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PECULIARITIES OF RESPIRATORY CELLULAR IMPLICATIONS IN STREPTOZOTOCIN-INDUCED DIABETES: A FOCUS ON TYPE I ALVEOLOCYTES

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Abstract. Introduction. The escalating global prevalence of diabetes mellitus has ignited concerns, given its multifaceted repercussions that extend beyond conventional metabolic considerations. Recent scientific inquiries have firmly established a profound association between diabetes mellitus and abnormalities within the respiratory system, unveiling a nuanced interplay that transcends mere glycemic control. This study delves into the intricate and far-reaching influence of diabetes on the respiratory system, recognizing it as a vulnerable target organ susceptible to systemic repercussions. Compelling epidemiological evidence underscores a heightened prevalence of respiratory complications among individuals grappling with diabetes. This observation prompts a meticulous exploration of the pathophysiological underpinnings of this intricate relationship, spanning from molecular intricacies to clinical manifestations.

The aim was to deepen our understanding of the impact of diabetes mellitus on the respiratory system by investigating the pathological alterations in type I alveolocytes within an experimental model of diabetes mellitus.

Methods. In this experimental model we used 88 male Wistar rats (170-210 g). The rats were divided into three groups: Group 1 (n=10) consisted of intact rats; Group 2 (n=40) served as the control group, and Group 3 (n=38) constituted the experimental group where diabetes was induced by intraperitoneal administration of streptozotocin (Sigma, USA) diluted in 0.1 M citrate buffer with pH 4.5 at a dosage of 60 mg/kg of body weight. The control group received an equivalent volume of 0.1 M citrate buffer solution with a pH of 4.5 via intraperitoneal injection. All procedures were performed under sodium thiopental anesthesia at a dose of 60 mg/kg of body weight. Tissue samples were collected at intervals of 14, 28, 42, and 70 days post streptozotocin injection.

For electron microscopy analysis, fragments of lung tissue were immersed in a 2.5% glutaraldehyde solution for fixation, followed by fixation in a 1% osmium tetroxide solution. After dehydration, the specimens were embedded in Epon Araldite. Sections, obtained through a "Tesla VS-490" ultramicrotome, underwent examination using a "PEM-125K" electron microscope.

Results. Throughout the experiment, discernible changes in type I alveolocytes were observed. At 14 days, nuclei exhibited rounding or oval shape with uniform chromatin distribution. Mitochondria displayed small size, and the Golgi apparatus (GA) and granulated endoplasmic reticulum (GER) showed no significant alterations. By 28 days, nuclei adopted an oval shape, chromatin localized peripherally, and mitochondria exhibited diverse morphologies. Increased micropinocytotic vesicles indicated heightened cellular activity. At 42 days, hyperhydration became pronounced, nuclei displayed lower electron density, and cellular components showed advanced changes. By 70 days, dystrophic-destructive changes included low electron density nuclei, disorganized mitochondria, and fragmented GA and GER.

Conclusion. This comprehensive ultrastructural analysis unveils the progressive impact of diabetes on type I alveolocytes, elucidating unique facets of pulmonary alterations over time. This study contributes to a growing body of knowledge, shedding light on the dynamic nature of pulmonary changes in diabetes mellitus, ultimately urging further exploration for a holistic understanding of its implications on respiratory health.

Keywords: Diabetes Mellitus, Lungs, Type I Alveolocytes, Ultrastructural Analysis, Experimental Model.

Introduction. The burgeoning prevalence of diabetes mellitus has become a global health concern, with its multifaceted impact extending beyond the traditionally associated metabolic disturbances. Recent scientific investigations have unveiled a significant connection between diabetes mellitus and respiratory system abnormalities, highlighting a complex interplay that extends far beyond the realm of glycemic control [1]. At its core, diabetes mellitus is characterized by aberrations in glucose metabolism, stemming from insufficient insulin production or impaired cellular response to insulin. Traditionally recognized for its implications on cardiovascular health, this metabolic disorder has increasingly garnered attention for its nuanced influence on pulmonary function. As we explore the intricate impact of diabetes on the respiratory system, it becomes apparent that the complex interplay between these seemingly distinct physiological systems offers a fertile ground for scientific investigation [2].

The respiratory system, often considered in the context of pulmonary and bronchial structures, is now emerging as a target organ affected by the systemic repercussions of diabetes mellitus. Recent epidemiological studies have unveiled a heightened prevalence of respiratory complications among individuals with diabetes, thereby prompting a paradigm shift in our understanding of the holistic implications of this metabolic disorder [3]. To comprehend the breadth of this association, it is imperative to dissect the pathophysiological underpinnings,

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starting from the molecular and cellular levels and progressing to the clinical manifestations observed in diabetic individuals.

