

DYNAMIC EVOLUTION OF OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN ISCHEMIC STROKE

ABSTRACT OF PhD THESIS

PhD: Ioana Varga

Scientific: Univ.Prof. Dr. Pascu Ion

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KEY WORDS: *stroke, oxidative stress, inflammation, markers, statins, omega-3 fatty acids*

INTRODUCTION

At the present time, description of cardiovascular diseases is realized in correlation with the two important biological processes, inflammation and oxidative stress, which detain a determinant etiopathogenetic role. In the last two decades, many studies revealed the role of inflammation, both in pathogenesis of atherosclerosis and in generation of brain tissue lesions, that appear during the ischemic stroke. More recently, there are experimental evidences that oxidative stress plays a major role, too, by production of free radicals, in the ischemic brain injury and in atherogenesis. Despite numerous defenses, the brain is vulnerable to oxidative stress resulting from ischemia and reperfusion. During ischemia many inflammatory mediators are released and high amounts of free radicals are formed by several mechanisms, which could be the target for new therapeutic strategies during the post-ischemic period. Enhanced antioxidant capacity (individually and total) after acute stroke, therefore may protect against the adverse effects of free radical production during ischemia and reperfusion. A number of individual components present in serum have been shown to possess antioxidant capacity, including albumin, uric acid, bilirubin and protein thiols, vitamin C and E, minerals known to be involved in antioxidant enzyme activation (selenium, iron, copper and zinc). Measurement of total antioxidant status (TAS) is regarded as more physiologically, more representative than individual antioxidants and is believed to be a useful measure of how much the antioxidants present can protect against oxidative damage.

Thus, many studies point an important anti-inflammatory and antioxidant effect for statins and more recently, are several similar studies for omega-3 fatty acids.

THE OBJECTIVES OF STUDY

- A. Examination of oxidative stress and inflammatory markers levels variation after ischemic stroke
- B. Analyze of dynamic evolution of these markers
- C. To highlight a possible correlation between these markers and clinical status of the patients with ischemic stroke, evaluated by NIHSS score
- D. Setting of any association between oxidative stress and inflammatory markers
- E. Determination of a possible correlation between the size of the infarct and levels of inflammatory and oxidative markers
- F. Detection of the predictive value of these markers regarding to the dynamic clinical evolution of these patients
- G. Study of the mode of how the therapy with statins versus that with omega-3 fatty acids influences the dynamic evolution of oxidative stress and inflammatory markers

PATIENTS AND METHOD

The prospective study included 55 patients with acute ischemic stroke and 19 controls, subjects defined as being free of major medical or surgical illness within 5 years. From the stroke group, we excluded patients who presented diseases that could modify the oxidative status.

All stroke patients were clinically assessed by NIHSS score (National Institutes of Health Stroke Scale) initial and 2 months after the onset of stroke. Cerebral CT scan was done in the first 24 hours after the admission in the hospital to exclude hemorrhagic stroke.

Blood tests were done twice: first time (T0) – in the first 72 hours after the stroke onset and second time (T1) – 2 months after, and included inflammatory markers (C reactive protein – CRP and fibrinogen), individual oxidative stress markers (albumin, uric acid, copper), total antioxidant status (TAS), lipid profile markers (total cholesterol, HDL-, LDL-cholesterol, triglycerides), markers of hepatocytolysis (transaminases ALT, AST) and myocytolysis (creatinphosphokinase – CPK).

The patients with ischemic stroke were divided in two groups, according to the clinical subtypes of cerebral infarction and the cerebral CT scan examination, in those with large-vessel (territorial) and with small-vessel (lacunar) infarcts, creating the possibility to assess the correlation between the size of the infarct and the oxidative or inflammatory status quantified by the studied markers.

