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SPRUCE NEEDLE POLYPRENOLS PROTECT AGAINST ATORVASTATIN-INDUCED MUSCLE WEAKNESS AND DO NOT INFLUENCE CENTRAL NERVOUS SYSTEM FUNCTIONS IN RATS

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Polyprenols (PPs) have been identified in almost all living organisms. The richest source of PPs is the needles of conifer trees. Endogenously, PPs, similarly to cholesterol, are synthesised in human and animal cells via the mevalonate pathway. Previous studies have demonstrated the antioxidant properties of PPs. To our knowledge, no studies have been published on the influence of PPs on muscle strength. We hypothesised that administration of PPs could prevent changes in muscle functioning caused by statins (weakness, etc.). In the present study, atorvastatin (80 mg/kg) was used as a model compound. PPs at doses 1, 10 and 20 mg/kg were administered. Both drugs were given per os *for 16 days. The influence of atorvastatin, PPs and their combination on behaviour, muscle strength, plasma cholesterol and creatine kinase activity was assessed in female Wistar rats. Our data demonstrated that atorvastatin considerably impaired muscle strength, whereas PPs protected that effect. Neither PPs, nor atorvastatin influenced plasma cholesterol levels, whereas PPs at dose 20 mg/kg elevated creatine kinase activity by about 25%. PPs at the tested doses did not alter behaviour, indicating safety of central nervous system functions. The obtained data suggest usefulness of PPs as a complement in statin therapy to reduce muscle-related side effects.*

Key words: *polyprenols, atorvastatin, muscle strength, behaviour.*

INTRODUCTION

Polyprenols (PPs) as linear polymers have been identified in almost all living organisms, and are also found in the human diet, in fruits and in beverages, such as tea, coffee and wine. The needles of conifer trees are one of the richest sources of PPs. Endogenously, free polyisoprenoid alcohols and their fatty acid esters are synthesised in human and animal cells via the cytoplasmic mevalonate pathway and serve as structural components of cellular membranes, modulating their physico-chemical properties like as fluidity and permeability (Chojnacki and Dallner, 1988; Wang *et al*., 2008). Up to now, the majority of studies on polyprenols and polyprenyl phosphates had been focused on their ability to prevent toxic injuries of the liver and to restore disturbed hepatic functions by lowering the levels of serum cholesterol through effects on its biosynthetic pathway (Fedotova *et al*., 2012; Pronin *et al*., 2014), as well as by protecting unsaturated membrane lipids from oxidative free radicals (Bizarri *et al*., 2003).

Comparatively little is known about the influence of PPs on central nervous system (CNS) functions. Some studies showed that orally used PP may reach the brain (Chojnacki and Dallner, 1983, 1988; Jakobsson *et al*., 1989), and induce anxiolytic and antidepressant effects (Fedotova *et al*., 2010). They also can protect against amyloid beta-induced impairment in cognitive functions (Fedotova *et al*., 2010) and can decrease deposition of amyloid beta in Alzheimer's disease model rats (Wang *et al*., 2014).

In the context of the mevalonate pathway, it is known that the widely used cholesterol-lowering drugs, such as statins, inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)

reductase and halt the production not only of cholesterol but also of other substances synthesised in a mevalonate-dependent pathway, for instance, long-chain PPs, ubiquinone and dolichols. The question of why statins, in addition to their beneficial lipid-lowering effect, also cause adverse reaction, is not up to now understood. The most devastating side effects of statins are related to skeletal muscle pathologies, ranging from mild to moderate muscle fatigue, weakness and pain, to fatal rhabdomyolysis (Abourjaily *et al.*, 2003). The overall incidence of myalgia, muscle aches, or cramps in clinical practice varies from 0.3% to 33% (Bays, 2006; Wilkinson *et al.*, 2014). In spite of evidence of degenerative processes, studies indicate that inhibition of cholesterol synthesis cannot be considered as the main cause of statin-induced myopathies. Some other factors can be essential, for instance, the increased activity of nitric oxide synthase and cyclooxygenase-2 enzyme (Werner *et al.*, 2004). Therefore, different drugs to protect against statin myotoxicity have been recently searched for. For example, L-carnitine, which is known as a free radical scavenger and antioxidant (Mazroa and Asker, 2010), and resveratrol, which inhibits inducible nitric oxide synthase (iNOS) induction in skeletal muscle (Soner *et al.* 2013), were shown to prevent myotoxic and apoptotic changes caused by atorvastatin in rats, raising possibility for their use in hypocholesterolemic therapy.