The molecular intricacies of diabetes mellitus involve a cascade of events that extend well beyond glucose dysregulation. Hyperglycemia, the hallmark feature of diabetes, triggers a milieu of biochemical alterations, including the formation of advanced glycation end-products (AGEs) and the activation of inflammatory pathways [4]. These molecular changes, in turn, contribute to a pro-inflammatory state and oxidative stress, with far-reaching consequences on various organ systems, including the respiratory apparatus.

The pulmonary ramifications of diabetes mellitus manifest in a spectrum of conditions ranging from mild pulmonary dysfunction to severe respiratory disorders. Chronic obstructive pulmonary disease (COPD), characterized by persistent airflow limitation, is notably linked to diabetes, suggesting a shared pathogenic basis [5]. Asthma, another prevalent respiratory ailment, exhibits intricate interactions with diabetes, further underscoring the need for a holistic approach to understanding the consequences of this metabolic disorder on respiratory health [6].

Beyond obstructive lung diseases, emerging evidence suggests that diabetes mellitus may exert influence on restrictive lung disorders, such as interstitial lung disease (ILD) [8]. The fibrotic changes observed in ILD bear semblance to the fibrotic manifestations encountered in diabetic complications, hinting at potential commonalities in the underlying mechanisms. Exploring these connections is paramount for unraveling the full scope of the impact of diabetes mellitus on pulmonary function.

Hence, our **aim** is to cultivate a more profound comprehension of the effects of diabetes mellitus on the respiratory system by examining the pathological changes in type I alveolocytes in experimental diabetes mellitus.

Methods. The study involved 88 male Wistar rats, characterized by a weight range of 170-210 g. The rats were categorized into three groups: Group 1 (n=10) comprised intact rats; Group 2 (n=40) served as the control group; and Group 3 (n=38) represented the experimental group with induced diabetes using intraperitoneal administration of streptozotocin (Sigma, USA) diluted in 0.1 M citrate buffer with pH 4.5, administered at a rate of 60 mg/kg of body weight. The control group received an intraperitoneal injection of an equivalent volume of 0.1 M citrate buffer solution with a pH of 4.5. All procedures were conducted under sodium thiopental anesthesia at a dose of 60 mg/kg of body weight. Tissue samples were collected at intervals of 14, 28, 42, and 70 days following streptozotocin injection.

For electron microscopy analysis, fragments of lung tissue were initially immersed in a 2.5% glutaraldehyde solution, followed by fixation in a 1% osmium tetroxide solution. Subsequent to dehydration, the specimens were embedded in Epon Araldite. Sections, obtained using a "Tesla VS-490" ultramicrotome, were examined using a "PEM-125K" electron microscope.

Results. The ultrastructural analysis revealed distinct changes in the Type I alveolocytes (A-I) over the course of the experiment. At the initial 14-day marked that the nuclei of the majority of Type I alveolocytes (A-I) exhibited a rounded or oval shape. Chromatin granules were predominantly and uniformly distributed across the entire nuclear area, as illustrated in Figure 1. Within the cytoplasm of A-I, a limited number of mitochondria were observed, characterized by their small size and either an elongated or rounded shape. The components of the Golgi apparatus (GA) and granular endoplasmic reticulum (GER) displayed no remarkable structural alterations. Importantly, the basement membrane maintained its characteristic structure consistently throughout this period.

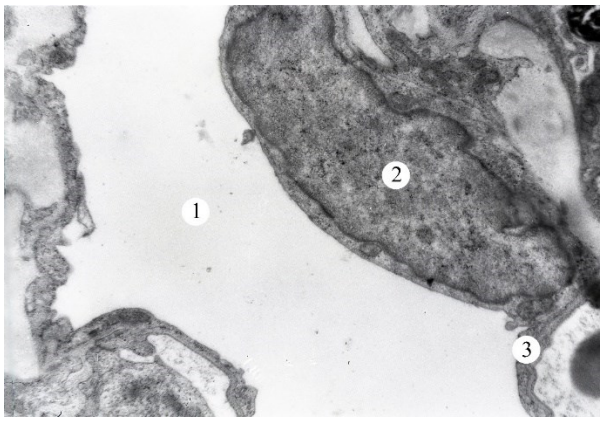

Figure 1. Ultrastructural changes of type I alveolocyte 14 days after the start of the experiment. Electron micrograph. Magnification: 9600.

Captions: 1 – alveolar lumen; 2 – nucleus of type I alveolocyte; 3 – peripheral part of type I alveolocyte.

As the study extended to 28 days, notable changes in the ultrastructure of Type I alveolocytes (A-I) were observed. The nuclei of A-I adopted an oval shape, characterized by a fine-grained matrix and a uniform distribution of chromatin. Interestingly, in individual cells, chromatin localization at the periphery of the nucleoplasm was evident. Mitochondria exhibited a rounded or elongated morphology with a relatively electron-dense matrix. Concurrently, some mitochondria appeared swollen, featuring single and reduced cristae.

