LIPOPROTEIN BIOMARKERS TO DETERMINE INFLAMMATION OF THE CAROTID PLAQUE IN ISCHEMIC STROKE

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1. Project Summary

Introduction

Stroke is the leading cause of acquired disability in our environment and the second cause of death. This morbimortality increases with recurrences after a first stroke and therefore secondary prevention after a first episode is of great importance. The medical and surgical strategies that can be applied to prevent recurrences depend on the type and cause of the stroke. Approximately 1 in 5 strokes are caused by atheromatous plaques (popularly called "cholesterol plaques") in the internal carotid artery. In the last decades it has been observed that inflammation of these plaques is one of the key processes related to the progression and risk of subsequent stroke. Our group, in collaboration with other international groups, observed that measuring the inflammation of carotid plaques with an imaging technique called positron emission tomography (PET) predicted patients at higher risk of recurrence after stroke allowing to identify which of them could benefit from more aggressive therapies such as surgery or stenting. Despite these promising results, PET is an expensive technique that is scarcely available in most centers, so its widespread implementation in a pathology as common as ischemic stroke associated with atheromatous plaques seems difficult. In this context, having plasma markers that are associated with inflammation measured by PET could be of great clinical utility since it would allow initial screening and monitoring of the response to treatment, avoiding unnecessary or repeated PET scans.

Objective of the study

To study plasma lipoprotein and inflammatory markers that correlate with the level of inflammation of carotid plaques measured by 18-FDG PET in patients with ischemic stroke.

Study design

We conducted a prospective observational study of patients with ischemic stroke and at least one atheromatous plaque in the internal carotid artery. The study started from patients enrolled in a parallel study to which 18-FDG PET of the carotid artery was performed.

Study procedures

All patients included in the study underwent 18-FDG carotid PET and a blood sample was obtained in the acute phase and at one year after stroke for the determination of inflammatory and lipoprotein plasma markers. Plasma markers were analyzed by ELISA, multiplex, chromatography and COBAS autoanalyzer techniques. Lipoproteins were determined physicochemically and their effect on cultured macrophages and mouse carotid arteries was analyzed. Patients have been followed up for at least one year and recurrences of stroke, other cardiovascular events and mortality have been recorded during follow-up.

2. Main Results

The study included 91 subjects (64 patients with recent ischemic stroke and 27 healthy subjects who served as the control population). All patients with ischemic stroke were followed up for an average of 14 months and an 18% recurrence rate was recorded. As explained in the "study procedures" section, all patients underwent a baseline analysis and one analysis at one year of follow-up to measure plasma inflammatory and lipoprotein markers and study their association with the main study variables (inflammation of carotid plaques and stroke recurrences). The main results obtained are summarized by points below and their importance is discussed in the section "Relevance and future implications".

- Biomarker analysis with ELISA: We have observed that patients with ischemic stroke have higher levels of oxidized LDL (a form of modified cholesterol) than healthy controls and that this increase is independent of the degree of carotid stenosis. This increased presence of oxidized LDL may be due to a release of this biomarker from the plaque. Increased ApoJ (a type of lipoprotein) values have also been found in patients with ischemic stroke, which could also come from the plaque. A significant increase in soluble LRP1 (sLRP1), which is increased in pathologies associated with cardiovascular risk, has also been observed in patients with higher levels of inflammation in carotid plaques, and this association has been found to be independent of the degree of stenosis and obesity.

- Multiplex biomarker analysis: Three plasma markers have been identified that predict with high sensitivity the risk of finding a highly inflamed plaque: sICAM-1, sVCAM-1 and fractalkine. In addition, sICAM-1 has been shown to independently predict the risk of recurrence of ischemic stroke.

- Determination of LDL(-) by chromatography: It has been observed that in patients who have had a recent ischemic stroke, the percentage of LDL(-) is not associated with the degree of inflammation seen by PET, but is independently associated with the degree of carotid stenosis. In contrast, total LDL concentration is not increased in patients and is not associated with the degree of stenosis. These interesting results point to the possibility that modified forms of LDL may have a greater influence on the progression of atherosclerosis than total LDL.

- PON and PAF-AH enzyme assays: Protective enzyme activities of platelet factor acetylhydrolase oxidase (PAF-AH) and paraoxonase (PON) have been found to be decreased in stroke patients. However, this could be due to a lower serum lipoprotein concentration in patients, since lipoproteins are the main transporters of these enzymes. No association has been found between these changes in enzyme activity and inflammation of carotid atheroma plaques.

- Characterization of lipoproteins in mouse carotid arteries ex vivo: During the study, mouse carotid arteries were incubated with human blood serum from patients with ischemic stroke and healthy controls. The results show that incubation with serum from healthy controls does not produce endothelial dysfunction in mouse carotid arteries, whereas incubation with serum from patients with ischemic stroke and carotid atheromatous plaques does. These results indicate that the endothelial dysfunction observed after incubation with patient serum is linked to circulating factors present in these patients that are not present in healthy controls. It has also been shown that the observed endothelial dysfunction disappears by both nonselective and selective inhibition of COX-1 and COX-2 isoforms.