Taking into account also the ability of PPs to protect membrane lipids from oxidative free radicals (Bizzarri *et al.*, 2003), we suggest that PPs could act as beneficial protectants of statin-induced muscle weakness and potentially influence CNS functions. We chose atorvastatin as a model compound of the statin series, because of its high lipophilicity and capability to penetrate muscle cells (Rosenson *et al.*, 2014) and also reach the brain (Sierra *et al.*, 2011). The ability to cross the blood-brain barrier was also demonstrated for PPs administered orally (Chojnacki and Dallner, 1988). To our knowledge, there is currently no data published on the influence of PPs *per se* and in combination with statin on muscle strength. Therefore, the main aims of the present research were: 1) to study the action of PPs *per se* on rat behaviour, muscle strength and biochemical markers (plasma cholesterol and creatine kinase levels), and 2) to ascertain whether PPs provide protection against changes caused by atorvastatin.

MATERIALS AND METHODS

Animals. Female Wistar rats weighing 230–245 g were obtained from the Laboratory of Experimental Animals, Rīga Stradiņš University, Latvia. The animals were housed in plastic cages (5 per cage) with food and water *ad libitum*, and kept in a controlled laboratory environment (temperature 22° C, humidity 50–60 %, 12 h light/dark cycle). Female rats were used, because female sex is considered as a risk factor to obtain statin-induced adverse effects (Sathasivam and Lecky, 2008). All efforts were made to minimise animal suffering and to reduce the number of animals used. The experiments were conducted in accordance with the EU Directive 2010/63/EU and local laws and policies on the protection of animals used for scientific purposes. The animal protocol for this study was approved by the Animal Ethics Committee of the Food and Veterinary Service, Rīga, Latvia, and the approved protocol was strictly adhered to.

Drugs and reagents. Atorvastatin (Atoris) was purchased from KRKA, Slovenia. Commercially available column chromatography purified PPs (polyprenols C55–C95, from *Picea abies* L. spruce needles) as > 95% substance were purchased from manufacturer JSC BioLat, Latvia. Blood cholesterol level measuring strips for a Accutrend GCT meter were purchased from Roche Diagnostics, Switzerland and a commercial kit for Creatine Kinase Activity Assay (MAK116) from Sigma-Aldrich, St. Louis, MO, USA. All other reagents were purchased from Sigma–Aldrich (St. Louis, MO).

Experimental design. Two experimental setups (1 and 2) were designed to limit experimental procedures in experimental animals (Fig. 1). All drugs were administered once daily by the oral route (*per os*) via orogastric cannula for 16 consecutive days and tested in several pharmacological tests. Control animals received saline (10 mL/kg per rat) and refined sunflower oil (2 mL/kg per rat). In setup 1, experimental animals were treated with PPs (at the doses of 1, 10 and 20 mg/kg) dissolved in refined sunflower oil in a volume of 2 mL/kg. Atorvastatin tablets were ground to powder, suspended in saline and administered at a dose of 80 mg/kg in a volume of 10 mL/kg. Rats were randomly divided into 8 groups (n = 9–10 per group), treated with PPs and atorvastatin alone, and their concomitant administra-

