Indian Journal of Forensic Medicine and Toxicology
Volume 16 No. 3, July-September 2022
DOI: http://dx.doi.org/xxxxx/ijfmt.xxxx.16322.68

Simvastatin Toxicity Induces Alteration of Bladder Thickness in Interstitial Cystitis Rat Model

Z K Yusuf[1], A D I Hasanuddin[2*], M N S Yusuf[3], M T Posumah[4], N Ibrahim[4], R Tianggara[4]

[1]Tutor at Pharmacology Department of Medical Faculty, Universitas Negeri Gorontalo, Gorontalo, Indonesia, [2]Tutor at Histology Department of Medical Faculty, Universitas Negeri Gorontalo, Gorontalo, Indonesia, [3]Tutor at Anatomy Department of Medical Faculty, Universitas Negeri Gorontalo, Gorontalo, Indonesia, [4]Student of Medical Faculty, Universitas Negeri Gorontalo, Gorontalo, Indonesia.

How to cite this article: Z K Yusuf, A D I Hasanuddin, M N S Yusuf et al. Simvastatin Toxicity Induces Alteration of Bladder Thickness in Interstitial Cystitis Rat Model. Indian Journal of Forensic Medicine and Toxicology 2022;16(3):388-392.

Abstract

Background: Interstitial cystitis (IC) is a chronic inflammatory of the bladder, while statin can increase its risk. Recently, the exact mechanism is yet known. We hypothesizedsimvastatin can induce alteration of bladder thickness.

Methods: Twenty-four female Wistar rats were aged 6-8 weeks old were divided into two groups and were treated with simvastatin 50 mg/kg BW or carboxymethylcellulose 0.5% by oral gavage for 30 days. Each group was then equally subdivided into three groups: control, Interstitial Cystitis (IC) day-0, and IC day-3. Either IC or control rat group was induced by intravesical instillation of protamine sulfate or buffered saline respectively. All animals in the control and IC day-0 group were sacrificed and collected for the bladder tissue in less than three hours following intravesical treatment, while animals in the IC day-3 group three days after. All collected tissue was prepared in hematoxylin-eosin staining and measured for the bladder thickness, namely the urothelial, suburothelial, and detrusor layer by image analyzer application.

Results: There was no significant difference between the groups receiving simvastatin and placebo in the thickness of the urothelium, suburothelium, and detrusor layers in all rat models, both control, IC0, and IC3 rats (all p values > 0.05). However, the thickness of the urothelium layer was consistently lower in the simvastatin group than in the placebo group in all rat models.

Conclusion: Mechanism of simvastatin toxicity on bladder tissue through urothelial denudation thus may alter the urothelial barrier function.

Keywords: Bladder Pain Syndrome; Denudation; Interstitial Cystitis; Protamin Sulfate; Statin; Toxicity; Urothelial.

Introduction

Statins are widely used to lower LDL cholesterol and prevention of cardiovascular disease.[1] Recently, statins have been found to have many health benefits despite their hypolipidemic effects, namelyanti-inflammatory, antiatherogenic, antifibrosis, and even anti-cancer. But on the other hand, this pleiotropic effect can trigger several toxicity such as increasing the incidence of new-onset diabetes

Corresponding Author: A D I Hasanuddin, Tutor at Histology Department of Medical Faculty, Universitas Negeri Gorontalo, Gorontalo, Indonesia.

Email: abdidzulikram@gmail.com

Contact No.: +6285233215280

mellitus type 2, neurological-neurocognitive effects, hepatotoxic, renal toxicity, and others.[2] Another statin toxicity is interstitial cystitis, while the exact mechanism is unrevealed.[3]

