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Strokes and traumatic spinal cord and brain injury

DAMAGE TO THE BLOOD-BRAIN BARRIER IN SUBARACHNOID HAEMORRHAGE: CLINICAL RELEVANCE, ROLE OF HYPERGLYCAEMIA AND EFFECT OF POTENTIATION OF ENDOGENOUS ANTIOXIDANT MECHANISMS. A TRANSLATIONAL STUDY

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1. Summary of the project

Spontaneous aneurysmal subarachnoid hemorrhage (SAH) is a devastating cerebrovascular disorder. SAH triggers a complex sequence of deleterious mechanisms that result in blood–brain barrier disruption (BBBd), with an essential role of hyperglycemia and oxidative stress. However, the relevance and mediators of early BBBd in SAH is poorly understood.

In this context we hypothesized that subjects with spontaneous aneurysmal SAH will present an early and diffuse alteration of BBB permeability that could be imaged through dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and that BBB disruption would be correlated with the release of circulating and CSF markers of BBB damage and associated with an increased severity of the bleeding. In addition, we anticipated that acute BBBd would be associated with early and delayed brain ischemic injury, diffuse parenchymal microstructural damage, and disturbed brain structural connectivity at long-term as well as poorer clinical outcome at 3 months. Moreover, we hypothesized that acute hyperglycemia and increased oxidative stress would be relevant mediators of BBBd both in humans and in a preclinical model of the disease, where the modulation of these deleterious mechanisms would lead to less brain injury and better neurological outcome. According to these hypotheses, the specific objectives of the study were: to evaluate the presence of early BBBd in SAH subjects through dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI); to evaluate the prognostic relevance of acute BBBd; to define the role of glucose-driven oxidative stress in the promotion of BBBd; and finally, to evaluate the effects of the administration of BBB-targeted antioxidants in a preclinical model of SAH through a theranostic approach under normoglycemia and hyperglycemia conditions.

In order to reach these objectives, we designed a translational study of the dynamics and interrelationships between hyperglycemia, oxidative stress and BBBd in acute SAH in humans and in a preclinical model. In humans, DCE-MRI BBB permeability was assessed longitudinally in a prospective cohort of 70 SAH subjects to evaluate its association with radiological and clinical prognostic variables at short and long term, with special focus on

ascertaining the effect of hyperglycemia and oxidative stress. In parallel, a preclinical model of experimental SAH in rats was developed and characterized through the longitudinal evaluation of BBBd (DCE-MRI), oxidative stress brain hallmarks (positron emission tomography and high field MRI imaging) and neurological function both in hyperglycemic and normoglycemic conditions. In this model, glucose-driven oxidative stress related to BBB injury was modulated through the administration of BBB-targeted antioxidants (uric acid).

2. Results

Clinical study

Patient recruitment began on 1 October 2018 and finished on 31 July 2021. At the end of the project, a total of 70 patients with adequate MRI data were successfully recruited and analyzed. Thus, in the final analysis we included a prospective cohort of 70 SAH patients evaluated longitudinally through DCE-MRI within 72+/-48h (T1) and at 3 months (T90) after clinical onset. Permeability maps were used to obtain the values of K-trans as a biomarker of increased BBB in the whole brain, in the grey and white matter and in arterial circulation defined territories. Poor clinical outcome was defined as a modified Rankin scale score >2 at 90 days. The association between K-trans values and clinical outcome was assessed with univariate and multivariate regression models adjusted for age, WFNS and mFisher grades at admission and aneurysm size. Of the included population, 63% were females, 70% had a WFNS I-III, 61% a modified-Fisher 4 and the median age was 55 years old. K-trans values were significantly higher at T1 in comparison with T90 in the whole brain, grey/white matter and across all arterial territories ($p<0.005$ for all comparisons). In adjusted models, elevated K-trans values were significantly associated with poor outcome [adjusted-OR (per 10% of unit increase) = 2.16, 95% CI=1.30-3.58, $p=0.003$]. According to these observations we concluded that SAH induces a diffuse increase in BBB permeability that can be assessed through DCE-MRI and that this increase is associated with poor clinical outcome, thus supporting the role of BBB disruption as a potential target for vasculoprotective therapies in SAH. In this cohort, we did not find an association between elevated K-trans values and glucose levels during the

