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Original Article

The effects of rosuvastatin and pravastatin on bone metabolism in diabetic rats

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Abstract

Aim: In this study, the effects of rosuvastatin and pravastatin on bone metabolism were evaluated. Comparison of the positive or negative effects of two different statins on biochemical parameters related to bone metabolism in 20 mg / kg / day diabetic rats will be contribute to the enrichment of the literature on this subject. In addition, information will be obtained about whether the use of statins will be beneficial in bone metabolism disorders that may occur due to aging or diabetes in DM patients.

Materials and Methods: In a diabetic rat model induced by Streptozotocin (STZ), the possible effects of Rosuvastatin and Pravastatin, both of which are hydrophilic, on biochemical parameters and histologycal examination related to bone metabolism (20 mg / kg) were examined in comparison with the control groups.

Results: In the intergroup comparisons, Phosphate (P) level was lower in the Pravastatin group than the controls (P = 0.017). However, there was no difference in the P level in the Rosuvastatin group compared to the control group and the diabetes group. The calcium (Ca) level was increased in the Rosuvastatin group then the the controls (P = 0.002). However, there was no significant change in Ca level in the Pravastatin group. The vitamin D2 level of rats was similar in all groups and was not statistically significant. There was no significant difference between the groups in terms of both osteoblastic activity and bone marrow cellularity.

Conclusion: In conclusion, although more extensive studies are needed, our study revealed that the serum Ca level was high in rats given rosuvastatin, and P levels were low in rats given pravastatin. But cytologically, there was no change in bone structure. Our study revealed that we should be a little more cautious about the information that statins have a positive effect on bone tissue.

Keywords: Bone metabolism; diabetic rats; pravastatin; rosuvastatin

INTRODUCTION

Today statins are among the most frequently used drug groups in the treatment of hyperlipidemia. These drugs are considered to be the first-line drugs of choice for lowering low-density lipoprotein cholesterol (LDL-C) levels in patients at high risk for cardiovascular disease. They prevent cholesterol synthesis by inhibiting the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase enzyme (1). Clinical studies have shown that statins are effective not only in the treatment of hypercholesterolemia, atherosclerosis and cardiovascular disease, but also in the treatment of other inflammatory-related diseases such as rheumatoid arthritis (2). It has been demonstrated that statins can inhibit bone resorption in vitro through inhibition of the mevalonate pathway, thus inhibiting osteoclast function (3). However, it has also been suggested that statins can act as bone anabolic agents that stimulate the activity of osteoblasts both in vitro and in vivo (4). The anabolic effects of statins can be abolished in vitro by restoring protein phenylation with the addition of HMG-CoA downstream products (5). Therefore, statins can affect bone metabolism through both antiresorptive and anabolic mechanisms that mediate the loss of proteins.

There are not enough comprehensive studies on the effects of statins on bone metabolism in diabetic patients. Due to the wide use of statins in the treatment of diseases, the positive or negative effects of these agents on bone metabolism should be examined in detail. The negative or positive effects of statins on bone fragility and metabolism are a complex issue and it is need to comprehensive studies on this subject.

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In this study, the effects of rosuvastatin and pravastatin on bone metabolism were evaluated. Comparison of the positive or negative effects of two different statins on biochemical parameters related to bone metabolism in 20 mg / kg / day diabetic rats will be contribute to the enrichment of the literature on this subject. In addition, information will be obtained about whether the use of statins will be beneficial in bone metabolism disorders that may occur due to aging or diabetes in DM patients. Thus, the results of this study and similar studies may contribute to the creation of additional options and strengthen the physician's hand in the prevention or treatment of one of the important complications of diabetes.

MATERIALS and METHODS

In a diabetic rat model induced by Streptozotocin (STZ), the possible effects of Rosuvastatin and Pravastatin, both of which are hydrophilic, on biochemical parameters and histologycal examination related to bone metabolism (20 mg / kg) were examined in comparison with the control groups. The animal experiment ethical approval of the study was given by Dicle University University, Health Sciences Research and Application Center (Date 2018, Number:11).

