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Interaction Study of Certain Dyes like Ferric oxide Red, Brilliant Blue and Soy Food Stuffs with Statins and Its Influence on Protein Binding and Intrinsic Association Constant 'K' of Statins

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ABSTRACT
Interaction of Atorvastatin, Simvastatin and Rosuvastatin with dyes like ferric oxide red, brilliant blue and soy food stuffs were carried out in BSA using RP-HPLC method. Developed method was validated as per ICH Guidelines. Protein binding and Intrinsic association constant 'K' of statin drugs were calculated in presence of dyes and food stuffs. The order of interaction in Atorvastatin, Simvastatin and Rosuvastatin were found to be ferric oxide > soy food stuffs > brilliant blue; Soy food stuff > ferric oxide > brilliant blue; brilliant blue > soy food stuffs > ferric oxide red respectively. Protein binding of Atorvastatin was found to be decreased by 46.16%, 38.91%, and 30.87% in presence of brilliant blue, ferric oxide and soy food stuffs respectively. In case of Simvastatin it was found to be decreased by 35.52%, 34.63% and 18.45% and in case of Rosuvastatin it was found to be decreased by 65.29%, 56.02%, 45.81% in presence of brilliant blue, ferric oxide red and soy food stuffs respectively. The intrinsic association constant of Atorvastatin, Simvastatin, and Rosuvastatin was found to be altered in presence of brilliant blue, ferric oxide red and soy food stuffs.

Keywords: RP-HPLC, Atorvastatin, Simvastatin, Rosuvastatin, soy food stuffs, brilliant blue, ferric oxide red.

INTRODUCTION

Statins were anti hyperlipidemic drugs which have been used to increase the LDL cholesterol levels and it plays a beneficial role in substantially reducing both cardiovascular morbidity and mortality. [1] Soy isoflavones are phytoestrogens which may also exert cardio protective effects. An interaction between isoflavones and statins appear to affect CYP3A4 gene transcription and cellular lipid metabolism. Taken together, the isoflavones are able to affect the complex drug metabolism pathways and these *in-vitro* interactions of isoflavones with statins suggest studies are warranted. [2] FDA is a federal body responsible for regulating colour additives used in food and formulations. As per FDA the acceptable daily intake (ADI) of brilliant blue is 12.5 mg/kg of body weight and in case of ferric oxide red it is 5 mg/kg of body weight. [3] Anything exceeding the limit may lead to harmful effects in humans. [4]

Binding of drug with plasma proteins affects drug distribution and elimination, as well as the pharmacological effect of the drug. Protein bound drug has low lipid solubility and is unable to cross the lipid barrier. The most important contribution to drug binding in plasma is made by albumin which comprises about one half of the total plasma proteins. Intrinsic association constant 'K' is a binding parameter that characterises the strength of binding between the drug molecules and binding sites. [5]

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MATERIALS & METHODS

Chemicals

Atorvastatin calcium, Simvastatin and Rosuvastatin calcium were obtained as free sample from Zydus Medicare ltd., and Glenmark. Soy isoflavones was obtained from Raptakos Brett & Co ltd. Dye stuffs like ferric oxide red and brilliant blue were obtained from Parshwanath dye stuff industry and Galaxy Corporation Mumbai. HPLC grade methanol was from Merck and all other reagents were of analytical grade.

Standard and sample solution preparations

Preparation of standard solution of brilliant blue

10 mg of brilliant blue was accurately weighed in to a 10 ml standard flask and made up to the required volume with the mobile phase to obtain a concentration of 1000µg/ml. This solution was suitably diluted to get a concentration of 10µg/ml.

Preparation of standard solution of ferric oxide red

10 mg of ferric oxide red was accurately weighed in to a 10 ml standard flask and made up to the required volume with

the mobile phase to obtain a concentration of 1000µg/ml. This solution was suitably diluted to get a concentration of 10µg/ml.

Preparation of standard solution of Soy isoflavones

10 mg of soy isoflavones was accurately weighed in to a 10 ml standard flask and made up to the required volume with the mobile phase to obtain a concentration of 1000µg/ml. This solution was suitably diluted to get a concentration of 24µg/ml.

Preparation of standard solution of statins

10µg/ml solution of Atorvastatin, Simvastatin and Rosuvastatin were prepared.

Preparation of sample solutions

Preparation of solution containing statin with soy isoflavone in Bovine Serum Albumin (BSA)

5 ml of soy isoflavone solution, 5ml of statin solution and 5 ml of BSA was added. The solutions were mixed thoroughly and kept aside for 30 minutes. Centrifuged at 4000rpm for 5 min and supernatant liquid is evaporated to dryness in nitrogen atmosphere and the residue is made up with mobile phase. The solution was then injected to analyse statin content by using HPLC.

Preparation of solution containing statin with brilliant blue in Bovine Serum Albumin (BSA)

5 ml of brilliant blue solution, 5ml of statin solution and 5 ml of BSA was added. The solutions were mixed thoroughly and kept aside for 30 minutes. Centrifuged at 4000 rpm for 5 min and supernatant liquid is evaporated to dryness in nitrogen atmosphere and the residue is made up with mobile phase. The solution was then injected to analyse statin content by using HPLC.

