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Original Research Article

Determination of the bioavailability and biodistribution of a single dose of oral cholecalciferol/Calcirol® soft gelatin capsule by pharmacoscintigraphy- CalSci study

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ABSTRACT

Background: It is required to study the bioavailability and biodistribution of specific cholecalciferol formulation before prescribing. Pharmacoscintigraphy is an established radiological-imaging technique that is used to map various drug formulations as they traverses the human body (biodistribution) in real-time. We evaluated the bioavailability and biodistribution pattern, transit time, and gastrointestinal clearance of a single dose of Calcirol® soft gelatin capsule 60,000 IU [an oral cholecalciferol (vitamin D) formulation] using pharmacoscintigraphy.
Methods: Six male healthy adult volunteers were administered a single oral dose of Calcirol® soft gelatin capsule labelled with technetium-99m. Post-dosing, serial venous blood samples were collected till day 27 for the estimation of the plasma levels of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol levels. Different pharmacokinetic parameters were calculated. Sequential static gamma imaging was performed to evaluate the biodistribution of Calcirol® soft gelatin capsule. Descriptive statistics was used. Various pharmacokinetic parameters were calculated from the concentration-time curves. Statistical analysis was carried out using Student's t-test. Suitable multivariate analysis was performed based on the distribution of data. All statistical analyses were performed using SAS® Software (v 9.4).
Results: The overall absorption of Calcirol® soft gelatin capsule was 93.23%, which was fully from the small intestine. It led to achieving a sufficient level of 25-hydroxycholecalciferol (>60 ng/ml) within 6 hours of oral intake. The levels of plasma 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol increased (maximum around 6 and 18 days, respectively). The small intestinal residence time was around 16 hours. No adverse event was noted.
Conclusions: This was the first pharmacoscintigraphy study in the world which demonstrated the favourable bio-distribution of the Calcirol softgels supporting its role in vitamin D supplementation.

Keywords: Bioavailability, Biodistribution, Calcirol®, Cholecalciferol, Pharmacokinetics, Pharmacoscintigraphy

INTRODUCTION

Vitamin D or cholecalciferol is a fat-soluble vitamin that acts as a steroid hormone. In humans, the primary source of cholecalciferol is ultraviolet B (from sunlight) induced conversion of 7-dehydrocholesterol to cholecalciferol in the skin. Cholecalciferol undergoes two hydroxylations in the body for activation; it is metabolized first to 25 hydroxyvitamin D (25OHD), then to the hormonal form 1,25-dihydroxyvitamin D [1,25(OH)2D]. 1,25(OH)2D is the ligand for the vitamin D receptor (VDR), a transcription factor, binding to sites in the DNA called vitamin D response elements. 25OHD which circulates at a much lower serum concentration than 25-

hydroxycholecalciferol, but has a much higher affinity to the vitamin D receptor.[1,2]

The main dietary sources of cholecalciferol are dairy products, eggs, and fish. Cholecalciferol influences bones, intestines, immune and cardiovascular systems, pancreas, muscles, brain, and the control of cell cycles. The different therapeutic indications of oral cholecalciferol include hypothyroidism, osteoporosis, osteomalacia, and vitamin D insufficiency/deficiency in healthy patients or those with chronic kidney disease. Aside from its known role in bone mineral metabolism, various studies have found a link between cholecalciferol supplementation and favourable prognosis for different diseases, such as hypertension, diabetes mellitus, cardiovascular diseases, cancer, rheumatoid arthritis, polycystic ovarian syndrome, multiple sclerosis, systemic lupus and some dermatological diseases.[3-10] Although less common, cholecalciferol has a few adverse effects, including hypercalcemia, hypercalciuria, nephrotoxicity, and hyperphosphatemia. However, the excessive use or overdose of cholecalciferol can lead to toxicity manifesting with nausea, vomiting, fatigue, reduced appetite, constipation, dehydration, irritability, weakness, and confusion.[11]