Interstitial cystitis is a complex disease and may involve a variety of unknown etiologies. One of the main processes that occur in the pathogenesis of interstitial cystitis is bladder urothelium barrier dysfunction, which can be caused by failure of urothelial cytodifferentiation, chronic inflammation in the suburothelial tissue, increased apoptotic cells and decreased proliferative cells. This dysfunction results in the leakage of water, urine, potassium, and other toxic substances in urine to the underlying tissue, triggering symptoms of urgency, frequency, and dysuria.[4] Based on this pathogenesis, we hypothesized that statins exert a side effect of interstitial cystitis through their antiproliferative mechanism. This is reinforced by evidence that statins inhibit the proliferative activity of stem cells in vitro, where there is an increase in senescence and apoptosis through upregulation of p16, p53, caspase 6, caspase 8, and caspase 9.[5] Therefore, in this study, we aim to investigate the effect of simvastatin on bladder thickness in the interstitial cystitis rat model.

Material and Method

Animal Preparation

This study used a total of 24 rat samples which were initially subjected to acclimatization for 10 days. After that, randomization was carried out using a simple random sampling method and divided equally into two groups, namely group C who only received a placebo carboxymethylcellulose (CMC) 0.5% (n = 12) and group S who received simvastatin 50 mg/kg BW (n = 12). All rats were kept in open, humid, well-ventilated cages, and life / light cycle 12 hours / 12 hours. Each cage consists of four to five rats. All rats received standard AD2 feed and free access to tap water ad libitum.

Simvastatin Treatment

Simvastatin was prepared from generic tablet form (Kimia Farma, Indonesia). The drug dosage was based on previous research where simvastatin 50 mg / Kg BW is a hypocholesterolemic dose in rats.[6,7] All groups received treatment for 30 days based on previous studies which found statin can induce senescence within 20 days in vitro.[8] The simvastatin tablets were turned into suspension form with 0.5% carboxymethylcellulose (CMC) as the solvent. Simvastatin suspension or 0.5% CMC was administered by oral gavage and was adjusted according to body weight each week. The duration of administration between simvastatin doses is every 24 hours during the day or afternoon.

Induction of Interstitial Cystitis

After completing simvastatin or placebo administration, each group of rats was further divided into three subgroups, namely control rats, Interstitial Cystitis (IC) day-0 rats, and IC day-3 rats. The IC group and the control group were induced by intravesical instillation of protamine sulfate and buffered saline, respectively based on previous studies.[9,10] Protamine sulfate (Sigma Aldrich, Japan) was dissolved in buffered saline with a concentration of 10 mg/ml, then put into an instillation tube in the form of a 1 ml spoit mounted to a sterile 22 / 24G vein catheter. Anesthetic experimental animals using ketamine injection 10% (60 mg / Kg) intraperitoneally. The rats were positioned dorsally recumbent and mildly massaged in the lower abdominal region to induce micturition. After identification of the external urethral ostium, the lubricated distal end of the instillation tube was inserted as deep as 3 mm in a cephalocaudal position parallel to the urethra, then rotate the proximal end of the instillation tube vertically about 180 degrees. After that, the distal end of the instillation tube was inserted 7 mm deep into the bladder. An amount of 0.6 ml of protamine sulfate or buffered saline was instilled with bolus for 30-45 seconds and was maintained in the bladder for 15 minutes while rotating the rats to homogenize the contact of instillation solution to the entire lumen of the bladder. Finally, the instillation tube is slowly pulled out from the urethra.

Tissue Preparation and Bladder Thickness Measurement

Tissue collection time was adjusted according to the rat model group. In the control and IC day-0 rat model, the animals were sacrificed less than 3 hours after the instillation procedure. As for the IC day-3 rat model, the animals have sacrificed three days after the instillation procedure. Initially, all rats were killed through the cervical dislocation technique then the bladder organs were taken. The tissue samples were fixed in 10% neutral formaldehyde solution overnight and then made in the form of paraffin blocks according to standard procedures. The paraffin blocks were cut using a microtome with a thickness of

5µm, followed by floating in a warm water container. After that, the specimens were placed on a slide and glued with a thin layer of albumen. The slides were then processed using the hematoxylin-eosin staining procedure.

