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THE INFLUENCE OF GLUCOSE CONCENTRATION ON THE ANTI-INFLAMMATORY PROPERTIES OF RESVERATROL IN ENDOTHELIAL CELLS AND ASTROCYTES - *IN VITRO* STUDIES ON THE BLOOD-BRAIN BARRIER MODEL [SUMMARY]

Due to the prevalence of diseases resulting from the obesity pandemic, including type 2 diabetes, as well as in individuals post-heart attack or stroke, abnormal blood glucose levels are increasingly observed, encompassing both hyper- and hypoglycemic states. This should be considered when evaluating the pharmacokinetics of drugs crossing the blood-brain barrier (BBB).

Chronic hyperglycemia can lead to increased production of reactive oxygen species (ROS) in astrocytes and endothelial cells. An excess of free radical species can activate the transcription factor NF-κB, which in turn stimulates the inflammatory response and increases the permeability of the BBB, among other things for glucose. Additionally, advanced glycation end products (AGEs), which appear as a result of chronic hyperglycemia, can stimulate the immune system to produce pro-inflammatory cytokines.

In the physiology of the central nervous system (CNS), the BBB plays a significant role, protecting the brain from harmful factors and enabling selective transport of substances from the blood to the cerebrospinal fluid. The ability to penetrate this barrier is influenced by many factors, such as the level of serum glycemia.

Inflammatory state emerging within the CNS due to sustained hyperglycemia can directly damage neurons, thus adversely affecting brain function. Diabetic encephalopathy, as it develops, poses a significant challenge for millions of individuals worldwide. Consequently, effective methods to mitigate the inflammatory response within the CNS are being sought.

It has been demonstrated that resveratrol (RSV) – a natural substance present, among others, in the skin of red grapes, nuts, mulberries, and black currants- possesses anti-inflammatory properties and, moreover, the ability to penetrate the BBB. These characteristics render RSV potentially suitable for application in anti-inflammatory therapy within the CNS.

The aim of the study was to assess the impact of hypo-, normo- and hyperglycemic states in the space corresponding to cerebral microcirculation vessels (MC- microvascular compartment) on the permeability of the BBB and the anti-inflammatory action of RSV at the brain level (BC – brain compartment). It seems significant in itself to assess whether glycemic fluctuations significantly disturb the penetration of substances with anti-inflammatory activity with a molar mass (M) of about 230 g/mol (Da) through the BBB, such as RSV (*trans*-RSV isomer, M = 228,25 Da), and whether they modify their action within astrocytes.

Data from the literature regarding the action of RSV in systems other than the BBB, such as the blood-placental barrier, suggest that optimal conditions for anti-inflammatory activity are represented by normoglycemia, while metabolic disturbances accompanying hyperglycemia disrupt it. The study described in the doctoral dissertation also evaluated how this situation presents in hypoglycemia.

A specially created model was used to conduct the experiment, simulating *in vitro* conditions on both sides of the BBB. This model consisted of two main compartments: the microcirculation (MC), whose interface with the BBB was covered with endothelial cells, and the brain compartment (BC), whose interface with the BBB was formed by astrocytes. The BBB model included cocultures of endothelial cells and astrocytes, which were separated by a semipermeable membrane with pores of 0.4 μm in diameter, preventing direct contact between the different cell types.

Three experimental groups were created: Group I simulating hypoglycemic conditions, Group II simulating normoglycemic conditions, and Group III simulating hyperglycemic conditions, with glucose concentrations of 40 mg% [2.2 mmol/L], 90 mg% [5.00 mmol/L], and 450 mg% [25 mmol/L], respectively. After 24 h of cell incubation under the specified glucose concentrations, measurements were taken for the concentrations of cytokines IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, INF-γ, TNF-α, and GM-CSF. It was also assessed whether the glucose concentration in MC alone affected differences in pro-inflammatory cytokine levels within BC. Subsequently, to induce an inflammatory response in a standardized manner, LPS was administered to BC at a concentration of 0.2 μM, and after additional 24 h, RSV was administered to MC at a concentration of 50 μM.

The assessment of the severity of the inflammatory state included an analysis of cytokine levels at both 12 and 36 h after LPS administration, followed by 24 h after RSV administration. Additionally, the concentration of RSV on the BC side was examined, allowing for the determination of the degree of RSV penetration through the BBB. All the above assays were conducted using the enzyme-linked immunosorbent assay method (ELISA) with kits compatible with human cells, according to the manufacturers' instructions. Moreover, the morphology of cells in co-culture was evaluated both before and after staining with hematoxylin and eosin.

RESULTS

In comparison to normoglycemia, the group with reduced glucose concentrations in the MC exhibited higher levels of pro-inflammatory cytokines in the BC. A similar association was observed in the hyperglycemic group, where greater cytokine concentrations were noted compared to Group II (normoglycemia). The cytokine profile varied depending on the study group, as detailed in publications No. 2 and 3, with statistical analysis taken into account.

Research findings confirm the permeation capability of BBB and the anti-inflammatory action of RSV in BC, manifested by the reduction of pro-inflammatory cytokine levels. Despite the decrease in cytokine concentrations across all groups, the most pronounced anti-inflammatory effect was observed in group II, indicating that normoglycemia provides an optimal environment for the anti-inflammatory actions of RSV ($p<0.05$). Moreover, the concentration of RSV in the BC was determined, both 12 and 24 h after its introduction into the MC in the three experimental groups. The highest concentration of RSV was detected in the environment with normal glucose concentration in the MC, and the lowest in the environment with hypoglycemia.

The above results indicate that maintaining proper glycemic control may be significant for the effectiveness of anti-inflammatory agents such as RSV. This may provide a basis for adjusting the dosage of RSV in individuals with diabetes as well as those who have experienced a stroke or heart attack, in cases of hypo- and/or hyperglycemia. Adjunctive therapy with RSV (as well as other substances with similar BBB permeating properties) is already being used in neurodegenerative diseases, but until now, the anti-inflammatory efficacy of such therapy has not been associated with glycemic disturbances, which are common in these neurological patients.