- Isolation and characterization of lipoproteins in cell cultures: Incubation experiments of LDL(-) with macrophages and endothelial cells in culture have been performed. It has been observed that in the presence of LDL(-) macrophages release significantly higher levels of sICAM-1, sVCAM-1, fractalkine and sLRP1 (which are

precisely the plasma markers that we have found to be elevated in the presence of inflamed plaques) than when cultured with the native LDL fraction. These results lead us to believe that high proportions of LDL(-) promote the progression of carotid plaques and provoke a chronic proinflammatory state within the lesion that can ultimately destabilize and provoke a release of some of the cytokines that we found elevated in the plasma of our patients.

3. Relevance and possible future implications

In the present study, funded by La Marató de TV3, we have discovered plasma inflammatory and lipoprotein markers that are elevated in patients who have suffered an ischemic stroke and have carotid atheromatous plaques. Furthermore, we have observed how some of these markers are associated with the vulnerability of carotid atheroma plaques and that they are predictors of the risk of recurrence. Finally, we have seen how these plasma factors induce inflammation in macrophages and endothelial dysfunction in culture and ex-vivo models. All these results are relevant from different points of view.

First, we have found that concentrations of sICAM, sVCAM, fractalkine and sLRP1 in patients who have just suffered an ischemic stroke predict the degree of inflammation of carotid atheromatous plaques measured with PET. This is very important, because in the last decade it has become clear how important inflammation is in the risk of recurrence in patients with stroke and carotid atherosclerosis. Up to five recent studies published in high-impact journals have described a strong association between inflammation measured with PET and the risk of recurrence in these patients. However, PET is an expensive test, scarcely available in many centers, requiring expertise for interpretation and using ionizing radiation (which makes it difficult to perform on a serial basis). Therefore, the molecules described in our study could be used in the future as a screening tool to determine which patients are candidates for PET or even for subsequent follow-up with serial measurements. Of course, if their usefulness is confirmed, they would allow a much more cost-efficient management of these patients. Consistently, in our study, despite having a relatively small number of patients, one of these molecules (sICAM) independently predicts the risk of recurrence in our cohort.

Secondly, we have observed that while patients with ischemic stroke and carotid atherosclerosis have lower total cholesterol and LDL levels than controls, mainly due to a more generalized use of statins, they have a higher proportion of modified forms of LDL and of some apolipoproteins such as apoJ. In addition, we have observed that the proportion of LDL(-) in patients is an independent predictor of the degree of carotid stenosis, whereas total LDL levels are not (which is what we are currently using to monitor our patients). This indicates that modified forms of LDL such as LDL(-) are likely to play a more relevant role in the genesis and progression of carotid atheromatous plaques than total LDL levels. Therefore, we will have to consider in the future whether it is sufficient to monitor these patients on the basis of total LDL levels as in the past, or whether we should start to look at their properties.

Third, our study provides insight into the underlying pathophysiological mechanisms of carotid atherosclerosis. We have been able to observe how incubation of macrophages and endothelial cells (two of the cell subtypes most strongly implicated in the progression of atherosclerosis) with LDL(-), generates the secretion of multiple inflammatory molecules including sICAM (which we have seen to be associated with inflammation and risk of recurrence). We have also seen that when we incubate ex-vivo mouse models of arterial wall with serum from these patients, endothelial dysfunction is induced that is not present in controls. This further demonstrates the involvement of all these plasma factors in endothelial dysfunction. Furthermore, we have observed how inhibition of COX isoforms can reverse this effect and therefore we could be looking at new therapeutic targets.

Finally, if we look at the world of vascular diseases with perspective, we see that we are on the verge of a paradigm shift in the management of certain patients with atherosclerotic disease. In the last decade we have seen that antiplatelet and statin therapy is insufficient to prevent recurrences and disease progression in patients with a persistent proinflammatory state. Therefore, the availability of inflammatory plasma markers (such as sICAM) and lipoprotein markers (such as the LDL(-) ratio) directly related to this inflammation and disease progression may be of great value for monitoring these patients, measuring the effect of treatments, and even designing new therapeutic targets.

4. Scientific literature generated

Plasma sICAM-1 as a Biomarker of Carotid Plaque Inflammation in Patients with a Recent Ischemic Stroke. Puig N, Camps-Renom P, Camacho M, et al. Trans Stroke Res. 2022; doi:10.1007/s12975-022-01002-x (online ahead of print). (IF 5.35)

Search for Reliable Circulating Biomarkers to Predict Carotid Plaque Vulnerability. Puig N, Jiménez-Xarrié E, Camps-Renom P, Benitez S. Int J Mol Sci. 2020; 21:8236. (IF 4.56).

Electronegative LDL Promotes Inflammation and Triglyceride Accumulation in Macrophages. Puig N, Montolio L, Camps-Renom P, et al. Cells 2020;9(3). (IF 5.66).