Several cholecalciferol formulations are available in the market and limited information is available on their bioavailability and biodistribution profiles.[13-15] It is important to understand the bioavailability and biodistribution of specific cholecalciferol formulation before prescribing such formulations therapeutically to ensure their optimal effectiveness and minimize toxicity. Pharmacoscintigraphy is an established radiological imaging technique that is used to map various drug formulations as it traverses the human body (biodistribution) in real time. Radiolabelling is generally achieved by the incorporation of an appropriate technetium-99m or indium-111 labelled radiopharmaceutical agent into the drug formulation. Combining this information with the pharmacokinetic data gives valuable information about the release and absorption mechanisms of drug formulations, including the ability of a formulation to reach a specific target location, the rate of erosion in comparison with in vitro dissolution data, and the impact of absorption windows on bioavailability.[12] This technique is also used for evaluating formulations at the preclinical and clinical stages of the development of drug delivery using novel drug carrier systems.[16-19]

Calcirol® Softgel is an oral cholecalciferol soft gelatin capsule formulation manufactured by Cadila Pharmaceuticals Ltd., Ahmedabad, India. This study was performed to evaluate the bioavailability and biodistribution pattern, transit time, and gastrointestinal clearance of a single dose of Calcirol® soft gelatin capsule using pharmacoscintigraphy, to estimate serial plasma cholecalciferol level after its administration, and assess its safety in six healthy human volunteers.

METHODS

This was a single center single-period interventional drug trial. The study duration was 27 days starting from the day of first check-in to the facility or dosing day.

Patients

Six male healthy adult (≥18 years) volunteers with body mass index between 18.5 and 24.9 kg/m^2, weighing at least 50 kg, having normal physical examination findings and normal laboratory test results (routine hematology, biochemistry, serology and electrocardiogram) within 21 days prior to the commencement of the study, without any significant medical or medication history, and willing to participate in this study were included. Vitamin D deficient patients, those having a significant history of hypersensitivity to the study drug or any ingredients of the drug formulation or any related products, those who had any significant medical illness or were hospitalized within the preceding 4 weeks, those who had consumed any over the counter or prescribed medications within the preceding 2 weeks, those who had taken any analgesic agent within the preceding 24 hours, those with a history of drug abuse or alcoholism (>2 units/day or 10 units/week) or smoking (>10 cigarettes/day or tobacco consumption of >4 packets/day), and those who had participated in any other clinical trial requiring repeated blood sampling or a blood donation program or blood loss of >450 ml, within the preceding 3 months were excluded.

Study drugs and procedure

Prior ethics committee approval was obtained before the commencement of the study. After taking the informed consent from the participants, all the patients. All patients were housed in a facility having an ambient temperature and relative humidity from around 2 hours prior to dosing to 24 hours after dosing. A single oral dose of Calcirol® soft gelatin capsule 60,000 IU (Cadila Pharmaceuticals Ltd., Ahmedabad, India) labelled with technetium-99m (freshly prepared on the study day) was administered to all volunteers in a sitting posture with about 240 ml of water under the supervision of trained study personnel who confirmed the dosing compliance. The volunteers were advised to take food before consuming the drug formulation and fast for at least 4 hours post-dosing. Water and fluid were restricted from at least 1 hour prior to dosing until at least 1-hour post-dosing. Volunteers were not permitted to consume alcohol or smoke or consume any grape fruit containing products or xanthine containing food or beverages from 48 hours prior to dosing till the end of the study. All volunteers remained in a seated or semi-inclined position till 2 hours post-dosing and were restricted from doing any stressful physical activity during their stay in the clinical facility. All subjects received a standard and uniform meal at scheduled times as per the study protocol throughout the study period and did not receive any other medications or nutritional supplements. Demographic characteristics, medical and medication

history, clinical examination, and breath alcohol analysis were performed for all volunteers at screening. Clinical examination and laboratory examination (routine hematology, biochemistry, serology, and electrocardiogram), were performed for all volunteers at the screening and the end of the study. All volunteers were advised to report any adverse event during the study period.

