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Original Article

Comparative *in vitro* anti-oxidative properties of statins

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Abstract

Introduction: This study seeks to determine and compare the *in vitro* anti-oxidative properties of 5 statins which aare available in the market for the treatment of dyslipidemia.

Methods: The ability of the statins to inhibit free radical formation by 2,2-Diphyenyl-1-Picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) in liver homogenates was determined.

Results: Out of the 5 statins tested, atorvastatin, simvastatin and rosuvastatin gave the highest inhibibition and lowest median effective concentration (IC_{50}). Pravastatin and fluvastatin were weak inhibitors of free radical formations.

Conclusion: This study shows that there is evidence on the anti-oxidative properties of statins in addition to their hypolipidemic properties.

1. Introduction

Nowadays, treatments of dyslipidemia go beyond lowering of blood lipid levels. Activation of the immune system by various cytokines, free radicals and pro-inflammatory substances which contribute greatly to the regression of atherosclerosis has been explored [1]. The *in vitro* anti-oxidative property of fenofibrate, a drug used for the management of high triglyceride levels, has been demonstrated[2]. A certain class of hypolipidemic drugs known as statin inhibits hydroxymethylglutaryl CoA reductase inhibitors, the rate-limiting enzyme for the synthesis of cholesterol in the liver. Rosuvastatin has been shown to prevent oxidative stress in humans[3]. Atorvastatin inhibits oxidative pathways upregulated in atheroma [4]. Fluvastatin and simvastatin scavenge superoxide dismutase and inhibit low-density lipoprotein (LDL) oxidation [5]. Currently, no studies on the *in vitro* anti-oxidative properties of the statins have been conducted. This study seeks to determine and compare in terms of potencies the *in vitro* anti-oxidative properties of 5 statins that are currently used for the management of dylipidemia.

2. Materials and Methods

2.1. Chemicals and Solvents

All solvents used were of reagent grades and purchased from Dakila Trading, Inc. Samples of simvastatin, atorvastatin, rosuvastatin, fluvastatin and pravastatin were given as gifts from Deackchem, Inc. Stock solutions of each drug in methanol were prepared and diluted accordingly with the solvent to prepare test samples with concenration ranges from 0 – 0.5 mg/mL.

2.2. Inhibition of 2,2-Diphyenyl-1-Picrylhydrazyl (DPPH)

Exactly 0.3 ml of each test sample (n = 5 trials per statin concentration) and 0.3 mL of methanol (i.e., control) was added to 2 mL portions of 1 mM DPPH (Sigma-Aldrich) in methanol in a series of test tubes. The mixtures were kept at room temperature for 30 minutes and then read for absorbance at 517 nm. The % inhibition (% I) of DPPH free radical was calculated, thus: % I = Ao – A/Ao, where Ao is absorbance of methanol solution while Ao is absorbance of the test sample after 30 minutes [6].

2.3. Inhibition of Lipid Peroxidation in Liver Homogenates

Livers of newly-slaughtered pigs were purchased from a local market. Livers were washed with ice-cold 0.9% NaCl. Liver homogenate was prepared using a blender in a ratio of 1 gram of wet tissue to 9 mL of 1.15% KCl. To 0.1 mL portions of liver homogenate were added 0.1 mL of 0.5 mg/mL statin sample solution (n = 5 trials) or 0.1 mL methanol (i.e., blank control), 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was made up to 4.0 mL with distilled water, and then heated at 95^{0}C for 60 minutes in a water bath. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (1:15, v/v) were added. After centrifugation at 4000 rpm for 10 minutes, the upper organic layer was measured for absorbance at 532 nm. 1,1,3,3-tetramethoxypropane (TMP) was used as an external standard and the level of lipid peroxides was expressed as nmol of malondialdehyde (MDA) [7].