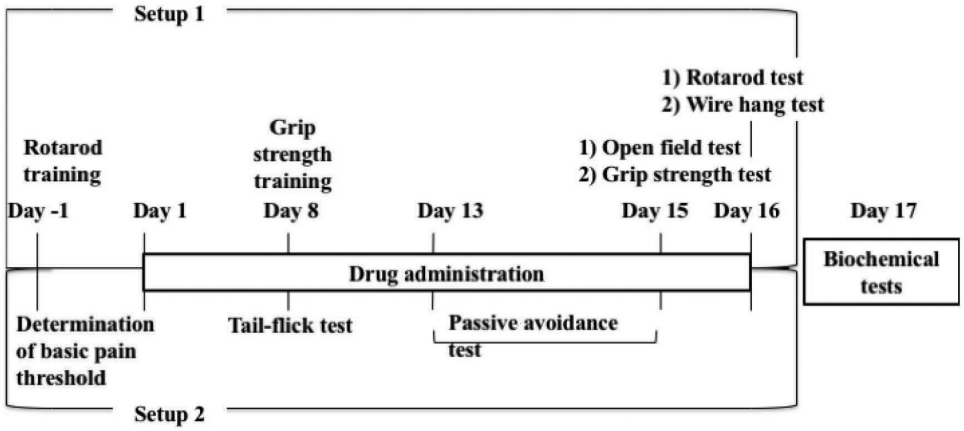

Fig. 1. Experimental setups.

tion. Atorvastatin or saline administrations were carried out in the morning, whereas PPs or refined sunflower oil in the afternoon. All behavioural tests (wire hang, open field, grip strength and rotarod tests) were conducted between 9:00 and 15:00 with an interval of 2 h between them. Setup 2 was carried out in a similar manner as setup 1, however, PPs were administered at a dose of 20 mg/kg, which demonstrated significant effects in several tests performed in setup 1. Rats were randomly divided into four groups (n = 9–10 per group), treated with PPs (20 mg/kg), atorvastatin (80 mg/kg) and their concomitant administration. Behavioural tests (passive avoidance and tail flick tests) were conducted between 9:00 and 15:00 with an interval of 2 h between them. At the end of the study, on day 17, rats were anaesthetised with ketamine (100 mg/kg) and xylazine (10 mg/kg), and blood for measurement of cholesterol level and creatine kinase (CK) activity was collected by cardiac puncture.

Wire hang test. This test was used to assess forelimb strength. The apparatus consisted of a stainless steel wire (90 cm length, 3 mm in diameter), fixed horizontally between two vertical supports and 60 cm above a soft padded surface. The wire hang test was carried out on day 16. The rat was forced to grasp the central position of the wire with its forepaws. The latency (s) to fall from the wire to the flat soft pad was measured. When the latency time was over 120 s, the rat was released from the wire, and the time was recorded as 120 s. The trial was conducted three times for each rat and the longest duration was the value used for evaluation. The resting pause between consecutive attempts was 3 min.

Grip strength test. A rat grip strength meter (model 47105, Ugo Basile, Italy) was used to assess forelimb strength. On day 8, animals were pre-trained for six training trials to establish reliable assessment of gripping ability, and on day 15 the grip strength test was performed. Animals were positioned by facing the T bar of the grip strength meter and the forelimbs of rats were placed on the tension bar. When the rat grasped the bar, the animal was gently pulled steadily by the root of the tail away from the T bar. The grip strength meter determined and recorded automatically the maximum force displayed by each animal in grams. The mean value of five consecutive measurements for each animal was calculated. Rats were allowed to recover for 30 s between the measurements.

Rotarod test. Locomotor coordination and balance were measured in rats on an accelerating rat rotarod apparatus (model 47700, Ugo Basile, Italy). A day before the beginning of the treatment, rats were pre-trained for five trials in the rotarod test. Each time the rat fell off the rotarod in the training trials, it was immediately placed back onto the treadmill to achieve 5 min stability. On day 16, animals were tested in the rotarod task. A gradually accelerating rotor mode was used to increase the speed slowly from 4 to 40 rpm over a 5 min period. The trial ended when the rat fell from the rod or after 5 min, which was used as the maximum time for the test. An hour break was given between four consecutive trials. The time (as latency in seconds to fall) and the speed on the rotarod treadmill were automatically registered. Results were expressed as the mean value of four trials.

Open field test. On day 15, general locomotor activity was evaluated in an open field apparatus (round arena, 98 cm in diameter, with 40 cm high walls) using a video-tracking programme with software Panlab Smart Version 2.5 (Pan-Lab, Spain). The arena was illuminated by a light (60 W) fixed 100 cm above the centre of the arena. At the beginning of the test, the rat was gently placed into the centre zone of the round arena and left to explore the arena freely for 5 min. Horizontal locomotor activity was quantified as the distance walked in cm and analysed for the whole arena (total distance).