GA was localized around the nucleus and manifested as unevenly expanded cisterns, vacuoles, and vesicles. The GER consisted of moderately expanded tubules, with ribosomes noted on the outer surface. A noticeable increase in the number of micropinocytotic vesicles was observed in the peripheral part of A-I. In specific areas of the thinned portion of A-I, sail-like protrusions directed into the alveoli were identified. The basement membrane exhibited localized thickening, and intercellular contacts retained their structural integrity. Moreover, microvilli were identified on the cytoplasmic surface of individual cells, directed into the lumen of the alveoli.

After 42 days of diabetes induction, hyperhydration in the respiratory components became more pronounced. A-I nuclei displayed lower electron-optical density, and chromatin was marginally localized . Shallow intussusceptions were noted in the nuclear membrane, and the perinuclear space was locally expanded. Mitochondria appeared swollen with disorganized cristae. GA and GER components were significantly expanded and deformed, with a reduced number of ribosomes on their membranes. Numerous micropinocytotic vesicles and varying-sized vacuoles were evident in the peripheral part of A-I.

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Intracellular edema led to cytoplasmic protrusions entering the alveoli.

The ultrastructural analysis at the 70-day mark indicated dystrophic-destructive changes in A-I. Nuclei of these cells exhibited low electron density and deformation, often with shallow nucleolemic intussusceptions. Mitochondria displayed a matrix of low electron-optical density and disoriented reduced cristae in the cytoplasm. Both GA and GER components showed significant expansion and fragmentation. The basal membrane exhibited indistinct contours and low electron-optical density in many areas. The peripheral sections of A-I displayed the fusion of micropinocytotic vesicles, forming large vacuoles. Sail-like protrusions into the alveolar lumen were still observed in individual A-I (Figure 2). Intercellular contacts remained intact. Notably, a substantial thickening of the alveolar wall was observed, attributed to the swelling of fibrous structures by the accumulating liquid. Interstitial tissue edema was particularly pronounced in regions adjacent to areas with compromised endothelial cell integrity in the hemocapillaries of the interalveolar septum.

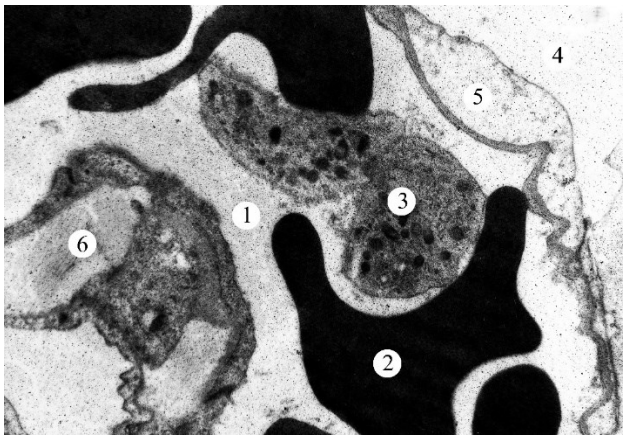

Figure 2. Respiratory department of the lungs 70 days after the start of the experiment. Electron micrograph. Magnification: 6400.
Captions: 1 – hemocapillary lumen; 2 – erythrocyte; 3 – platelet; 4 – alveolar lumen; 5– sail-like protrusion of the peripheral part of type I alveolocyte; 6 – interstitium.

Discussion. The current study presents a detailed ultrastructural analysis of A-I in an experimental model of diabetes mellitus, aiming to contribute to the broader understanding of the intricate interplay between diabetes and respiratory system abnormalities. The multifaceted impact of diabetes extends beyond its well-established metabolic implications, as evidenced by emerging connections to pulmonary dysfunction.

The observed changes in A-I nuclei, particularly their shape and chromatin distribution, suggest an early response to the induction of diabetes. At the 14-day mark, nuclei were predominantly round or oval, with even chromatin distribution, indicating an initial adaptation. The limited structural changes in Golgi apparatus (GA) and granular endoplasmic reticulum (GER) components and the preserved basement membrane underscore the resilience of A-I at this stage.

As the study progressed to 28 days, the ultrastructural alterations became more pronounced. A-I nuclei adopted an oval shape with a fine-grained matrix, accompanied by chromatin localization at the nucleoplasm periphery in individual cells. Mitochondria exhibited varied morphologies, including swelling and reduced cristae. The expansion of GA and GER components, along with the increased presence of micropinocytotic vesicles, indicated heightened cellular activity and potential adaptive responses.