Pharmacoscintigraphy study

Sequential static gamma imaging was performed at baseline (within 5 minutes of dosing), 0, 0.5, 1, 2, 4, 6, 12, and 24 hours post-dosing for 2 minutes to determine the bioavailability and biodistribution of the drug formulation. The imaging was stopped as soon as the radioactive soft gelatin capsule reached the large intestine. Additional images were obtained at the investigator's discretion without causing any discomfort to the volunteers.

Pharmacokinetic study

10 ml of venous blood sample was collected from each volunteer before dosing (0 hour). Post-dosing, 4 ml of venous blood samples were collected at the following time points: 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, 3rd day, 6th day, 9th day, 12th day, 15th day, 18th day, 21st day, 24th day, and 27th days. All blood samples were collected in ethylenediaminetetraacetic acid vacutainers from a heparinized cannula placed on the dorsal aspect of the hand of each volunteer. The blood samples were stored and processed for the estimation of plasma 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol levels using a validated high performance liquid chromatography technique. Various pharmacokinetic parameters [concentration at time 0 (C_0), maximum concentration (C_{max}), time to reach the maximum concentration (T_{max}), half-life ($t_{1/2}$), volume of distribution (V_d), clearance (Cl), elimination rate constant (k_{el}), and area under the concentration time curve from (trough to day 27 and trough to infinity) (AUC0-t and AUC0-inf)] of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol were calculated using professional software.

Statistical analyses

Descriptive statistics was used. Various pharmacokinetic parameters were calculated from the concentration-time curves. Statistical analysis was carried out using Student's t-test. Suitable multivariate analysis was performed based on the distribution of data. All statistical analyses were performed using SAS® Software (v 9.4).

RESULTS

General characteristics

A total of eight healthy volunteers were screened and six were enrolled. There was no protocol deviation during the study period and no volunteers were discontinued from the study. The mean age of the volunteers was 22.66±2.70 years and the mean body mass index was 21.71±2.33 kg/m^2.

Pharmacoscintigraphy study

The radiolabelled Calcirol® soft gelatin capsule stayed inside the stomach as a single entity without any release or leakage of radioactivity from the capsule within first 15 min of dosing. The capsule could not be captured in the first dynamic gamma image implying that the esophageal transit time was very short (<5 seconds). The capsule was found to be dispersed throughout the stomach (oblong and end-on forms of radioactivity were noted). The two spots of radioactivity indicated the disintegration of the capsule from the capsule shell (30 and 60 minutes). At 30 minutes, the capsule started disintegrating and the radiolabelled drug started to disperse into the media. At 2 and 3 hours post-imaging, the radioactivity was observed at the right of the gastric cavity as a single entity and some light spots in the stomach and intestinal region appeared, indicating the small intestinal arrival time. Hence total gastric residence time was somewhere 0-2 hours and the intestine arrival time as 2 hours. Hence the total gastric residence time was around 2 hours. From 2 to 20 hours, the capsule travelled within the small intestine. No sign of radioactivity was found after 24 hours. Hence, complete absorption of the capsule happened from the small intestine without reaching the colon and the small intestinal residence time was around 16 hours (extrapolated) (Figure 1). The overall absorption of Calcirol® soft gelatin capsule was 93.23% within a timeframe of 12-24 hours. This led to achieving a sufficient level of 25-hydroxycholecalciferol (>60 ng/ml) within a very short time of around 6 h after oral intake. The overall dispersion pattern of Calcirol® soft gelatin capsule in each volunteer is shown in Table 5. The percentage absorption of Calcirol® soft gelatin capsule in each volunteer is shown in Table 6.

