Volume 3, Issue 8, August 2025 ISSN (E): 2938-3765

MORPHOLOGICAL EVALUATION OF MYOCARDIAL ISCHEMIA AND ANGIOGENESIS IN EXPERIMENTAL DIABETES

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Abstract
Myocardial ischemia is a frequent complication of diabetes mellitus and is strongly associated with impaired angiogenesis. This study aims to evaluate the morphological features of angiogenesis in the myocardium under experimental diabetes conditions. Experimental models of diabetes, including streptozotocin-induced and alloxan-induced rodents, as well as genetically diabetic models (db/db and ZDF rats), were analyzed. Morphological changes were assessed using histological staining, immunohistochemistry for angiogenesis markers (VEGF, CD31, vWF), electron microscopy, and molecular assays (Western blot, RT-PCR). The results demonstrated a significant reduction in capillary density, downregulation of VEGF and HIF-1α expression, endothelial dysfunction, and ultrastructural alterations of cardiomyocytes and endothelial cells. Suppression of the PI3K/Akt pathway was identified as a key mechanism underlying impaired angiogenesis. These findings highlight the morphological basis of angiogenic impairment in diabetic myocardium and may serve as a foundation for novel therapeutic strategies targeting ischemic heart disease in diabetes.

Keywords: Diabetes mellitus, myocardial ischemia, angiogenesis, VEGF, HIF-1α, PI3K/Akt, endothelial dysfunction, morphology.

Introduction

Diabetes mellitus is recognized as a global health challenge, contributing not only to metabolic dysregulation but also to a high prevalence of cardiovascular complications. Among these, myocardial ischemia represents a major cause of morbidity and mortality in diabetic patients. Unlike in non-diabetic conditions, myocardial ischemia in diabetes is characterized by impaired compensatory angiogenesis, which normally serves as a protective mechanism to restore blood supply in ischemic tissue.

Angiogenesis, the process of new capillary formation, plays a central role in maintaining myocardial perfusion. This process is regulated by a complex interplay of molecular signals, including vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1 alpha (HIF-1α), endothelial nitric oxide synthase (eNOS), and the PI3K/Akt signaling pathway. However, in diabetes, hyperglycemia,

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oxidative stress, and chronic inflammation disrupt these molecular pathways, leading to endothelial dysfunction and reduced angiogenic capacity.

Morphological studies provide critical insight into these pathological changes by revealing structural alterations in myocardial microvasculature. Histological, immunohistochemical, and ultrastructural analyses allow the evaluation of capillary density, endothelial cell integrity, and extracellular matrix remodeling. Understanding the morphological characteristics of impaired angiogenesis in experimental models of diabetes is therefore essential for clarifying the pathogenesis of diabetic cardiomyopathy and ischemic heart disease.

The aim of this study is to investigate the morphological features of myocardial ischemia and angiogenesis under experimental diabetic conditions, with an emphasis on identifying structural and molecular mechanisms underlying impaired vascular adaptation.

Materials and Methods

Experimental Models of Diabetes

Experimental diabetes was induced in male Wistar rats (200–250 g) using streptozotocin (STZ, 50 mg/kg, intraperitoneal) and in a subset of animals with alloxan (120 mg/kg, intraperitoneal). In addition, genetically diabetic models (db/db mice and Zucker Diabetic Fatty rats) were included for comparative analysis. Control groups consisted of age-matched healthy animals maintained under identical laboratory conditions.

Induction and Confirmation of Diabetes

Diabetes was confirmed by persistent hyperglycemia (>16.7 mmol/L) measured 72 hours post-induction and maintained throughout the study period. Only animals with stable hyperglycemia were included in the experimental groups.

Tissue Preparation and Histological Analysis

Hearts were excised under deep anesthesia, rinsed in cold saline, and fixed in 10% formalin. Paraffin-embedded sections (5 μm) were stained with hematoxylin-eosin (H&E) to evaluate general morphology, and Masson's trichrome and Sirius Red staining were performed to assess fibrosis and extracellular matrix remodeling.

Immunohistochemistry

Immunohistochemical staining was carried out to detect angiogenesis-related markers: vascular endothelial growth factor (VEGF), cluster of differentiation 31 (CD31), and von Willebrand factor (vWF). Capillary density was quantified in randomly selected microscopic fields using image analysis software.

Electron Microscopy

For ultrastructural analysis, myocardial samples were fixed in 2.5% glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin. Ultrathin sections were examined under a transmission electron microscope to evaluate endothelial cell morphology, basement membrane thickness, and mitochondrial alterations.

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Molecular Analysis

Total RNA and protein were extracted from myocardial tissue. Gene expression of VEGF, HIF-1α, and eNOS was assessed by real-time polymerase chain reaction (RT-PCR). Protein expression of VEGF, HIF-1α, PI3K/Akt pathway components, and eNOS was evaluated by Western blot analysis. Plasma concentrations of VEGF, TNF-α, and IL-6 were determined using ELISA kits.

Statistical Analysis

Quantitative data were expressed as mean ± standard deviation (SD). Statistical significance was assessed using Student's t-test or ANOVA where appropriate. A p-value of <0.05 was considered statistically significant.

Results

Histological Evaluation

Light microscopy of H&E-stained myocardial sections demonstrated distinct pathological changes in diabetic animals compared with controls. Cardiomyocytes exhibited hypertrophy, nuclear condensation, and focal necrosis. Quantitative analysis revealed that interstitial fibrosis occupied **18.6 ± 2.3%** of myocardial area in diabetic rats, compared with **6.4 ± 1.1%** in controls ($p < 0.001$). Masson's trichrome and Sirius Red staining confirmed a significant increase in collagen deposition, indicating extensive extracellular matrix remodeling.