The slides were examined using an Axio Imager. A2 microscope using a 20X magnification objective lens. Each slide was photographed to randomly obtain five different images that clearly show the full thickness of all bladder tissue layers, namely the urothelial, suburothelial, and detrusor layers. The width of each layer was calculated using the ImageJ NIH application. Five measurements on each slide were then averaged to obtain the representative data.

Statistical Analysis

All collected data were analyzed by SPSS version 17.0 with a 95% confidence interval ($\alpha = 0.05$). Data for the bladder layer thickness is expressed in terms of mean ± standard deviation, or median ± standard error of mean if not normally distributed. Independent Sample T-test or Mann Whitney method was used to compare the thickness of each bladder layer among treatment groups in the same mouse model. A p-value ≤ of 0.05 was considered significant.

Result and Discussion

The results of tissue thickness measurements at various layers can be seen in Table 1. In the control rat model without interstitial cystitis induced, it was revealed that the thickness of the urothelium layer in the simvastatin group was lower than in the placebo group, although not significantly different (0.298 ± 0.023 mm vs. 0.321 ± 0.122 mm, p = 0.564). The same thing was also observed in the mouse model of interstitial cystitis on day 0 (almost significant, p=0.061) and day 3 (p=0.538). However, the thickness of the urothelium layer was consistently lower in the simvastatin group than in the placebo group in all rat models. The thickness of the suburothelial layer in the group receiving simvastatin was always higher than the group receiving placebo, except for the rat model of interstitial cystitis on day 0. Meanwhile, the thickness of the detrusor layer in the group receiving simvastatin was always lower than the group receiving placebo, except for the 3rd-day interstitial cystitis rat model. Figure 1 also shows that there are differences in tissue thickness between the treatment groups, both in control, IC0, and IC3 rats.

Table 1: Differences in bladder tissue thickness at different layers of treatment groups

Group	Urothelial Thickness (µm)		Suburothelial Thickness (µm)		Detrusor Thickness (µm)	
	Mean (SD)	P value[#]	Mean (SD)	P value[#]	Mean (SD)	P value[#]
C-C	321 (122)*	0.564[$]	907 (233)	0.657	2,186 (464)	0.814
S-C	298 (23)		978 (195)		2,087 (664)	
C-IC0	290 (49)	0.061	983 (166)	0.386[$]	1,741 (327)	0.467
S-IC0	205 (55)		809 (142)*		1,510 (499)	
C-IC3	344 (39)	0.538	919 (209)	0.549	1,901 (439)	0.395
S-IC3	327 (031)		1,037 (309)		2,426 (1.,060)	

C-C, Carboxymethylcellulose-Control Rat; C-IC0, Carboxymethylcellulose -Interstitial Cystitis Rat Day 0; C-IC3, Carboxymethylcellulose-Interstitial Cystitis Rat Day 3; S-C, Simvastatin-Control Rat; S-IC0, Simvastatin-Interstitial Cystitis Rat Day 0; S-IC3, Simvastatin-Interstitial Cystitis Rat Day 3.

