illness; hypersensitivity to cholecalciferol, ergocalciferol, or vitamin D metabolites; and on any other medication including vitamins and minerals, within 14 days prior to study-drug administration, were excluded from the study.

2.2. Treatment schedule

After an overnight fast of at least 10 h, participants were administered either cholecalciferol 5 mL nanoemulsified oral solution (test formulation) or cholecalciferol oral soft gelatin capsule (reference formulation) containing 60,000 IU of vitamin D3 with 240 ± 2 mL of water. Participants were served a controlled meal ≥ 4 h post-dose and at appropriate times thereafter, in each period. No fluids were permitted 1 h before and after dosing, except that given at the time of dosing. Water was permitted as and when required at all other times. In each study period, participants were confined to the clinical facility from at least 12 h before dosing until after the 48-h post-dose blood withdrawal. They were further informed to return to the clinical facility for all subsequent blood collections. A total of 14 blood samples were drawn at baseline (pre-dose) and 1, 2, 4, 6, 8, 10, 16, 24, 36, 48, 72, 96, 120 h post-dose in each treatment period. After 48 h, blood samples were drawn by venipuncture on ambulatory basis or using intravenous catheter to avoid multiple skin punctures. The samples were collected in tri-potassium ethylenediaminetetraacetic acid tubes (1 × 6 mL), centrifuged (2000 ± 5 g, 10 min, 4 ± 2 °C), aliquoted in polypropylene tubes, and stored at −20 ± 5 °C for further analysis. Concentrations of cholecalciferol in plasma samples were determined using a validated liquid chromatography - Tandem Mass Spectrometry (liquid chromatography–mass spectrometry/mass spectrometry) bioanalytical methodology.

2.3. Objectives and assessments

The primary objective of the study was to compare the area under the concentration-time curve up to 120 h ($AUC_{0–120h}$) values of nanoemulsion vs. capsules under fasting condition. The secondary objectives were to monitor the safety and tolerability and compare the maximum observed drug concentration (C_{max}) and time to observe maximum drug concentration (T_{max}) of a single oral dose of the two formulations.

2.4. Statistical analysis

2.4.1. Pharmacokinetic parameters

Data from participants who completed all periods of study were considered for pharmacokinetic (PK) and statistical analysis. The PK parameters were estimated by the non-compartmental model using Phoenix WinNonlin (version 6.4 or higher). These parameters included $AUC_{0–120h}$, C_{max}, T_{max}, apparent first-order terminal elimination rate constant (K_{el}), and terminal half-life ($T_{½el}$). All concentration values below the limit of quantification (BLQ) were set to zero for the estimation of PK parameters. The PK results were analyzed using baseline corrected data. Descriptive statistics were computed for each PK parameter at each sampling time. Inferential statistical analyses were performed using Statistical Analysis System (SAS) version 9.2. Analysis of variance (ANOVA) was performed on log transformed $AUC_{0–120h}$ and C_{max} and untransformed T_{max}, K_{el}, and $T_{½el}$ of cholecalciferol using PROC general linear model procedures.

The 90% geometric confidence intervals (CIs) were constructed for ratios of least squares mean (LSM) of nanoemulsion and capsule

[3] DePURA - a recently developed oral nanoemulsion formulation of 60,000 IU of cholecalciferol (water-miscible vitamin D3); marketed by Sanofi India Limited under the brand name DePURA.

[4] Uprise D3 - a soft gelatin capsule formulation containing 60,000 IU of conventional fat-soluble cholecalciferol marketed by Alkem under the brand name Uprise D3.

formulations for log-transformed $AUC_{0–120h}$ and C_{max}. Intra- and inter-participant coefficient of variation (CV%) were also calculated. A drug potency correction was done, as the difference in the measured drug content of the test was greater than 5% from the capsule formulation. The 90% CI for C_{max} and AUC_{0-120} were presented for both potency-corrected and uncorrected data.

2.4.2. Sample size determination

It was assumed that the true standard deviation (SD) was within 0.325 and CV% of AUC_{last} was 33%, data from 20 participants would have provided a maximum imprecision of 19.3% for the estimation of the treatment ratio (i.e., 90% CI not wider than 0.80 and 1/0.80 = 1.24 times the observed ratio) with 90% assurance for log-transformed PK of the nanoemulsion. A total of 30 participants were planned to be enrolled assuming that four participants were on stand-by.

