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Efficacy of Simvastatin on Chronic Renal Failure Patients - A Spectroscopic Approach

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The effect of simvastatin in chronic renal failure patients spectroscopically is investigated. A total of 10 chronic renal failure patients were assigned to receive simvastatin (zocor) 20 mg/d, added to their ongoing treatment, for 6 months. Blood samples were obtained at baseline and after 3 and 6 months of observation for the determination of their lipid profiles and study on biochemistry of blood using FTIR spectroscopy. The FTIR spectra of blood sera of the patients were recorded before and after drug therapy over the region 4000-400 cm^{-1}. Simvastatin therapy reduced urea by 22 %, LDL cholesterol by 21.5 % and triglycerides by 25.7 % and creatinine by 20 %. These data suggest that in chronic renal failure patients, simvastatin profoundly affect the lipid profile, as well as the triglyceride levels.

Key Words: Chronic renal failure, Simvastatin drug therapy, FTIR spectroscopy, Efficacy.

INTRODUCTION

The inhibitors of 3-hydroxyl-3-methyl glutaryl CoA reductase, the so called statins, are effective in controlling hypercholesterolemia, even in the more advanced stages of renal failure and in patients who are on maintenance of dialysis. The anti lipidemic effect of statins combines with other effects - antioxidant, antiinflammatory, antiimmunomodulatory and antithrombatic as a result of the inhibition of the mevalonate pathway induced by these agents. Also because of the non-lipid dependent effects, statins could have an antiatherosclerotic and reno productive effect. Ongoing large trials will establish more clearly whether such effects are present in renal patients.

In chronic renal failure, the risk for cardiovascular complications also is very high, but it is more difficult to distinguish the role of dyslipidemia from that of many other coexisting risk factor (*e.g.*, cardiomyopathy, high blood pressure, fluid overload, anaemia, vascular calcification and hyperhomocysteinemia).

All statins induce a marked reduction of LDL-cholesterol and the most powerful statins even induce a marked reduction of triglycerides in patients with nephritic syndrome. Clinical and pharmacokinetic studies

have demonstrated that, even patients who have chronic renal failure and are on maintenance of dialysis, statins are well tolerated and effective, providing equivalent control of lipid level to the seen in matched control subjects. However, there is a dearth of information regarding the goals, efficacy and safety of statin treatment among dialysis patients[1-7]. Panchi *et al.*[1] investigated the *in vivo* and *in vitro* effects of simvastatin on cytokine production in predialysis chronic renal failure patients. Nishokawa *et al.*[2] studied the effects of simvastatin on the lipid profile of hemodialysis patients.

Baigent and coworkers[3] assessed the effects of simvastatin and the cholesterol-absorption inhibitor ezetimibe among patients with chronic disease. The effects of HMG-CoA reductase inhibitors in hypercholesterolemic patients on hemodialysis was studied by Wanner and his group[4]. The safety and efficacy of simvastatin in hypercholesterolemic patients undergoing chronic renal dialysis was studied by Saltissi and his team[5]. Mathis *et al.*[6] concluded that stains and diet therapy should be used as treatments in renal transplant recipients with dyslipidemia. The effect of two lipid lowering drugs simvastatin and probucol on lipid profile in 12 hemodialysis paitnets was studied by Fiorini and his group[7]. Though an extensive work has already been carried out on the safety and efficacy of simvastatin, no work has been performed using spectroscopic method and hence the present study aims to employ FT-IR to analyze the efficacy of the simvastatin drug therapy.

EXPERIMENTAL

Healthy blood samples were collected from healthy adult volunteers whose kidney function is normal and the excretory values of the following fall well within the permissible level (blood urea 10-40 mg/dl, S. creatinine upto 1.4 mg/dl, triglycerides < 200 mg/dl, LDL cholesterol < 130 mg/dl). Renal failure blood samples before and after dialysis were collected from Government General Hospital, Royapettah, Chennai. Blood samples were obtained from the patients after 14 h of food intake. After centrifugation of blood in refrigerated centrifuge, the plasma aliquots were transported to the laboratory in a portable freezer and kept at 20ºC until analyzed. Using the conventional method, the sample could be prepared by spreading a small volume of serum on a IR-transparent material, allowing to dry and measuring the absorption spectrum of the film. The accuracy of the method may be compromised by any variation in the amount of serum successfully deposited on the KBr window, particularly with the manual sample preparation. In order to make up for this variation and to assess its impact on the overall accuracy of the method, a standard solution is added to each serum sample. The solution is chosen in such a way that it

responds to IR radiation at the point where serum sample contains no absorption peak. Shaw *et al.*[8] reported that the IR absorption spectrum of the thiocyanate ion (SCN) includes absorption at 2060 cm^{-1} in a spectral region where serum samples and subsequently normalizing all of the spectra to equal intensities therefore compensated for the imprecision in the film preparation.

