SUPPLEMENTARY MATERIAL

Molecular Rationale Delineating the Role of Lycopene as a Potent HMG-CoA Reductase

Inhibitor: *In vitro* and *In silico* study

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Abstract

The present study initially aims to depict the molecular rationale evolving the role of lycopene in inhibiting the enzymatic activity of β -hydroxy- β -methylglutaryl CoA (HMG-CoA) reductase via *in vitro* and *in silico* analysis. Our results illustrated that lycopene exhibited strong HMG-CoA reductase inhibitory activity (IC₅₀ value of 36 ng/ml) quite better than Pravastatin (IC₅₀=42 ng/ml) and strong DPPH free radical scavenging activity (IC₅₀ value = 4.57 ± 0.23 μ g/ml) as compared to ascorbic acid (IC₅₀ value = 9.82 ± 0.42 μ g/ml). Moreover, the K_i value of lycopene (36 ng/ml) depicted via Dixon plot was well concurred with an IC₅₀ value of 36 ± 1.8 ng/ml. Moreover, molecular informatics study showed that lycopene exhibited binding energy of -5.62 Kcal/mol indicating high affinity for HMG-CoA reductase than HMG-CoA (ΔG : -5.34 kcal/mol). Thus, *in silico* data clearly demonstrate and support the *in vitro* results that lycopene competitively inhibit HMG-CoA reductase activity by binding at the hydrophobic portion of HMG-CoA reductase.

Keywords: Lycopene, Hyperlipidemia, HMG-CoA reductase, Antioxidant.

Experimental

Chemical reagents: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Hi-Media Laboratories, Mumbai, India. Lycopene and HMG-CoA reductase assay kit were procured from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals used in this study were of analytical grade.

DPPH radical scavenging activity of lycopene

The DPPH radical scavenging capacity of the lycopene from MP Biomedicals (India) was determined by the method of (Brand-Williams et al, 1995). Further, IC₅₀ value represented the concentration of the lycopene that caused 50% inhibition of DPPH radicals and was calculated by interpolation of linear regression analysis.

In vitro HMG-CoA reductase inhibitory activity of lycopene

The HMG-CoA reductase assay kit with the catalytic domain of the human enzyme (recombinant GST fusion protein expressed in *E. coli*) procured from Sigma-Aldrich (St. Louis, MO, USA) was used according to the manufacturer's instructions, to analyse the HMG-CoA reductase inhibitory activity of lycopene (Iqbal et al, 2014a). The concentration of the purified human enzyme stock solution was 0.52–0.85 mg protein/mL. Reference statin drug pravastatin was used as positive control. To characterize HMG-CoA reductase inhibition under defined assay conditions, reactions containing 4 µL of NADPH (to obtain a final concentration of 400 µM) and 12 µL of HMG-CoA substrate (to obtain a final concentration of 400 µM) in a final volume of 0.2mL of 100mM potassium phosphate buffer, pH 7.4 (containing 120mM KCl, 1mM EDTA, and 5mM DTT), were initiated (time 0) by the addition of 2 µL of the catalytic domain of human recombinant HMG-CoA reductase and incubated in Eppendorf BioSpectrometer (equipped with thermostatically controlled cell holder) at 37 °C in the presence or absence (control) of 1 µL aliquots of lycopene dissolved in DMSO (5%). The rates of NADPH consumed were monitored every 20 sec for up to 15 mins by scanning spectrophotometrically.

Enzyme kinetics studies

In order to determine the kinetic properties of HMG-CoA reductase after addition of lycopene, the activity was assayed by using various concentrations of HMG-CoA (100, 200, 300, 400, and 500 µM) in the absence and presence of different concentrations of Lycopene. *K_m* and mode of inhibition was determined by double-reciprocal Lineweaver-Burk plot analysis according to Michaelis-Menten kinetics, and *K_i* was determined by Dixon plot (Lineweaver and Burk, 1934; Dixon, 1953).

Molecular docking studies

HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, a key precursor of cholesterol biosynthesis. The PDB structure of the HMG-CoA in complex with HMG-CoA reductase was retrieved from the Protein Data Bank (PDB ID: 1DQ9) (Brookhaven Protein Data Bank, <http://www.rcsb.org>). The pdb file was energy minimized. The substrate HMG-CoA (ligand) was also exported in the form of a single sdf file. The separate ligand files of lycopene and Pravastatin were obtained as sdf files from Pubchem database. Molecular docking was performed by using Autodock 4.2 version.

Statistical Analysis

For the entire assays, samples were analysed in triplicate and the results were expressed as mean $\pm$ S.D. IC₅₀ value was calculated by Origin version 6.0 Professional software.

References

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Figure S. S.1: DPPH free radical scavenging activity of lycopene and ascorbic acid. S.2A: *In-vitro* HMG-CoA reductase inhibitory activity of lycopene and pravastatin. Spectrophotometric time-scans demonstrating the ability of Pravastatin (S.2B) and lycopene (S.2C) to inhibit HMG-CoA reductase activity. S.3A: Lineweaver-Burk double-reciprocal. S.3B: Dixon plot of lycopene against β -hydroxy- β -methylglutaryl-CoA reductase. Each value in the figure is represented as mean $\pm$ SD (n = 3). S.4A,B&C: Binding pattern of HMG-CoA, lycopene and Pravastatin within the active site of HMG-CoA Reductase. S.4D: Superimpose image (S.4D) lycopene (Yellow), Pravastatin (Magenta), HMG-CoA (Green) within the active site of HMG-CoA Reductase.