The Evaluation Of Analgesic Effects Of Rosuvastatin In Hot Plate Test

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***Abstract*—Statins are widely used for the prevention of cardiovascular disease. Recent studies have focused on the antiinflamatory effects on the inflammatory disease. Rosuvastatin has been evaluated whether to have analgesic effects in mice in hot plate test.**

Keywords—Rosuvastatin; Hot Plate; Analgesia

INTRODUCTION

Statins exhibit their pharmalogical effects by competitive inhibition trough binding with active sites of enzymes. Enzyme active site for substrate binding is *hydrophobic pocket* and it can be modified to allow access and binding of statins molecules hydrophobic ring[1]. Studies of crystal structure of enzyme active sites showed that there are some differences in the way statins bind to HMG-CoA reductase[2]. For example, rosuvastatin binds to enzyme with an additional hydrogen bond and a polar interaction with active site[3]. Rosuvastatin, of all statins, 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[4]. Reduction of derivatives of mevalonic acids results with decreased risk of cardiovascular diseases and very significant pleotropic effect of statins[5]. Rosuvastatin comes in the form of calcium salt[6]. During the clinical trial, it has been marked as *superstatin* with its proven activity in lowering cholesterol levels[7].

Aim of this paper was to investigate if rosuvastatin exhibits analgesic action (hot plate method) through effect of acute sensitivity on thermal stimulus on *in vivo* models and effect of chronic sensitivity on thermal stimulus on *in vivo* models[8-10].

METHODS

Materials

Study was held with mice of either sex weighting 20 – 30 g. Animals were randomly used in the experiments. Sensitivity to thermal stimulation was tested by a hot plate test. (Hot Plate Model DS-37) Panel surface temperature is set at 55 ± 0.5 ° C. By using the hot plate method, the time latency measured in seconds requires the animal to raise and lick the paw. Latency time is defined as the time interval between zero point when the animal was placed on the warm surface of the plate and the time when the animal began to lick the paw or jump, avoiding the pain caused by thermal stimulation. To prevent skin and tissue damage, the maximum length of the paw on the hot plate is 30 seconds. The measurement test for each animal is 60 and 120 minutes. Three groups of animals were included in the experiment: acute application, chronic application and mechanism of action. The animals received the test substance per os, and naloxone (Abbot) and L-NAME (L-nitro arginine methyl ester; Sigma-Aldrich) were intraperitoneally (i.p.). Rosuvastatin (Belupo) was dissolved in phosphate buffered saline, L-NAME in saline, and naloxone was used in the form they are manufactured.

Experimental protocols

Animals were divided into three gropus.

Group A: Acute application gropus

After marking the animals with picric acid and weighing, the animals were divided into 5 subgroups (3 females + 3 males).

- Control group (vehicle group), n=6
- Rosuvastatin 5 mg/kg, n=6
- Rosuvastatin 10 mg/kg, n=6
- Rosuvastatin 20 mg/kg, n=6
- Rosuvastatin 40 mg/kg, n=6.

Group B: Chronical application gropus

For the purposes of chronical evaluation, rosuvastatin was administered to animal for three consecutive days at the same time. After the third day, the sensitivity to thermal stimulation was measured by the hot plate method. The animals are divided into 5 subgroups (3 females + 3 males).

- Control group (vehicle group), n=6
- Rosuvastatin 5 mg/kg, n=6
- Rosuvastatin 10 mg/kg, n=6
- Rosuvastatin 20 mg/kg, n=6
- Rosuvastatin 40 mg/kg, n=6.

Group C: Investigation of mechanism groups

Experimental animals were intraperitoneally injected naloxone at a dose of 0.5 mg / kg and L-NAME 10 mg / kg. Sensitivity to thermal stimulation by hot plate method was investigated. For each animal, time before and after drug administration (after 60 and 120 minutes) is defined. The animals are divided into 5 subgroups (3 females + 3 males).

- Control group (vehicle group), n=6
- Nalokson 0.5 mg kg, n=6
- *L*-NAME 10 mg/kg, n=6
- Rosuvastatin (40 mg/kg) + Nalokson (0.5 mg /kg), n=6
- Rosuvastatin (40 mg/kg) + *L*-NAME (10 mg/kg), n=6.

Statistics

The data were expressed as the mean ±SEM using Mann-Whitney U test. The level of significance was set at $p < 0.05$.