2.5. Ethics

All clinical work was conducted in compliance with Good Clinical Practices (GCP) and Good Laboratory Practices (GLP) as referenced in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (ICH E6 [R2]), local regulatory requirements, and the recommendations laid down in the most recent version of the World Medical Association and Declaration of Helsinki. Before beginning the associated study procedures, the study was approved by the Ethics Committee (Human Care Independent Ethics Committee, Thane, India), and all participants signed a written informed consent (CTRI/2018/05/013839 http://ctri.nic.in/Clinicaltrials/advsearch.php).

3. Results

A total of 25 healthy Indian adults (1 woman and 24 men), with mean ± SD age of 32 ± 7 years, were randomized in the study. Although all the 25 participants completed all the study periods, 1 participant was not included in the PK population as all the inclusion criteria were deemed to be not fulfilled.

3.1. Pharmacokinetics

The mean ± SD concentration-time profiles for baseline-corrected cholecalciferol for nanoemulsion vs. capsules are shown in Fig. 1. The PK parameters of cholecalciferol for both nanoemulsion and capsules are summarized in Table 1. Mean ± SD C_{max} was higher for nanoemulsion vs. capsules (127.10 ± 26.24 ng/mL vs. 91.82 ± 29.27 ng/mL). The $AUC_{0–120h}$ calculated for the nanoemulsion was higher than that of the capsules (mean ± SD: 5599.73 ± 1060.59 h*ng/mL vs. 4199.61 ± 1224.99 h*ng/mL). For both the formulations, T_{max}, $T_{½el}$, and K_{el}, were comparable (Table 1).

3.2. Bioavailability

The treatment comparison (nanoemulsion vs. capsules) ratios for $AUC_{0–120h}$ and C_{max} are presented in Table 2. The relative bioavailability of the nanoemulsion was significantly higher than that of the capsules by ~36% (p = 0.0001) based on $AUC_{0–120h}$. Similarly, the C_{max} of the nanoemulsion was significantly higher by ~43% (p = 0.0001) than that of the capsules. The intra-participant variability for $AUC_{0–120h}$ and C_{max} were 23.22% and 26.51%, respectively. Additionally, the inter-participant variability for $AUC_{0–120h}$ and C_{max} were 13.66% and 14.47%, respectively.

3.3. Safety

No adverse effects were observed among participants except mild pharyngitis in one participant, following the administration of cholecalciferol capsule and considered to be unrelated to the formulation.

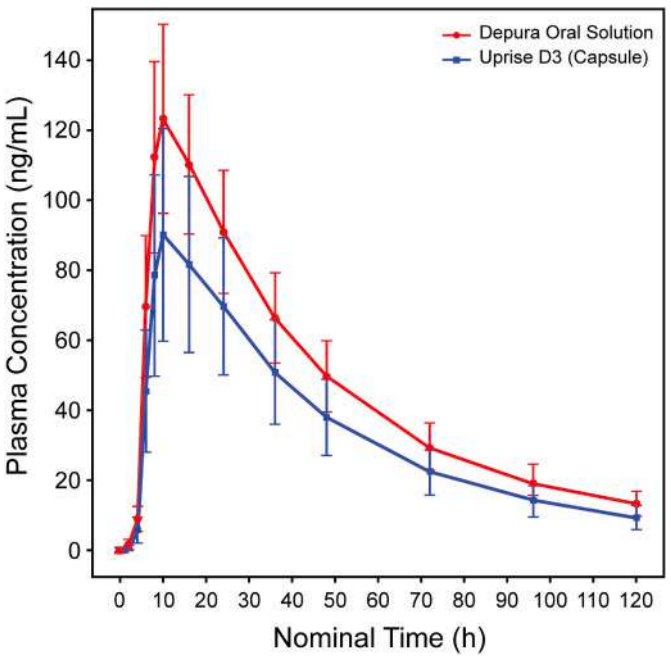

Fig. 1. Mean (SD) concentration-time profile for baseline-corrected cholecalciferol for each treatment (linear scale).

