Abstract
Rosuvastatin or 7- [4-(4-fluorophenyl)-6-(1-methylethyl) -2 - (methylsulfonyl-methyl-amino)-pyrimidin-5-yl] -3,5-
dihydroxy-hept-6-enoic acid is the newest drug of the statin on the market. On the market is since 2003., in a form of
the calcium salt. Also, rosuvastatin is marked as superstatin in reduction of cholesterol. Compared to the others
hipolipemic drugs, statins shows others pleitropic effects. It means that they show analgesic, anti-inflammatory,
antioxidant, antibacterial and antifungal activity. The presence of the pyrimidine ring in the molecule of rosuvastatin
justifies above mentioned pharmacological actions. This paper presents a diffusion method, as a screening method for
potential antimicrobial and antifungal effects of rosuvastatin. The test was perform against five different American
Type Culture Collection (ATCC): Pseudomonas aeruginosa (ATCC 9027), Escherichia coli (ATCC 8739), Candida
albicans (ATCC 10231), Staphylococcus aureus (ATCC 6538) and Staphylococcus epidermidis (ATCC 12228).

Key words: Rosuvastain, Antibacetrial effect, Diffusion method.

Introduction
Statins are known as inhibitors of 3-hydroxyl-3-methyl-glutaryl reductase (HMG-CoA). By the structure, statins are
analogues HMG-CoA in one part of molecule, which is based on their properties to inhibite HMG-CoA. Because of
their effectiveness, their consumption increas significantly, and they become most frequently drugs (6, 1, 2).
Natural statins have been isolated by fermentation from the microorganismus (lovastatin, simvastatin, pravastatin).
Synthetic statins are fluvastatin, atorvastatin and rosuvastatin (3).
Rosuvastatin or 7- [4-(4-fluorophenyl)-6-(1-methylethyl) - 2 - (methylsulfonyl-methyl-amino)-pyrimidin-5-yl] -3,5-
dihydroxy-hept-6-enoic acid is the newest drug of the statin on the market (5). The most common dose in the primary prevention is 10 mg and 20 – 40 mg in the secondary prevention. Tmax is about 3 hours, and half-life elimination ($t_{1/2}$) is 20.8 hours. Elimination of rosuvastatin is by the liver, and 90% excretion is in the faeces and 10% by the kidneys (6, 7). The existance of the pyrimidine ring in the molecule explain several interesting characteristics of rosuvastatin. Pyrimidine derivates are also used as anti-inflammatory, antimalerial drugs, cardiovascular, anti-tumor, diuretic and anti-viral drugs (8, 9, 10, 11).

The aim of this study is to investigate potential antimicrobial action of rosuvastatin and identify spectrum of action.

**Material and methods**

Antibacterial and antifungal activity of rosuvastatin was evaluated against four different bacteria Pseudomonas aeruginosa (ATCC 9027, lot 483-296-4), Esherichia coli (ATCC 8739, lot 483-296-4), Staphylococcus aureus (ATCC 6538, lot 485-155-4), Staphylococcus epidermidis (ATCC 12228, lot 371-87-5) and one unghi Candida albicans (ATCC 10231, lot 443-277-4). All test organism were stored at +4°C. For diffusion method, we used microbiological assay of antibiotics according to Eurpean Pharmacopoea (EP, monograph 2.7.2. Microbiological Assay of Antibiotics).

The potency of rosuvastatin is estimated by comparing of growth of sensitive microorganisms produced by known concentrations of the rosuvastatin and reference substances as positive control and solvent as negative control.

The culture media for Staphylococcus aureus and Staphylococcus epidermidisthat we used is according to EP Medium A. Medium A containing 6g peptone, 4g pancreatic digest of casein, 1.5g beef extract, 3g yeast extract, 1g glucose monohydrate, 15g agar and to 1000ml water. After weighing all components exept agar, we added water to 1000ml, and stand at room temeprature for 15 minutes with stirring occasionally, then add agar and heated in an apparatus for melting and sterilization of microbiological media. Sterilization is carried out at 121°C for 15 minutes. pH value of this medium after sterilisation have to be about 8.

For Esherichia coli, media containing 2g ammonuim chloride, 3g sodium chloride, 0.4g di-potassium hidrogen phosphate, 3g sodium citrat, 3g lactose, 15g agar and to 1000ml water. Sterilization is carried out at 121°C for 15 minutes. pH value of this medium after sterilisation have to be about 7.

For Pseudomonas aeruginosa we used casein soybean digest agar (Trypticase, Merck) and for Candida albicans sabouraud dextrose agar (Merck) (4).
All components that we used for media preparing are pro analysis gradient grade (Merck).