RESULTS

Acute application gropus

Rosuvastatin (5, 20 mg/kg) did not produce any statistically significat analgesic effect compared to controls after 60th and 120th minutes in mice. On the other side rosuvastatin (10, 40 mg/kg) produced a statistically significant analgesic effect compared to control group after 60th and 120th minutes in mice ($p < 0.05$) (Fig. 1; Fig. 2; Fig. 3; Fig. 4).

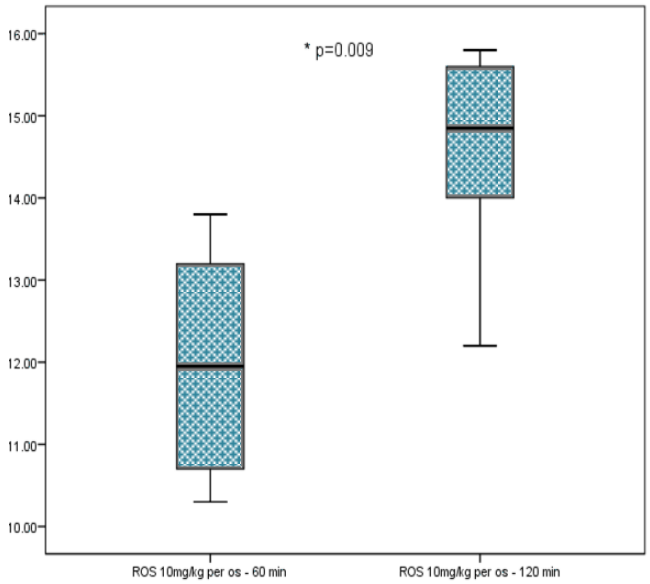

Fig. 1. Analgesic effect of rosuvastatin 10 mg/kg after acute application in 60th and 120th minutes in hot plate test

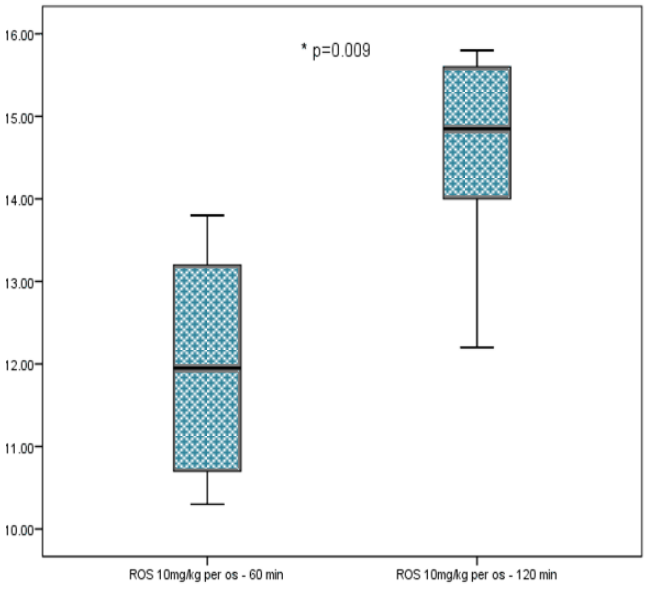

Fig. 2. Analgesic effect of rosuvastatin 40 mg/kg after acute application in 60th and 120th minutes in hot plate test

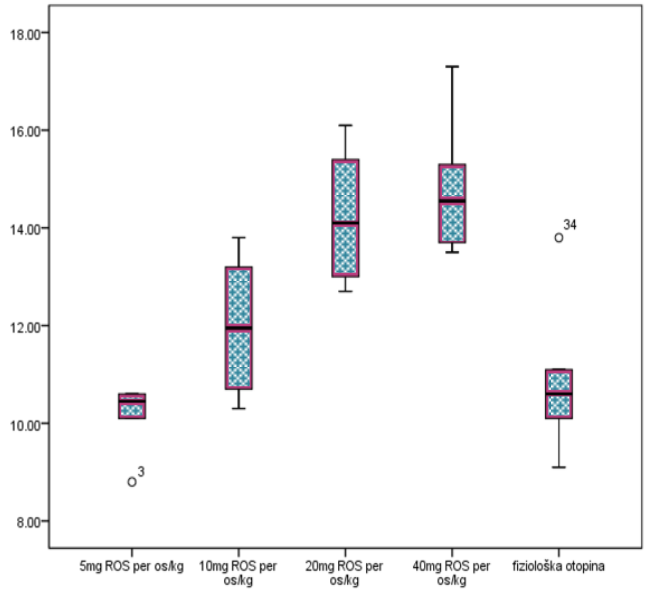

Fig. 3. Analgesic effect of rosuvastatin 5, 10, 20 and 40 mg/kg after acute application in 60th minute in hot plate test