Rats

Sixty 8-10 week old male Wistar Albino rats were divided into 4 groups with 10 in each. Groups; 1- Control group, 2- Diabetic group, 3- Rosuvastatin group (20 mg / kg / day), 4- pravastatin group (20 mg / kg /day).

To induce diabetes, a single dose of nicotinamide (110 mg / kg) was administered first to the abdominal cavity of rats, 15 minutes later STZ (60 mg / kg) dissolved in citrate buffer was injected into the abdominal cavity. Placebo (citrate buffer) was given to the control group only in the same way. After 48 hours after STZ administration, fasting blood glucose level was checked in blood samples taken from the tail vein, and those with a blood glucose level higher than 14mM (250 mg / dl) were included in the study as diabetic. Diabetic rats were divided into 5 groups with 10 in each. The daily feed and water consumption of the rats was determined and normal tap water was given to the 1st and 2nd groups. The 3rd group was given 20 mg / kg rosuvastatin in the water that they could drink daily, and the 4th group was given 20 mg / kg pravastatin daily by dissolving in the amount of water they could drink. By practicing this way for eight weeks, the subjects belonging to all groups were weighed every week and their weight was followed. At the end of the eight-week experimental period, the rats were sacrificed by cardiac puncture under ketamine anesthesia following a 12-hour fasting and blood and bone samples were taken.

Biochemical Parameters

Calcium, magnesium, phosphorus, ALP, 1,25 dihydroxy vitamin D, 25 hydroxy Vitamin D and PTH values were measured in blood samples. In addition, parameters related to fasting blood glucose, glycated hemoglobin (HbA1c), insulin resistance and lipid metabolism in blood samples; triglyceride, total cholesterol, HDL, LDL and VLDL-cholesterol levels were measured.

Histopathological examination

Histopathological examination (evaluation of osteoblastic and osteoclastic activity) was performed on bone samples. Femur samples dissected from rats were fixed in 10% formalin for 24 hours. After the fixation process, they were decalcified in 15% formic acid and embedded in paraffin blocks. Serial sections of 4 micron thickness were taken from the paraffin blocks and the preparations were stained with hematoxylin eosin and examined under a light microscope. In histopathological examination; Bone marrow cellularity, fibrosis and new bone formation (osteoblastic activity) scores were evaluated. The ratio of osteoblasts to the total cell number was determined for the osteoblastic activity score; 0-25% was interpreted as no (Score 1), 26-50% mild (Score 2), 51-75% moderate (Score 3), 76-100% advanced (Score 4). Bone marrow cellularity was interpreted as 0.25% absent (Score 1), 26-50% mild (Score 2), 51 75% moderate (Score 3), 76-100% advanced (Score 4). Fibrosis was interpreted as absent (Score 0), mild (Score 1), moderate (Score 2), and significant (Score 3).

Statistical Analysis

IBM SPSS Statistics version 25 was used for the evaluation of statistical data. Shapiro-Wilk test was used for normality status of continuous variable data. Kruska Wallis test was used between groups because it was non-parametric. Then Tamhan test was used for non-parametric evaluation in post-hock tests for comparison within groups. Student t test was used to compare the two groups of continuous variables. Results were considered significant for $p < 0.05$ values.