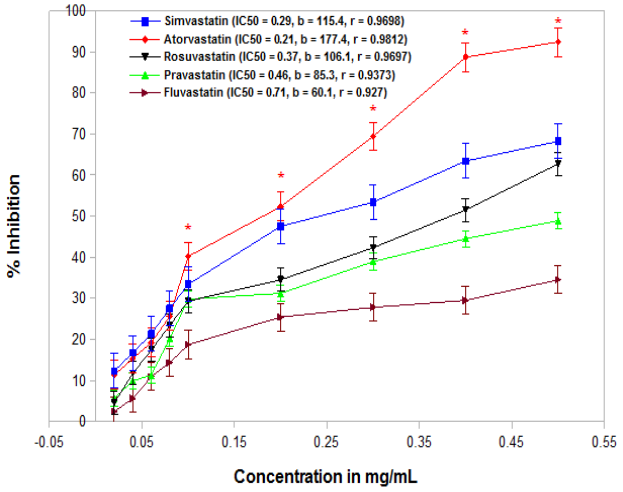
3. Results

The ability of statins to scavenge or inhibit free radical formation by DPPH at 517 nm was explored to assess and compare *in vitro* anti-oxidative effects. Figure 1 shows that among the 5 drugs tested, atorvastatin gave the lowest median inhibitory concentration (IC_{50}). Inhibition was concentration dependent (pearson r > 0.9) in all the test samples, with atorvastatin giving the highest linearity and slope (b). The weak inhibitory effects of pravastatin and fluvastatin even at the maximum concentration of 0.5 mg/mL suggest weak *in vitro* free radical scavenging properties.

The second *in vitro* assay used in this study is the ability of statins to inhibit the formation of free radicals from polyunsaturated fatty acids in liver homogenates. The end-product of this reaction is the formation of MDA which conjugates with TBA to form a reduced

colored complex known as thiobarbituric acid reactive species (TBARS). Table 1 agrees with the findings in Figure 1 as both atorvastatin and simvastatin gave the highest % inhibition and gave the lowest formation of MDA.

Figure I: Comparative *In Vitro* Inhibition of DPPH Free Radical Formation Among Statins



*p < 0.001 vs. the rest of the statins and corresponding % inhibition at 0.02 - 0.20 concentrations; ** p < 0.01 vs rosuvastatin, pravastatin and fluvastatin; n = 5/concentration/statin

**p < 0.001 vs simvastatin, rosuvastatin, pravastatin and Fluvastatin; ** p < 0.01 vs rosuvastatin, pravastatin and fluvastatin; n = 5/concentration/statin

Table 1: Comparative % Inhibition of TBARS and MDA Formation among Statins

Statin	% Inhibition of TBARS Formation	MDA Formation in nM/mg
Atorvastatin	91.4 ± 3.1*	0.32 ± 0.03*
Simvastatin	89.2 ± 4.3*	0.27 ± 0.02*
Rosuvastatin	77.5 ± 2.8	0.51 ± 0.05
Pravastatin	28.4 ± 4.5	0.83 ± 0.11
Fluvastatin	19.7 ± 4.0	0.92 ± 0.09

*p < 0.01 vs rosuvastatin, pravastatin and fluvastatin; n = 5

4. Discussions

The role of oxidation in accelerating atherosclerosis can be coursed through the formation of free radicals which interact with low-density lipoproteins (LDL) to form foam cells in the coronary arteries. The density of foam cell formation corresponds to the density of plaque formation during atheroslerosis. This study shows that 3 statins, namely atorvastatin, simvastatin and rosuvastatin exhibit strong *in vitro* anti-oxidative properties, pleiotropic effects which may aid in reducing the harmful effects of free radical formation in addition to their LDL-lowering effects. Unlike atorvastatin, rosuvastatin and simvastatin, both pravastatin and fluvastatin do not contain proton donors in their chemical structures (carboxyl hydrogen and amino hydrogen) which are needed to reduce MDA and sequester DPPH free radical formation. Based on the findings from this study, it is recommended that *in vivo* anti-oxidative enzymatic assays, using liver homogenates, be conducted for atorvastatin, simvastatin and rosuvastatin to fully determine the mechanisms by which their anti-oxidative properties are conveyed.

5. Conclusions

This study was able to demonstrate the high *in vitro* anti-oxidative properties of atorvastatin and simvastatn in terms of their free radical scavenging effects. Simvastatin showed moderate anti-oxidative properties. On the other hand, pravastatin and fluvastatin gave weak inhibitory effects to *in vitro* free radical formation.

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