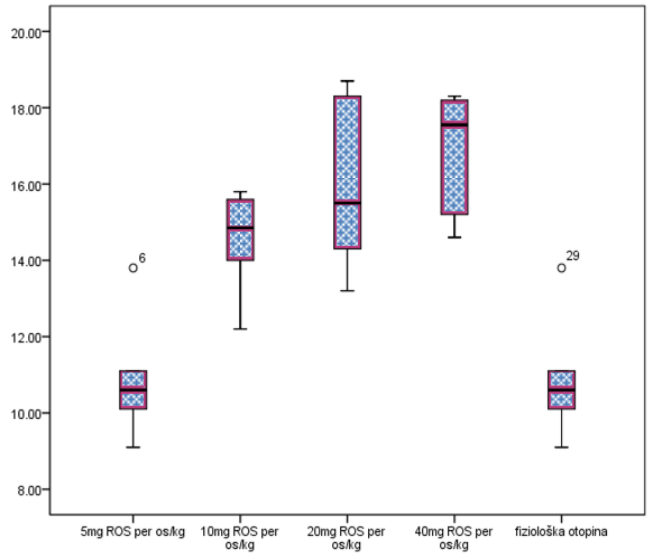

Fig. 4. Analgesic effect of rosuvastatin 5, 10, 20 and 40 mg/kg after acute application in 120th minute in hot plate test

Chronic application gropus

Rosuvastatin (5, 20, 40 mg/kg) did not produce any statistically significat analgesic effect compared to controls after 60th and 120th minutes in mice. On the other side rosuvastatin (10 mg/kg) produced a statistically significant analgesic effect compared to control group after 60th and 120th minutes in mice ($p < 0.05$) (Fig. 5; Fig. 6; Fig. 7).

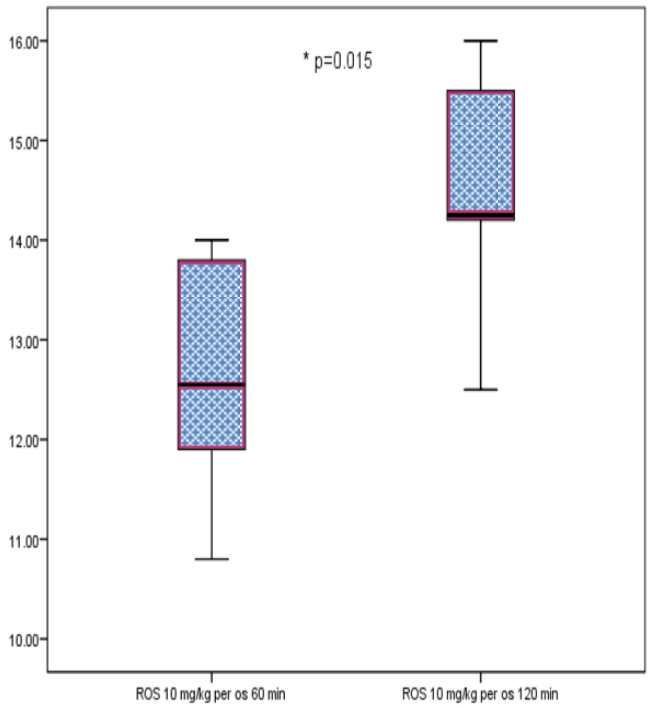

Fig. 5. Analgesic effect of rosuvastatin 10 mg/kg after chronical application in 60th and 120th minute in hot plate test

