Original Article

Histological and Biochemical Alterations Underlying Statin-Associated Pancreatic Toxicity in a Rat Model

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Abstract

Objective: To investigate the histological and biochemical changes in the pancreas associated with simvastatin-induced toxicity using a rat model, focusing specifically on changes in pancreatic weight, vascular congestion, necrosis, fibrosis, and inflammatory markers.

Methodology: A 12-week laboratory-based experimental control trial conducted at the Department of Anatomy of a public sector medical college in Rawalpindi, in collaboration with the National Institute of Health (NIH) Islamabad and Armed Forces Institute of Pathology (AFIP) Rawalpindi from Dec 2021 to Dec 2022. Twenty male Sprague-Dawley rats weighing 250 ± 50 grams, aged 60 ± 5 days were assigned to the control group and another twenty with similar age and weight ranges were assigned to the experimental group. For a duration of 12 weeks, the experimental group was given 60 mg/kg/day of simvastatin orally, whereas the control group was given water and a conventional diet. The following characteristics were measured: body weight, pancreatic weight, biochemical parameters (cytokines, enzymes), and histological alterations (congestion, necrosis, fibrosis). Version 23 of SPSS was used to analyze the data. Qualitative variables were expressed as percentages and frequencies. The statistical significance was defined as $P < 0.05$. Chi-square analyses were used to compare the qualitative factors across the groups.

Results: Compared to controls, the simvastatin group showed significantly increased mean pancreatic weight ($p=0.043$), congestion (55% vs 0%, $p<0.001$), higher levels of CRP ($p<0.030$), IL-6 ($p<0.040$), and TNF-α ($p<0.040$). Trends of mild necrosis (25% vs 5%, $p=0.182$) and interstitial fibrosis (15% vs 0%, $p=0.231$) were observed but not statistically significant.

Conclusion: Simvastatin profoundly impacts pancreatic histomorphology, particularly through increased congestion and inflammatory markers, with potential implications for necrosis and fibrosis development. These findings warrant further investigation into mechanisms of toxicity and close monitoring of patients for pancreatic adverse effects during statin therapy.

Keywords: Simvastatin, pancreatic toxicity, histology, inflammation, congestion, necrosis, fibrosis, cytokines.

Introduction

Statin therapy has revolutionized the dyslipidemia management, however it continues to be a source of concern for its potential side effects.[1] Statins related myopathies are well known and documented, however statin's effect on the pancreas remains unclear.[2] Pancreas controls glucose homeostasis through insulin and glucagon secretion from its endocrine islets. Acinar cells of pancreas provide essential digestive enzymes. Disturbing this intricate balance can have unfavourable consequences for both exocrine and endocrine functions.[3]

There is scarcity of the existing literature suggesting a potential link between statin use and effects on pancreas. Pancreatitis has been attributed to inflammatory reactions, cellular toxicity and accumulation of toxic metabolites.[4] However, this remains unclear as only few studies identified a significant association. Furthermore, the histomorphological basis of this potential risk is not clearly understood, reaching definitive conclusions. The association of statin therapy and related pancreatic toxicity is a complex phenomenon. Some evidences suggest a modest increased risk of acute pancreatitis. Its particularly more evident with higher doses and certain statins.[5] As the precise mechanisms are not fully understood, few of the potential contributors include direct cytotoxicity, disturbance of cholesterol dependent cell signalling, and mitochondrial dysfunction within the pancreatic cells.[6] Despite certain potential risks, cardiovascular benefits of statins outweigh the pancreatic toxicity concerns. It calls for necessary careful risk benefit analysis and of statin therapy.[7]

Despite the growing research on statin associated adverse effects, there remains a significant gap in understanding of the specific histological and biochemical changes that occur in the pancreas following statin exposure. [8]The majority of existing studies have focused on clinical outcomes or in vitro cellular changes, leaving a critical need for in vivo investigations that can clarify the structural and functional alterations in pancreatic tissue. This study aims to address this knowledge gap by providing a comprehensive analysis of pancreatic histomorphology and associated biochemical markers in a rat model of

simvastatin toxicity. Ongoing research is vital to reveal the specific mechanisms underlining statin associated pancreatic toxicity, identify risk factors of patients, and to adopt strategies for ideal mitigation. Future clinical trials can explore statin alternatives for cardiovascular benefits or co-administration of protective agents to decrease pancreatic adverse effects while maintaining a balance of cholesterol lowering efficacy.