RESULTS

As seen in Table 1, mean ± standard deviation values and minimum-maximum values of the independent variables of the groups are shown. In rats with diabetes, it is seen that insulin level is low and blood glucose level is high. In the intergroup comparisons, Phosphate (P) level was 6.60 ± 0.68 in the control group and 5.40 ± 0.22 in the Pravastatin group (Table 2). The decreasing difference was statistically significant (P = 0.017). However, there was no difference in the P level in the Rosuvastatin group compared to the control group and the diabetes group. In our study, the Ca level was 9.50 ± 0.46 in the control group and 10.67 ± 0.15 in the Rosuvastatin group (Table 2). The increase in calcium level in the rosuvastatin group was statistically significant (P = 0.002). However, there was no significant change in Ca level in the Pravastatin group. In our study, the vitamin D2 level of rats was similar in all groups and was not statistically significant. Moreover, as seen in Table 3, the results were similar in terms of parameters in rats given Pravastatin and Lovastatin. In the bone cytology study of rats in our study, there was no significant difference between the groups in terms of both osteoblastic activity and bone marrow cellularity (Table 4).

Table 1. Distribution of biochemical data of all groups

Parameters	Groups	Mean±SD	Min-Max	P [α]
Insulin(µg)	1	14.67±1.76	13.3-17.8	<0.001
	2	7.814±0.61	7.1-8.8	
	3	8.40±0.92	7.1-9.7	
	4	7.90±0.87	6.9-9.4	
Glucose(mg/dL)	1	99.75±2.81	95.6-103.4	<0.001
	2	465.39±14.99	451.1-495.1	
	3	428.40±14.16	409.6-445.5	
	4	476.27±17.88	446.4-501.6	
Home IR (mg/dL)	1	3.61±0.39	3.23-4.34	<0.001
	2	8.98±0.79	8.21-10.39	
	3	8.89±1.06	7.75-10.47	
	4	9.28±0.99	8.20-11.31	
CA (mg/dl)	1	9.50±0.46	9.16-10.21	0.413
	2	10.33±0.75	9.20-11.30	
	3	10.67±0.15	10.40-10.80	
	4	10.58±0.83	9.40-12.10	
MG (mg/dl)	1	2.38±0.28	2.10-2.90	0.673
	2	2.62±0.62	2.10-3.60	
	3	2.45±0.26	2.20-2.80	
	4	2.45±0.22	2.20-2.80	
P (mg/dl)	1	6.60±0.68	5.95-7.67	<0.001
	2	5.57±0.31	451.1-495.1	
	3	5.68±0.24	5.40-5.98	
	4	5.40±0.22	5.03-5.75	
Vitamin D2 (µg/g)	1	0.21±0.07	0.13-0.30	0.002
	2	0.29±0.02	0.27-0.36	
	3	0.29±0.27	0.25-0.32	
	4	0.28±0.01	0.27-0.30	

The mean difference is significant at the 0.05 level, α-Multiple Comparisons of the Tamhane tes, Groups:1-control (no drug,no STZ),2-STZ group,3-Provastatin group,4-Rovastatin goup. Home IR- Homeostatic Model Assessment-Insulin Resistance, CA- Calcium,MG- Magnesium, P- Phosphate

Table 2. Multiple Comparisons of the Tamhan test for biochemical parameters

Dependent Variable	Groups		Mean Difference (I-J)	Std. Error	Sig. [α]	95% Confidence Interval	
						Lower Bound	Upper Bound
Insulin	1	2	6.8571*	0.7031	<0.001	4.366	9.348
		3	6.2714*	0.7501	<0.001	3.765	8.778
		4	6.7714*	0.7406	<0.001	4.273	9.270
	2	1	-6.8571*	0.7031	<0.001	-9.348	-4.366
		3	-0.5857	0.4200	0.722	-1.944	0.772
		4	-0.0857	0.4026	1.000	-1.378	1.206
Glucose	1	2	-365.6286	5.7654	<0.001	-387.182	-344.076
		3	-328.6429	5.4565	<0.001	-348.978	-308.308
		4	-376.514*	6.8406	<0.001	-402.289	-350.740
	2	1	365.6286*	5.7654	<0.001	344.076	387.182
		3	36.9857*	7.7940	0.003	12.488	61.483
		4	-10.8857	8.8185	0.809	-38.747	16.976
		3	47.8714*	8.6197	0.001	20.523	75.220

