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Basic nutritional investigation

Dietary fats significantly influence the survival of penumbral neurons in a rat model of chronic ischemic by modifying lipid mediators, inflammatory biomarkers, NOS production, and redox-dependent apoptotic signals

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ABSTRACT

Objective: Brain stroke is the third most important cause of death in developed countries. We studied the effect of different dietary lipids on the outcome of a permanent ischemic stroke rat model.
Methods: Wistar rats were fed diets containing 7% commercial oils (S, soybean; O, olive; C, coconut; G, grape seed) for 35 d. Stroke was induced by permanent middle cerebral artery occlusion. Coronal slices from ischemic brains and sham-operated animals were supravitally stained. Penumbra and core volumes were calculated by image digitalization after 24, 48, and 72 h poststroke. Homogenates and mitochondrial fractions were prepared from different zones and analyzed by redox status, inflammatory markers, ceramide, and arachidonate content, phospholipase A2, NOS, and proteases.
Results: Soybean (S) and G diets were mainly prooxidative and proinflammatory by increasing the liberation of arachidonate and its transformation into prostaglandins. O was protective in terms of redox homeostatic balance, minor increases in lipid and protein damage, conservation of reduced glutathione, protective activation of NOS in penumbra, and net ratio of anti-to proinflammatory cytokines. Apoptosis (caspase-3, milli- and microcalpains) was less activated by O than by any other diet.
Conclusion: Dietary lipids modulate NOS and PLA2 activities, ceramide production, and glutathione import into the mitochondrial matrix, finally determining the activation of the two main protease systems involved in programmed cell death. Olive oil appears to be a biological source for the isolation of protective agents that block the expansion of brain core at the expense of penumbral neurons.

Introduction

Brain stroke is the third leading cause of death in developed countries, behind cancer and heart disease [1]. Cerebral ischemia generally results in immense distress and residual impairments to patients, and is viewed as one of the leading causes of

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decreased quality of life. In humans, ischemic stroke approximately accounts for 90% of all strokes and specially affects the territory of the middle cerebral artery [2]. Cerebral stroke is one of the diseases primarily linked to nutritional factors [1].

Occlusion of the middle cerebral artery (MCAO) is the model most used worldwide experimental to induce stroke in rats, and it is has been proven as an effective and reproducible tool for the investigation of neuroprotective drugs as it closely resembles stroke injury in human patients [2]. Cerebral ischemia has been considered as untreatable pathology with no effective therapeutic protocols [1]. Human ischemic stroke is heterogeneous in its manifestations, causes, and anatomic sites of occurrence. Consequently, a wide variety of animal models have been developed to assess stroke-related pathologies to find better approaches to the study of neuronal injury, especially the recruitment of neurons from the surrounding tissues (penumbral zone or region) into ischemic core [1]. So far, it is known that there is a relatively short window of opportunity during which a population of penumbral neurons remains viable for a variable number of hours following stroke onset [3]. Thus, penumbra is defined as a moderately hypoperfused region that retains structural integrity but has lost function [4,5]. Even with the implementation of therapeutic interventions this penumbra area will die and become recruited into the core ischemic zone [5]. Only 2% of stroke patients receive tissue plasminogen activator as thrombolytic agent [6]. The lack of effective therapeutic agents establishes a high unmet medical need for the development of stroke preventive strategies [7].

A number of previous studies have demonstrated that oxidative stress [8–14] and inflammation [15–17] play a crucial role in the pathogenesis of brain stroke [8,12,13]. These factors strongly influence many other signals that converge into the regulation of pro-apoptotic cascade [8,10,12,13], in which phospholipase A2 [18], production of prostaglandins and cytokines [15–17], activation of nitric oxide synthethase activity [19–22], and ceramide overproduction [23,24] are the most crucial events affecting the final result.

Previous statistical evidence and experimental results clearly demonstrated that the quality and quantity of dietary fats and fatty acids significantly influence the incidence, prevalence, and outcome of brain stroke and ischemic heart disease [25,26]. Fatty acids may modulate inflammatory response [27,28], modify the antioxidant status of many tissues [29–31], and regulate ceramide generation [32]. In addition, several studies have confirmed that other components of dietary fats such as polyphenols play an important role in the prevention of stroke and cardiovascular illnesses or they attenuate the detrimental effects caused by ischemia [33–41].