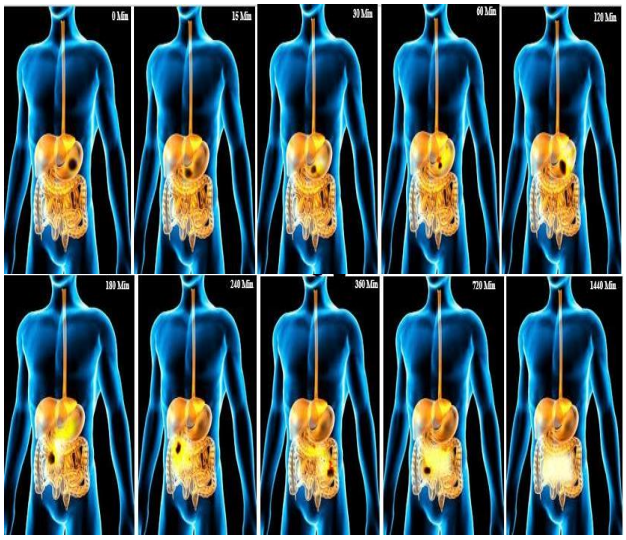

Figure 1. Pharmacoscintigraphy study with Cacirol® soft gelatin capsule.

Table 1: The concentration-time profile of 25-hydroxycholecalciferol.

Time (day)	Plasma concentration (ng/ml)	Log plasma concentration	AUC (ng.day/ml)	AUC total (ng.day/ml)
0	25.55±3.20	1.41	0	0
0.25	66.73±18.84	1.82	11.54	11.54
0.5	80.47±16.99	1.91	18.40	29.94
1	82.68±15.50	1.92	40.79	70.72
2	86.80±17.11	1.94	84.74	155.46
3	87.75±19.23	1.94	87.28	242.74
6	89.46±11.36	1.95	265.82	508.55
9	88.48±9.88	1.95	266.92	775.47
12	85.52±9.51	1.93	261.00	1036.47
15	83.17±12.10	1.92	253.03	1289.49
18	79.12±13.52	1.90	243.43	1532.92
21	80.15±9.65	1.90	238.90	1771.82
24	72.48±12.84	1.86	228.95	2000.77
27	63.82±14.10	1.80	204.45	2205.22

The results are expressed as the mean±standard deviation. AUC: area under the concentration-time curve.

Table 2: Net increase in plasma 25-hydroxycholecalciferol level with respect to baseline.

Time (day)	Plasma concentration (ng/ml)	Standard deviation	Log plasma concentration	AUC (ng.day/ml)	AUC total (ng.day/ml)
0	0.00	0.00	0	0.00	0.00
0.25	41.18	19.54	1.61	5.15	5.15
0.5	54.92	17.11	1.74	12.01	17.16
1	57.13	15.79	1.76	28.01	45.17
2	61.25	16.79	1.79	59.19	104.36
3	62.20	19.69	1.79	61.73	166.09
6	63.92	12.45	1.81	189.18	355.26
9	62.93	11.29	1.80	190.28	545.54
12	59.97	10.65	1.78	184.35	729.89
15	57.62	12.44	1.76	176.38	906.26
18	53.57	13.87	1.73	166.78	1073.04
21	54.60	9.32	1.74	162.25	1235.29
24	46.93	13.92	1.67	152.30	1387.59
27	38.27	14.94	1.58	127.80	1515.39

Table 3: The concentration-time profile of 1,25-dihydroxycholecalciferol.

Time (day)	Plasma concentration (pg/ml)	Log plasma conc.	AUC (pg.day/ml)	AUC total (pg.day/ml)
0	9.57±2.99	0.98	0	0
0.25	21.90±7.74	1.34	3.93	3.93
0.5	30.38±8.88	1.48	6.54	10.47
1	40.00±12.32	1.60	17.60	28.06
2	41.98±11.39	1.62	40.99	69.06
3	45.27±11.14	1.66	43.63	112.68
6	48.13±13.44	1.70	123.25	241.91
9	54.20±10.11	1.73	155.73	410.73
12	57.68±10.16	1.76	167.83	578.56
15	61.25±13.96	1.79	178.40	756.96
18	89.87±28.50	1.95	226.68	983.63
21	82.80±21.96	1.92	259.00	1242.63
24	68.11±24.86	1.85	205.05	1309.81
27	56.75±22.15	1.75	192.43	1666.56

Table 4: Net increase in plasma 1,25-dihydroxycholecalciferol level with respect to baseline.