Preparation of solution containing statin with ferric oxide red in Bovine Serum Albumin (BSA)

5 ml of ferric oxide red solution, 5ml of statin solution and 5 ml of BSA was added. The solutions were mixed thoroughly and kept aside for 30 minutes. Centrifuged at 4000 rpm for 5 min and supernatant liquid is evaporated to dryness in nitrogen atmosphere and the residue is made up with mobile phase. The solution was then injected to analyse statin content by using HPLC.

Chromatographic analysis

LC system of Shimadzu class LC 10 AT apparatus equipped with PDA detector was used for the analysis of statins.

For Atorvastatin, analyses were carried out at 245nm on Phenomenex Gemini C_{18} column (150×4.6mm, 5µ). Mobile phase consisted of 0.4% v/v Triethylamine: Methanol in the ratio of 10:90% v/v. The pH was adjusted to 4 using orthophosphoric acid. The mobile phase was delivered at 1ml/min.

In case of Simvastatin analyses were carried out at 238nm on Phenomenex Gemini C_{18} column (150×4.6mm, 5µ). Mobile phase consisted of 0.1% v/v Triethylamine: Acetonitrile in the ratio of 30:70% v/v. The pH was adjusted to 7.5 using orthophosphoric acid. The mobile phase was delivered at 1.1ml/min.

In case of Rosuvastatin analyses were carried out at 240nm on Phenomenex Gemini C_{18} column (150×4.6mm, 5µ). Mobile phase consisted of 0.05M Formic acid: Acetonitrile in the ratio of 55:45% v/v. The mobile phase was delivered at 1ml/min.

Protein binding

Preparation of reagents

Preparation of pH 7.2 buffer solution

50 ml of 0.2 N potassium hydrogen phosphate is added to 34.7 ml of 0.2 N NaOH solution and made up to 200 ml with distilled water.

Preparation of 2.8×10^{4} M solution of Egg albumin

Accurately weighed 0.315 g of egg albumin flakes was dissolved in 25ml of distilled water. It was shaken well (till flakes are completely dissolved) and was kept aside.

General procedure

25 ml of drug solution is taken in to a beaker and a boiling tube open on both sides was taken. A semi permeable membrane was tied onto the neck of the boiling tube. The egg albumin solution 10 ml was taken inside the boiling tube tied with semi permeable membrane. The boiling tube was then immersed into the beaker containing the drug solution.

Immediately at zero time 1ml of the drug solution was pipetted out from the beaker and is replaced with 1ml of water and the absorbance is noted at specified wavelength. Readings are taken at 0, 10, 30, 45 min, 1h, 1.15, 1.30, 1.45, 2 h.

Intrinsic association constant

The procedure for estimating the intrinsic association constant was the same as that of protein binding. Different concentrations of drug solutions were prepared. By using the Scatchard's plot the 'K' value was estimated.

RESULTS AND DISCUSSION

Interaction study of Statin with food stuff (soy isoflavone) and dyes in BSA by RP-HPLC method:

The concentration of standard Atorvastatin in BSA was reduced to an extent of 4.2% in presence of brilliant blue and 19.1% in presence of ferric oxide red and 12.7% in presence of food stuff when compared with standard Fig. 1.

The concentration of Simvastatin in BSA was reduced to an extent of 8.4% in presence of brilliant blue and 10.2% in presence of ferric oxide red and 18.3% in presence of food stuff when compared with standard Fig. 2.

The concentration of Rosuvastatin in BSA was reduced to an extent of 11.1% in presence of brilliant blue and 5.04% in presence of ferric oxide red. The peak area of standard Rosuvastatin in BSA showed a decrease of 6.2% in presence of food stuff when compared with standard Fig. 3.

The Effect of dyes and food stuffs on Protein binding of statins

The protein binding of Atorvastatin was decreased by 46.16%, 38.91% and 30.87% in presence of brilliant blue, ferric oxide red and food stuffs respectively. The protein binding of Simvastatin was decreased by 35.52%, 34.63% and 18.45% in presence of brilliant blue, ferric oxide red and food stuffs respectively. The protein binding of Rosuvastatin was decreased by 65.29%, 56.02% and 45.81% in presence of brilliant blue, ferric oxide red and food stuffs respectively Table 1.

Estimation of 'K' value of Statins in presence of certain dyes and food stuffs

Intrinsic association constant 'k' was calculated for Statins individually and in combination with various dyes and food stuffs are as shown in Tables 2 & 3.

