Proceedings S.Z.M.C. Vol: 35(2): pp. 78-82, 2021. PSZMC-798-35-2-2021

Effect of Atorvastatin Alone and in Combination with Aspirin on Uric Acid Handling of Normal Rats

[1]Usman Aslam, [2]Muhammad Ubaidullah Khan, [1]Sadia Chiragh, [3]Abdul Karim, [4]Mushtaq Ahmed

[1]Department of Pharmacology, Al-Aleem Medical College, Lahore
[2]Allama Iqbal Medical College, Lahore
[3]Department of Pharmacology, Shalamar Medical and Dental College, Lahore
[4]Department of Pharmacology, Rahbar Medical College, Lahore

ABSTRACT

Introduction: Low doses of aspirin and statins are usually given in conjunction to patients with coronary artery disease. Aspirin at low doses is known to cause hyperuricemia in these patients which can further worsen their condition, while statins can theoretically counteract this effect. However, this interaction needs to be tested. **Aims & Objectives:** To estimate the effect of atorvastatin and low dose of aspirin alone and in combination on serum uric acid level and urinary uric acid excretion in normal rats. **Place and duration of study:** Post Graduate Medical Institute, Lahore from July, 2018 to August, 2018. **Material & Methods:** Twenty-four healthy Sprague Dawley rats were distributed equally into four groups (Normal control, Aspirin, Atorvastatin and Combination groups). They were given distilled water, aspirin (6.75mg/kg), atorvastatin (5mg/kg) or combination of the same doses of aspirin and atorvastatin for 4 weeks. One ml blood and twenty-four-hour urine sample was collected on week 0 and 4 for estimation of uric acid and creatinine concentrations. Fractional excretion of uric acid was calculated. **Results:** At the end of four weeks the Aspirin group had higher serum uric acid level and lower fractional excretion of uric acid as compared to the Normal control group. Atorvastatin group had lower serum uric acid and higher urinary uric acid level as compared to Aspirin group while combination had higher serum uric acid level versus Normal control. fractional excretion of uric acid decreased from 0-4 weeks in Aspirin and Combination groups only. **Conclusion:** The role of atorvastatin in lowering serum uric acid levels in group receiving atorvastatin alone and in combination group emerged non-significant.

Key words: Atorvastatin, Aspirin, Uric Acid Homeostasis, Sprague Dawley rat.

INTRODUCTION

Hyperuricemia is a state of disturbed homeostasis that results in raised levels of serum uric acid, owing to either increased production of uric acid in vivo or decreased renal excretion of uric acid. Raised levels of serum uric acid are for long identified as a sole metabolic risk factor for gout.[1] However, it can progress to involve renal and vascular systems too.[2]

Hyperuricemia in an individual having otherwise normal homeostasis of uric acid, can evolve as a side effect of certain drug intake. These drugs include anti-tubercular, diuretics, cytotoxic chemotherapy, immunosuppressive agents, nicotinic acid and aspirin at low doses. Aspirin, an irreversible inhibitor of COX in platelets, has a bimodal effect on uric acid excretion.[3] At high doses, aspirin decreases tubular reabsorption of uric acid hence uricosuric. However, at low to moderate doses, aspirin tends to decrease tubular secretion of uric acid leading to retention of uric acid and hyperuricemia.

Hyperuricemia is observed to promote coronary heart disease by exerting oxidative stress, inflammatory reactions, oxidation modification of low-density lipoprotein-cholesterol (LDL-C),[4] and reducing adiponectin production. Hyperuricemia also significantly reduces endothelial progenitor cell proliferation, further worsening tissue perfusion and ischemia.[5] Low dose aspirin attracts prophylactic use in patients surviving an acute cardiac event despite its hyperuricemic effect.

Another group of drugs prescribed to such patients prophylactically is statins, which are HMG-CoA reductase inhibitors and improve lipid profiles. In

addition to this, statins are known to have an early protective effect on vascular endothelium in patients with ST-elevation myocardial infarction (STEMI).[6]A study showed that atorvastatin therapy in Post Coronary Intervention STEMI patients significantly reduced the levels of serum uric acid levels.[7]

Aspirin and statins are routinely given in conjunction to patients with coronary artery disease, however the definite role of statins in lowering serum uric acid levels and its interaction with low doses of aspirin remain somewhat unclear and needs to be further investigated.

MATERIAL AND METHODS

This study was conducted at Post Graduate Medical Institute (PGMI), Lahore after approval of Institutional Review Committee for Basic Sciences from July, 2018 to August, 2018. Sample size of six rats in each group was calculated at 5% level of significance and 90% power of study.[8] Twenty-four male Sprague Dawley rats were selected according to inclusion criteria of 7-8 weeks age and 150-200 g weight from the breeding room of PGMI Animal House. They were kept in stainless steel cages for one week for acclimatization and randomly divided into four groups of six rats each by simple lottery method.

