Abstract

Traumatic brain injury (TBI) poses a significant socioeconomic challenge. Consequently, there is an intensive search for therapies that could minimize the long-term sequelae of TBI. One proposed approach is the application of the ketogenic diet (KD), which finds clinical use in the management of drug-resistant epilepsy. Therapeutic efficacy in TBI may depend on numerous factors, including sex and the timing of intervention. Therefore, this study investigated the impact of KD and the sex of the subjects on glial scar formation following TBI.

Beyond therapeutic strategies, it is crucial to develop and explore novel diagnostic tools that enable rapid and precise assessment of TBI-associated changes, including the identification of disease markers and alterations induced by KD administration. This study evaluated the potential of instrumental atomic and molecular spectroscopy methods as tools for investigating glial scar formation. Analysis was performed on samples from experimental animals, comprising brain sections and mineralizates of selected internal organs. The research employed Fourier transform infrared microspectroscopy (FTIRM), Raman spectroscopy (RS), total reflection X-ray fluorescence (TXRF), and synchrotron radiation X-ray fluorescence (SR-XRF).

A primary objective was to identify molecules and elements whose tissue accumulation changes due to TBI and to determine how KD modifies these alterations depending on the sex of the subjects. FTIRM analyses demonstrated that glial scar development induces distinct changes in the biomolecular composition of the cerebral cortex. A decrease was observed in the content of compounds containing phosphate groups, cholesterol and its esters, compounds containing carbonyl groups, and lipids, particularly unsaturated ones. Conversely, regarding protein secondary structure, an increase in the relative content of β-sheet structures was observed during the early stage of glial scar formation. Data obtained via RS proved valuable in assessing the impact of KD on cortical regions not directly involved in the primary injury. KD effects were particularly pronounced in females, who exhibited increased cytochrome C/DNA ratios, lipid esterification, amide III levels, and lipid unsaturation degrees; similar, albeit less intense, changes were also observed in males. Brain section analyses were also conducted using SR-XRF, allowing for a detailed temporal assessment of glial scar physiology. The most significant observations concerned changes in the distribution of elements such as calcium, iron, and copper. However, no significant modifying effect of KD was found in this regard. Given the potentially pleiotropic effects of KD, the diet's impact on internal organs was also evaluated. The liver, kidneys, and spleen were analysed, and their mineralizates were examined using TXRF. The results confirmed the burdening effect of KD on internal organs; the kidneys appeared particularly sensitive, showing significant deviations in calcium and phosphorus levels compared to the control group.

The application of spectroscopic methods provided new insights into the systemic response of rats to KD under TBI conditions. Each of the employed spectroscopic techniques proved useful in characterizing the profile of observed changes. FTIRM enabled the precise delineation of the injury boundaries. The impact of the ketogenic diet on TBI progression and the molecular composition of the cerebral cortex was elucidated using RS. Meanwhile, TXRF provided information regarding changes in the elemental composition of internal organs. Finally, SR-XRF analyses highlighted the dynamics of elemental accumulation within the forming glial scar, contributing to a better understanding of the post-traumatic glial response.