From the research it has been proved that a significant interaction of statin drugs with dyes (brilliant blue, ferric oxide red) and soy food stuffs were found. Hence the statins (Atorvastatin, Simvastatin, and Rosuvastatin) should not be taken along with food stuffs containing soy isoflavones as it influences the protein binding of statin drugs and also the

Table 1: Protein binding of Statins in Presence of dyes and food stuffs

Name of the Statin	Time interval in minutes	Protein Binding			% Decrease in protein binding		
		with Brilliant blue	with ferric oxide red	with food stuff	By brilliant blue	By ferric oxide red	By food stuffs
Atorvastatin	120	0.1897	0.2152	0.2719	46.16	38.91	30.87
Simvastatin	100	0.1434	0.0997	0.1244	35.52	34.63	18.45
Rosuvastatin	120	0.5394	0.2372	0.3031	65.29	56.02	45.81

Table 2: 'K' Value of Statins

S. No	Name of the statin	Total concentration of statin in moles (B)	Concentration of unbound drug (A)	$(B-A)\times10^{-4}$	$r = B - A/.8\times10^{4}$ M	$r/A\times10^{4}$	$K\times10^{4}$
		1.63×10^{4} M	0.1622×10^{-4}	1.467	0.524	3.23	
1	Atorvastatin	1.95×10^{4} M	0.13675×10^{-4}	1.813	0.647	4.74	4.5
		2.35×10^{4} M	0.1862×10^{-4}	2.163	0.920	4.94	
		1.63×10^{4} M	0.1212×10^{-4}	1.508	0.538	4.44	
2	Simvastatin	1.95×10^{4} M	0.1188×10^{-4}	1.831	0.654	5.50	6.25
		2.35×10^{4} M	0.1405×10^{-4}	2.209	0.789	5.61	
		1.63×10^{4} M	0.1694×10^{-4}	1.460	0.521	3.07	
3	Rosuvastatin	1.95×10^{4} M	0.1571×10^{-4}	1.792	0.682	4.34	4.54
		2.35×10^{4} M	0.1637×10^{-4}	2.186	0.740	4.52	

Table 3: Estimation of 'K' Value of Statins in presence of certain dyes and food stuffs

S. No	Name of the Statin	'K' Value of			
		Statin alone	Statin with Brilliant blue lake	Statin with Ferric oxide red	Statin with Soy food stuffs
1	Atorvastatin	4.5×10^{4}	5.22×10^{4}	4.34×10^{4}	4.24×10^{4}
2	Simvastatin	6.25×10^{4}	7.14×10^{4}	5.94×10^{4}	7.5×10^{4}
3	Rosuvastatin	4.54×10^{4}	9.52×10^{4}	3.65×10^{4}	6.25×10^{4}

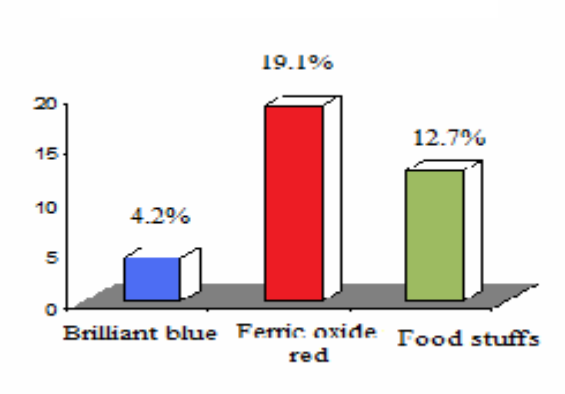

Fig. 1: % decrease in concentration of Atorvastatin

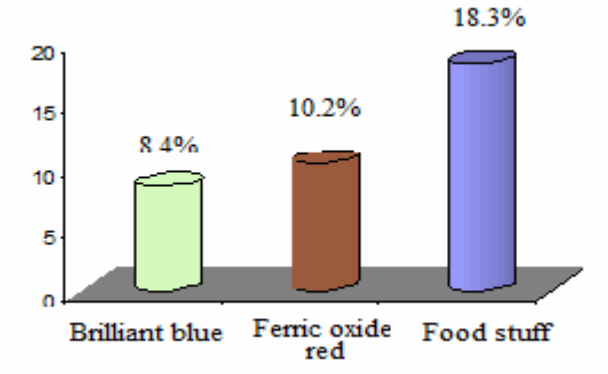

Fig. 2: % decrease in concentration of Simvastatin

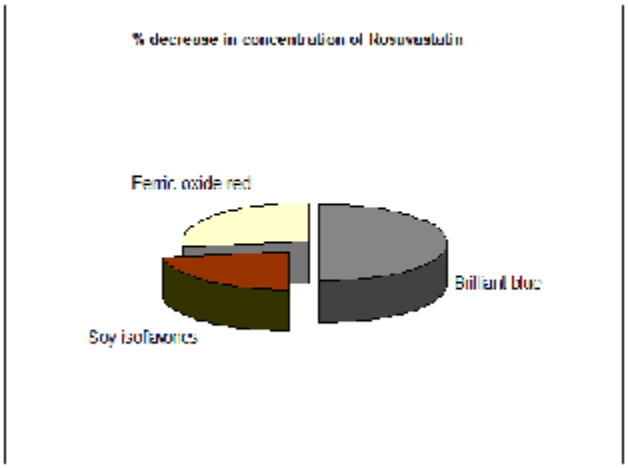

Fig. 3: % decrease in concentration of Rosuvastatin

protein binding of statin drugs was found to get altered in presence of dyes.

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