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EFFECTS OF ADMINISTRATION OF SIMVASTATIN ON THE THRESHOLD OF THERMAL PAIN TOLERANCE IN ADULT MALE RATS WITH HFD

Zahra Hajimashhadi[1], Mahboubeh Kooskbaghi[2] and Hamid Sepehri[3*]

[1]*Department of Physiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
[2]*General Medical Student, Student Research Committee, Neuroscience Research Center, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
[3]*Neuroscience Research Center, Department of Physiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*

Background: *Increased consumption of high-fat diets has become a global health problem. Obesity by result of various endocrine changes and release of inflammatory cytokines that may contribute to the pathogenesis of pain. Simvastatin is a statin that inhibits the 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase and has anti-inflammatory effects.* ***The aim of the present study*** *was to assess the antinociceptive activity of simvastatin following the use of high-fat diet in Male rats.* ***Methods and materials:*** *Forty adult male Wistar rats divided into six groups: control, high-fat diet, high-fat diet treated with 2 and 20 mg/Kg simvastatin and normal diet treated with 2 and 20 mg/kg simvastatin. Rats were maintained on a high-fat or normal diet for 45 days. Simvastatin was daily given through gavage from two weeks after starting of the study and continued for 30 days. Hot plate and blood biochemistry tests were performed to evaluate the threshold of thermal pain t tolerance.* ***Results:*** *Total cholesterol, LDL, and triglyceride, and body weight in the group that maintained a high fat diet was significantly higher compared with the other groups, especially the control group. But serum TNF-α and IL-6 were not found in a detectable production in all groups. High fat diet significantly reduced thermal pain reaction time. Simvastatin administration significantly decreased thermal hyperalgesia.* ***Conclusion:*** *The results indicate that simvastatin administration is a suitable way for reducing heat hyperalgesia following use of a high fat diet, but there is need to do further research.*

INTRODUCTION

Obesity is a rapidly growing worldwide health problem that increases the risk for many diseases[1]. Accumulating evidence strongly suggests that pain and obesity are significantly related to each other[2]. It has been suggested that obesity influences pain perception and obesity can be a risk factor for increased pain thresholds[3]. It has been shown that obese subjects may have a different pain perception and may react differently to analgesics[4] but the mechanism of this relation and increase pain perception not yet identified. It is well established that a high-fat diet (HFD) can lead to overweight and ultimately to obesity, as well as promoting low-grade chronic inflammation associated with increased levels of such mediators as Tumor necrosis factor (TNF-α,) IL-1, and IL-6[5&6]. Statins, or HMG-CoA reductase inhibitors, are widely used agents in the treatment of dyslipidemia and the prevention of cardiovascular disease[6]. It is well-established that this class of medications has a good effect on the lipid profile, In addition to the known lipid-lowering effects,

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*Corresponding author: Hamid Sepehri, E-mail: Hamid.Sepehri@yandex.com

statins are now accepted to have anti-inflammatory and immunomodulatory effects[7]. It is well established that anti-inflammatory effects of statins, reduce the release of several inflammation mediators, including TNF-α, IL-6 (interleukin-6) and IL-8 (interleukin-8)[8]. Simvastatin is reported to have analgesic properties and also potentiate the action of other analgesics[9&10]. It has reported that statins have prevented nociception which are observed in inflamed joints and can act as analgesic by inhibition of cytokines[11].

Recent study has focused on the ability of statins to modulate pain perception in high fat diet rats by inhibition of TNF-α, and IL-6.

MATERIALS AND METHODS

Animals

In this study, 48 male Wistar rats aged 10 weeks and weighing 180-250 grams were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2°C) and humidity (55±5%) with 12:12 h light and dark cycle and randomly divided into six groups 8 animals as follows:

- Group1 Control (C): standard laboratory diet without simvastatin.
- Group2 ($C.S_2$): standard laboratory diet with simvastatin 2 for 4 week
- Group3 ($C.S_{20}$): standard laboratory diet with simvastatin 20 for 4 week
- Group3 (HFD): High-Fat Diet (HFD) and without simvastatin for 6 weeks.
- Group4 ($H.S_2$): High-Fat Diet (HFD) and simvastatin 2 mg/kg of body weight/day for 4 weeks.
- Group5 ($H.S_{20}$): High-Fat Diet (HFD) and simvastatin 20 mg/kg of body weight/day for 4 weeks.