This research investigates this critical knowledge gap, identifying potentially important insights into statin-induced histological changes in the rat pancreas. Through a comprehensive histological analysis, we unveil the visual evidence of direct pancreatic toxicity associated with statin exposure. These structural abnormalities, demonstrably disrupting pancreatic physiology, provide a morphological foundation for potential pancreatitis development. The findings of this study hold significant implications for developing therapeutic strategies for patients undergoing long-term statin treatment. By elucidating the histo-morphological landscape of statin-induced pancreatic toxicity, we pave the way for a more nuanced understanding of this potential side effect and, consequently, the development of targeted interventions to mitigate its risk.

Methodology

This novel research study was carried out in the Anatomy Department at a public medical college in Rawalpindi, in collaboration with esteemed research institute National Institute of Health (NIH) Islamabad from Dec 2021 to Dec 2022. Employing a laboratory-based experimental control trial traversing 12-week period, the study scheme was reviewed and permitted by the institutional Ethical Committee on Animal Experiments before commencing (ID AMC/375).

Simvastatin was administered for 12 weeks to two equal groups of forty male Sprague-Dawley rats, each weighing 250 ± 50 grams, aged 60 ± 5 days. Group B was the experimental group and group A was the control group. During the course of the experiment, the rats were euthanized and their pancreases were taken for analysis. The control group (A) was fed a standard diet and water through an oral gavage tube, while the simvastatin group (B) received a standard diet plus 60 mg/kg/day of simvastatin orally every day via a gavage tube. The body weight of all the animals was recorded at the end of the study just before the sacrifice of animals, and the weight of the pancreas was recorded in grams using a digital precision balance.

Histological Parameters

Congestion (increase in blood volume showing presence of RBCs) in the vascular lumen was recorded as present or absent in the pancreatic stroma. Necrosis in acinar cells was accessed at 40X on a semi-quantitative scale using the following criteria of necrosis.[7] Three slides per specimen were observed; Score 0: Not present; Score 1: Mild, less than 25% of the parenchyma involved; Score 2: Moderate, 25–50% of the parenchyma involved; Score 3: Severe, more than 50% of the parenchyma involved Interstitial fibrosis was accessed at 10X on a semi-quantitative scale using criteria modified from sections stained with Masson's trichrome. [8] Mean was taken for the scoring of three slides per specimen. Score 0: No connective tissue between the lobules; Score 1: mild, presence of thick fibrous septa in less than 15% of the pancreatic tissue per slide. Score 2: moderate, presence of thick fibrous septa in 15 to 30% of pancreatic tissue per slide; Score 3: severe, presence of thick fibrous septa or presence of fibrous tissue in the lobules in greater than 30% of pancreatic tissue per slide.

Necrosis in acinar cells was accessed at 40X on the semi-quantitative scale.[7] Three slides per specimen were observed.

Biochemical Parameters

Cytokines Assay and Enzymes Assay (Blood Chemistry Profile) were measured as follows;

Using a commercial kit from Pierce-Endogen (Rockford, IL), the sandwich ELISA technique was used to measure the levels of TNF-α, IL-6, and CRP in plasma. The data are reported as picograms per millilitres of plasma and were obtained using the controls and standards supplied by the manufacturer. Control samples were evaluated alongside experimental samples on separate analysis days in order to watch for any plate-to-plate fluctuation and guarantee uniformity in cytokine values. Serum Amylase was measured using an Amylase colorimetric assay kit of Centronic GmbH/ Germany (LOT GF10201G)). Serum LDH was measured using an LDH colorimetric assay kit of LABLIT (LOT LIQ-1164-CM).

Data Analysis

Data analysis was done by using Statistical Package for the Social Sciences (SPSS) software version 23. Frequencies and percentages were expressed as qualitative variables. $P \leq 0.05$ defined statistical significance.Chi-square tests compared qualitative variables between groups.