Passive avoidance test. A day before testing (on day 13), animals were habituated to the step-through passive avoidance apparatus (model 7550, Ugo Basile, Italy), consisting of light and dark compartments. The acquisition trial was conducted on day 14, when each rat was placed in the light compartment. As soon as the rat entered the dark compartment, the door was closed and the rat received an inescapable foot-shock (0.5 mA, 2 s) through the grid floor. The step-through latency, i.e. time spent in the light compartment before entering the dark chamber, was measured in seconds. The retention test was carried out 24 h after the acquisition trial (i.e., on day 15). No foot-shock was applied in this trial. The difference in step through latencies between retention and acquisition days was calculated. The maximum testing limit for the step-through latency test was 240 s for both training and retention days.

Tail flick test. Analgesic activity of the tested drugs was assessed by using an Analgesy-Meter (model LE 7106, Panlab, Spain). A day before start of the drug treatment, the individual tail flick latency was determined as a pain threshold. On day 8, 2 h after drug administration, a concentrated burning light was directed onto the tail of the animal. The time in seconds taken for the animal to withdraw its tail after exposition to the heat was considered as the latent period. Baseline tail flick latency was from 2.5 to 4.5 s. A cut-off time of 10 s was used to prevent any possible tissue damage. The mean values of the two tail flick latencies measured with 5 min interval were used for analysis. Each animal was used as its own control. Antinociception was quantified as the percentage of maximal possible effect (%MPE = [(postdrug latency-predrug latency)/(cut-off time (10 s) – predrug latency)] × 100).

Assessment of cholesterol level and creatine kinase activity. A few drops of the collected blood were taken to measure the blood cholesterol level using an Accutrend GCT meter (Roche Diagnostics, Switzerland) expressed in mmol/L. For the determination of creatine kinase activity, the blood was collected into tubes containing heparin. The tubes were immediately centrifuged at 3000 rpm for 10 min and stored at –80 °C until the assay. Creatine kinase activity determination in rat blood plasma samples was performed by stan-

dard spectrophotometric analysis according to the instructions of the manufacturer (Sigma-Aldrich, USA) using a commercial kit for Creatine Kinase Activity Assay (MAK116). The absorbance was read at 340 nm at 37 °C using a spectrophotometer (INFINITE M200 PRO NanoQuant, Tecan Group Ltd., Switzerland). The data were calculated as units/L. One unit of creatine kinase activity was defined as the amount of enzyme that transfered 1.0 μmol of phosphate from phosphocreatine to adenosine diphosphate (ADP) per min at pH 6.

Statistical analysis. GraphPad Prism 6 software (GraphPad Software Inc., USA) was used for the statistical analysis. All data were expressed as the mean ± SEM. Significant differences among experimental groups were assessed by one-way ANOVA followed by a Uncorrected Fisher's LSD post-test for *in vivo* tests, and by Kruskal-Wallis followed by a Dunn's Multiple Comparison test for biochemical data. In all cases, differences with $p < 0.05$ were considered statistically significant.

RESULTS

Influence on muscle strength/tone and coordination. Rat hanging time (Fig. 2) was not altered by administration of PPs at 1 and 10 mg/kg doses, while a dose of 20 mg/kg prolonged hanging time by about 2-fold more than in the control group. Atorvastatin at the dose of 80 mg/kg significantly (about by 3-fold) decreased the hanging time compared with the control group, indicating that atorvastatin reduced muscle strength. PPs at all tested doses significantly prolonged rat hanging time when reduced by atorvastatin, thereby restoring muscle strength to the control level. In the grip strength test (Fig. 3), administration of PPs at all tested doses did not show effect on rat grasping strength compared with the control group. When treated with atorvastatin at the dose of 80 mg/kg, rats exhibited marked reduction in grasping strength compared with the control group. PPs administered at dose of 20 mg/kg reversed the atorvastatin effect by increasing the grasping strength to the control level. In the accelerating rotarod test (data not shown), PPs at all doses, atorvastatin at the dose of 80 mg/kg and concomitant administration of PPs at all doses with atorvastatin showed no influence on rat falling latency compared with that of the control group.