Based on previous evidence, we decided to investigate in detail the influence of different types of commercial oils as dietary lipid source in relation to the outcome of ischemic stroke in a MCAO rat model from 24 to 72 h poststroke by determining: 1) the redox status (antioxidant enzymes and levels of lipid-soluble and water-soluble antioxidants), 2) the inflammatory response (prostaglandin and cytokine production), 3) activity of phospholipase A2 and nitric oxide synthetase isoforms, 4) production of ceramide, and 5) activation of cellular death cascades (caspase-3 and milli- and microcalpains) to contribute with experimental evidence that supports nutritional recommendation for the prevention of damages produced by brain stroke.

Materials and methods

Chemicals

All chemicals used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany), or Carlo Erba (Milan, Italy). Dietary commercial oils were from Molinos Río de La Plata SAIC and Platafarm SA (La Plata, Argentina).

Animals and treatments

Certified pathogen-free male Wistar rats were used. The rats were maintained under controlled temperature conditions (25°C) with relative humidity of 60%, forced ventilation and a normal photoperiod of 12 h darkness and 12 h light. Animals were handled in accordance with the internationally recommended practices of the ILAR (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council) and the National Institutes of Health regulations [42]. Solid food and drinking water were provided ad libitum. The experimental diets were prepared in our laboratory according to the recommendations for Wistar rats [43]. The experimental protocol was reviewed and approved by the Bioethics Committee of the School of Medical Sciences; UNLP (COBIMED, protocol code # 00382/11). Male Wistar pups (three weeks old, weight 48 ± 3 g) were used. Animals were ramdomly asigned to four experimental groups (12 animals for each experiment) and after weaning were fed on specific diets for 35 d. The diets used were supplemented with a different oil as lipid source: soybean (S), olive (O), cocconut (C), or grape-seed (G). During the feeding period clinical examination, body weight, foot intake, and water consumption were controlled daily [44]. All isocaloric diets were prepared in identical manner with the addition of one of the different commercial oils (70 g/kg diet) as detailed previously [30,31]. General composition and fatty acid composition of the diets were reported in detail in previous papers [30,31].

Surgical procedure

To avoid individual differences among animals, the experiments were run under equivalent conditions. On day 35 all rats fasted overnight with free access to water, and the surgical procedure was implemented at 0800 h. Anesthesia was conducted as recomended elsewhere [45]. Feedback-controlled heating lamp and pad were used to maintain animal temperature at 37°C. Stroke was induced by permanent middle cerebral artery occlusion model (MCAO) following the procedure of Longa et al. [46]. None of the animals experimented subarachnoid hemorraghe. This rat model of permanent ischemia is the most commonly used due to many reasons, including up to two-week survival poststroke, high reproducibility (involving frontoparietal cortex and lateral caudoputamen) and its resemblance to humans in cerebrovascular anatomy and physiology [12,41,47]. Transient occlusion is considered inappropriate for the replication of spontaneous or thromboslysis-induced stroke [2,48].

Obtaining samples

We performed different sets of experiments. In one type, after various times poststroke (24, 48, and 72 h) rats were sacrificed by rapid decapitation without the use of anesthesia. To obtain plasma samples, blood was collected into heparinized (10 mUI/mL) sterile polystyrene test tubes and centrifuged at 2°C, 1000 g for 15 min. The brains were excised, cleaned, weighed and frozen for 5 min at –20°C. Coronal slices of 1 mm were performed beginning at 2 mm from the anterior tip of the frontal lobe. Sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTZ) at 37°C for 20 min with gentle orbital agitation. Slices were photographed and analyzed using the Software Erdas Image v8.0 (Infosat Geomática, Buenos Aires, Argentina) that can digitalize and quantify infarct area, penumbra, and undamage hemisphere (contralateral). After digitalization was complete, image analysis was compiled to obtain stroke core, penumbral zone and undamaged tissue volumes in mm^3. Sham-operated animals were also run as reference. In all cases, the values obtained were corrected by edema using the following fomula [8]:

$$\frac{[\text{total infarct volume} - (\text{volume of intact ipsilateral hemisphere} - \text{volume of intact contralateral hemipshere})]}{(\text{contralateral hemisphere volume})} \times 100$$