One group was kept as control and administered distilled water 5 ml/ Kg, second group was administered aspirin (Highnoon Pharmaceuticals, Pakistan Ltd) in a dose of 6.75 mg/ 5 ml/Kg[9] and third group was administered atorvastatin (Highnoon Pharmaceuticals, Pakistan Ltd) 5 mg/ 5 ml/ Kg.[8] Fourth group was administered aspirin 6.75 mg and atorvastatin 5mg/ 5 ml. Both drugs were dissolved in distilled water, fresh solutions were prepared daily, and administered as a single daily dose in the morning. All drugs were given for a period of four weeks. Each rat was weighed at the beginning of study and then weekly to adjust the dose.

At the beginning and end of study blood and twenty-four-hour urine samples were collected. Blood sample was collected by cardiac puncture under light chloroform anesthesia (Scharlab S.L. European Union CE Label, Spain) using 5 ml disposable syringe (BD, Pakistan) with 23-gauge needle. One ml blood was collected and put in a clot activator vacutainer (Biovac, Pakistan). After half an hour it was centrifuged at 2500 revolutions per minute for 10 minutes and serum was separated and stored at -20°C. For urine sampling, each rat was put in a separate cage for twenty-four hours and collected urine was centrifuged at 2500 revolutions per minute for 10 minutes. Supernatant was taken and stored at -20°C.

Both serum and urine samples were analyzed for uric acid and creatinine levels. On day of estimation, stored samples were thawed to room temperature and kept in an incubator (DIN-12880-K1, Germany) for 5 to 10 minutes at 37°C. uric acid was estimated by enzymatic colorimetric method and creatinine was estimated by Jaffe method with diagnostic kits (Linear Chemicals, Spain) using spectrophotometer (Pictus B, Diatron, Japan). Fractional excretion of uric acid (FEUA) was calculated by following formula:

$$\text{FEUA} = \frac{(\text{serum creatinine} \times \text{urinary uric acid})}{(\text{urinary creatinine} \times \text{serum uric acid})} \times 100$$

Statistical analysis:

The data collected was processed by using SPSS 20. Data was checked for normality and homogeneity of variance with Shapiro Wilk and Kolmogorov Smirnov test and was found non-normal, therefore nonparametric statistical tests were applied for analysis. It was presented in the form of tables as median (interquartile range). Kruskal-Wallis test was used to find the difference of results amongst all groups. Mann-Whitney U test was used to find difference among individual groups. Wilcoxon signed-rank test was used to find the significance in results over the time within each group. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

Administration of aspirin alone and with atorvastatin for four-weeks, caused a non-significant rise in serum uric acid level from baseline. At the end of study, serum uric acid was significantly higher in the Aspirin group as compared to Normal control and Atorvastatin groups. Combination groups also had higher levels of serum uric acid than Normal control group (Table-1).

At the end of study, urinary uric acid levels non-significantly decreased in the Aspirin group from baseline but significantly lower than Atorvastatin group (Table-2).

Serum and urine creatinine were estimated to calculate FEUA which decreased in Aspirin and Combination groups at the end of study from baseline. FEUA was significantly lower only in Aspirin group versus Normal control group at termination of study (Table-3).

Serum Uric Acid (mg/dl)			
Group	Week 0	Week 4	*p* value Wilcoxon test
Normal Control	2.15 (2.008-2.33)	1.98 (1.95-2.15)	0.225
Aspirin	2.25 (2.10-2.33)	2.45[§] (2.35-2.50)	0.071
Atorvastatin	2.20 (2.10-2.33)	2.10[±] (2.08-2.23)	0.276
Combination	2.20 (1.98-2.33)	2.35[ǂ] (2.08-2.53)	0.221
p value Kruskal Wallis ANOVA	0.884	0.007	

[ǂ]p value ≤ 0.05,[§]*p* value ≤ 0.01 vs Normal control group
[±]p value ≤ 0.01 vs Aspirin group

Table-1: Effect of aspirin, atorvastatin and their combination on serum uric acid level of normal rats (n=6). Data is expressed as median (interquartile range).

Urinary Uric Acid (mg/dl)			
Group	Week 0	Week 4	*p* value Wilcoxon test
Normal Control	37.90 (33.45-44.35)	42.20 (39.75-44.58)	0.528
Aspirin	42.90 (34.40-47.08)	37.40 (34.65-40.33)	0.249
Atorvastatin	41.0 (38.50-57.53)	46.00 [ǂ] (42.0-50.25)	0.599
Combination	40.50 (36.75-46.0)	41.00 (33.75-43.25)	0.343
p value Kruskal Wallis ANOVA	0.751	0.046	

[ǂ]*p*value ≤ 0.01 vs Aspirin group

Table-2: Effect of aspirin, atorvastatin and their combination on urinary uric acid level of normal rats (n=6). Data is expressed as median (interquartile range).