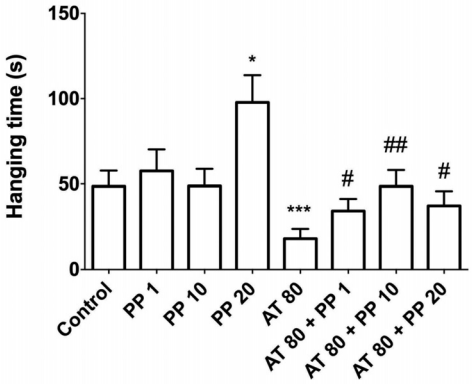

Fig. 2. Muscle strength in wire hang test in rats. Hanging time (s) was measured on day 16 after peroral administration of polyprenols (PPs) at 1, 10 and 20 mg/kg, atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. One-way ANOVA followed by Uncorrected Fisher's LSD post-test. * $p < 0.05$ and *** $p < 0.001$ vs. control; # $p < 0.05$ and ## $p < 0.01$ vs. AT 80 mg/kg.

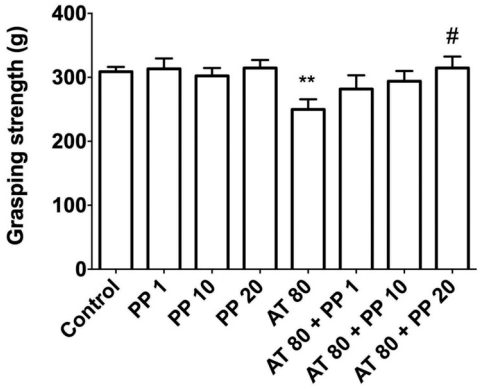

Fig. 3. Muscle strength in the grip strength test in rats. Grasping strength (g) was measured on day 15 after peroral administration of polyprenols (PPs) at 1, 10 and 20 mg/kg, atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. One-way ANOVA followed by Uncorrected Fisher's LSD post-test. ** $p < 0.01$ vs. control; # $p < 0.05$ vs. AT 80 mg/kg.

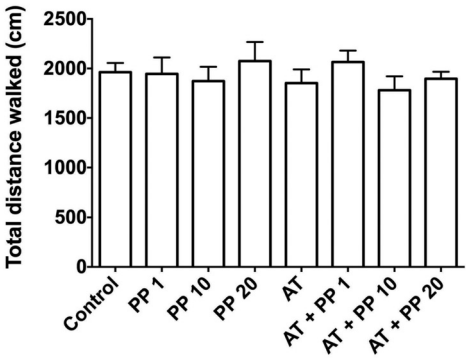

Fig. 4. Locomotor activity in the open field test in rats. The total distance walked (cm) was measured on day 15 after peroral administration of polyprenols (PPs) at 1, 10 and 20 mg/kg, atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. One-way ANOVA followed by Uncorrected Fisher's LSD post-test. No significant differences between variants were found.

Influence on general locomotor activity. PPs at the doses of 1, 10 and 20 mg/kg did not influence the total distance walked by rats compared with that in the control group (Fig. 4). Administration of atorvastatin at dose of 80 mg/kg and also the combined administration of both PPs and atorvastatin did not affect rat locomotor activity (Fig. 4).

Influence on learning/memory. Data obtained in the passive avoidance response test (Fig. 5) demonstrated no significant changes in step-through latencies in rats treated with PPs (20 mg/kg), or atorvastatin (80 mg/kg), or the combined administration of both drugs, compared with values in the control group.

Analgesic activity. In the tail flick test (Fig. 6), PPs at the dose of 20 mg/kg did not show analgesic activity and did

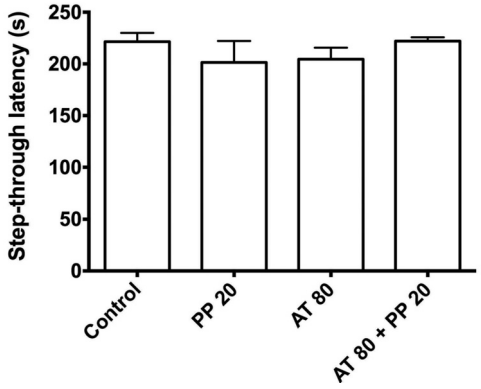

Fig. 5. Influence on memory in passive avoidance response test in rats. Difference of step-through latency (s) was measured on day 15 after peroral administration of polyprenols (PPs) at 20 mg/kg , atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. One-way ANOVA followed by Uncorrected Fisher's LSD post-test. No significant differences between variants were found.