Results

Animals in the control group (A) remained active for the entire duration. The mean animal weight just before dissection was 294.18±10.82gm. The mean ±SD weight of the pancreas was 0.750±0.082 gm in control group A. The control group (A) did not display major structural pancreatic changes (Fig 1). None of the animals in the control c showed congested blood vessels. Necrosis was not observed in any of the animals in the control group. Interstitial fibrosis was absent in all animals of the control group. Animals in the experimental group (B) remained active for the entire duration. The mean animal weight of the experimental group just before dissection was 307.64±7.05gm. The change in weight between the groups was statistically significant ($p<0.001$). The weight of the pancreas was significantly higher in experimental group B as compared to control group A ($p = 0.043$). The experimental group (B) exhibited marked pancreatic alterations. The pancreatic tissue showed congestion in eleven (55%) rats of

the experimental group. The frequency of congestion was significantly higher in experimental group B as compared to control group A ($p< 0.001$). In the experimental group, mild necrosis of acinar cells was observed in three (25%) rats while it was observed in 1(5%) rat in the control group. The difference was statistically insignificant ($p=0.182$). In experimental group B, Interstitial fibrosis was present in 3 (15%) rats. The frequency of interstitial fibrosis was higher in experimental group B as compared to control group A but the difference was statistically insignificant ($p= 0.231$). Significant differences were found in CRP, PCT, and IL-6 but not in LDH values between experimental and controls ($P \leq 0.05$).

Discussion

When we compared rats given simvastatin to those that weren't, we found some important differences. Our study revealed that simvastatin, a commonly prescribed cholesterol-lowering medication, can cause noticeable changes in the pancreas of rats.

This aligns with previous evidence that increasing body weight can lead to augmented abdominal organ weight and associated morbidity risks.[9] The specific mechanisms underlying this pancreatic weight increase warrant further investigation but may relate to inflammation and fatty infiltration of the tissue. Findings of this study are important because they give us visual and measurable evidence that statins might affect the pancreas in ways we didn't fully understand before. This information could help doctors better monitor patients taking statins and potentially prevent pancreas-related side effects.

Histological examination revealed structural changes in the experimental group's pancreas. Vascular congestion was observed in 55% of samples, a significantly higher proportion than the control group. Possible explanation for this finding may include local inflammation and acinar cell damage leading to vascular endothelial dysfunction. This is validated by deranged cytokines assay in our results. This may cause vasodilation, increased permeability, and congestion.[10] Another possible explanation can be the release of pro-inflammatory mediators and pancreatic enzymes that activate coagulation cascades.

This can precipitate microvascular thrombosis and congestion.[11]

Similar mechanisms have been proposed to underlie vascular complications in acute pancreatitis, which carry high morbidity if unmanaged.[10] The congestion seen here may indicate early signs of simvastatin-associated pancreatitis.

Though statistically insignificant, mild necrosis affected more experimental samples (Figure 2). The short 12-week study duration likely limited the progression to severe necrosis. However, the increased pancreatic weight and congestion suggest the potential for eventual necrotizing pancreatitis. Necrosis arises from unrelenting cellular insults leading to apoptotic and necrotic death.[12] The trend warrants monitoring with prolonged simvastatin use.

Table 1: Comparison of histological and biochemical parameters between the control and experimental groups

Parameter	Control group A	Experimental group B	p-value
Gross			
Animal Weight (gm)	294.18±10.82gm	307.64±7.05	< 0.001
Pancreas Weight (gm)	0.75±0.094	0.805±0.068)	0.043
Histological			
Congestion	0 (0%)	11 (55%)	< 0.001*
Necrosis	1(5%)	5(25%)	0.182
Interstitial fibrosis	0 (0%)	3 (15%)	0.231
Biochemical			
CRP (mg/ml)	0.4	0.7	<0.030*
Interleukin 6 (pg/ml)	18.5	23.5	<0.040*
TNF-α (pg/ml)	19.5	45	<0.040*
LDH (IU/L)	60	83	0.176

*p-value≤0.05

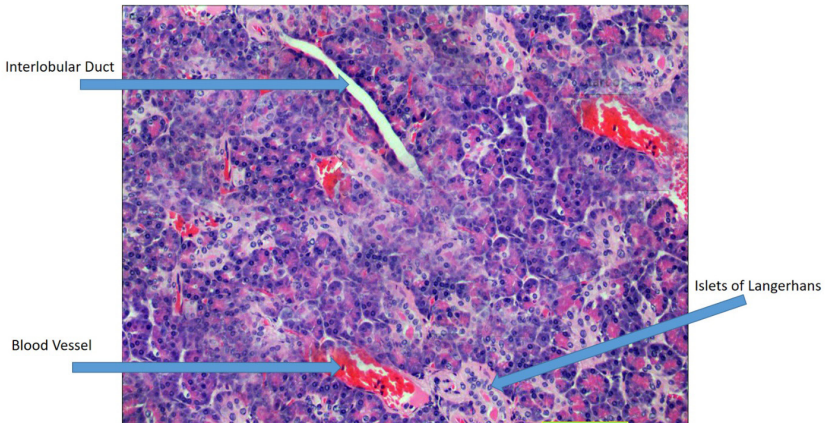

Figure-1: Photomicrograph of a histological section of the pancreas of rat in group B showing necrosis and fibrosis