Time (day)	Plasma concentration (ng/ml)	Standard deviation	Log plasma concentration	AUC (ng.day/ml)	AUC total (ng.day/ml)
0	0.00	0.00	0	0.00	0.00
0.25	12.33	8.55	1.09	1.54	1.54
0.5	20.82	9.43	1.32	4.14	5.69
1	30.43	12.45	1.48	12.81	18.50
2	32.42	11.54	1.51	31.43	49.92
3	35.70	11.45	1.55	34.06	83.98
9	44.63	10.78	1.65	127.03	324.63
12	48.12	11.08	1.68	139.13	463.76
15	51.68	15.01	1.71	149.70	613.46
18	80.30	28.51	1.90	197.98	811.43
21	73.23	22.16	1.86	230.30	1041.73
27	47.18	22.26	1.67	163.73	1408.26

Table 5: Overall dispersion pattern of Calcirol® soft gelatin capsule in each volunteer.

Time	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6
0 minute	Stomach	Stomach	Stomach	Stomach	Stomach	Stomach
15 minutes	Stomach	Stomach	Stomach	Stomach	Stomach	Stomach
30 minutes	Stomach	Stomach	Stomach	Stomach	Stomach	Stomach
1 hour	Stomach	Stomach	Stomach	Stomach	Stomach	Stomach
2 hours	Stomach	Stomach	Stomach	Stomach	Small intestine	Small intestine
3 hours	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine
4 hours	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine
6 hours	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine
12 hours	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine	Small intestine
24 hours	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time
Gastric residence time	2 hours	2 hours	2 hours	2 hours	1.5 hours	1.5 hours
Intestinal arrival time	2 hours	2 hours	2 hours	2 hours	1.5 hours	1.5 hours
Intestinal residence time	12 hours	12 hours	12 hours	12 hours	12 hours	12 hours
Colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time	No colon arrival time

Table 6: Percentage absorption of Calcirol® soft gelatin capsule in each volunteer.

Time	Volunteer 1		Volunteer 2		Volunteer 3		Volunteer 4		Volunteer 5		Volunteer 6	
	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed
0 minute	Stomach	0	Stomach	0	Stomach	0	Stomach	0	Stomach	0	Stomach	0
15 minutes	Stomach	2.34	Stomach	1.83	Stomach	2.83	Stomach	2.13	Stomach	3.81	Stomach	2.8
30 minutes	Stomach	6.14	Stomach	8.74	Stomach	4.74	Stomach	5.14	Stomach	7.24	Stomach	5.2
1 hour	Stomach	8.01	Stomach	9.91	Stomach	9.41	Stomach	7.71	Stomach	11.21	Stomach	10.4
2 hours	Stomach	11.65	Stomach	12.12	Stomach	10.82	Stomach	11.21	Small intestine	20.11	Small intestine	21.76

Continued.

Time	Volunteer 1		Volunteer 2		Volunteer 3		Volunteer 4		Volunteer 5		Volunteer 6	
	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed	Location	% absorbed
3 hours	Small intestine	28.12	Small intestine	25.32	Small intestine	30.32	Small intestine	33.51	Small intestine	43.51	Small intestine	42.65
4 hours	Small intestine	65.72	Small intestine	68.27	Small intestine	65.87	Small intestine	71.15	Small intestine	61.15	Small intestine	51.25
6 hours	Small intestine	70.11	Small intestine	71.22	Small intestine	75.54	Small intestine	74.32	Small intestine	69.22	Small intestine	68.21
12 hours	Small intestine	72.65	Small intestine	81.72	Small intestine	85.12	Small intestine	82.32	Small intestine	79.13	Small intestine	78.43
24 hours	No colon arrival time	94.47	No colon arrival time	92.24	No colon arrival time	94.24	No colon arrival time	93.21	No colon arrival time	93.12	No colon arrival time	92.12
Gastric residence time	2 hours		2 hours		2 hours		2 hours		1.5 hours		1.5 hours	
Intestinal arrival time	2 hours		2 hours		2 hours		2 hours		1.5 hours		1.5 hours	
Intestinal residence time	12 hours		12 hours		12 hours		12 hours		12 hours		12 hours	
Colon arrival time	No colon arrival time		No colon arrival time		No colon arrival time		Colon arrival time		No colon arrival time		No colon arrival time	

Table 7: The percentage increase in different pharmacokinetic parameters of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol.