4. Discussion

The $AUC_{0–120h}$ and C_{max} values were significantly higher for nanoemulsion solution than for the reference capsule formulation, indicating higher bioavailability of cholecalciferol with nanoemulsion solution than with soft gelatin capsules. Single doses of both the treatments were well-tolerated as no new safety concerns were observed.

The present study results were in agreement with the findings of Kadappan et al.,[15] in a simulated GIT system and in other in-vivo study in mice, that demonstrated nanoemulsion-based delivery system is superior to coarse emulsions in terms of bioavailability of vitamin D. Walia et al.[18] in their study also observed an increased bioavailability, using a fish oil-based nanoemulsion of vitamin D in a simulated GIT, compared with the non-encapsulated vitamin D. Similarly, nanoemulsion preparations of vitamin A have also shown greater absorption and higher plasma levels following supplementation as against standard oil preparation in children and adults.[19] Besides, in a two-way, open-label, randomized single-dose, crossover design in children with chronic cholestasis, bioavailability of Tocofersolan (a water-soluble derivative of natural d isomer of α-tocopherol) was shown to be significantly higher than that of fat-soluble preparation of vitamin E (dl-α- Tocopheryl acetate).[20] In contrast, Nandgaya et al.,[21] in an open-label, randomized, single-dose, three-treatment study conducted in healthy adult men, showed vitamin D3 oral solution formulated with nanotechnology to be bioequivalent to conventional vitamin D3 tablet and capsule (bioequivalence acceptance limit of 80–125%). However, AUC_{0-28d} and C_{max} with cholecalciferol oral solution formulated with nanotechnology was higher when compared with conventional vitamin D3 tablet and capsule.[21]

The vitamin D3 in conventional oral formulations is absorbed via lipid digestion and absorption pathway. Bile from liver and lipases/co-lipases from pancreas convert orally consumed vitamin D3 into nano-sized micelles. These nanoparticles being water miscible cross the unstirred water layer covering the enterocytes and facilitate vitamin D3 absorption.[16] Similar to the body mechanism, DePURA nanoemulsion

Table 1
Summary of pharmacokinetic parameters for baseline corrected cholecalciferol for each treatment.

Parameter (Unit)	Cholecalciferol oral solution (Test formulation)			Cholecalciferol soft gelatin capsule (Reference formulation)		
	Mean	SD	CV%	Mean	SD	CV%
AUC_{0-120h} (h*ng/mL)	5599.73	1060.59	18.94	4199.61	1224.99	29.17
C_{max} (ng/mL)	127.10	26.24	20.64	91.82	29.27	31.88
$T_{1/2el}$ (h)	38.72	5.77	14.91	36.44	5.94	16.32
K_{el} (h^{-1})	0.0183	0.0027	14.8921	0.0195	0.0032	16.3479
T_{max} (h)[a]	10.0	24–8[a]		10.0	16–8[a]	

AUC_{0-120h}, area under the concentration-time curve up to 120 h; C_{max}, maximum observed drug concentration; CV, coefficient of variation; h, hour; K_{el}, terminal elimination rate constant; SD, standard deviation; T_{max}, time to reach maximum drug concentration; $T_{½el}$, terminal half-life.
[a] Presented as median (range).

Table 2
Treatment comparison ratios for cholecalciferol capsules and cholecalciferol oral solution using baseline corrected cholecalciferol.

Parameter (Unit)	Geometric LSM		Ratio[a] (%)	90% Geometric CI[b]		Intra-Participant CV (%)	Inter-Participant CV (%)	P-values		
	Test formulation N = 24	Reference formulation N = 24		Lower (%)	Upper (%)			Sequence	Period	Treatment
AUC_{0-120} (h*ng/mL)	5483.84	4019.67	136.43	121.73	152.90	23.22	13.66	0.7954	0.2677	0.0001
C_{max} (ng/mL)	124.12	86.90	142.83	125.46	162.59	26.51	14.47	0.7717	0.4693	0.0001