Fractional Excretion of Uric Acid (FEUA)			
Group	Week 0	Week 4	*p* value Wilcoxon test
Normal Control	55.99 (38.30-77.70)	54.39 (47.46-65.92)	0.753
Aspirin	75.25 (45.42-96.83)	28.20[§] (17.62-41.81)	0.046
Atorvastatin	56.25 (43.30-77.83)	42.45 (35.21-52.90)	0.116
Combination	66.93 (53.23-101.17)	33.97 (24.23-64.80)	0.046
p value Kruskal Wallis ANOVA	0.563	0.032	

[§]*p* value ≤ 0.01 vs Normal control group

Table-3: Effect of aspirin, atorvastatin, and their combination on fractional excretion of uric acid of normal rats (n=6). Data is expressed as median (interquartile range).

DISCUSSION

Considering the routine practice of prescribing aspirin and atorvastatin to patients after an acute cardiac event, it was hypothesized that atorvastatin therapy can counteract the hyperuricemia induced by aspirin, but results of this study do not support this hypothesis.

This study was designed to investigate the role of statins i.e., atorvastatin, in decreasing serum uric acid levels and increasing urinary uric acid in Sparing Dawley rats. For this, 24 of these male rats were randomly organized into 4 groups of 6 rats in each. A normal control group, a group receiving aspirin, a group receiving atorvastatin and a group receiving both drugs. Aspirin administered to animals was equivalent to human low dose aspirin, while administered doses of atorvastatin were equivalent to human high dose therapy which regime is mostly followed after an acute cardiac event.[10]

Variables studied were serum and urinary uric acid and FEUA. Serum uric acid levels in humans surge either by increased production of uric acid by metabolism of purines or by decreased excretion of uric acid by renal apparatus. Apart from cytotoxic drugs, which raise SUA by increasing purine metabolism, all other drugs which induce hyperuricemia raise SUA by decreasing its renal excretion. Hence, the fractional excretion of uric acid is indicated as an important factor to assess the interaction among these two adopted drugs.[11]

At the end of this 4-week study, our results showed that the Aspirin group had higher serum uric acid and lower FEUA as compared to the Normal control group. Decline in fractional excretion of uric acid due to aspirin over time was also statistically significant (p value=0.046). Raman et al. have demonstrated similar reduction in FEUA of patients with two different doses of aspirin given for four weeks.[12] Aspirin has a unique dose dependent interaction with urate monocarboxylate exchanger (URAT-1).[3] At low doses, aspirin acts as an exchange substrate for the transporter and promotes reuptake of uric acid.

Atorvastatin did not produce any significant change in serum uric acid or excretion of uric acid as compared to control. A few studies on human subjects proposed that atorvastatin can lower serum uric acid levels,[13,14,15] improve renal function[16] and can play protective role against the deleterious effects of hyperuricemia on vascular endothelium.[6]

Atorvastatin given in combination had higher serum acid as compared to Normal control group and lower FEUA from baseline (*p* value=0.046). The

later finding being statistically significant, shows that atorvastatin was not able to counteract the hyperuricemic effect of aspirin in normal rats. The bioavailability and pharmacokinetics can differ among non-human primates and humans and do not show a linear correlation.[17] Other studies which showed that atorvastatin lowered serum uric acid levels and improved uric acid handling, were conducted on human subjects with comorbidities and previously raised serum uric acid. Hence, these contrasting findings can probably be an outcome of species difference.

As the impact of atorvastatin on uric acid homeostasis in Sparing Dawley rats has not been explored extensively so far, we do not have any other study available to compare our findings to. Also, these findings regarding atorvastatin influence on uric acid homeostasis in Sparing Dawley rats opens up a new frontier to explore. There is a possibility to discover a different mechanism related to pharmacokinetics and pharmacodynamics in rats, which can further extend our understanding of how one drug can behave differently in humans and other mammals and primates.

This study was limited by having a small number of animal subjects and studying them for a short duration.

CONCLUSION

The results could not affirm the hypothesis of nullifying the hyperuricemic effect of low dose aspirin with the addition of atorvastatin in normal rats.

Acknowledgement:
Authors are grateful to staff of animal house and Chemical Pathology laboratory of PGMI, Lahore for their cooperation. Special thanks to Dr. Amer Hassan Siddiqui for helping in statistical analysis.

