celular desencadeiam respostas inflamatórias mediadas por TLR4 e amplificadas por TREM-1. Isso resulta em aumento da permeabilidade vascular, recrutamento de neutrófilos e células mononucleares, e prejuízo na vasodilatação, promovendo morte celular por apoptose. Além disso, HMGB1 contribui significativamente para a inflamação e trombose na anemia falciforme, ativando inflamassoma NLRP3 em plaquetas. **Conclusão:** TREM-1 desempenha um papel crucial como um possível modulador da resposta imunológica em condições de inflamação crônica, sendo ativado por HMGB1. Tanto TREM-1 quanto HMGB1 estão significativamente elevados em estados inflamatórios crônicos e podem piorar clinicamente a AF, especialmente durante crises vaso-oclusivas. Como perspectivas futuras, estudos e ensaios clínicos que visem inibir/bloquear a via TREM-1, são necessários para confirmar esses achados e desenvolver novas estratégias terapêuticas promissoras na redução da inflamação e danos teciduais na AF.

https://doi.org/10.1016/j.htct.2024.09.090

DEVELOPMENT OF AN AUTOMATED FLUORESCENCE MICROSCOPY ASSAY FOR QUANTIFYING FACTORS AFFECTING RED BLOOD CELL (RBC) SICKLING IN SICKLE CELL DISEASE (SCD)

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Sickle cell disease is the most common monogenetic disease in the world, characterized by a point mutation in the sixth codon of the beta-globin gene, which generates mutant hemoglobin S (HbS). Under conditions of deoxygenation, HbS polymerizes, distorting the discoid morphology of an erythrocyte into a sickle-like shape, responsible for causing vaso-occlusive episodes associated with pain attacks, hemolytic anemia and early mortality. Although the main inhibitor of HbS polymerization is fetal hemoglobin (HbF), many additional factors can contribute to cell sickling, including extrinsic factors from the surrounding interacting cells in the microvasculature environment. However, the lack of straightforward functional assays to quantitate how these factor affect cell sickling, hamper a more profound understanding of SCD physiopathology. Therefore, our objective was to develop an automated fluorescence microscopy assay for quantifying factors affecting RBC Sickling in SCD, without the need of special incubators or microfluidic devices. In order to stablish a self-generated hypoxic environment, we cultured HS-5 stromal cells in 96-well plates at a low-density, and placed a small round (5 mm diameter) glass coverslip above the cell monolayer, thus limiting oxygen diffusion underneath the coverslip. Before placing the coverslips, erythrocytes from patients with different types of SCD (HbSS, HbSC and S-Beta Thalassemia) were fluorescently-labeled with DiD'Oil (red) and seeded along with HS-5 cells. A nuclear dye (Hoechst 33342, blue) and a hypoxia-activated fluorescent marker (Image-iT hypoxia, green) were added, thus allowing the identification of HS-5 cell nuclei and hypoxic regions. Immediately after coverslip placement (0h), and after 1h and 24h, a total of 25 sites from each well were acquired with a 20x objective, using transmitted-light and the three fluorescence channels, using an ImageXpress^{Micro} XLS High-Content-Screening-HCS system (Molecular Devices). Images were exported and analyzed using the open-source software CellProfiler, in order to delineate and segment RBCs. Shape-related morphometric features were extracted for each RBC (including, cell area, perimeter, form-factor, roundness and eccentricity), and used to classify and quantify different subpopulations of erythrocytes. Two form-factor thresholds (of 0.60 and 0.85) were used to classify RBCs into sickled (form-factor < 0.60), abnormally-shaped, and round cells (form-factor > 0.85). An eccentricity threshold of 0.60 was also used to separate round cells from elliptical/sickled cells (eccentricity > 0.60). Data analysis was carried using the open-source software Knime. In the three SCDs evaluated, the percentage of cells with eccentricity > 0.60 increased as a function of time, indicating that sickling occurred. In agreement, the percentage of cells with lower form-factors decreased with time. Importantly, the standard deviation of the percentages of RBCs in each morphological class, as calculated from three replica wells, were around 2.2%, for all three SCD patients and time-points evaluated. The use of advanced imaging tools and analysis software allowed a detailed and quantitative assessment of morphological changes in different hypoxic conditions. The 2D microscopy assay presented here is highly-reproducible and allows a quantitative analysis of the influence of different factors in the kinetics of RBC sickling, thus constituting an important tool to explore the pathophysiology of sickle cell disease ex vivo. **Support:** Grant #2022/12856-6, São Paulo Research Foundation (FAPESP).

https://doi.org/10.1016/j.htct.2024.09.091

IN VITRO EFFECTS OF HEME ON THE ACTIVATION OF MICROVASCULAR ENDOTHELIAL CELLS, HMEC-1

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Introduction: Sickle cell anemia (SCA) is a hereditary condition characterized by morphological changes in erythrocytes, which increase their fragility and susceptibility to rupture, releasing intracellular components, including the heme