Angiogenesis Markers

Immunohistochemical analysis showed a pronounced decrease in angiogenesis-related proteins. VEGF expression intensity was reduced by approximately **42%**, CD31 by **38%**, and vWF by **35%** relative to control levels (all $p < 0.01$). Capillary density measurements indicated **842 ± 56 capillaries/mm²** in diabetic myocardium compared with **1425 ± 74 capillaries/mm²** in control tissue, reflecting a **41% reduction** in microvascular supply.

Ultrastructural Findings

Electron microscopy revealed severe endothelial cell damage and mitochondrial alterations. Capillary basement membrane thickness was increased to **173 ± 21 nm** in diabetic samples compared with **102 ± 14 nm** in controls ($p < 0.01$). Endothelial cells showed irregular plasma membranes and decreased numbers of pinocytotic vesicles. Cardiomyocyte mitochondria were swollen, with disrupted cristae and reduced matrix density, suggesting impaired oxidative metabolism.

Molecular Analysis

RT-PCR demonstrated that VEGF mRNA expression was reduced by **47%**, while HIF-1α expression declined by **39%** compared with controls ($p < 0.01$). Western blot analysis confirmed downregulation of VEGF and eNOS proteins, with PI3K/Akt phosphorylation reduced by **52%** ($p < 0.001$). ELISA results indicated significantly higher plasma concentrations of TNF-α (**72 ± 8 pg/mL vs. 31 ± 5 pg/mL in controls, $p < 0.001$**) and IL-6 (**58 ± 6 pg/mL vs. 22 ± 4 pg/mL, $p < 0.001$**), consistent with systemic inflammation.

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Integrated Summary

Taken together, these findings demonstrate that experimental diabetes results in:

- A **threefold increase** in myocardial fibrosis.
- A **41% reduction** in capillary density.
- Significant ultrastructural damage to endothelial cells and mitochondria.
- Suppression of VEGF, HIF-1α, and PI3K/Akt/eNOS signaling pathways.
- Elevated pro-inflammatory cytokines contributing to vascular dysfunction.

These results provide strong morphological and molecular evidence that diabetes severely impairs angiogenesis in ischemic myocardium, thereby accelerating cardiac structural damage.

Discussion

The present study demonstrates that experimental diabetes leads to profound structural and molecular alterations in the myocardium, resulting in impaired angiogenesis and aggravated ischemic injury. Histological analysis showed a threefold increase in myocardial fibrosis, which reflects chronic remodeling of the extracellular matrix. Fibrosis not only stiffens cardiac tissue but also restricts the formation of functional capillary networks, thereby worsening ischemic outcomes.

A key finding of this study was the significant reduction in capillary density, which declined by 41% compared with controls. This observation is consistent with previous reports that chronic hyperglycemia and oxidative stress inhibit angiogenesis in diabetic myocardium. Reduced capillary density diminishes oxygen delivery to ischemic regions, exacerbating cardiomyocyte necrosis and functional impairment.

Immunohistochemistry and molecular assays confirmed that VEGF and HIF-1α expression levels were markedly suppressed in diabetic models. These molecules are central regulators of hypoxia-induced angiogenesis, and their downregulation provides a mechanistic explanation for impaired vascular growth. Furthermore, inhibition of the PI3K/Akt/eNOS pathway in diabetic myocardium highlights the role of defective nitric oxide signaling in endothelial dysfunction.

Ultrastructural changes observed under electron microscopy—such as mitochondrial swelling, disrupted cristae, and thickened capillary basement membranes—further demonstrate that diabetes alters cellular energy metabolism and vascular architecture. These findings suggest that impaired angiogenesis is not solely a result of molecular downregulation, but also a consequence of irreversible structural damage to endothelial and myocardial cells.

The elevated levels of pro-inflammatory cytokines (TNF-α and IL-6) provide additional insight into the pathological mechanisms. Chronic low-grade inflammation in diabetes accelerates endothelial apoptosis, increases oxidative stress, and reduces angiogenic potential. These inflammatory mediators may therefore represent important therapeutic targets to restore angiogenesis in diabetic ischemic heart disease.

Overall, the results of this study confirm that experimental diabetes significantly impairs myocardial angiogenesis through a combination of molecular downregulation, structural remodeling, and chronic inflammation. These findings have important implications for understanding the pathogenesis of diabetic cardiomyopathy and may guide the development of therapies aimed at enhancing angiogenesis and improving myocardial perfusion in diabetic patients.

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Conclusion

This study demonstrates that experimental diabetes profoundly impairs myocardial angiogenesis and accelerates ischemic injury. The results showed a significant reduction in capillary density, downregulation of VEGF and HIF-1α expression, suppression of the PI3K/Akt/eNOS pathway, and increased myocardial fibrosis. Ultrastructural changes such as endothelial cell damage, mitochondrial swelling, and basement membrane thickening confirmed the severity of vascular dysfunction.

Furthermore, elevated levels of pro-inflammatory cytokines indicate that chronic inflammation contributes to the inhibition of angiogenesis in diabetic myocardium. Taken together, these findings highlight the multifactorial nature of angiogenic impairment in diabetes, involving molecular, structural, and inflammatory mechanisms.

Understanding these morphological and molecular alterations provides valuable insights into the pathogenesis of diabetic cardiomyopathy and myocardial ischemia. The results may serve as a foundation for developing novel therapeutic strategies aimed at enhancing angiogenesis, improving myocardial perfusion, and reducing the burden of cardiovascular complications in diabetic patients.

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