The 42-day interval marked a notable progression in the hyperhydration of respiratory components, suggesting a cumulative effect of diabetes induction. A-I nuclei displayed lower electron density, indicating a potential compromise in cellular health. Swollen mitochondria with disorganized cristae and the expansion and deformation of GA and GER components pointed towards advanced cellular changes. The presence of intracellular edema, cytoplasmic protrusions into the alveoli, and the fusion of micropinocytotic vesicles highlighted a shift towards pathological alterations.

By the 70-day mark, dystrophic-destructive changes in A-I became apparent, indicating a culmination of cellular responses to prolonged diabetes induction. Nuclei exhibited low electron density and deformation, with shallow nucleolemic intussusceptions. Mitochondria displayed further disorganization, and GA and GER components showed extensive fragmentation. The thickening of the alveolar wall, attributed to fibrous structure swelling, and pronounced interstitial tissue edema underscored the severity of structural alterations in advanced stages.

Comparing these findings with existing literature reveals both consistencies and unique observations [8]. The observed early changes align with previous studies indicating altered nuclear morphology and chromatin distribution in response to diabetes induction. The increased presence of micropinocytotic vesicles and sail-like protrusions is consistent with cellular adaptations reported in diabetes-related pulmonary studies.

However, the distinct stages observed in this study, particularly the 70-day mark with pronounced dystrophic-destructive changes, contribute novel insights. The identified structural alterations, such as the fusion of micropinocytotic vesicles and sail-like protrusions, enrich our understanding of the progressive impact of diabetes on A-I ultrastructure.

This study contributes to the growing body of knowledge, emphasizing the need for continued research to unravel the intricate mechanisms underlying the complex interplay between diabetes mellitus and respiratory system abnormalities.

Conclusion

The ultrastructural analysis of type I alveolocytes in experimental diabetes mellitus provides a comprehensive depiction of the evolving cellular responses over time. The observed changes unveiling unique aspects of diabetes-induced pulmonary alterations.

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ОСОБЛИВОСТІ ЗАЛУЧЕННЯ КЛІТИН РЕСПІРАТОРНОГО ВІДДІЛУ ЛЕГЕНЬ ПРИ СТРЕПТОЗОТОЦИН-ІНДУКОВАНОМУ ДІАБЕТІ: ФОКУС НА АЛЬВЕОЛОЦИТАХ І ТИПУ

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Вступ. Переконливі епідеміологічні дані підкреслюють підвищену поширеність респіраторних ускладнень серед осіб, які борються з діабетом. Це спостереження спонукає до ретельного дослідження патофізіологічних основ цього складного взаємозв'язку, яке полягає у вивченні від молекулярних тонкощів і до клінічних проявів.

Мета: дослідження патологічних змін в альвеолоцитах I типу у межах експериментальної моделі цукрового діабету.

Методи. У цій експериментальній моделі 88 самців щурів Вістар (170-210 г) були розділені на інтактну, контрольну та експериментальну групу, в яких діабет індукували внутрішньоочеревинним введенням стрептозотоцину (Sigma, США), розведеного в 0,1 М цитратному буфері з pH 4,5 при дозуванні 60 мг/кг маси тіла. Контрольна група отримувала еквівалентний об'єм 0,1 М цитратного буферного розчину з pH 4,5 шляхом внутрішньочеревної ін'єкції. Усі процедури проводили під наркозом за допомогою введення тіопенталу натрію у дозі 60 мг/кг маси тіла. Забір тканин здійснювали з інтервалами в 14, 28, 42 і 70 днів після ін'єкції стрептозотоцину. Для електронно-мікроскопічного аналізу використовували стандартну методику.

Результати. Протягом усього експерименту спостерігалися помітні зміни в альвеолоцитах I типу. Через 14 днів ядра мали округлу або овальну форму з рівномірним розподілом хроматину. Мітохондрії мали невеликий розмір, а апарат Гольджі (АГ) і гранульований ендоплазматичний ретикулум ГЕР не показали значних змін. До 28 дня ядра приймали овальну форму, хроматин локалізувався периферично, а мітохондрії демонстрували різноманітну морфологію. Збільшення мікропіноцитозних везикул свідчить про підвищену клітинну активність. Через 42 дні гіпергідратація стала вираженою, ядра показали нижчу електронну щільність, а клітинні компоненти показали прогресивні зміни. На 70 добу дистрофічно-деструктивні зміни включали ядра з низькою електронною щільністю, дезорганізовані мітохондрії, фрагментовані (АГ) і ГЕР.

Висновок. Проведений ультраструктурний аналіз розкриває прогресуючий вплив діабету на альвеолоцити I типу, з'ясовуючи унікальні аспекти легеневих змін через певний період часу.

Ключові слова: цукровий діабет, легені, альвеолоцити I типу, ультраструктурний аналіз, експериментальна модель.

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