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Intracerebral injection of oil cyst content of human craniopharyngioma (oil machinery fluid) as a toxic model in the rat brain

Martha Lilia Tena-Suck[a,b], Ma. Elena Hernández-Campos[a], Alma Ortiz-Plata[c], Citlaltepetl Salinas-Lara[b], Ana Laura Colín-González[d], Abel Santamaría[d,*]

[a] Sección de Estudios de Posgrado e Investigación de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City, Mexico
[b] Departamento de Neuropatología, Instituto Nacional de Neurología y Neurocirugía. Mexico City, Mexico
[c] Laboratorio de Neuropatología Experimental, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico
[d] Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico

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ABSTRACT

Craniopharyngiomas (CPs) are benign epithelial cystic tumors of the sellar and suprasellar region with a high survival rate and high recurrence in children. CPs contain dense oily fluid, but little is known yet about this content and its contribution to tissue damage and tumoral growth. In this study, we developed a simple experimental model produced by intracortical injection to rats of the cyst fluid content collected from human CPs to explore its possible contribution to brain tissue damage. The cyst fluid of the CPs ("oil machinery fluid") was collected during surgical removal, briefly preserved and further tested in rats through intracortical infusion. The group receiving "oil machinery fluid" presented increased reactive oxygen species formation, oxidative damage to lipids and reactive gliosis accompanied by augmented immunoreactivity to peroxiredoxin and thioredoxin reductase 1 at 15, 30 and 45 days post-injection. Other markers of inflammation and cell damage were stimulated at all post-lesion days tested. There was also a body weight gain. The persistence of tissue damage and oxidative stress suggests that "oil machinery fluid" exerts progressive alterations similar to those observed in patients with CPs, supporting the concept that some components of cyst fluid may contribute to brain tissue damage in these patients.

Introduction

Craniopharyngiomas (CPs) account for 2–5% of all primary intracranial tumors. Despite their benign histological appearance, they are often associated with an unfavorable prognosis and their optimal treatment remains controversial. This type is the most common non-neuroepithelial brain tumor, which is a histologically benign, but still aggressive, pituitary tumor accounting for up to 10% of pediatric intracranial tumor cases. CPs are tumors associated with significant morbidity; however, gross total surgical removal provides favorable results in terms of recurrences. If resection cannot be achieved safely, adjuvant radiotherapy is beneficial in preventing tumor re-growth (Kiehna and Merchant, 2010). Most patients who have been treated for CPs are growth hormone (GH) deficient (GHD). GH replacement therapy (GHRT) may stimulate tumor re-growth, and one of the concerns about long-term GHRT is the risk of subsequent tumor progression (Olsson et al., 2012). Noteworthy, patients with CPs located in the pituitary and/or hypothalamus are susceptible of developing obesity and many metabolic complications. These features include excessive weight gain due to increased adiposity, increased food intake, and pronounced hyperinsulinemia and hyperleptinemia (Roth et al., 2011).

The pathophysiogenic mechanisms underlying hypothalamic obesity are complex and multifactorial. For instance, children after surgical removal of CPs are at risk of rapid weight gain and the development of metabolic syndrome. Further studies need to be conducted to improve our understanding of the mechanisms of

Abbreviations: α-MSH, alpha-melanocyte stimulating hormone; CMHL, combined medial hypothalamic lesion; e-NOS, endothelial nitric oxide synthase; GFAP, glial fibrillary acidic protein; GH, growth hormone; GHD, growth hormone deficient; GHRT, replacement therapy; HIF-1α, hypoxia-inducible factor 1-alpha; MDA, malondialdehyde; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2 transcription factor; OF, oil machinery fluid; PBS, phosphate-buffered saline; CPs, craniopharyngiomas; ROS, reactive oxygen species; RNS, reactive nitrogen species; S.E.M., standard error of mean; TrxR1, thioredoxin reductase 1; TNF-α, tumor necrosis factor alpha; TBARS, thiobarbituric acid-reactive substances.

* Corresponding author at: Laboratorio de Aminoácidos Excitadores, Av. Insurgentes Sur 3877, Instituto Nacional de Neurología y Neurocirugía, México D.F. 14269, México.
E-mail address: absada@yahoo.com (A. Santamaría).

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these events in order to design improved effective treatment and prevention. In the meantime, the estimation of free radical formation and malondialdehyde concentrations in cyst fluid ("oil machinery fluid") of CPs seems to be prognostic for the tumor recurrence (Arefyeva et al., 2001). This finding suggests that "oil machinery fluid" might somehow contribute to adiposity and tissue damage throughout oxidative stress, although the chemical composition of this fluid remains unknown.