Home IR	1	2	-5.37429*	0.33758	<0.001	-6.5126	-4.2360
		3	-5.27714*	0.42942	<0.001	-6.7863	-3.7680
		4	-5.67286*	0.40348	<0.001	-7.0771	-4.2686
	2	1	5.37429*	0.33758	<0.001	4.2360	6.5126
		3	0.09714	0.50324	1.000	-1.5071	1.7014
		4	-0.29857	0.48130	0.991	-1.8233	1.2261
CA(mg/dl)	1	2	-15.3571	14.6934	0.914	-71.8418	41.127
		3	-1.1714*	0.1821	0.002	-1.822454	-0.5204
		4	-1.0857	0.3588	0.080	-2.27679	0.10537
	2	1	15.35714	14.6934	0.914	-41.127577	71.84186
		3	14.1857	14.6924	0.938	-42.301	70.6729
		4	14.27142	14.695	0.937	-42.2068	70.7497
		3	-0.08571	0.3193	1.000	-1.28208	1.11065
Mg(mg/dl)	1	2	-0.24285	0.25648	0.937	-1.116834	0.63112
		3	-0.07142	0.14846	0.998	0.538053	0.39519
		4	-0.07142	0.13677	0.997	-0.505953	0.36309
	2	1	0.24285	0.25648	0.937	-0.631120	1.11683
		3	0.17142	0.25408	0.988	-0.7015848	1.04444
		4	0.17142	0.24743	0.986	-0.701824	1.04468
P(mg/dl)	1	2	1.032*	0.28470	0.036	0.0607	2.0050
		3	0.9257	0.27508	0.063	-0.0461	1.8975
		4	1.198*	0.27251	0.017	0.2253	2.1718
	2	1	-1.0328	0.28470	0.036	-2,0050	-0.0607
		3	-0.1071	0.15045	0.983	-0.5849	0.3706
		4	0.1657	0.14570	0.860	-0.3010	0.6324
Vit D2(µg/g)	1	2	-0.08	0.02	0.100	-0.18	0.0136
		3	-0.080	0.02	0.132	-0.17	0.01937
		4	-0.07	0.02	0.160	-0.17	0.02515
	2	1	0.08	0.03	0.100	-0.01	0.18508
		3	0.01	0.015	0.999	-0.04	0.05338
		4	0.010	0.01223	0.968	-0.0322	0.05229

*The mean difference is significant at the 0.05 level. [α]-Multiple Comparisons of the Tamhane tes. Groups:1-controls(no drug,no STZ),2-STZ groups,3-Provastatins Group,4-Rovastatin goup. Home IR- Homeostatic Model Assessment-Insulin Resistance, CA- Calcium,MG- Magnesium, P- Phosphate

Table 3. Results of rats given Pravastatin and Lovastatin

Parameters	Provastatin (Mean±SD)	Rovastatin (Mean±SD)	P[β]
Home IR	8.887 ± 1.064	9.284 ± 0.990	0.485
CA (mg/dl)	10.671 ± 0.149	10.586 ± 0.831	0.793
MG (mg/dl)	2.457 ± 0.269	2.452 ± 0.222	1.000
P(mg/dl)	5.677 ± 0.245	5.402 ± 0.224	0.051
Vit D2(µg/g)	0.292 ± 0.026	0.284 ± 0.013	0.710

[β]-student t test
Home IR- Homeostatic Model Assessment-Insulin Resistance, CA- Calcium,MG- Magnesium, P- Phosphate

Table 4. Multiple Comparisons of the Tamhane test for osteoblastic activity and bone marrow cellularity