Rats were maintained on a high-fat or normal diet for 45 days. Body weight was measured in all rats from beginning until behavioral assessment. Simvastatin was daily given through gavage from two weeks after starting of the study and continued for 30 days. The Control group had ad libitum access to a standard rat diet and HFD groups to the same diet containing 50% cholesterol[12]. The study was performed according to the guidelines for laboratory animal use and care set by the Animal Ethics Committee of Golestan University of Medical Sciences (http://goums.ac.ir) with ethical code 1397.45.

Hot Plate Test

The hot plate test was used to calculate analgesic activity by the method explained by Eddy and Leimbach[13] with minor modifications. Rat were retained on a hot plate having a stable temperature of 50°C. The time taken for either paw licking or jumping was recorded. Each rat was individually placed on the hot plate in order to find the animal's reaction to the latency until rat showed first signs of discomfort (hind paw lifting, hind paw licking, or jumping was recorded.

Blood analysis

At the end of the experimental period, all animals were anesthetized with ketamine/xylazine HCl (75/10 mg/kg intraperitoneal). Blood samples were collected from the aorta without anticoagulant, left for 10 min and then, centrifuged for 15 min at 3,500 r/min to obtain serum which was stored at -20°C until biochemical analysis for determination of serum Total Cholesterol, Triglyceride (TG), lowdensity lipoprotein (LDL). TNFα and IL-6 concentrations in serum were determined using an enzyme-linked immunosorbent assay (ELISA) performed with the Quantikine® ELISA Immunoassay kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. Results from duplicate samples were averaged to obtain the final concentration of TNFα and IL-6 from each sample.

Statistical analysis

Data were analyzed by one-way ANOVA and were expressed as the means and respective standard error. This analysis allows checking as to whether there are any statistically significant differences ($p< 0.05$) between the mean latency times of the groups. To determine which groups are different, the data were analyzed by Tukey test.

RESULTS AND DISCUSSION

Results

Effect of HFD on body weight and lipids and cytokines

Effect of a high-fat diet and simvastatin treatment on weight and serum lipid parameters. Table 1 depicts the levels of body weight, serum TC, TG, and LDL levels of groups C, HFD and HFD with Simvastatin. Total cholesterol ($p< 0.001$), LDL ($p< 0.01$), triglyceride ($p< 0.05$) and body weight ($p< 0.05$) were significantly higher in the high fat diet group compared to the control group. Administration of simvastatin at doses of 2 and 20 mg in the high-fat diet groups significantly reduced fat indices ($p< 0.01$) and body weight ($p< 0.05$) compared to the high-fat diet group at the end of experiments. While there was no significant difference between the treatment and control groups. We evaluated serum TNF-α and IL-6 that have been reported to be affected by diet in some studies but we did not found a detectable production of these cytokines in the groups.

Effect of a high-fat diet and simvastatin treatment on Pain perception in hotplate test

The results in figure 1 show that the consumption of high-fat diet for 42 days decreased the reaction time in the hot plate test from 18 to 10 second. Post hoc analysis revealed significantly difference between the mean reaction time in HFD and control groups ($p< 0.05$), oral administration of simvastatin (20 mg/kg) in the normal diet group, increased the latency response compared to the control group, but was not significant ($p> 0.05$), whereas administration of simvastatin by dosage of 2 and 20 mg/kg in rats by high fat diet caused significant ($p< 0.001$) decrease in the mean reaction time. It should be noted that there was no significant difference between the mean pain response threshold in the high-fat diet groups treated with different doses of simvastatin and the control group ($p> 0.05$).

Table 1: Effect of HFD on body weight and lipids of control, hyperlipidemia, Simvastatin treatment groups.