A volume of 1 mL of serum was diluted with an equal volume of 4 mg/L aqueous potassium thiocyanate (KSCN) solution. 20 µL of each diluted sample was spread evenly over the surface of a circular KBr window (9 mm diameter and 2 mm thickness). Infrared spectra in the region 4000-400 cm^{-1} were recorded on a Perkin-Elmer spectrum-one FTIR spectrometer equipped with an air-cooled DTGS (Deuterated triglycine sulphate) detector. It has already mentioned that the strong absorption band of water in the mid IR region is hindered and to eliminate the same, the serum samples are air dried to form a thin uniform film on the KBr pellet[8,9]. Infrared transparent KBr material without the sample was scanned as background for each spectrum and 16 scans were co added at a spectra resolution of ± 1 cm^{-1}. The collected signal was transferred to the Pc. The data were processed by windows based data program-spectrum software.

The spectra were base line corrected and they were normalized to acquire identical area under the curves and the maximum absorbance values of the corresponding characteristics bands were noted.

RESULTS AND DISCUSSION

Abnormalities in the composition of lipoproteins are particularly common in patients with chronic renal disease (CRD). They may also play a role in the pathogenesis of cardiovascular disease. The special report from the National Kidney Foundation Task Force[10,11] on cardiovascular disease in hyperlipidemic patients with chronic renal disease identifies 3 renal target populations: Patient with CRI and those undergoing hemodialysis or peritonial dialysis. However, target lipid levels and management of lipid abnormalities are the same for proteinuric patients. In end stage renal disease (ESRD) patients on hemodialysis, the classes and doses of lipid lowering agents used to manage lipid abnormalities differ from those used to manage lipid abnormalities[12,13] differ from those used in patients on peritonial dialysis.

In general, the prevalence of hyperlipidemia appears to increase with worsening of renal function. However, the level of renal dysfunction at which elevated lipid levels become more prevalent than in normal individuals is not well defined. Attman and Alaupovic[14] reported elevated triglyceride and VLDL-cholesterol level in a group of patients with mild to moderate CRI. Total cholesterol levels were the same as those in controls.

Using the high-cholesterol stratergy, LDL-cholesterol is the major serum lipid component to focus on, although it does not reflect the entire risk of the dyslipidemia profile in CRD patients. VLDL is nothing but of LDL, only the variations in the lipid to protein ratio and hence the band corresponding to LDL can be considered for the quantification of VLDL present in the blood sera of CRD patients spectroscopically.

The infrared spectrum is an essence of reflection of the infrared colour pattern characteristic of the sample. The basis of quantisation is that each constituent contributes a unique absorption pattern to the overall spectrum governed by the unique set of molecular vibrational characteristic of each distinct molecular species. The quantitative information is carried by the relative intensitites of vibrational frequencies of, the various constituents contributing to the unique absorption profile of each serum specimen.

The IR spectrum of serum includes spectral contribution from protein, cholesterol, triglycerides, urea, glucose and other more dilute contributes a complex set of several absorptions falling within the mid-IR spectral region[15-17], it is impossible to find any single absorption band that can serve as the basis to quantify any single component; coincident absorptions from other species would degrade or completely sabotage the effort.

A vibrational band assignment of the absorption bands of the spectra is done with the idea of the group frequency of various constituents of the serum samples. A statisfactory assignment of the vibrational bands has been carried out by interpreting the well established infrared spectra of blood and serum[16,17]. Fig. 1 presents the FTIR overlaid spectra of a chronic renal failure patient to find the efficacy of simvastatin.