Dependent Variable	Groups		Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval	
						Lower Bound	Upper Bound
Osteoblastic activity	1	2	-0.143	0.319	0.999	-1.17	0.88
		3	-0.286	0.360	0.971	-1.46	0.89
		4	-0.429	0.233	0.442	-1.17	0.31
	2	1	0.143	0.319	0.999	-0.88	1.17
		3	-0.143	0.404	1.000	-1.42	1.13
		4	-0.286	0.297	0.932	-1.27	0.70
Bone Marrow Cellularity	1	2	0.571	0.202	0.167	-0.21	1.35
		3	-0.286	0.452	0.991	-1.81	1.24
		4	<0.001	0.286	1.000	-0.90	0.90
	2	1	-0.571	0.202	0.167	-1.35	0.21
		3	-0.857	0.404	0.386	-2.41	0.70
		4	-0.571	0.202	0.167	-1.35	0.21

* The mean difference is significant at the 0.05 level, [a]-Multiple Comparisons of the Tamhane test.
Groups:1-control (no drug,no STZ),2-STZ group,3-Provastatin Group,4-Rovastatin goup

DISCUSSION

In our study, the effects of rosuvastatin and pravastatin on bone metabolism were evaluated. We investigated the comparison of positive or negative effects of two different statins on biochemical parameters related to bone metabolism in rats with diabetes, and whether rovusastatin and pravastatin use is beneficial in bone metabolism disorders. Our results showed that the P level was significantly lower in the Pravastatin group, and the Ca level was significantly increased in the Rosuvastatin group. Our study also showed that there was no significant change in osteoblastic activity and bone marrow cellularity in the bone cytology study of rats.

Statins inhibit HMG-CoA reductase, a proximal enzyme in the mevalonate pathway. As a result of HMG-CoA reductase inhibition, statins have been shown to reduce cholesterol biosynthesis and inhibit the synthesis of prenyl groups that are important for membrane targeting of small GTP az proteins involved in osteoclast function (6). It has been shown that statins strongly inhibits osteoclast-mediated resorption in the mouse skull in vitro (7). Some studies have shown that statins can also inhibit bone resorption through inhibition of the mevalonate pathway in vitro and thus inhibit osteoclast function (6,8). Besides the cholesterol-lowering effects of statins, another benefit is their effect on bone metabolism. The possible link between statins and bone health was first reported in 1999 that statin increases bone formation by stimulating bone morphogenic protein-2 (BMP-2) production in rodent bone cells (4). Statins have both antiresorptive and anabolic effects, including proliferation, differentiation, preservation of osteoblasts, and reduction of osteoclast formation (9,10).

Although several observational studies in humans have reported lower fracture risk or higher bone mineral density in statin users, some studies have reported conflicting results (11-13).

In this study, we found that P level was significantly lower in rats given Pravastatin. In addition, we found that the Ca level increased significantly in the Rosuvastatin group. Studies have shown that Ca is an important electrolyte that plays a role in bone and joint function, especially in elderly people (14). Horecka et al. Showed that simvastatin may contribute to a decrease in Ca concentration in plasma (15). Ipekci et al. found that a patient using atorvastatin had hypercalcemia, and Ca level increased again when atorvastatin was discontinued. They suggested that the high Ca level in this case was the use of atorvastatin (16). Another rat study showed that atorvastatin can significantly increase serum Ca concentration levels in rats (17). Similar to the results of this study, Ca levels were significantly increased in rats given Rosuvastatin. However, vitamin D2 level was similar in all groups. Our results are in line with the results of studies showing a lower incidence of fractures after statin use. When other causes are ruled out, the use of statin may cause hypercalcemia in diabetic rats. In our study, there were no significant changes in bone cytology results, osteoblastic activity and bone marrow cellularity of the rats. It is obvious that more comprehensive studies are needed on this subject.

CONCLUSION

In conclusion, although more extensive studies are needed, our study revealed that the serum ca level was high in rats given Rosuvastatin, and P levels were low in rats given Pravastatin. But cytologically, there was no change in bone structure. Our study revealed that we should be a little more cautious about the information that statins have a positive effect on bone tissue.

Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: The animal experiment ethical approval of the study was given by Dicle University University, Health Sciences Research and Application Center (Date 2018, Number:11).

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