Parameters	25-hydroxycholecalciferol	1,25-dihydroxycholecalciferol
C_{max}	89.46 ng/ml	89.87 pg/ml
T_{max}	6 days	18 days
$t_{1/2}$	48.42 days	14.05 days
V_d	14836.09 ml	6445.41 ml
$AUC_{0\text{-}inf}$	6664.04 ng.day/ml	2782.54 pg.day/ml

AUC: area under the concentration-time curve.; C_{max}: Maximum Concentration; T_{max}: Time to achieve maximum concentration; $t_{1/2}$: Half-life; V_d: Volume of distribution

Table 8: Results of laboratory investigations of each volunteer.

Test name	Unit	Reference value	Volunteer 1		Volunteer 2		Volunteer 3		Volunteer 4		Volunteer 5		Volunteer 6	
			Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study
Hematology														
Hemoglobin	g/dl	11.1-14.8	14.4	14	14.9	16.3	14.6	13.2	14	13.6	14.2	13.2	15.5	15.4
Total RBC	millions/cmm	4.1-5.2	4.77	4.65	4.55	4.66	4.2	4.22	4.41	4.40	5.13	5.6	4.74	

Continued.

Test name	Unit	Reference value	Volunteer 1		Volunteer 2		Volunteer 3		Volunteer 4		Volunteer 5		Volunteer 6	
			Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study
Total leucocyte count	10^3/µl	5-15	5.44	5.40	5.66	5.50	5.77	5.70	6.85	6.90	3.35	3.40	5.59	5.50
Platelet count	10^3/µl	200-550	219	222	200	242	174	189	187	180	150	156	256	242
Differential leukocyte count														
Neutrophils	%	40-80	53	55	70	69	45	48	58	59	40	42	72	70
Lymphocytes	%	20-40	43	42	22	23	52	54	34	34	56	50	23	25
Eosino phils	%	1-6	1	1.5	2	1	1	2	2	1.5	2	1	2	1
Monocytes	%	2-10.	3	1.5	6	03	2	1.5	6	04	2	2	3	2.5
Basophils	%	0-2	00	00	00	00	00	00	00	00	00	00	00	00
Biochemistry														
BUN	mg/dl	7-18	10.5	10	15	16	8.5	8	13	14	8.5	9.5	8	9
Serum creatinine	mg/dl	0.2-0.4	0.7	0.8	0.8	0.9	0.7	0.6	0.6	0.7	0.8	0.7	0.6	0.6
SGPT	U/l	<45	13	12	49	50	20	19	41	42	26	25	22	20
SGOT	U/l	<35	28	29	42	40	36	35	38	39	63	65	30	36
alkaline phosphatase	U/l	60-300	74	75	77	75	104	101	100	102	91	99	102	101
serum bilirubin														
total bilirubin	mg/dl	0.2-1.3	0.56	0.50	0.74	0.80	0.87	0.80	0.73	0.75	0.81	0.80	1.42	1.30
Direct bilirubin	mg/dl	<0.3	0.19	0.20	0.21	0.20	0.29	0.28	0.23	0.24	0.25	0.26	0.35	0.36
Indirect bilirubin	mg/dl	0-0.6	0.37	0.40	0.53	0.55	0.58	0.50	0.5	0.5	0.56	0.57	1.07	1.08
Serum electrolytes														
Sodium	mmol/l	135-145	132	134	140	141	132	133	142	141	132	141	136	141
Chloride	mEq/l	98-106	104	106	99	101	104	103	101	103	104	101	100	103
Potassium	mmol/l	3.5-5.0	3.4	3.6	3.7	4.0	4.8	4.6	4.4	4.3	4.2	4.4	5.0	4.5
Phosphorous	mg/d	2.5-4.5	3.5	3.1	4.8	4.4	3.8	3.2	3.8	4.0	4.11	4.2	4.6	4.7
Calcium	mg/dl	8.6-10.3	8.12	8.3	8.8	9.1	9.6	9.9	10.1	10.1	9.2	9.3	9.6	10.2
Serology														
HIV I			Non- reactive		Non-reactive		Non-reactive		Non- reactive		Non- reactive		Non- reactive	
HIV II			Non- reactive		Non-reactive		Non-reactive		Non- reactive		Non- reactive		Non- reactive	
HBV			Non- reactive		Non-reactive		Non-reactive		Non- reactive		Non- reactive		Non- reactive	
HCV			Non- reactive		Non-reactive		Non-reactive		Non- reactive		Non- reactive		Non- reactive	
VDRL			Non- reactive		Non-reactive		Non-reactive		Non- reactive		Non- reactive		Non- reactive	
Urine analysis														
Color			Pale yellow		Pale yellow		Pale yellow		Pale yellow		Pale yellow		Pale yellow	
pH		5.0-8.0	5.5	5.4	5.7	5.5	6.2	6.6	7.0	6.9	5.4	5.5	7.2	7.0
RBC	/hpf	0.0-0.99	00	00	00	00	00	00	00	00	00	00	00	00