* Data were not normally distributed expressed in median ± standard error

[#] T-independent test

[$] Mann-Whitney test

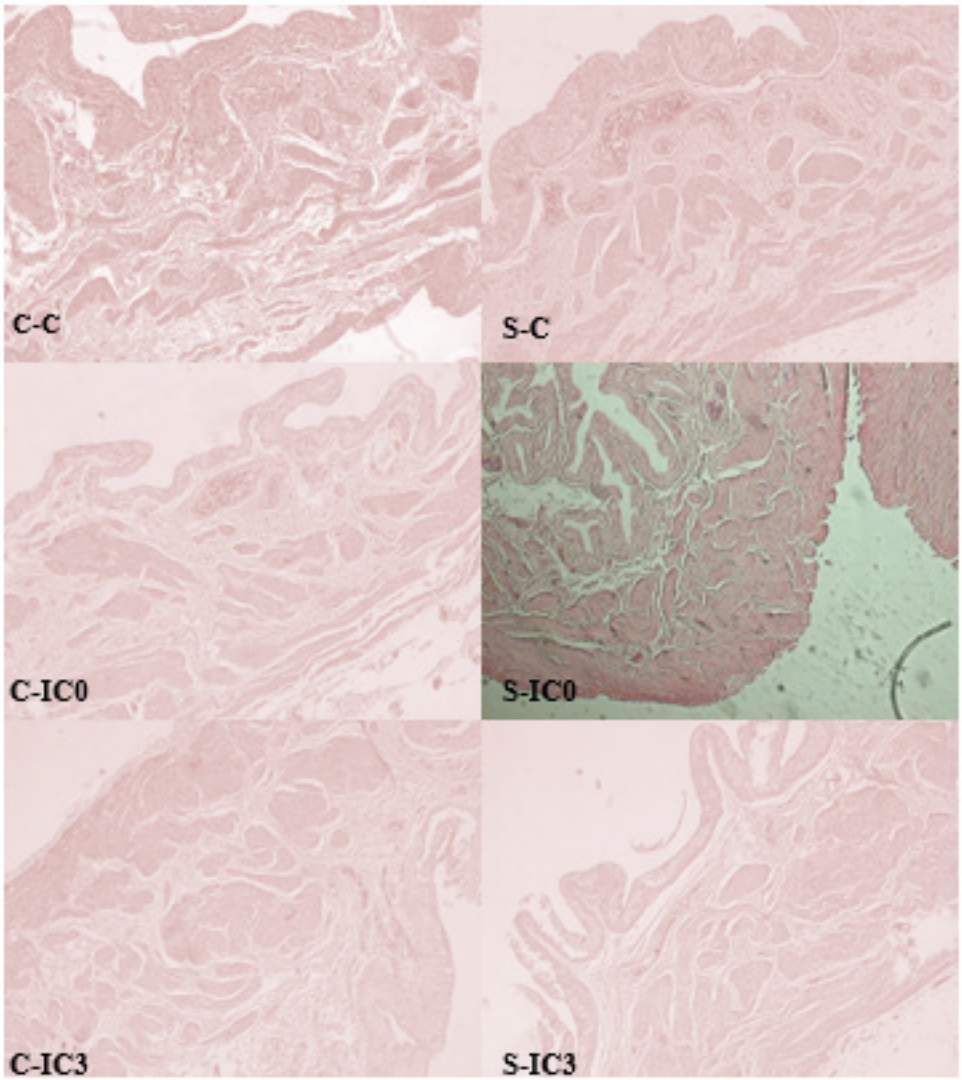

Figure 1: Representative Image of the Bladder Tissue Showing All Layers in the Treatment Group.

C-C, Carboxymethylcellulose-Control Rat; C-IC0, Carboxymethylcellulose -Interstitial Cystitis Rat Day 0; C-IC3, Carboxymethylcellulose-Interstitial Cystitis Rat Day 3; S-C, Simvastatin-Control Rat; S-IC0, Simvastatin-Interstitial Cystitis Rat Day 0; S-IC3, Simvastatin-Interstitial Cystitis Rat Day 3.

In this study, no significant difference was found between the thickness of various layers of bladder tissue between those receiving simvastatin and those receiving placebo. However, in the urothelium layer, the group receiving simvastatin always had a lower mean thickness value than the group receiving the placebo. Denudation or thinning of the urothelium is one of the typical features that indicate bladder urothelium barrier dysfunction in interstitial cystitis. The presence of this dysfunction results in the leakage of water, urine, potassium, and other toxic substances in the urine to the suburothelial tissue and the underlying bladder wall, triggering symptoms of urgency, frequency, and dysuria.[4] On the one hand, the difference in urothelial tissue thickness was almost significant in a mouse model of interstitial cystitis induced acutely by protamine sulfate. Instillation of protamine sulfate has been known to make umbrella cell exfoliation in the first two days of the administration, thereby exacerbating the dysfunction of the previously thinned urothelial barrier.[11] The impact of this dysfunction of the urothelium barrier was seen in this study, while only on day 3 after protamine sulfate instillation, the group receiving simvastatin had greater detrusor tissue thickness than the group receiving placebo. This urothelial barrier dysfunction can be caused by several factors, including failure of cytodifferentiation of the basal

layer and urothelial intermediates in response to damage to the overlying umbrella cells that may occur in this study.[12,13]