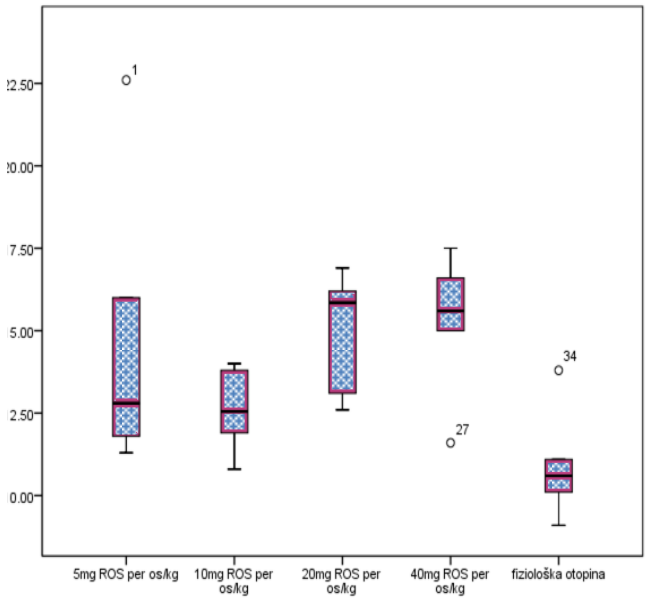

Fig. 6. Analgesic effect of rosuvastatin 5, 10, 20 and 40 mg/kg after chronical application in 60th minute in hot plate test

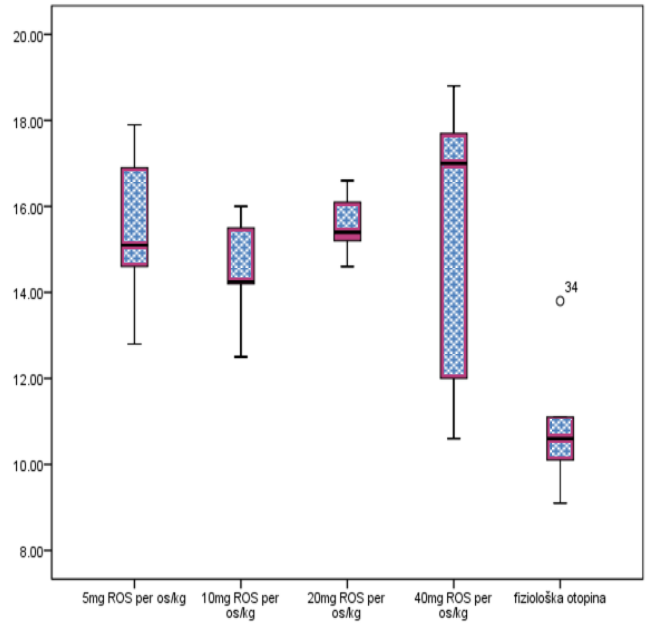

Fig. 7. Analgesic effect of rosuvastatin 5, 10, 20 and 40 mg/kg after chronical application in 120th minute in hot plate test

Investigation of mechanism groups

Combination of the administered dose naloxone (0.5 mg/kg) i.p. with a dose of rosuvastatin 40 mg / kg per os, half an hour after naloxone, no effect compared to the control group, by measuring the latency time after 60 minutes, while after 120 minutes there is a statistically significant difference ($p < 0.05$) (Fig. 8).

The enhanced analgesic effect was obtained in combination of the administered dose of L-NAME (10

mg/kg) i.p. with a dose of rosuvastatin 40 mg/kg per os, half an hour after L-NAME compared to the rosuvastatin administered at 40 mg/kg, during the 60 and 120 minutes ($p < 0.05$) (Fig. 9; Fig. 10).

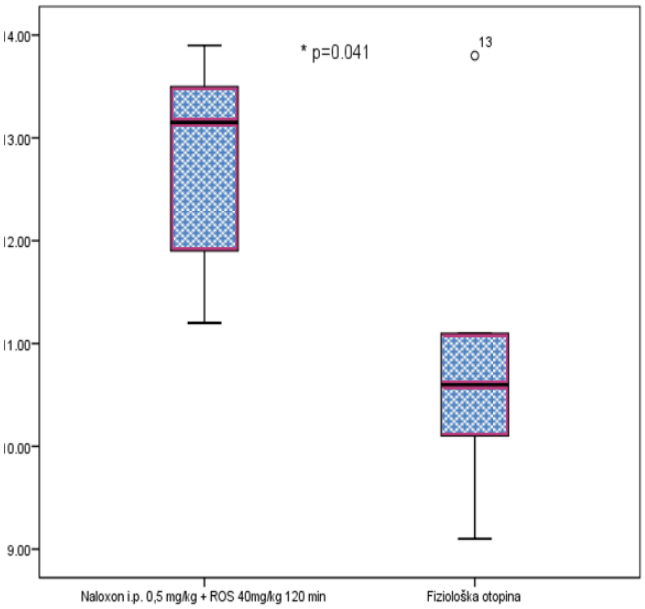

Fig. 8. Effect of naloxone (0.5 mg/kg) in combination with rosuvastatin (40 mg/kg) in 120th minute in hot plate test