	Control	High-fat	High-fat + 2 mg Simvastatin	High-fat + 20 mg Simvastatin
Total cholesterol	60 ± 11	140 ± 25***	70 ± 8	62 ± 15
LDL (mg/dl)	6 ± 1.3	12 ± 2.5**	6 ± 2.5	5.4 ± 0.4
Triglyceride (mg/dl)	58.6 ± 11	84 ± 9*	60 ± 8	55 ± 5
Body weight (g)	220 ± 7	245 ± 13*	235 ± 8	224 ± 5

Mean value was significantly different from control group: *$p< 0.05$, **$p< 0.01$.

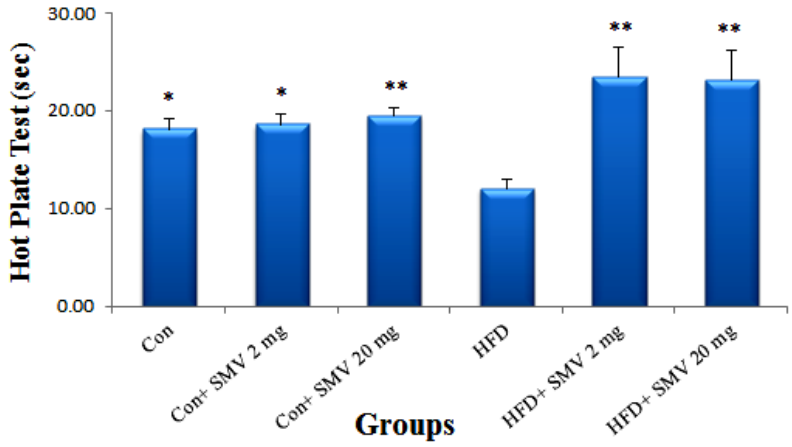

Fig. 1: Effect of HFD and simvastatin on hot plate test in rat. Each column represents the mean values of the reaction time group and respective standard error (mean ± SE, $n= 6$); **$p< 0.01$ and *$p< 0.05$ compared with HFD.

Discussion

Changes in dietary components can modulate pain perception. In this study, we evaluated the effect of a HFD administered for 6 weeks on pain perception in hot plate and biochemical parameters related to the lipid profile and cytokines. We also examined the potential effect of simvastatin as antilipdemic drug on pain perception on this animal. Studies have shown an association between HFD and an increase in weight and total cholesterol and triglyceride levels[14], as well as elevated cytokines[15&16]. In this study, high-fat diet was successfully induced hyperlipidemia in rats. Body weight and serum concentrations of TC, TG and LDL were significantly increased. Administration of simvastatin significantly decreased body weight and serum levels of TC, LDL and TG in high fat groups. High fat diet significantly reduced thermal pain threshold. Many study indicated relation between obesity and pain. but this relation is controversial some of them similar to our finding reported an increase pain sensitivity[17]. Similar to our finding Zongbin Song *et al.*[18] observed an increase in pain perception in mechanical and cold allodynia in rats that were maintained on a high-fat diet (HFD) for 6 weeks prior to Dorsal Root Ganglion (DRG) inflammation. High-fat diet increased pain behaviors even in the absence of body weight gain[18]. Simvastatin administration significantly decreased thermal hyperalgesia in HFD groups but this was not significant in the control group. While many reports indicated the analgesic effects of statins. Shi *et al.*[19] found that systemic daily administration of statin from days 0 to 14 completely reversed the mechanical allodynia and thermal hyperalgesia in neuropathic pain animal[19]. Statin also showed antinociceptive activities in acetic acid – and formalin – induced nociception in mice[20].

In the hot plate test, atorvastatin showed antinociceptive activity only with high doses (100 mg/kg during 1 or 3 days)[21].

The lack of analgesic effect of simvastatin in our study may be due to the low prescribed dose (2 mg/Kg). These results partially agree with previous reports, which did not demonstrate any significant antinociception with the 1, 3 and 10 mg/kg doses of atorvastatin[22]. The hyperalgesia induced by the HFD seems to depend on the enhanced levels of pre-inflammatory factors in the tissues[23&24]. Many studies have reported elevated levels of cytokines in tissue or serum during obesity[16&25]. But we did not found a detectable production of interleukin-6 and TNF-α in plasma. Probably if we measured the cytokines such as Cortez *et al.* in the adipose tissue we would observed an increased[15]. Otherwise the antinociceptive effect of statin may be due to the inhibition of the cytokine and prostaglandin release[26]. The daily administration of statin effectively induced antinociception by decreasing local production of pro-inflammatory cytokines[11]. Other studies have shown that statins could also antinociceptive effect by upregulate the nitric oxide synthesis[26]. However, the mechanisms involved in the inhibition of pain by simvastatin have not been completely elucidated.