Continued.

Test name	Unit	Reference value	Volunteer 1		Volunteer 2		Volunteer 3		Volunteer 4		Volunteer 5		Volunteer 6	
			Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study	Screening	Post-study
WBC	/hpf	0.0-0.99	00	00	00	00	00	00	00	00	00	00	00	00
Glucose			Negative		Negative		Negative		Negative		Negative		Negative	
Protein			Negative		Negative		Negative		Negative		Negative		Negative	

Pharmacokinetic study

The concentration time profile of 25-hydroxycholecalciferol is enumerated in Table 1. The net increase in plasma 25-hydroxycholecalciferol level with respect to the baseline is 64.05 ng/ml (Table 2).

The concentration-time profile of 1,25-dihydroxycholecalciferol is enumerated in Table 3. The net in-crease in plasma 1,25-dihydroxycholecalciferol level with respect to the baseline is enumerated in Table 4.

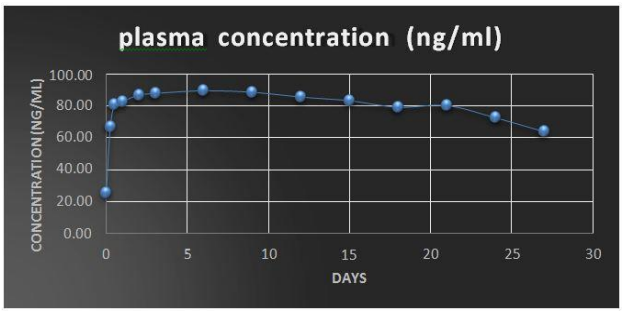

Figure 2: Mean concentration-time profile of 25-hydroxycholecalciferol in six healthy volunteers.

The percentage increase in different pharmacokinetic parameters of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol are enumerated in Table 7 and Figure 2.

Safety

The Calcirol® soft gelatin capsule was well tolerated by all volunteers and no safety issue was noted. The results of the laboratory examination were normal at the end of the study for all volunteers (Table 8).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the bioavailability and biodistribution pattern, transit time, and gastrointestinal clearance of a single dose of Calcirol® soft gelatin capsule using pharmacoscintigraphy. We found that the bioavailability of Calcirol® soft gelatin capsule was good and 93.23% of the formulation was absorbed through the small intestine. It led to achieving a sufficient level of 25-hydroxycholecalciferol (>60 ng/ml) within just 6 hours of oral intake. There was a substantial and persistent increase in the levels of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol as compared to the baseline. No adverse event was noted in the volunteers.