AUC_{0-120h}, area under the concentration-time curve up to 120 h; CI, confidence interval; C_{max}, maximum observed drug concentration; CV, coefficient of variation; LSM, least squares mean.
Test formulation represents Cholecalciferol oral solution.
Reference formulation represents Cholecalciferol soft gelatin capsule.
[a] Calculated using LSM according to the formula: eDifference X 100.
[b] 90% Geometric CI using ln-transformed data.

designed using Aqueol technology traps solubilized vitamin D3 in a nano-lipid system. This system has a distinct stable hydrophilic surface that protects the breakdown of nanoparticles in presence of high concentration bile and lipases during its passage through the GIT. It delivers vitamin D3 directly at the site of absorption without depending on lipid digestion process like the traditional system. Vitamin D nanoemulsion has also been shown to have more prominent hepato-protective effect against high fat diet-induced liver injury in rats compared with conventional oral vitamin D.[22] Another advantage of nanoemulsion is better compliance, as it does not require consumption of milk or clarified butter for absorption.[23,24] Nanoemulsion process facilitate smooth paracellular, transcellular, and persorption of vitamin D through the intestinal mucus layer ensuring higher bioavailability than conventional formulations, independent of the amount of fat in the gut.[16] This study affirms the clinical viability and efficacy of DePURA nanoemulsion observed in two of the earlier studies in children and adults using serum 25-(OH)D as surrogate biomarker.[24,25]

This being a crossover study, each participant was his/her own control and, thus, eliminated the inter-participant variability that largely influences assessment of product bioequivalence.[26,27] Baseline correction helped to achieve an estimate of the actual drug availability from the drug product by eliminating the endogenous levels of the drug product in the plasma.[28] A number of factors influence the absorption of vitamin D in human GIT, including the complexity of food matrix.[29] The bioavailability under fed condition has not been evaluated in the current study. Although absorption from nanoemulsion is not affected by fast/fed conditions, it would be useful to repeat the study in fed condition to mimic clinical settings.

5. Conclusions

The DePURA nanoemulsion oral solution showed a greater bioavailability compared with Uprise D3 soft gelatin capsule, under fasting conditions in healthy human participants. Further clinical studies could be carried out in participants with malabsorption syndromes to extend the efficacy superiority of nanoemulsion preparations over conventional fat-soluble vitamin D3 in diverse individuals with vitamin D deficiency.

Data availability statement

Qualified researchers may request access to level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: http://www.vivli.org.

Funding/sponsorship

The study was funded and supported by Sanofi, India. This support from industry not only includes direct financial support for the study but also support for other study related services such as statistical analysis.

Sanofi also contributed to the study design, conduct of the study, analysis of samples or data, interpretation of findings or the preparation of the manuscript.

Informed consent (Patient/Guardian)

Not applicable.

Institutional ethical committee approval

All clinical work was conducted in compliance with Good Clinical Practices (GCP) and Good Laboratory Practices (GLP) as referenced in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (ICH E6 [R2]), local regulatory requirements, and the recommendations laid down in the most recent version of the World Medical Association and Declaration of Helsinki. Before beginning the associated study procedures, the study was approved by the Ethics Committee (Human Care Independent

Ethics Committee, Thane, India), and all participants signed a written informed consent (CTRI/2018/05/013839 http://ctri.nic.in/Clinicaltrials/advsearch.php).

CRediT authorship contribution statement

Raman Kumar Marwaha: Conceptualization, Methodology, Writing – review & editing. **Manish Verma:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Ajit Walekar:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Rakesh Sonawane:** Visualization, Writing – review & editing. **Chirag Trivedi:** Conceptualization, Methodology, Visualization, Writing – review & editing.

Declaration of competing interest

RKM received honoraria during conduct of the study. MV and RS are ex-employees of Sanofi. AW and CT are employees of Sanofi and may hold shares and/or stock options in the company.

Acknowledgement

Medical writing support was provided by Rukhsar Wasta and editorial assistance was provided by Sonal More (both from Tata consultancy services India Ltd.), which was funded by Sanofi, India. Editorial support was provided by Anahita Gouri (Sanofi, India) and Rohan Mitra (ex-employee of Sanofi, India).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jor.2022.10.017.

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