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№ **49** (лютий, 2025)

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THERAPEUTIC POTENTIAL OF CONDITIONED MEDIA FROM MESENCHYMAL STEM CELLS IN REGULATING NO PRODUCTION IN KIDNEY DISEASE

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Background. Nitric oxide (NO) is a vital signaling molecule that plays an essential role in numerous physiological processes, including the regulation of blood pressure, immune response, and neurotransmission. It is widely recognized for its vasodilatory properties, which help in the relaxation of blood vessels, and it acts in concert with other gaseous molecules like hydrogen sulfide (H₂S) and carbon monoxide (CO). NO is synthesized from the amino acid L-arginine by a group of enzymes known as nitric oxide synthases (NOS). These enzymes catalyze the conversion of L-arginine into L-citrulline and NO, with three major isoforms identified: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). These isoforms are expressed in various tissues, including the kidneys, where they contribute to maintaining renal function by regulating blood flow, glomerular filtration, and interstitial pressure. NO production in the kidney is crucial for maintaining the balance of vasodilation and vasoconstriction, and it is produced by different kidney cell types, such as endothelial cells, mesangial cells, and podocytes. Podocyte injury is a hallmark of membranous nephropathy (MN), a condition characterized by the deposition of immune complexes beneath the glomerular visceral epithelial cells. The immune-mediated damage to podocytes leads to proteinuria and progressive kidney dysfunction. In recent years, there has been growing interest in the potential therapeutic use of cryopreserved biological agents (CfBA), such as conditioned media from mesenchymal stem cells (MSC-CM), as a potential treatment for autoimmune kidney diseases like MN. These agents are believed to have regenerative properties and may help modulate various biological pathways, including those involving NO [1, 2, 3, 4].

Objective. The aim of this study was to evaluate the impact of MSC-CM on the nitric oxide (NO)-ergic system in the kidneys of rats with autoimmune membranous nephropathy (AIN). Specifically, the study sought to determine how MSC-CM affects the activity of NOS isoforms and the levels of NO metabolites in both kidney tissues and the bloodstream, to assess its potential as a therapeutic agent for managing kidney diseases, particularly those involving immune-mediated glomerular injury.

Methods. The study was conducted using a rat model of autoimmune nephritis (AIN), which was induced according to the well-established Heymann W.R. method. A total of 42 male rats were included in the experiment. The treatments began on the 60th day of the experiment, with MSC-CM administered intramuscularly at 2-day intervals. This treatment protocol resulted in five injections, which were given on days 60, 62, 64, 66, and 68 of the study period. To assess the effect of MSC-CM on the NO-ergic system, the activity of NOS enzymes was measured spectrophotometrically by monitoring the oxidation of NADPH₂. The levels of stable NO metabolites in the blood were measured using the modified Griess reaction, a widely recognized and reliable assay for detecting nitric oxide-related compounds. The results from both the NOS activity assay and the NO metabolite measurements were used to evaluate how MSC-CM influenced NO synthesis and its associated physiological effects in the kidney [5].

Results. In the untreated rats with AIN, there was a significant imbalance in the activity of NOS isoforms within the kidney tissues. Notably, the activity of inducible NOS (iNOS) was found to be elevated by 83.3% compared to baseline levels ($p < 0.001$). This increase in iNOS activity correlated with a substantial rise in the concentration of stable NO metabolites in the bloodstream, which was 68.2% higher than the baseline levels ($p = 0.008$). This increase in NO metabolites was approximately 4.5 times higher than the increase in total NOS activity, suggesting a dysregulation in NO production in response to the autoimmune nephritis. The increased iNOS activity and elevated NO metabolite levels in the blood are indicative of an excessive inflammatory response that contributes to kidney damage in AIN.

Upon administration of MSC-CM, a significant reduction in iNOS activity was observed, suggesting that MSC-CM exerts a modulatory effect on the excessive activity of this inducible isoform. Furthermore, MSC-CM administration resulted in a marked increase in the activity of calcium-dependent NOS isoforms (nNOS and eNOS), which are typically associated with more regulated and constitutive NO production. This indicates that MSC-CM may help restore the balance between inducible and constitutive NOS isoforms, promoting a more controlled and homeostatic production of NO in the kidneys. This finding suggests that MSC-CM may have a beneficial role in alleviating the dysregulated NO production that is characteristic of autoimmune nephropathy.

Conclusions. MSC-CM was the most effective treatment in normalizing the excessive activity of iNOS in rats with AIN. Relative to untreated rats, MSC-CM reduced iNOS activity by 54.5% ($p < 0.001$), demonstrating a significant reduction in the hyperactive inflammatory response. In addition, MSC-CM had a remarkable ability to enhance the activity of the constitutional NOS isoforms, nNOS and eNOS, with an improvement of 57.9% compared to untreated rats ($p = 0.05$). These isoforms are essential for maintaining normal renal function and regulating vascular tone. Moreover, MSC-CM was associated with a significant reduction in the concentration

of stable NO metabolites in the blood, which decreased by 41.3% ($p=0.018$). These findings underscore the potential of MSC-CM as a promising therapeutic agent for restoring balance to the NO-ergic system in autoimmune nephropathy and other kidney diseases.

In conclusion, MSC-CM demonstrated the most substantial effects in normalizing NOS activity and NO production, outpacing the effects of other potential treatments. The ability of MSC-CM to regulate NO synthesis and improve the balance of NOS isoform activity suggests that it could serve as an effective therapeutic strategy for patients with autoimmune nephropathy, potentially improving kidney function and reducing inflammation.

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ТЕРАПЕВТИЧНИЙ ПОТЕНЦІАЛ КОНДИЦІОНОВАНОГО СЕРЕДОВИЩА МЕЗЕНХІМАЛЬНИХ СТОБУРОВИХ КЛІТИН У РЕГУЛЯЦІЇ ПРОДУКЦІЇ NO ПРИ ЗАХВОРЮВАННЯХ НИРОК

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