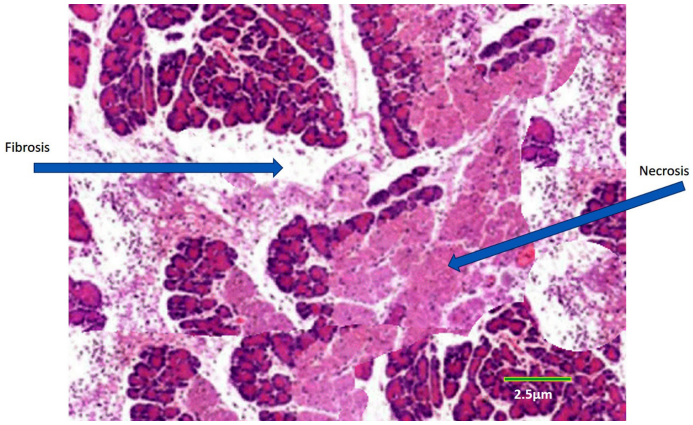

Figure-2: Photomicrograph of a histological section of the pancreas of rat in group B showing necrosis and fibrosis.

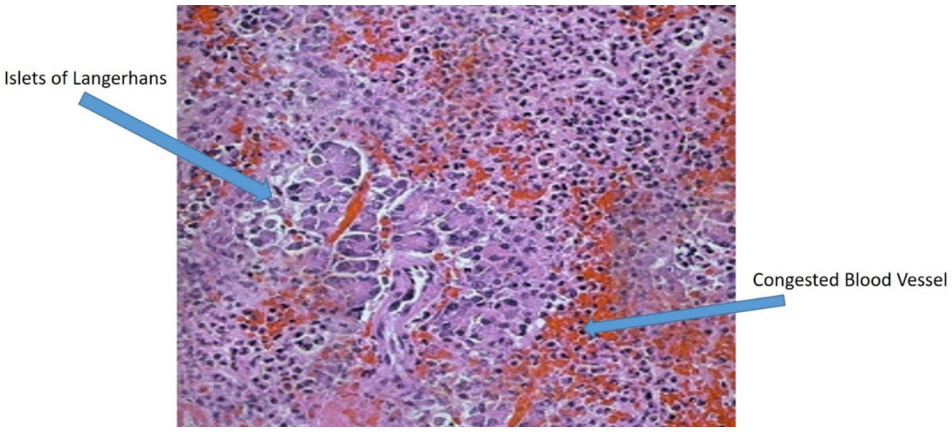

Figure-3: Photomicrograph of a histological section of the pancreas of rat in group B showing congestion

Interstitial fibrosis was not significantly elevated, though human studies link pancreatic fibrosis to aging, obesity, and chronic inflammation.[13] The brief study period may have precluded advanced fibrotic remodeling. However, the inflammation could prime the pancreas for eventual scarring with extended simvastatin exposure. Further studies are needed to characterize long-term fibrosis risk.

From this conversation, it's evident that the structural changes offer clear visual evidence that statins disrupt the microanatomy of the pancreas. The inflammation and fatty replacement are particularly noticeable, as they can disrupt the delicate functions of both the exocrine and endocrine aspects of the organ. Interleukin-6 (IL-6) is a cytokine, a type of signaling molecule that plays a crucial role in the immune response and inflammation. While traditionally associated with immune regulation, recent research has unveiled its involvement in various physiological processes beyond immunity, including metabolic regulation and pancreatic function.[14] Pancreatic beta cells are essential for maintaining glucose homeostasis by secreting insulin, a hormone that regulates blood sugar levels. Any disturbance in the integrity or function of intestinal beta cells can lead to metabolic disorders such as diabetes. Several studies have shown a link between IL-6 and intestinal beta cell health. Chronic low-grade inflammation marked by elevated levels of cytokines such as IL-6 is associated with the development of type 2 diabetes. These inflammatory cytokines can cause beta cell dysfunction and apoptosis, and play a role in the onset and progression of diabetes, particularly IL-6, which is associated with insulin resistance, a state of low cellular response of insulin is related.[15] Insulin resistance is often diagnosed before the onset of type 2 diabetes when elevated levels of IL-6 can disrupt insulin signalling in endothelial cells, causing the body to produce and release excess insulin is activated, and can eventually damage the beta cells IL-6 may also directly affect intestinal function. Some studies have shown that IL-6 can increase or decrease insulin secretion from beta cells, depending on the situation. In some cases, IL-6 can help beta cells grow and survive, suggesting that it may be safe.[16] IL-6 plays a multifaceted role in the inflammatory response in the pancreas. Conditions such as pancreatitis often show increased levels of IL-6, leading to tissue damage and dysfunction. Chronic pancreatitis can lead to loss of pancreatic beta cells and decreased insulin secretion. In addition, genetic studies have identified the association of polymorphisms of the IL-6 gene with insulin metabolism and increased susceptibility to type 2 diabetes, further confirming the association with IL-6 between symptoms and pancreatic function is emphasized. [17]