REFERENCES

1. Fenando A, Widrich J. Gout [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546606/
2. Jin M. Uric acid, hyperuricemia and vascular diseases. Front Biosci. 2012; 17(1):656 659.
3. Ben Salem C, Slim R, Fathallah N, Hmouda H. Drug-induced hyperuricaemia and gout. Rheumatol. 2017; 56(5):679-688.
4. Yang Y, Tian J, Zeng C, Wei J, Li L, Xie X et al. Relationship between hyperuricemia and risk of coronary heart disease in a middle-aged and elderly Chinese population. J Int Med Res 2017; 45(1):254-260.
5. Chen I, Kuo C, Wu C, Tsai H, Lin C, Li S et al. Chronic hyperuricemia impairs blood flow recovery in the ischemic hindlimb through suppression of endothelial progenitor cells. Oncotarget. 2018; 9(10):9285-9298.
6. Gavazzoni M, Gorga E, Derosa G, Maffioli P, Metra M, Raddino R. High-dose atorvastatin versus moderate dose on early vascular protection after ST-elevation myocardial infarction. Drug Des, Devel Ther. 2017; 11:3425-3434.
7. Yan L,Ye L, Wang K, Zhou J, Zhu C. Atorvastatin improves reflow after percutaneous coronary intervention in patients with acute ST-segment elevation myocardial infarction by decreasing serum uric acid level. J. Zhejiang Univ. Med Sci. 2016; 45(5):530-535.
8. Xilifu D, Abudula A, Rehemu N, Zhao L, Zhou X, Zhang X. Effect of rosuvastatin on hyperuricemic rats and the protective effect on endothelial dysfunction. Exp Ther Med. 2014; 8(6):1683-1688.
9. Ghosh A, Tilak A, Pandey M, Dhumal V, Singh A, Bondekar A. Effect of chronic administration of low dose aspirin on haloperidol induced catalepsy in rats. J Pharmacol Pharmacother. 2011; 2(3):198-199.
10. Pignone M, Earnshaw S, Tice J, Pletcher M. Aspirin, statins, or both drugs for the primary prevention of coronary heart disease events in men: A cost–utility analysis.Ann. Intern. Med. 2006; 144(5):326-236.
11. Chen C, Lü J, Yao Q. Hyperuricemia-related diseases, and xanthine oxidoreductase (XOR) Inhibitors: An overview. Med Sci Monit. 2016; 22:2501-2512.
12. Raman D, Rajak D, Raman D, Kumar D, Kumari D. Assessment of renal function after administration different doses of aspirin [Internet]. Medicalsciencejournal.com. http://www.medicalsciencejournal.com/archives/2019/vol5/issue1/5-1-61
13. Ogata N, Fujimori S, Oka Y, Kaneko K. Effects of three strong statins (atorvastatin, pitavastatin, and rosuvastatin) on serum uric acid levels in dyslipidemic patients. Nucleos, NucleotNuc. 2010; 29(4-6):321-324.
14. Derosa G, Maffioli P, Reiner Ž, Simental-Mendía L, Sahebkar A. Impact of statin therapy on plasma uric acid concentrations: A systematic review and meta-analysis. Drugs. 2016; 76(9):947-956.
15. Paschos P, Athyros V, Tsimperidis A, Katsoula

A, Grammatikos N, Giouleme O. Can serum uric acid lowering therapy contribute to the prevention or treatment of nonalcoholic fatty liver disease?. Curr Vasc Pharmacol. 2018; 16(3):269-275.
16. Deedwania P, Stone P, Fayyad R, Laskey R, Wilson D. Improvement in renal function and reduction in serum uric acid with intensive statin therapy in older patients: A post hoc analysis of the SAGE trial. Drugs Aging. 2015; 32(12):1055-1065.
17. Musther H, Olivares-Morales A, Hatley O, Liu B, Rostami Hodjegan A. Animal versus human oral drug bioavailability: Do they correlate?. Eur J Pharm Sci. 2014; 57:280-291.

The Authors:
Dr. Usman Aslam
Senior Demonstrator,
Department of Pharmacology,
Al-Aleem Medical College, Lahore.

Muhammad Ubaidullah Khan
Final Year Student,
Allama Iqbal Medical College, Lahore.

Prof. Sadia Chiragh
Department of Pharmacology,
Al-Aleem Medical College, Lahore.

Prof. Abdul Karim
Department of Pharmacology,
Shalamar Medical and Dental College, Lahore.

Prof. Mushtaq Ahmed
Department of Pharmacology,
Rahbar Medical College, Lahore.

Corresponding Author:
Dr. Usman Aslam
Senior Demonstrator,
Department of Pharmacology,
Al-Aleem Medical College, Lahore.
E-mail: dr.usmanaslam@yahoo.com