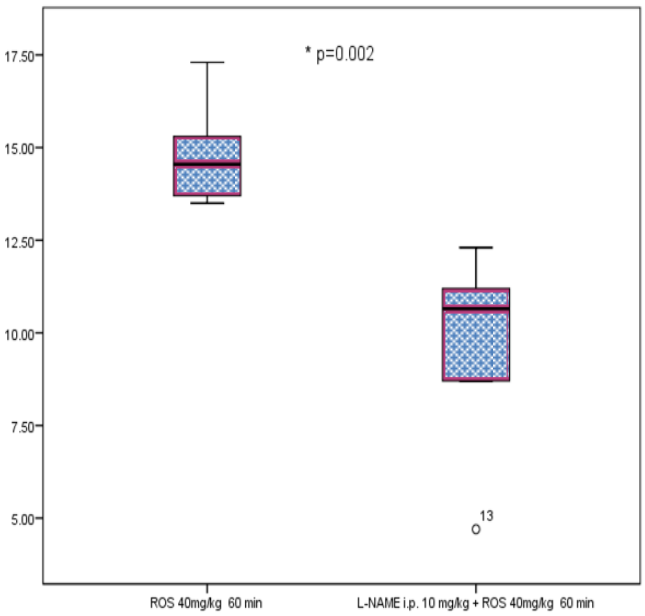

Fig. 9. Effect of L-NAME (10 mg/kg) in combination with rosuvastatin (40 mg/kg) in 60th minute in hot plate test

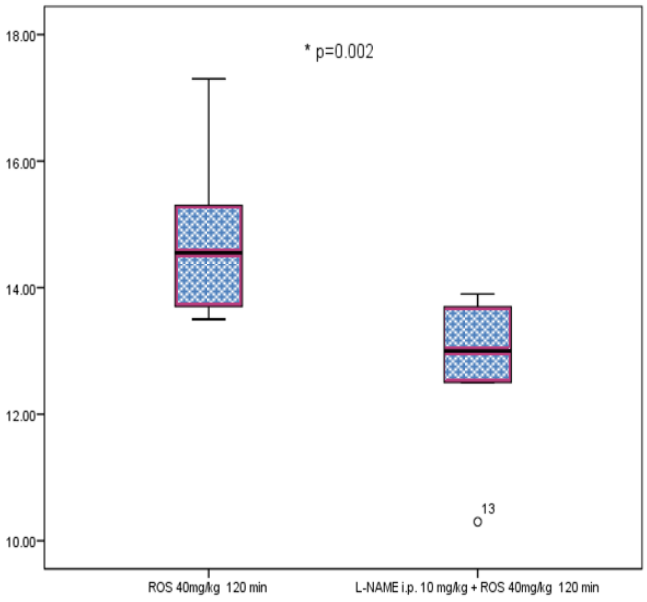

Fig. 10. Effect of L-NAME (10 mg/kg) in combination with rosuvastatin (40 mg/kg) in 120th minute in hot plate test

DISCUSSION

Several studies reporting antiinflamatory effect of statins, but there are not enough data related to their analgesic effects. In this study, we have studied analgesic effect of rosuvsatin in hot plate test in mice. According to results, rosuvastatin produced analgesic effects on acute and on chronicallly application.

In our study, naloxone (0.5 mg/kg) inhibited analgesic effect of rosuvastatin partially. That means that opioiderig system has not a role in the analgesic effect of rosuvastatin.

On the other side, L-NAME (10 mg/kg), it can be said that the analgesic effect of rosuvastatin might be related with the stimulation of nitrogen oxide (NO) production.

CONCLUSIONS

Finally in concluision of this study, contribution of nitregric system in analgesic effect of rosuvastatin. The results of this study and all other data for statins open the possibility of using rosuvastatin in the treatment of inflammatory processes and nociceptive pain.

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