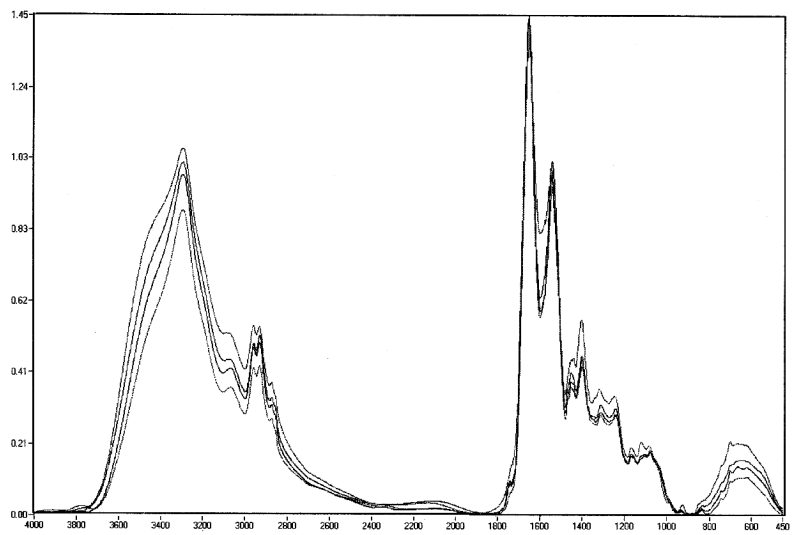

Fig. 1. FTIR overlaid spectra of chronic renal failure patient

A build up of the toxins namely, urea and creatinine in the blood occurs in the case of renal failure subjects and hence the present work aims to quantify these two blood contituents to characterize the blood samples of renal failure subjects and to study the efficacy of simvastatin therapy on chronic renal failure patients waiting on kidney transplant. The molecular structures of urea and creatinine are presented in Fig. 2.

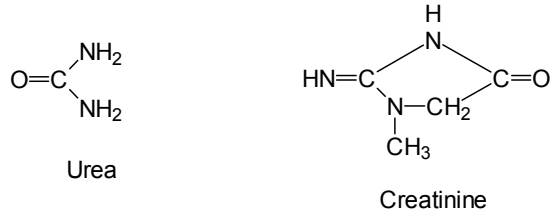

Fig. 2. Molecular structures of urea and creatinine

Quantification of low density lipoprotein (LDL-C) was derived from two spectral regions encomposing strong lipid absorptions, namely 1800-1700 cm^{-1}, which contains the C=O stretching vibration[18] and 3000-2800 cm^{-1} which includes the acyl CH_2 stretching modes. The spectral regions were identified as 3200-2800 cm^{-1} for triglycerides by Shaw *et al.*[19].

Shaw *et al.*[20] predicted the spectral region 1500-900 cm^{-1} for protein, 1800-1400 cm^{-1} for creatinine 3550-3100 cm^{-1} for urea in their urine films study. On the basis of the above study and the structure of urea and creatinine the specific assignments of bands have been achieved by interpreting the previously well established IR spectra of serum samples. In analogy with the above views, the absorption peaks at 3296, 3060, 1647 and 1446 cm^{-1} are assigned to urea, triglycerides, creatinine and LDL, respectively. The absorption crests at 1400 and 1452 cm^{-1} stem from the CH_2 groups in α and β anomers and the bending of the C–H groups, respectively. The essential band at 1547 cm^{-1} is attributed to amide-II from N–H bending vibrations that are strongly coupled to the C–N stretching vibration of protein amide groups. Absorption band at 1647 cm^{-1} is attributed to amide-I (C=O) band.

The vibrational band at 3296 cm^{-1} is due to the N–H stretching vibration of the secondary amides of protein. The asymmetric stretching vibrations of methyl group of proteins and lipids are found to be present near 2960 cm^{-1}. The other two bands found to be present near 2930 and 2872 cm^{-1} are due to the asymmetric and symmetric stretching vibrations of the methylene group, respectively.

In order to find the efficacy of simvastatin on chronic renal failure patients, the absorption values of the above mentioned predominant peaks in the FTIR spectra of pre and post drug therapy were noted and the % of efficacy were calculated (Table-1) using the formula

$$\% \text{ of Efficacy} = [(\text{Pre} - \text{Post})/\text{Pre}] \times 100$$

TABLE-1
ABSORBANCE OF SPECIFIC MODES OF VIBRATION - TO FIND THE EFFICACY OF SIMVASTATIN IN RENAL FAILURE PATIENTS
(values in mg/dl)