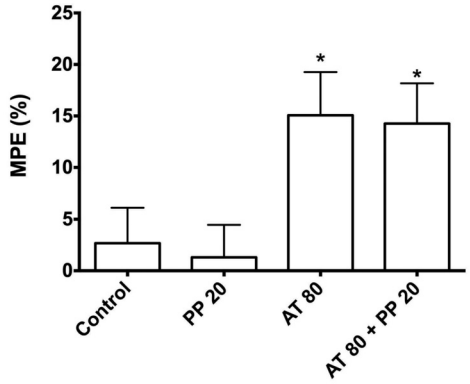

Fig. 6. Analgesic activity in tail flick test in rats. The maximal possible effect (MPE, %) was measured on day 8 after peroral administration of polyprenols (PPs) at 20 mg/kg, atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. One-way ANOVA followed by Uncorrected Fisher's LSD post-test. * $p < 0.05$ vs. control.

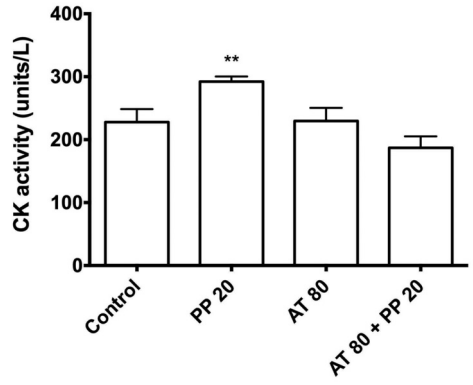

Fig. 7. Creatine kinase (CK) activity in rat plasma (units/l). CK determined spectrophotometrically after peroral administration (for 16 days) of polyprenols (PPs) at 20 mg/kg, atorvastatin (AT) at 80 mg/kg and their combination. Control rats were treated with vehicle (saline + oil). N = 9–10 per group. Values are means ± SEM. Kruskal-Wallis test followed by the Dunn's Multiple Comparison test. ** $p < 0.01$ vs. control.

not alter the maximal possible effect (MPE), compared to that in the control group. Atorvastatin at the dose of 80 mg/kg, however, produced a marked increase in the MPE compared with the control group. Concomitant administration of PPs (20 mg/kg) with atorvastatin (80 mg/kg) did not alter the atorvastatin-induced analgesic effect.

Influence on plasma cholesterol level and creatine kinase activity. Blood cholesterol level was not changed in any tested group (data not shown). A significant increase (by about 25%) in plasma creatine kinase activity was observed after the administration of PPs at dose of 20 mg/kg, compared with that in the control group (Fig. 7). The creatine kinase activity after treatment with atorvastatin (80 mg/kg) or concomitant with its administration with PPs (20 mg/kg) was comparable to that of the control group.

DISCUSSION

Most studies on biological effects and mechanisms of PPs are related to their peripheral action and are associated with hepatoprotective activity due to cholesterol-lowering, antioxidant, membrane-protecting properties (Cantagrel and Lefeber, 2011; Hartley and Imperiali, 2012; Pronin *et al.*, 2014). A recent report (Milenkovic *et al.*, 2013) demonstrated that PPs may interact with cellular signalling cascades regulating activity of transcription factors and expression of genes, particularly by the influence on microRNA.

The present study was aimed at examining the pharmacological action of PPs (from *Picea abies L.* spruce needles) in rats after a 16-day treatment, with a focus on muscle strength, behaviour and several biochemical parameters in rat blood. We especially aimed to clarify whether and how PPs may alter these activities in statin-treated rats, considering that the mechanism of action of statins involves not only inhibition of cholesterol biosynthesis, but also that of polyprenols/dolichols (Buhaescu and Izzedine, 2007). The doses of polyprenols reported previously in the literature in Alzheimer's disease models were 8.6 mg/kg in rats (Fedotova *et al.*, 2012) and 20, 40 and 80 mg/kg in mice (Wang *et al.*, 2014). In our study, we chose similar doses (10 and 20 mg/kg) and a lower dose (1 mg/kg) of PPs. The atorvastatin dose of 80 mg/kg and the treatment regimen for 16 days were in the mid range of values in the literature (Madsen *et al.*, 2008).