molecule. Release of heme during intravascular hemolysis is closely associated with the vascular inflammatory response in this condition, contributing to the development of vaso-occlusive processes in the microcirculation. **Objective:** To evaluate the influence of heme on the activation of human microvascular endothelial cells, particularly with regard to the expression of surface adhesion molecules, the production of reactive oxygen species (ROS), and activation of caspase-1. **Materials and methods:** HMEC-1 (Human Microvascular Endothelial Cells) were used and incubated with varying concentrations of heme (0, 25, 50, and 100 μM) for 3 hours (37°C). Specific antibodies were used to assess the expression of adhesion molecules ICAM-1 (CD54), VCAM-1 (CD106), and E-selectin (CD62E) on the cells using flow cytometry. ROS production was measured using the 2,7-dichlorofluorescein diacetate (DCFH-DA) probe and caspase-1 activation was evaluated using the FAM-FLICA probe, and both were analyzed by flow cytometry. Data were analyzed using FlowJo, and statistical analysis was performed using ANOVA with Sidak's multiple comparisons post-tests, using Prism software. **Results:** Heme induced significant and dose-dependent increases in the expressions of ICAM-1 ([25 μM] $p = 0.011$; [50 μM] $p = 0.002$; [100 μM] $p = 0.007$, $N = 5$), VCAM-1 ([25 μM] $p = 0.023$; [50 μM] $p = 0.011$; [100 μM] $p = 0.02$, $N = 5$), and E-selectin ([25 μM] $p = 0.024$; [50 μM] $p = 0.011$; [100 μM] $p = 0.001$, $N = 5$) on the HMEC-1 cell surface. The evaluation of ROS production demonstrated a significant and progressive increase with increasing heme concentrations ([25 μM] $p < 0.001$; [50 μM] $p = 0.023$; [100 μM] $p = 0.020$, $N = 6$). Additionally, the analysis of caspase-1 activation revealed a significant and dose-dependent increase in response to heme concentration ([25 μM] $p = 0.023$; [50 μM] $p < 0.001$; [100 μM] $p = 0.015$, $N = 5$). **Discussion and conclusion:** The heme molecule was found to induce the expression of the adhesion molecules ICAM-1, VCAM-1, and E-selectin by HMEC-1 cells. Additionally, heme prompted oxidative stress, with the generation of ROS, and activation of caspase-1, indicating the involvement of the inflammasome in this process of cell activation. Similar results have been observed by our group, previously, in macrovascular Human Umbilical Vein Endothelial Cells (HUVEC). However, this study is novel in that we have investigated this effect specifically in microvascular HMEC-1 cells. These data suggest that heme, released during intravascular hemolysis and present in sickle cell anemia, may activate microvascular endothelial cells in small blood vessels such as venules, thereby contributing to the recruitment of circulating cells and the development of vaso-occlusive processes.

https://doi.org/10.1016/j.htct.2024.09.092

REVISÃO BIBLIOGRÁFICA DA EFICÁCIA DO MITAPIVAT NO TRATAMENTO DE ANEMIA HEMOLÍTICA, ANEMIA FALCIFORME E TALASSEMIA

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Objetivos: Investigar a eficácia terapêutica do Mitapivat, um fármaco ativador de primeira classe da enzima piruvato quinase (EPQ), que tem sido pesquisado como opção inovadora no tratamento de anemia hemolítica, doença falciforme e talassemia. **Material e métodos:** Revisão de literatura realizada em junho de 2024 utilizando a plataforma Pubmed. Foi adotado o período de busca de 2019 a 2024 com a localização de 51 artigos. Destes, 8 foram utilizados, atendendo aos critérios de serem disponíveis gratuitamente na íntegra e publicados em inglês. **Resultados:** No tratamento de pacientes com doença falciforme, com deficiência da EPQ eritrocitária e com talassemia, o Mitapivat aumentou o nível e a resposta de hemoglobina com consequente redução de hemólise e melhora no quadro de anemia, além de aumento na atividade hematopoiética em comparação aos pacientes que receberam placebo. Além disso, em pacientes com anemia falciforme o fármaco evitou a falcização das hemácias, com melhoria no quadro da doença. **Discussão:** O Mitapivat é uma pequena molécula ativadora da enzima piruvato quinase biodisponível por via oral. A ativação da EPQ específica para hemácias aumenta a síntese de adenosina trifosfato (ATP) para manter a homeostase energética, com integridade e deformabilidade da membrana plasmática, bem como reduz a produção de espécies reativas de oxigênio (EROs) e a concentração de 2,3-bifosfoglicerato (2,3-BPG). Nos pacientes com anemia hemolítica por deficiência na EPQ eritrocitária o aumento na síntese de ATP promovido pelo fármaco faz com que a hemácia tenha sua vida útil aumentada e, portanto, reduz hemólise e o quadro anêmico. Na doença falciforme, o fármaco, através da redução de 2,3-BPG, promove maior oxigenação o que consequentemente inibe a polimerização da Hemoglobina S e evita a falcização das hemácias, melhorando assim o quadro de anemia. Na talassemia, a redução na formação de EROs também é um fator prolongador da vida de hemácias, melhorando assim os sintomas anêmicos. Reações adversas, como dor de cabeça, insônia e náusea também foram relatadas pelos pacientes que receberam o fármaco, porém os efeitos não são graves, sendo de grau 1 ou grau 2. **Conclusão:** Os estudos analisados vem mostrando que o Mitapivat possui um bom perfil de segurança e forte eficácia clínica em um amplo espectro de anemias hemolíticas hereditárias, com baixo grau de efeitos adversos. Além disso, foi observado que doenças que requerem transfusões sanguíneas recorrentes têm a sua necessidade reduzida devido aos efeitos benéficos do Mitapivat, melhorando a qualidade de vida dos pacientes. Um diferencial desse medicamento é que ele também já mostrou resultados positivos no tratamento de pacientes com alfa-talassemia, doença que apresenta poucas opções terapêuticas, quando comparado à beta-talassemia. Por fim, embora alguns desses estudos ainda necessitam de mais testes, o Mitapivat demonstra ter potencial significativo de aumentar os níveis de hemoglobina nos pacientes e por consequência melhorar os sintomas de anemias hemolíticas hereditárias.

https://doi.org/10.1016/j.htct.2024.09.093