Conclusion

Mechanism of simvastatin toxicity on bladder tissue through urothelial denudation thus may alter the urothelial barrier function.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Clearance: The Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University has confirmed the proposal and research protocol (No.375/UN4.6.4.5.31/PP36/2020).

Source of funding: Internal grant funds of UniversitasNegeriGorontalo

Reference

1. C Baigent, A Keech, Patricia M Kearney, L Blackwell. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. The Lancet. 2005 Oct;366(9493):1267–78.
2. Bitzur R, Cohen H, Kamari Y, Harats D. Intolerance to statins: mechanisms and management. Diabetes Care. 2013 Aug;36 Suppl 2:S325-330.
3. Huang CY, Chung SD, Kao LT, Lin HC, Wang LH. Statin Use Is Associated with Bladder Pain Syndrome/ Interstitial Cystitis: A Population-Based Case-Control Study. Urol Int. 2015;95(2):227–32.
4. Jhang J-F, Kuo H-C. Pathomechanism of Interstitial Cystitis/Bladder Pain Syndrome and Mapping the Heterogeneity of Disease. Int Neurourol J. 2016 Nov;20(Suppl 2):S95-104.
5. Izadpanah R, Schächtele DJ, Pfnür AB, Lin D, Slakey DP, Kadowitz PJ, et al. The impact of statins on biological characteristics of stem cells provides a novel explanation for their pleiotropic beneficial and adverse clinical effects. Am J Physiol Cell Physiol. 2015 Oct 15;309(8):C522-531.
6. Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, et al. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol. 2001 Jan;21(1):115–21.
7. Crespo MJ, Quidgley J. Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats. World J Diabetes. 2015 Aug 25;6(10):1168–78.
8. Peric D, Barragan I, Giraud-Triboult K, Egesipe A-L, Meyniel-Schicklin L, Cousin C, et al. Cytostatic Effect of Repeated Exposure to Simvastatin: A Mechanism for Chronic Myotoxicity Revealed by the Use of Mesodermal Progenitors Derived from Human Pluripotent Stem Cells: Simvastatin Repeated-Dose Toxicity In Vitro. STEM CELLS. 2015 Oct;33(10):2936–48.
9. Lavelle J, Meyers S, Ramage R, Doty D, Bastacky S, Apoddaca G, et al. Protamine sulfate-induced cystitis: a model of selective cytodestruction of the urothelium. Urology. 2001 Jun;57(6 Suppl 1):113.
10. Reis LO, Sopena JMG, Fávaro WJ, Martin MC, Simão AFL, Reis RB dos, et al. Anatomical features of the urethra and urinary bladder catheterization in female mice and rats. An essential translational tool. Acta Cir Bras. 2011;26(suppl 2):106–10.
11. LW Crock, 2013. Urine Trouble: A Molecular and Anatomical Examination of Bladder Pain. Arts Sci. Electron. Theses Diss. 132.
12. Malik, S.T., Birch, B.R., Voegeli, D., Fader, M., Foria, V., Cooper, A.J., Walls, A.F., Lwaleed, B.A., 2018. Distribution of mast cell subtypes in interstitial cystitis: implications for novel diagnostic and therapeutic strategies? J. Clin. Pathol. 71, 840–844.
13. Southgate, J., Varley, C.L., Garthwaite, M.A.E., Hinley, J., Marsh, F., Stahlschmidt, J., Trejdosiewicz, L.K., Eardley, I., 2007. Differentiation potential of urothelium from patients with benign bladder dysfunction. BJU Int. 99, 1506–1516.