In this study, following supplementation of cholecalciferol (Calcirol®) soft gelatin capsule, the levels of plasma 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol increased (t_{max} around 6 and 18 days, respectively). The $t_{1/2}$ was long (12 days and 14 days, respectively) and the AUC_{0-27} was within the therapeutic range. The slow rise in the blood plasma concentration was due to the large volume of distribution of the formulation. A drug with a high volume of distribution indicates higher drug distribution in the tissues than binding to plasma or getting distributed only in blood. If the drug is used for intracellular targets, then a high volume of distribution value is desirable. This is applicable for cholecalciferol to exert its several pleiotropic effects at the cellular level. We also found that Calcirol® soft gelatin capsule was very well absorbed through the small intestine. The gastric residence time was very short (around 1 hour) and the small intestinal residence time was around 16 hours. The efficient absorption of cholecalciferol is dependent on the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase. In turn, bile acids initiate the emulsification of lipids, pancreatic lipase hydrolyses the triglycerides into monoglycerides and free fatty acids, and bile acids support the formation of lipid-containing micelles, which diffuse into the enterocytes.[20]

Vitamin D (cholecalciferol) is considered a pluripotent prohormone because of its role in various physiological domains, such as immune system, cell proliferation, differentiation and apoptosis, regulation of insulin secretion, cardiac activity, regulation of blood pressure, and brain development along with its major role in bone health.[7,20] Various guidelines recommend a different dose of vitamin D as the recommended dietary allowance. The Institute of Medicine committee's 2011 report recommends daily adequate dietary vitamin D supplementation of 400, 600, and 800 IU for infants, age group of 1-70 years, and elderly people (>70 years), respectively. This supplemental dose is recommended as an add-on to minimal sunlight exposure.[21]

According to the Endocrine Society Clinical Practice Guideline, vitamin D deficiency is defined as a 25-hydroxycholecalciferol level of <20 ng/ml and vitamin D insufficiency as a 25 hydroxycholecalciferol level of 21-29 ng/ml.[22] Vitamin D deficiency is widely present throughout the world.[23] Its prevalence among the adult population is >40% both in the United State of America and Europe in the population of age of >50 years.[24] In developing countries like India, the prevalence of vitamin D deficiency varies from 51.3-100%.[25] The important causes of vitamin D deficiency are inadequate exposure to sunlight, increased melanin content in the body, and the use of sunscreen. Other causes are inadequate intake of vitamin D rich foods and defective intestinal vitamin D absorption; co-administration of drugs interacting with vitamin D metabolism, the presence of hepatic and/or renal diseases, old age, and obesity.[26] Vitamin D deficiency leads to imperfect bone mineralization (leading to rickets and osteomalacia), fractures, and other conditions, such as myocardial infarction, and malignancies.[27] All these conditions warrant cholecalciferol supplementation. Hence, it is extremely important to understand the bioavailability of specific cholecalciferol formulations before prescribing the same for vitamin D deficiency.

The most common treatment regime for vitamin D deficiency is 60,000 IU weekly for 8 weeks.[27] Cholecalciferol softgels are one of the widely used formulations used to supplement vitamin D in the body.[28] The advantage of this formulation is that it promotes rapid dispersion of capsule content and drug dissolution, thereby allowing greater uniformity of content between dosage units. The favorable bioavailability and biodistribution patterns of Calcirol® soft gelatin capsule were found to be good and can be recommended for vitamin D supplementation in individuals having vitamin D insufficiency or deficiency based on clinical indications. The convenience, superior dosing accuracy, and rapid onset of action of Calcirol® soft gelatin capsule contribute to a strong preference over conventional solid cholecalciferol formulations across a wide range of patient groups.

The strength of our study includes the use of pharmacoscintigraphy and a clear visualization of the absorption pattern of Cholecalciferol. Also, this study included a pharmacokinetic study protocol involving six healthy volunteers. The limitations include the small sample size and the absence of a comparator arm. Notwithstanding these limitations, because of the favorable bioavailability and biodistribution pattern as found in this study, Calcirol® soft gelatin capsule can be recommended for vitamin D supplementation.

CONCLUSION

This was the first pharmacoscintography study in the world to demonstrate the favorable biodistribution of Calcirol® soft gelatin capsules, supporting its use for vitamin D supplementation.

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