Rosuvastatin (Sigma-Aldrich) was obtained from the manufacturer as pure drug and dissolved in methanol (Merck p.a.) to give initial concentration 1g/l. Positive control and methanol as negative control are included. As a positive control we used vancomycin (Fluka), ciprofloxacin (Fluka), gentamcin sulfate (Sigma-Aldrich), ampicilin sodium salt (Sigma-Aldrich), fluconazole (Sigma-Aldrich) and metronidazole (Fluka). After incubation, all petri dishes are scanned on automatic colony counter and zone growth Scan Interscience.

**Results and discussion**

The antibacterial activity of the rosuvastatin against to Gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis), Gram-negative bacteria (Pseudomonas aeruginosa and Esherichia coli) and fungi (Candida albicans) is given in Table 1 and figures 1-6.

**Table-1: Antibacterial activity (Inhibition zone measured in mm, including hole 6 mm in diametar) of Rosuvastatin.**

<table>
<thead>
<tr>
<th>Sample / Organisms</th>
<th>Staphylococcus aureus (ATCC 6538)</th>
<th>Staphylococcus epidermidis (ATCC 12228)</th>
<th>Pseudomonas aeruginosa (ATCC 9027)</th>
<th>Esherichia coli (ATCC 8739)</th>
<th>Candida albicans (ATCC 10231)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>23.7; 22.4</td>
<td>11.3; 12.0</td>
<td>11.1; 12.0</td>
<td>0</td>
<td>37.6; 36.3</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>35.0; 33.3</td>
<td>28.0; 28.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>32.6; 38.2</td>
<td>30.0; 29.6</td>
<td>37.4; 37.0</td>
<td>31.1; 32.2</td>
<td>NA</td>
</tr>
<tr>
<td>Ampicilin sodium salt</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>28.0; 33.0</td>
<td>NA</td>
</tr>
<tr>
<td>Gentamicin sulfate</td>
<td>NA</td>
<td>NA</td>
<td>15.2; 15.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>37.6; 37.6</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

*NA- not applicable

**Picture 1.** Inhibition zone against Staphylococcus aureus; A- for methanol (position 1 and 4) and for vancomycin (position 2 and 3); B- for rosuvastatin (position 1 and 4) and for ciprofloxacine (position 2 and 3).
Picture 2. Inhibition zone against Staphylococcus epidermidis: A- for rosuvastatin (position from 1 to 4); B- for vancomycin (position 1 and 4) and for ciprofloxacin (position 2 and 3); C- for methanol (position from 1 to 4).

Picture 3. Inhibition zone against Pseudomonas aeruginosa: A- for rosuvastatin (position from 1 to 4); B- for ciprofloxacin (position 1 and 4) and for methanol (position 2 and 3); C- for gentamicin sulfate (position 1 and 4).

Picture 4. Inhibition zone against Escherichia coli: A- for rosuvastatin (position 1 and 4) and for methanol (position 2 an 3); B- ampicilin sodium salt (position 1 and 4) and for ciprofloxacin (position 2 an 3).
Picture 5. Inhibition zone against against Candida albicans: A- for rosuvastatin (position from 1 to 4); B- for fluconazole (position 1 and 4) and for methanol (position 2 and 3); C- for metronidazole (position 1 and 4).

From the result in Table 1, and scanned pictures of petri dishes (1-5), we can see that we have corresponding inhibition zone on Gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis) and Gram-negative bacteria (Pseudomonas aeruginosa and Esherichia coli). It is considerably smaller at Staphylococcus epidermidis with regard to Staphylococcus aureus and positive antibiotic control (Vancomycin and Ciprofloxacine). But is not negligible. The result of antimicrobial activity on Gram-negative bacteria are different. OnPseudomonas aeruginosa inhibition zone are significantly less then on positive antibiotic control, and on Esherichia coli, rosuvatatin does not have any antibacterial activity. But, very important is antifungal activity of rosuvastatin. The inhibition zone are the same as a positive control fluconazole. For rosuvastatin average inhibition zone iz about 37.0mm, and for fluconazole is 37.6mm. Interesting is that metronidazole as positive control does not have any antifungal activity on this American Type Culture Candida albicans (ATCC 10231). Positive antifungal activity of rosuvastatin leads us to reflect of the topical use of rosuvastatin. Also, testing should be done at dillution method, to confirm minimum inhibitory concentration (MIC in µg/ml) and minimum bactericidial concentration (MBC in µg/ml) of rosuvastatin. Given to demonstrated antibacterial activity on Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa test sholud be done against different clinical isolates, with both methods, diffusion and dilution, especially against strains in which resistance is (diagnosed diabetic wounds caused with Pseudomonas and fungal diseases). Methanol as solvent has no antibacterial and antifungal activity.

Concluding remarks

The study suggests the observed antimicrobial effect of rosuvastatin. Rosuvastatin have unexpected antimicrobial effect in vitro, and interesting is antifungal activity on Candida albicans. In summary, results of this study and the future study have to include investigation of mechanism by which rosuvastatin exhibits antibacterial and antifungal activity. Further investigation have to include MIC and MBC to confirm antibacterial and antifungal activity. These points need more study, and could be a matter of future work.

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References:


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