first 72 hours after bleeding onset. However, in this sample of patients higher glucose was associated with poor clinical outcome independently of confounders in adjusted models and this association was mediated (31%) by neuroaxonal injury evaluated at 72 hours through the analysis of neurofilament light chain (NFL) circulating levels and of MRI derived diffusion tensor indices (specifically, fractional anisotropy in the white matter). No association was found between DCE-MRI derived K-trans values or glucose levels with circulating concentrations of matrix metalloproteases or markers of oxidative stress (uric acid).

Preclinical study

The analysis of the effect of hyperglycemia following experimental SAH was evaluated in a preclinical model in rat. A total of 80 rats were subjected to mild hyperglycemia (N=120), severe hyperglycemia (N=28) and normoglycemia (N=94) through the administration of dextrose 30 minutes before the SAH onset. Both mild and severe hyperglycemic rats showed a significant increase of blood glucose levels in relation to normoglycemia at the time of SAH onset that was reverted 30 minutes later. These studies showed that hyperglycemia increased the mortality rate during the first 24 hours in relation to normoglycemic rats. In addition, severely hyperglycemic animals showed a lower survival rate at day 3 in comparison with both mild hyperglycemia and normoglycemia. Due to the high mortality shown by the rats with severe hyperglycemia after SAH, we evaluated the effect of mild and severe hyperglycemia in relation to normoglycemia after SAH at day 1 and the effect of mild hyperglycemia in comparison to normoglycemia at days 1 and 3 with MRI, neurological studies, and PET after SAH. We found that mild and severe hyperglycemia worsened the SAH outcome at day 1 after SAH onset, showing an increase of stroke volume, mass effect and neurological outcome in comparison to normoglycemic rats. Additionally, rats with hyperglycemia showed higher BBB disruption compared to normoglycemic animals. Besides, hyperglycemic rats showed a significant increase of infarction volume and midline displacement at day 3 in relation to day 1 and normoglycemic rats evidencing the role of hyperglycemia in the worsening of SAH evolution.

In addition, we conducted PET imaging evaluations to study the expression of metalloproteinases (MMPs) (proteases related with BBBd) under hyperglycemic and

normoglycemic conditions after SAH. We found that hyperglycemic animals showed a higher uptake of the radiotracer [18F]BR-351 reflecting an increased expression of MMPs in both the ipsilateral hemisphere and infarction compared to normoglycemic animals. In vivo studies were supported by ex-vivo gel-zymography studies that showed a significant increase of MMP-9 at day 1 and MMP-2 at day 3 in hyperglycemic rats when compared with normoglycemia. Overall, these studies observed the effect of hyperglycemia on MMP activation at different days after preclinical SAH. In addition, oxidative stress was evaluated by PET with the radiotracer [18F]-FSPG in mild-hyperglycemic (N=9) and normoglycemic animals (N=8) at days 1 and 3 after SAH. These results did not show any effect of hyperglycemia on oxidative stress increase after SAH. At the time of completing this final report, the interventional studies based on the administration of a BBB-targeted antioxidants (uric acid in different preparations including liposomes) were ongoing and therefore we cannot draw reliable conclusions.

3. Relevance and possible clinical applicability of the final results

Several findings should be highlighted regarding their relevance and clinical applicability.

First, we have found that patients that suffer an acute subarachnoid hemorrhage (SAH) have an acute disruption of the blood brain barrier (BBB) that can be tracked though dynamic contrast enhanced MRI (DCE-MRI). This BBB permeability increase occurs within the first days after the bleeding and improves at 3 months. The acute BBB disruption is diffuse and not focal and is independently associated with poor clinical outcome at 3 months. This finding is potentially relevant as for the first time it identifies through neuroimaging techniques a potentially deleterious physiopathological mechanism in this disease. According to our findings, DCE-MRI could have potential as a prognostic factor and as a potential surrogate neuroimaging measure in interventional studies aimed to modulate BBB disruption in the acute phase of the disease. We did not find a direct association between glucose levels at hospital admission and DCE-MRI measures of BBB disruption. However, we found that hyperglycemia was independently associated with poor clinical outcome in this disease and that this negative association was significantly

mediated through neuroaxonal injury. This finding is relevant from a physiopathological point of view as it gives further support to the potential protective role of modulating the glucose-driven deleterious effects in the acute phase of the disease and also supports the use of neuroaxonal injury markers for the evaluation of the effect of interventions.