Unfortunately, rodent models that mimic the metabolic sequel of obese craniopharyngioma patients are still scarce. There is a growing need to develop experimental models to determine the cellular and molecular mechanisms participating in this disorder. One such attempt involved a rodent model developed to mimic the complex neuroanatomical and metabolic disturbances commonly seen in these patients (Roth et al., 2011). In that report, authors compared the metabolic phenotype of animals subjected to three distinct types of hypothalamic lesions. However, only the one produced by the combined medial hypothalamic lesion (CMHL) affecting the ventromedial, dorsomedial and arcuate nuclei exhibited all key features observed in obese patients with the presence of CPs. These observed features included excessive weight gain linked to adiposity, increased food intake, and pronounced hyperinsulinemia and hyperleptinemia, all accompanied by reduced plasma levels of alpha-melanocyte stimulating hormone (α-MSH) and reduced ambulatory activity. The authors concluded that this model best mimics the complex metabolic abnormalities observed in obese CP patients through complex hypothalamic damage. However, the possible contribution of CP cyst fluid was not studied. Since this fluid contains pro-oxidant components and several unknown proteins and factors potentially contributing to cell damage, and invades the tissue after removal and/or rupture of CPs, we believe that the fluid itself should be considered for detailed toxicological studies to characterize its physiopathological profiles. The aim of this study was to investigate whether human "oil machinery fluid" obtained during surgical removal of CPs plays a toxic role in the normal rat brain through the development of a simple toxic experimental model. We determined biochemical markers of oxidative stress and immunoreactivity against some proteins involved in redox activity and inflammatory responses in the brain of rats infused with this fluid as a simple approach to an initial basic characterization of a possible toxic role of cyst fluid content.

Materials and methods

CPs surgical removal and cyst fluid collection

The cyst oil fluid ("oil machinery fluid") of CPs was obtained during surgery of patients during tumor resection, and then transferred for application to animals. The fluid content was collected and pooled from 6 patients diagnosed with CPs (4 females and 2 males), ranging from 18 to 50 years of age. All patients had approved surgical removal of CPs in the Instituto Nacional de Neurología y Neurocirugía. Once removed, cysts were drained with surgical syringes and liquids were then collected and immediately frozen and pooled in one single source (3–5 ml collected as total volume from each drain).

Animals

This study protocol was approved by the Ethical Committee of Experimental Animals of the Instituto Nacional de Neurología y Neurocirugía (INNN, Mexico) and followed criteria of the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (2003). The protocol also conformed to local and international guidelines regarding the ethical use of animals, and all efforts were made to minimize the number of animals used and their suffering. Twelve week-old male Wistar rats (weighing about 260 g) were obtained from the Animal Vivarium of the INNN, and maintained under controlled environmental conditions of temperature ($25 \pm 1\,^{\circ}C$), relative humidity (50–60%) and lighting (12:12 light–dark cycles). Rats had free access to water and food during the investigation.

Experimental groups

After a week of acclimation and feeding, rats ($N = 325$) were digitally randomized and divided into different experimental groups that included non-lesioned rats, sham and animals injected with CPs cyst fluid. In a more detailed description, Group 1 represented the non-lesioned animals (for comparative purposes only, but it was not shown because it revealed no major differences when compared with Sham group) and comprised a total of 25 animals (10 for biochemical analyses and 15 more for histological and histochemical experiments). In Group 2 (Sham), 15 animals were used for the estimation of lipid peroxidation and reactive oxygen species (ROS) formation (5 rats per time tested), and 135 more for histological and histochemical analyses (5 for each immunoreactivity assay per time tested). The same number of animals was used for biochemical analyses ($n = 15$) and histological and histochemical assays ($n = 135$) in Group 3, representing those animals receiving the oil machinery fluid (OF). All groups were weighed before surgery, briefly anesthetized with chloroform, and euthanized by quick decapitation.

Surgical procedures and schedules

Stereotactic surgeries were carried out following stereotactic coordinates from a stereotaxic atlas (Paxinos and Watson, 1998). Under anesthesia (sodium pentobarbital, 50 mg/kg i.p.) and aseptic conditions, rats underwent a stereotaxic craniotomy (3 mm) centered over the following coordinates with reference to Bregma: anterior/posterior −3.0 mm, medial/lateral 6.0 mm. All rats were subjected to stereotactic surgery and infused with 5 μl of liquid (saline solution or CPs cyst fluid) in the cortex (fronto-parietal left hemisphere). The right fronto-parietal lobe was considered as control for each group. Rats were euthanized at different times after stereotactic surgery (15, 30 and 45 days) in order to evaluate time-course effects. The times for evaluation of all these changes were strictly chosen on the basis of the establishment of a parallelism with the development of alterations of CPs and the observed neurodegeneration in patients through a wide chronology. In addition, the selection of the lesioned brain area (cortex) was made to demonstrate that cyst fluid is capable of exerting toxic features in a remote area from that typically localized by CPs (ventromedial, dorsomedial and arcuate nuclei). Consequently, this first approach is not precisely designed to produce an accurate model of CPs, but mainly to investigate the toxic properties of cyst fluid.

For histological examination, animals of each group were anesthetized (2.5 g/kg urethane) and perfused transcardially with saline, followed by 4% paraformaldehyde in PBS (pH 7.2–7.4). The left fronto-temporal lobe was removed and immediately ice-cooled to the temperature needed for the reactive ROS assay. After a brief period (5 min), the brain tissue was submitted to successive ethanol washes (dehydration by alcohol from 70% to 100% and further xylene bath) for dehydration and then embedded in paraffin. Gross coronal sections (from 3 mm anterior to 6 mm posterior to the lesion site) were first serially obtained with a brain matrix coronal cutter (Electron Microscopy Sciences, Hatfield, PA, USA); then, sections (4 μm thick) were cut with a microtome. The paraffin-embedded sections of perfused/fixed brains were stained with hematoxylin and eosin for histological analysis under light