IN-VITRO ANTIINFLAMMATORY AND ANTIOXIDANT ACTIVITY OF ROSUVASTATIN

Larisa Alagić-Džambić*, Ervina Bečić, Fahir Bečić and Mirsad Džambić
Bosnia and Herzegovina.

*Corresponding Author: Larisa Alagić-Džambić
Bosnia and Herzegovina.

ABSTRACT
Statins exhibit their pharmalogical effects by competitive inhibition through binding with active sites of enzymes. Rosuvastatin, of all statines, has the most binding interactions with the enzyme, and being the most potent statin, it is presumed that the strength of enzyme binding directly influences its potency. Reduction of derivatives of mevalonate results with decreased risk of cardiovascular diseases and very significant pleotropic effect of statins. Rosuvastatin comes in the form of calcium salt. During the clinical trial it has been marked as superstatin with its proven activity in lowering cholesterol levels.[6] Aim of this paper was to examine the antiinflammatory and antioxidant activity

KEYWORDS: Rosuvastatin, Antiinflammatory, Antioxidant, DPPH.

INTRODUCTION
Rosuvastatin calcium is a synthetic lipid-lowering agent for oral administration. It inhibits HMG-CoA reductase, the rate-limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol. The chemical name for rosuvastatin calcium is bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino]-pyrimidin-5-yl]-3,5-dihydroxyhept-6-enolic acid] calcium salt. During clinical trials, it is labeled as superstatin with its proven activity in lowering cholesterol levels.[1] The aim of this work was to examine the pleiotropic properties of rosuvastatin through in-vitro antiinflammatory by denaturing effects of proteins and antioxidant activity.

MATERIAL AND METHODS
DPPH (α, α-diphenyl-β-picrylhydrazine) antioxidant activity method is based on the reduction of relatively stable DPPH radicals in the presence of an antioxidant compound capable of donating H atom. Free DPPH radicals give maximum absorption at 517 nm (purple color). In the presence of antioxidants, an absorption drop occurs, resulting in a decrease in color intensity, the same from purple to light yellow, whereby the color intensity is directly proportional to the antioxidant potential.[2]

The percentage of antioxidant activity was calculated by using following formula:

% inhibition = [(Acontrol solution-Atest solution) / Acontrol solution] x100

The results are presented as the value of EC50 and this is actually the concentration of antioxidants required for the 50% DPPH reduction. In determining the antioxidant activity by DPPH method, the absorbance of 0.2 mM methanolic solution of rosuvastatin was mixed with the same ratio of 0.2 mM DPPH solution. The control solution is a mixture of methanol and DPPH. The measurement was done 30 minutes after addition of DPPH, at 517 nm. For determination concentration that inhibits DPPH by 50% (EC50), a dilution series was made for each sample individually.

Rosuvastatin was screened for antiinflammatory activity using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi with slight modification. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in phosphate buffer. Denaturation was induced by keeping the reaction mixture at 60° + 1°C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer). Percentage of inhibition of denaturation was calculated from control where no drug was added. The Ibuprofen was used as standard drug.[3,4,5]

The percentage inhibition of denaturation was calculated by using following formula:

% of Inhibition = 100 X {Vt / Vc – 1}
Where,
Vt = Mean absorbance of test sample.
Vc = Mean absorbance of control

RESULTS AND CONCLUSION
Antioxidant activity
For EC50 determination, rosuvastatin and trolox solutions were prepared in a concentration range 25-120%. From linearity data, correlation coefficient for trolox solution is 0.9959 (Figure 1). EC50 for rosuvastatin solution is 0.005mM (Table 1). If we compare inhibition % between this solution, we can see that all rosuvastatin solution have % INH from 45%-50%. For troxol solutions, %INH are from 22%-64% (Figure 2).

Table 1: Antioxidant activity for Rosuvastatin solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>(% INH)</th>
<th>EC50 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>0.733</td>
<td>49.48</td>
<td>0.005</td>
</tr>
<tr>
<td>DPPH&lt;sub&gt;60&lt;/sub&gt;</td>
<td>1.451</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TROLOX</td>
<td>0.201</td>
<td>86.15</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Figure 1: Calibration curve for Trolox solution.

Figure 2: Screening of antioxidant activity.
In-vitro antiinflammatory activity
The antiinflammatory activity of rosuvastatin on the in vitro model was assayed by inhibition of albumin denaturation. Rosuvastatin solutions at concentrations of 10, 50, 100, 200 and 400 μg / ml showed inhibition of denaturation ranging from 91.6% to 98.5%. Ibuprofen, as the drug cure, prepared at a concentration of 200 μg/ml, was denatured with 98.5% (Table 2).

Table 2: Screening of antiinflammatory activity.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibition of denaturation in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>/</td>
</tr>
<tr>
<td>Rosuvastatin solution 10μg/ml</td>
<td>98.5</td>
</tr>
<tr>
<td>Rosuvastatin solution 50μg/ml</td>
<td>98.3</td>
</tr>
<tr>
<td>Rosuvastatin solution 100μg/ml</td>
<td>98.5</td>
</tr>
<tr>
<td>Rosuvastatin solution 200μg/ml</td>
<td>98.5</td>
</tr>
<tr>
<td>Rosuvastatin solution 400μg/ml</td>
<td>91.6</td>
</tr>
<tr>
<td>Ibuprofen solution 200μg/ml</td>
<td>98.5</td>
</tr>
</tbody>
</table>

CONCLUSION
According to presented results, Rosuvastatin has shown significant antioxidant and anti-inflammatory activity. The results of this study open possibility of using rosuvastatin in the treatment of antiinflammatory and antioxidant processes and challenge for further research.

REFERENCES