In the preclinical model, we have characterized a model of experimental subarachnoid hemorrhage using neuroimaging techniques that included DCE-MRI (to assess the permeability of the BBB) and PET (to assess the activity of metalloproteases and of oxidative stress and neuroinflammation surrogate markers). We found that hyperglycemia is deleterious in this experimental model and that this deleterious effect is in part explained by higher BBB disruption (higher DCE-MRI derived K-trans values, higher expression of MMPs) and higher local oxidative stress (PET studies). The characterization of an experimental SAH model through non-invasive neuroimaging techniques has an important translational value as it allows the design and performance of interventional studies with longitudinal evaluations in surrogate biomarkers that can be translated to the human beings (as demonstrated with DCE-MRI). At the time of completing this final report, the interventional studies based on the administration of a BBB-targeted antioxidant (uric acid in different preparations including liposomes) were ongoing and therefore we cannot draw reliable conclusions.

4. Publications and communications derived from this research

Communications

The association between initial hyperglycemia and poor outcome in aneurysmal subarachnoid hemorrhage patients is modified by the initial clinical severity. 15th International Conference on SubArachnoid Hemorrhage (ISAH 2019). June 25-28, 2019 Muziekgebouw Amsterdam. Poster presentation.

Synthetic MRI in subarachnoid hemorrhage. European Stroke Organisation and World Stroke Organization Conference (ESO-WSO 2020). November 7-9, 2020. Virtual conference. Poster presentation.

Blood-brain barrier disruption in spontaneous subarachnoid hemorrhage: a dynamic contrast-enhanced MRI study. European Stroke Organisation and World Stroke Organization Conference (ESO-WSO 2020). November 7-9, 2020. Virtual conference. Poster presentation.

Magnetic Resonance Imaging evaluation of the role of hyperglycemia in experimental subarachnoid haemorrhage. 24-28 August 2020, 15th European Molecular Imaging Meeting. Poster presentation.

Longitudinal evaluation of blood-brain barrier disruption in spontaneous subarachnoid haemorrhage: a dynamic contrast enhanced MRI study. September 1-3, 2021, ESOC 2021. Virtual conference. Oral communication.

White matter injury in spontaneous subarachnoid haemorrhage: predictors and clinical relevance. September 1-3, 2021, ESOC 2021. Virtual conference. Oral communication.
Multimodal imaging evaluation of the role of hyperglycemia in experimental subarachnoid haemorrhage. 15-18 March 2022, 17th European Molecular Imaging Meeting, Thessaloniki. Oral communication.

Publications

Montejo C, Laredo C, Llull L, Martínez-Heras E, López-Rueda A, Torné R, Garrido C, Bargallo N, Llufriu S, Amaro S. Synthetic MRI in subarachnoid haemorrhage. Clin Radiol. 2021; 76(10):785.e17-785.e23. Epub 2021 Jun 27. doi: 10.1016/j.crad.2021.05.021.

Torné R, Hoyos J, Llull L, et al. Edema Resolution and Clinical Assessment in Poor-Grade Subarachnoid Hemorrhage: Useful Indicators to Predict Delayed Cerebral Infarctions?. J Clin Med. 2021;10(2):321. Published 2021 Jan 17. doi:10.3390/jcm10020321.

Publications under preparation

Blood-brain barrier disruption in spontaneous subarachnoid hemorrhage: a dynamic contrast-enhanced MRI study. 2022. Under preparation.

Neuroaxonal injury mediates glucose-driven brain damage in spontaneous Subarachnoid Hemorrhage. 2022. Under preparation.

Multimodal Imaging evaluation of the role of hyperglycemia in experimental subarachnoid haemorrhage. 2022. Under preparation.