Sample	Status	Wavelength (cm^{-1})			
		Urea	TGL	LDL	Creatinine
		3296	3060	1446	1647
1	Pre	0.8898	0.3461	0.4104	0.3638
	Post 1	0.7845	0.3038	0.3653	0.3267
	Post 2	0.6905	0.2559	0.3209	0.2914
	% of Eff	-22.3	-26.1	-21.8	-19.9
2	Pre	0.7947	0.3759	0.5107	0.3946
	Post 1	0.7001	0.3270	0.4545	0.3516
	Post 2	0.6183	0.2871	0.3983	0.3167
	% of Eff	-22.2	-23.6	-22.0	-19.7
3	Pre	0.8019	0.4105	0.4947	0.5002
	Post 1	0.7105	0.3571	0.4401	0.4482
	Post 2	0.5848	0.3058	0.3832	0.4012
	% of Eff	-19.5	-25.5	-22.5	-19.8
4	Pre	0.9146	0.4213	0.4973	0.5219
	Post 1	0.8094	0.3707	0.4452	0.4655
	Post 2	0.7061	0.3142	0.3879	0.4212
	% of Eff	-22.7	-25.4	-22.0	-19.3
5	Pre	0.8774	0.4836	0.5219	0.5103
	Post 1	0.7800	0.4207	0.4139	0.4546
	Post 2	0.6791	0.3679	0.4071	0.4118
	% of Eff	-22.6	-23.9	-21.9	-19.3
6	Pre	0.7999	0.4965	0.5093	0.4207
	Post 1	0.7112	0.4309	0.4533	0.3744
	Post 2	0.6207	0.3729	0.3978	0.3399
	% of Eff	-22.4	-24.9	-21.8	-19.2
7	Pre	0.8841	0.4777	0.4953	0.5776
	Post 1	0.7789	0.4161	0.4408	0.5181
	Post 2	0.6834	0.3549	0.3869	0.4638
	% of Eff	-22.7	-25.7	-21.9	-19.7
8	Pre	0.8143	0.3979	0.4203	0.3948
	Post 1	0.7206	0.3489	0.3741	0.3537
	Post 2	0.6303	0.2968	0.3282	0.3162
	% of Eff	-22.6	-25.4	-21.9	-19.9
9	Pre	0.7916	0.3213	0.4599	0.3717
	Post 1	0.6997	0.2795	0.4097	0.3330
	Post 2	0.6112	0.2384	0.3592	0.3008
	% of Eff	-22.8	-25.8	-21.8	-19.2
10	Pre	0.8073	0.3446	0.4592	0.3714
	Post 1	0.7145	0.2998	0.4087	0.3339
	Post 2	0.6272	0.25332	0.3582	0.2942
	% of Eff	-22.3	-26.5	-21.9	-20.7

After completion of the study, simvastatin significantly reduced 22 % urea, 20 % creatinine, 25.7 % triglycerides and 21.5 % LDL from the baseline.

Clinical analysis

Urea is the major end product of protein metabolism in human. It constitutes the largest fraction of the non-protein nitrogen component of blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently the circulating levels of urea depends upon protein intake, protein catabolism and kidney function. Elevated serum urea concentrations are observed in impaired kidney function, liver disease, congestive cardiac failure, diabetes infections and diseases which impair kidney function. The estimation of urea in serum involves the enzyme catalyzed reactions. The reagent and aqua-4 are allowed to attain room temperature. Equal amount of reagent and aqua-4 are added and mixed gently[21,22] and aspirate standard followed by samples. The absorbance change ΔA for the standard and unknown samples are determined by using the formula ΔA = A1-A2. The concentration of urea is calculated as

$$\text{Urea (mg/dl)} = \Delta A \text{ of test}/\Delta A \text{ of standard} \times \text{concentration of standard (mg/dl)}$$

Creatinine is a waste product formed in muscle from the high energy storage compound, creatinine phosphate. The amount of creatinine is fairly constant and is primarily a function of muscle mass. It is removed from plasma by glomercular filteration and then excreted in urine without any appreciable re-absorption by the tubules. Creatinine is an useful indicator of renal function. Elevated creatinine level in serum is usually associated with various renal disease. In the earlier stage of renal disease, creatinine clearance test is a sensitive index of impaired renal function. Creatinine reacts with alkaline picorate to produce an orange-yellow colour (Jaffe's reaction). Specificity of the assays has been improved by introduction of an initial rate method. However, cephalosporin ibiotics are still major interferants. The absorbance of orange-yellow colour formed is directlyproportional to creatinine concentration and is measured photometrically at 500-520 nm[23]. The two reagents picric acid reagent and NaOH reagent are mixed and kept for 15 min before use. The serum is mixed with the reagents and the initial absorbance A1(20 s after mixing) and final absorbance A2 (80 s after mixing) were noted ΔA = A2-A1. The creatinine concentration is calculated by the formula

$$\text{Creatinine (mg/dl)} = \Delta A \text{ of test}/\Delta A \text{ of standard} \times \text{concentration of standard (mg/dl)}$$

Normally, triglycerides, HDL-cholesterol, total-cholesterol are estimated and LDL-cholesterol is calculated. These parameters represent a routine practical aspect of lipid profile which is useful in determination of risk factor or health status of a subject[24].

HDL are separated from other lipoprotein fractions by treating serum with phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated; cholesterol content of which is estimated by enzymatic method. The levels of LDL, HDL, TC and TG in blood sera were measured using Pointe-180 photometer.