We showed that atorvastatin at a dose of 80 mg/kg significantly decreased muscle strength in both tests (grip strength and wire hang). A particularly dramatic atorvastatin effect was observed in the wire hang test, when muscle weakness was about 3-fold more than in the control animals. PPs at the dose of 20 mg/kg in the wire hang test considerably increased the rats' hanging time. In our experiments, PPs at all tested doses significantly protected against atorvastatin-induced alterations by restoring muscle strength. At the same time, neither atorvastatin nor PPs influenced muscle tone and coordination in the accelerating rotarod test.

The mechanisms of atorvastatin myopathies can be mostly explained by their direct influence on the mevalonate path-

way leading to the inhibition of cholesterol biosynthesis and endogenous polyisoprenoid production, resulting in lowering of the concentrations of metabolites necessary for cellular processes. Thus, the deficiency of molecules like ubiquinone (provides mitochondrial electron transport), isoprenoid pyrophosphates (protein prenylation) and dolichol (protein glycosylation) is considered as essential for the development of statin-induced myopathy (Baker, 2005; Manoukian *et al*., 1990). However, since myotoxicity does not occur *in vitro* when cholesterol is lowered by inhibiting squalene synthetase, this mechanism seems less plausible (Bitzur *et al.*, 2013). Thus, the exact cause of statin-induced myopathy remains elusive. As reviewed by Bitzur *et al.* (2013), statins may cause myopathy via impairing calcium signaling. There are several case reports of induction of inflammatory myopathies (i.e., polymyositis and dermatomyositis) by statins, but many (25 cases of a histologically distinct statin myopathy) muscle biopsies demonstrated necrotising myopathy without significant inflammation. Other studies showed that atorvastatin (10 mg/kg for 2 months in rats) may induce a down-regulation of the expression of proteins essential for skeletal muscle function (Camerino *et al*., 2011).

As our experiments were carried out in normolipidic rats, it was already predictable that atorvastatin would not influence the cholesterol level. It was also not influenced by PPs. Atorvastatin also did not alter creatine kinase activity. At the same time, polyprenols at a dose of 20 mg/kg caused an elevation (by about 25 %) in creatine kinase activity and an increase in muscle strength. We measured total creatine kinase activity, bearing in mind that nearly all creatine kinase activity in plasma is derived from skeletal muscle (Ballard *et al*., 2013). In the past, an elevated creatine kinase protein level in blood has been used as a "diagnostic tool" to verify statin induced muscular side effects. However, in the light of recent data, it is no longer considered as a hallmark of atorvastatin-induced myopathies (Abdelbaset *et al*., 2014; Ballard *et al*., 2013. Furthermore, physical exercise may elevate creatine kinase protein concentration in plasma much more than statins alone (Ballard *et al.*, 2013; Laaksonen, 2013). At present, we cannot explain why, in combination with atorvastatin (when PPs restored muscle tone in atorvastatin-treated animals), the activity of creatine kinase remained at control values. One explanation might be that PPs, by elevation of the creatine kinase activity, may intensify intracellular energy transportation and adenosine triphosphate (ATP) generation, leading to normalisation of energy processes impaired by atorvastatin.

Another question the study sought to answer was: whether PPs are safe and whether they may influence CNS functions. Up to now, little is known about the central effects of both endogenous and exogenous PPs. There are some interesting studies that demonstrate that the content of polyisoprenoid alcohols is greatly increased in tissues during life. For example, a 100-fold increase in the human brain has been observed in 80-year-old individuals vs. newborns. Moreover, equal amounts of dolichols and phospholipids have been noted in senile pituitary glands. The organism might attempt to protect the brain from oxidative stress and lipid peroxidation by increasing the level of dolichyl phosphate and coenzyme Q10 in the brain (Surmacz and Swiezewska, 2011).

Regarding the bioavailability of exogenous PPs supplied orally, only about 0.05% of the total amount was found in rat organs (Cantagrel and Lefeber, 2011), with the highest uptake in the liver and stomach, and about 10-fold less in the brain (Jakobsson *et al*., 1989). However, the altered physiological conditions or pathological processes may enhance PPs uptake in the brain. Only some studies have been carried out on the neuropharmacological actions of PPs in animals. For example, in rats with β-amyloid peptide (25-35)-induced amnesia, polyprenols at a peroral dose of 8.6 mg/kg for 28 days demonstrated significant improvement of spatial learning (Fedotova *et al*., 2012).