Conclusion

It can be concluded that the 6-week high-fat diet reduced thermal pain threshold in hotplate. Administration of simvastatin reversed the heat hyperalgesia without any detectable change in serum interleukin-6 and TNF-α.

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نشرة العلوم الصيدلية
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تأثير تناول السيمفاستاتين على عتبة تحمل الالم الحراري فى ذكور الجرذان البالغين ذوي نظام عذائى عالى الدهون

زهرة حجيمشهدى[1] – محبوبه كوسقباجى[2] – حميد سيبيهرى[3]

[1]قسم الفسيولوجى ، كلية الطب ، جامعة جولستان للعلوم الطبية ، جورجان ، إيران

[2]طالب طب عام ، لجنة أبحاث الطلاب ، مركز أبحاث العلوم العصبية ، جامعة جولستان للعلوم الطبية ، جورجان ، إيران

[3]مركز أبحاث العلوم العصبية ، قسم الفسيولوجى ، كلية الطب ، جامعة جولستان للعلوم الطبية ، جورجان ، إيران

الخلفية: أصبحت زيادة استهلاك الأنظمة الغذائية الغنية بالدهون مشكلة صحية عالمية. السمنة كنتيجة لتغيرات الغدد الصماء المختلفة وإطلاق السيتوكينات الالتهابية، قد تساهم في التسبب في الألم. Simvastatin عبارة عن ستاتين يثبط اختزال الإنزيم المساعد ٣-هيدروكسي ميثيل-جلوتاريل A (HMG-CoA) وله تأثيرات مضادة للالتهابات.

كان الهدف من هذه الدراسة هو تقييم النشاط المضاد للألم لسيمفاستاتين بعد استخدام نظام غذائي عالي الدهون في ذكور الجرذان.

الطرق والمواد: تم تقسيم أربعون فأرا بالغا من ذكور ويستار إلى ست مجموعات: المجموعةالضابطة ، ومجموعة النظام الغذائي عالي الدهون ، ومجموعةالنظام الغذائي عالي الدهون المعالج بـ ٢ و ٢٠ مجم/كجم من سيمفاستاتين ومجموعةالنظام الغذائي العادي المعالج بـ ٢ و ٢٠ مجم/كجم من سيمفاستاتين. تم الحفاظ على الفئران على نظام غذائي عالي الدهون أو طبيعي لمدة ٤٥ يومًا. تم إعطاء السيمفاستاتين يوميًا من خلال التزقيم بعد أسبوعين من بدء الدراسة ولمدة ٣٠ يومًا. تم إجراء اختبار اللوح الساخن والإختبارات الكيميائية الحيوية للدم لتقييم عتبة تحمل الألم الحراري.

النتائج: كان الكوليسترول الكلي ، الدهون منخفضة الكثافة ، والدهون الثلاثية ، ووزن الجسم في المجموعة التي حافظت على نظام غذائي عالي الدهون أعلى بشكل ملحوظ مقارنة بالمجموعات الأخرى ، وخاصة المجموعة الضابطة. لكن لم يتم العثور على TNF-α و IL-6 بكميات قابل للاكتشاف في جميع المجموعات. النظام الغذائي عالي الدهون قلل بشكل كبير من وقت رد فعل الألم الحراري. وقد أدى تناول السيمفاستاتين لخفض فرط التألم الحراري بشكل ملحوظ.

الخلاصة: تشير النتائج إلى أن إعطاء سيمفاستاتين هو وسيلة مناسبة للحد من فرط التألم الحراري بعد استخدام نظام غذائي غني بالدهون ، ولكن ما زال هناك حاجة إلى مزيد من البحث.