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice. The serum is mixed well and centrifuged at 3500-4000 rpm for 10 min. The clear supernatant immediately separated it is added with the working reagent mixed and incubated for 10 min at 37 ºC and the absorbance of test and standard against reagent blank at 505 nm is noticed. The serum HDL-cholesterol is calculated for formula, absorbance of test × 238.1[25,26]. The values of LDL-cholesterol can be calculated, if the value of triglyceride is known by using Friedewald's equation:

LDL-cholesterol = Total chol – (HDL cho + Triglycerides/5)

Serum triglyceride estimation is an important parameter in the investigation of hyperlipidemia. Elevated levels may be found in atherosclerosis diabetes mellitus, glycogen storage disease like Van Gierke's disease, secondary hyperlipoproteinaemia, alcoholism and nephritic syndrome.

Fresh, clear fasting serum with no hemolysis should be used. It is mixed with the reagent and incubated at 37ºC for 15 min and the absorbance of test and standard against reagent blank at 546 nm and the triglycerides values are calculated using the formula,

Absorbance of test × 689.6

All the parameters were measured at the end of the study and summarized in Table-2. Treatment with simvastatin resulted in a significant reduction in urea (22 %), creatinine (20 %) LDL cholesterol (21.5 %) and triglycerides (25.7 %) and increases HDL (14.8 %). Our results suggest that simvastatin beneficially alters the atherogenic lipid profile in these patients and significantly decreases the density of LDL particles produced resulting a shift from small, dense LDL to more buoyant and less atherogenic particles. These data confirm the marked efficacy and safety of simvastatin in CRD patients to attain their LDL-C goals.

TABLE-2
CLINICAL ANALYSIS - TO FIND THE EFFICACY OF SIMVASTATIN IN CHRONIC RENAL FAILURE PATIENTS
(values in mg/dl)

Ple	Status	Urea	TGL	LDL	Creatinine
1	Pre	125	217	169	4.1
	Post 1	111	189	151	3.7
	Post 2	98	160	132	3.3
	% of Eff	-21.6	-26.3	-21.9	-19.5

Ple	Status	Urea	TGL	LDL	Creatinine
2	Pre	153	229	171	8.4
	Post 1	136	198	152	7.5
	Post 2	119	170	134	6.6
	% of Eff	-22.2	-25.8	-21.6	-21.4
3	Pre	78	187	149	5.0
	Post 1	67	163	133	4.5
	Post 2	61	139	117	3.9
	% of Eff	-21.8	-25.6	-21.5	-21
4	Pre	95	179	163	7.3
	Post 1	84	156	136	6.6
	Post 2	74	133	127	5.8
	% of Eff	-22.1	-25.6	-22.1	-20.5
5	Pre	101	222	179	5.7
	Post 1	89	193	159	5.1
	Post 2	78	164	140	4.6
	% of Eff	-22.8	-26.1	-21.8	-19.3
6	Pre	66	204	153	6.7
	Post 1	56	177	138	6.0
	Post 2	53	151	122	5.4
	% of Eff	-21.2	-25.9	-20.3	-19.4
7	Pre	75	213	161	5.9
	Post 1	68	185	144	5.3
	Post 2	59	158	128	4.7
	% of Eff	-21.3	-25.8	-20.5	-20.3
8	Pre	81	187	159	5.8
	Post 1	73	163	142	5.2
	Post 2	64	138	126	4.6
	% of Eff	-20.9	-26.2	-20.8	-20.7
9	Pre	101	173	149	4.8
	Post 1	89	751	133	4.3
	Post 2	80	128	119	3.8
	% of Eff	-20.8	-26.0	-20.1	-20.8
10	Pre	69	163	152	5.1
	Post 1	62	142	135	4.6
	Post 2	55	121	121	4.0
	% of Eff	-20.3	-25.8	-20.4	-21.6

Conclusion

In general population, primary strategy of every anti-hyperlipidemic therapy is a dietary approach. However, proteinuric or ESRD patients on hemodialysis or peritoneal dialysis need different dietary recommendations because these approaches are frequently insufficient to correct

dyslipidemia. In such patients, a pharmacological approach to lipid lowering is often necessary. The most effective pharmacological agents for reducing LDL-cholesterol are statins. The safety profile of simvastatin on renal failure patients is highly favourable and it has been proved systematically using FTIR spectroscopy in the present study. Simvastatin therapy reduced urea by 22 %, LDL cholesterol by 21.5 % and triglycerides by 25.7 % and creatinine by 20 %. These data suggest that in chronic renal failure patients, simvastatin profoundly affect the lipid profile, as well as the triglyceride levels.

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