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Determining the effect of sterols and polyunsaturated fatty acids on the magnitude of dipole potential with a novel high-throughput flow cytometric method

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Introduction: Dipole potential (DP) is an intramembrane potential with a magnitude of around 300 mV, which greatly affects the function and conformation of transmembrane proteins. The magnitude of the DP is mainly determined by the lipid composition and particularly the sterol concentration of the membrane. Thus, diseases characterized by elevated cholesterol or 7-dehydrocholesterol (7DHC) levels, such as hypercholesterolemia (HC) or Smith-Lemli-Opitz syndrome (SLOS), can be associated with elevated DP, which may play a role in their pathophysiology.

Aim of the study: In such cases decreasing the DP could be favourable, however, no known physiological method to lower DP has been described. Measurement of the DP, especially in living cells, is difficult, yet we were able to develop a novel flow cytometric method to study the DP in models of such diseases and also in clinical specimens.

Methods: We have measured the magnitude of DP in membrane-biophysical models of HC and SLOS with our novel emission ratiometric (Rem) approach using the environment sensitive fluorophore F66 and flow cytometry. The models were created in JY lymphocytoid, THP-1 monocytoid and HEK-293-T human embryonal kidney cell lines treated with methyl-beta-cyclodextrin/sterol complexes. The effects of omega-3 (alpha-linolenic acid), omega-6 (gamma-linolenic acid) fatty acids and stearic acid on DP were determined in disease models using our novel method.

Results: In models of HC and SLOS loading the cells with cholesterol and 7DHC resulted in dose-dependent increases in DP ($\Delta\text{Rem}_{\text{max}}$ -14%, and -8%, respectively). Demonstrating the importance of a diet rich in polyunsaturated fatty acids, ω -3 fatty acid decreased, while ω -6 or saturated fatty acid increased the magnitude of DP ($\Delta\text{Rem}_{\text{max}}$ +10%, and -5%, respectively). In combined treatments ω -3 fatty acid attenuated, while ω -6 fatty acid amplified the effects of cholesterol on DP.

Conclusion: The results of our novel flow cytometric method confirmed the positive effect of ω -3 fatty acid in reducing the adverse membrane biophysical alterations observed in HC. In our ongoing studies, we are determining alterations in the DP in samples from hypercholesterolemic and hypertriglyceridemic patients compared to control individuals.

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