Our study showed that PPs at the doses of 1, 10 and 20 mg/kg did not influence rat locomotor activity tested in the open field test, neither did they alter the learning/memory processes in the passive avoidance response test. Our results are in close agreement with those showing a lack of activity of PPs in non-spatial tests (Fedotova *et al*., 2012). In our experiments, the CNS functions in the open field and memory tests were also not altered by atorvastatin. However, results on the influence of statins on CNS functions are conflicting in human studies. On the one hand, experimental studies supported links between cholesterol intake and amyloid synthesis, and observational studies indicated that patients receiving statins had a reduced risk of dementia (Wagstaff *et al*., 2003). In addition, numerous studies have demonstrated that statins may ameliorate neurodegenerative symptoms in Alzheimer's and Parkinson's diseases, stroke and multiple sclerosis. These observations suggest that neuroprotection is dependent on cholesterol rather than isoprenoid depletion (van der Most *et al*., 2009). On the other hand, statins were shown to cause behavioural alterations (severe irritability, homicidal impulses, threats to others, depression and violence, paranoia, antisocial behaviour), cognitive and memory impairments, sleep disturbances and sexual dysfunction (Tuccori *et al*., 2014).

In the tail flick test, atorvastatin demonstrated considerable analgesic activity by prolonging tail flick latency by about 3.5 times. PPs at a dose of 20 mg/kg *per se* lacked this activity and did not alter the analgesic action of atorvastatin. Analgesic and anti-inflammatory activities of atorvastatin have been shown previously in different analgesia tests (Garcia *et al*., 2011; Dwajani *et al*., 2012; Jaiswal and Sontakke, 2012).

CONCLUSION

In summary, it can be concluded that PPs may act as a successful protector of atorvastatin-induced muscle weakness, while it did not alter behaviour and memory. These results suggest that combination of PPs with atorvastatin may be

helpful for reducing muscle-related side effects in patients receiving long-term atorvastatin therapy.

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EGĻU SKUJU POLIPRENOLI AIZSARGĀ MUSKUĻUS NO ATORVASTATĪNA IZRAISĪTĀ VĀJUMA UN NEIETEKMĒ CENTRĀLĀS NERVU SISTĒMAS FUNKCIJAS ŽURKĀM

Poliprenolus (PP) identificē gandrīz visos dzīvajos organismos, un to visbagātākais avots ir skuju koki. PP, līdzīgi kā holesterīns, sintezējas endogēni cilvēka un dzīvnieka šūnās mevalonāta bioķīmiskajā ciklā. Iepriekš aprakstītas PP antioksidanta īpašības, taču līdz šim nav datu par PP ietekmi uz muskuļu spēku. Mūsu hipotēze: PP ievadīšana var pasargāt no negatīvām izmaiņām muskuļu funkcionēšanā, ko izraisa statīni (muskuļu vājums utt.). Veiktajā pētījumā kā modeļvielu izmantojām atorvastatīnu (80 mg/kg). PP ievadījām devās 1, 10 and 20 mg/kg. Abas vielas ievadījām 16 dienas *per os.* Novērtējām atorvastatīna, PP un abu vielu kombinācijas ietekmi uz *Wistar* žurku uzvedību, muskuļu spēku, plazmas holesterīna līmeni un kreatīnkināzes aktivitāti. Mūsu rezultāti parāda, ka atorvastatīns būtiski samazina muskuļu spēku, toties PP aizsargā pret šo efektu. Ne PP, ne atorvastatīns neietekmē plazmas holesterīna līmeni, bet PP devā 20 mg/kg paaugstina kreatīnkināzes aktivitāti par apmēram 25%. PP nevienā devā neizmaina uzvedības reakcijas, kas liecina par to lietošanas drošību centrālās nervu sistēmas līmenī. Iegūtie dati ļauj domāt par PP kā papildus līdzekļa lietderību pacientiem, kam nozīmēta ilgstoša statīnu terapija, lai novērstu to izraisītās blaknes muskuļos.