Elevated CRP levels are a marker of systemic inflammation. In both type 1 and type 2 diabetes mellitus, chronic low-grade inflammation is frequently observed and is linked to higher CRP levels. This inflammation can compromise beta cell integrity by driving oxidative stress, causing cytokine-induced damage, and facilitating immune cell infiltration into the pancreatic islets, as demonstrated in our study. Elevated levels of inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), are commonly observed in chronic inflammatory states and have been implicated in beta cell dysfunction. These cytokines contribute to beta cell impairment through multiple mechanisms: they disrupt insulin secretion pathways, induce endoplasmic reticulum stress, and promote beta cell apoptosis. The derangement of C-reactive protein (CRP) in the context of pancreatic injury reflects a complex interplay of inflammatory mediators, tissue damage, cellular activation, and oxidative stress. Although CRP levels can serve as a useful biomarker for evaluating the severity of pancreatic injury and monitoring the progression of pancreatitis, it is crucial to recognize that CRP elevation is not specific to pancreatic injury and may be elevated in a variety of other inflammatory conditions.[18] Monitoring LDH levels may provide valuable information in the assessment of pancreatic injury or disease. However, LDH is a relatively nonspecific marker of cellular damage, and its interpretation should be considered alongside other clinical and laboratory parameters [19] as evident in our study. In summary, while LDH is not specific to pancreatic tissue, changes in its levels can indicate pancreatic injury or damage, including conditions such as acute pancreatitis, chronic pancreatitis, and pancreatic cancer. Monitoring LDH levels may be useful in the evaluation and management of pancreatic diseases, but it should be interpreted in the context of the clinical presentation and other diagnostic findings. Simvastatin, a widely prescribed cholesterol-lowering medication, is generally well-tolerated. However, there's a potential link between simvastatin toxicity and pancreatic effects which may manifest as biochemical derangements and prompting cautious consideration and individualized treatment approaches.

It is important to note that while animal studies provide valuable insights into the effects of medications like simvastatin, findings in rodents may not always directly translate to humans. Therefore, further research, including clinical studies, is needed to fully understand the effects of simvastatin on pancreatic tissues and its relevance to human health and disease. Additionally, individuals should consult healthcare professionals for personalized medical advice and information regarding the use of simvastatin or any other medication.

Limitations

It's important to note that while animal studies provide valuable insights into the effects of medications like simvastatin, findings in rodents may not always directly translate to humans. Therefore, further research, including clinical studies, is needed to fully understand the effects of simvastatin on pancreatic tissues and its relevance to human health and disease. Additionally, individuals should consult healthcare professionals for personalized medical advice and information regarding the use of simvastatin or any other medication.

Conclusion

This research establishes that statins have a significant effect on the structure of the pancreas. Rats treated with simvastatin displayed noTable alterations, such as increased congestion,

inflammation, and fatty infiltration compared to the control group. Additionally, concerning patterns were observed in terms of necrosis and fibrosis. These findings highlight the need for careful monitoring of pancreatic function in patients undergoing long-term statin therapy and indicate the importance of further research into the mechanisms of statin-induced pancreatic toxicity.

Conflict of Interest

We declare no conflict of interest that could have influenced the work reported in this paper.

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This research project did not receive any funding.

Authors' Contribution

AQ: Study design, concept, data acquisition, manuscript writing; HGK: Manuscript writing, final data approval, analysis, interpretation; MMK: Final manuscript approval, critical review; FU: Critical review, data analysis, interpretation; NM: Microphotograph analysis; KA: Critical review, data interpretation.

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