First, we assessed the values of these markers, compare with those from the control group and appreciated its sense of variation immediately after the onset of stroke and then its dynamic evolution 2 months after, trying to establish an evolution curve. We analysed a possible correlation of these parameters with the clinical status evaluated by NIHSS score or with the size of the infarct, or between oxidative and inflammatory markers. We administered to all the patients included in the study one of the two different therapeutic classes: 40 mg Sortis (Atorvastatin) to 35 patients and 1 g Omacor [460 mg eicosapentaenoic acid ethyl ester (EPA) plus 380 mg docosahexaenoic acid ethyl ester (DHA)] to a lot of 20 patients. We appreciate how each of these therapies influences the dynamic evolution of oxidative, inflammatory and lipid profile markers and the possible side effects – hepatic cytolysis and myocytolysis.

Statistical analyses were performed using the program STATISTICA Six Sigma version 8.0. StatSoft Inc. U.S.A.

RESULTS AND DISCUSSIONS

VARIATION OF OXIDATIVE STRESS MARKERS

Values of oxidative stress markers at T=0 in patients with stroke were significantly lower comparing with controls, for all the oxidative markers except uric acid, which presented insignificant higher levels.

Dynamic evolution of oxidative stress markers. We obtained for albumin and TAS a variation in “U” shape-curve, with reference levels in controls, then an evident decreasing, significant at the onset of stroke and further with higher levels than initial, but still lower than in control lot, 2 months later. The curve respects almost the same shape for copper and presents initial high values at T=0, then with a trend to return to control levels at T=1, for uric acid.

Dynamic evolution of correlations between individual oxidative markers and TAS at control-T=0-T=1. Levels of albumin correlated the best with those of TAS, from all the individual oxidative stress markers, initial and two months after the ischemic stroke.

Correlation of oxidative markers – clinical status (NIHSS). There was no association between NIHSS score and any of the studied oxidative stress markers at T=0 and T=1.

Correlation of oxidative markers with the size of infarct. Patients with territorial infarcts presented values of TAS, albumin and uric acid, statistically insignificant lower comparing with those with lacunar infarcts, probably, by a higher consumption of antioxidants at the place of brain tissue injury, proportional with the size of infarct.

Oxidative stress markers prognostic value.

Short-term prognostic value. We remarked low serum levels for albumin and TAS with negative prognostic value referring to the clinical evolution – with aggravation of the neurological deficit, recurrent stroke or death - of the patients with ischemic stroke, in the first 7 days after stroke onset.

Long-term prognostic value.

Functional outcome. Values just less above the level of statistical significance for uric acid ($p=0.07$) and TAS ($p=0.07$) and significant higher variations in uric acid ($p<0.05$) were associated with a favorable evolution – a NIHSS score lower than 3, two months after the ictus appearance.

Vascular outcome. Albumin mean value was lower in patients who presented major vascular events – stroke or vascular death in the first year after stroke onset.

VARIATION OF INFLAMMATORY MARKERS IN ISCHEMIC STROKE

Values of inflammatory markers at T=0 in patients with stroke. Levels of CRP were significantly ($p<0.0001$) and those of fibrinogen insignificantly higher, comparing with controls.

Dynamic evolution of inflammatory markers. We observe for CRP a variation in “overturned U” shape-curve - “look in the mirror” comparing with that obtained for the oxidative markers.

Correlation between oxidative stress and inflammatory markers. Values of the two inflammatory markers didn't correlated with those of TAS at both determinations.

Correlation inflammatory markers – clinical status (NIHSS). We detected a significant association between levels of CRP and NIHSS score ($r^2=0,3156$) at the initial moment (T=0).

Correlation of inflammatory markers with the size of infarct. CRP presented significant higher levels in patients with territorial than those with lacunar infarcts ($p=0.01$), by an inflammatory proces, probably more intense, proportional with the size of infarct. Mean values of fibrinogen were equal in both subgroups of patients.

Inflammatory markers prognostic value.

Short-term prognostic value. None of the two studied inflammatory markers presented prognostic value regarding to the regression or progression evolution, in the first week after the stroke onset.

Long-term prognostic value.

Functional outcome. We didn't remark any prognostic value for CRP concerning with the long-term evolution. On the other hand initial increased levels of fibrinogen were associated with an unfavorable evolution, with a NIHSS score less than 3, two months after ictus appearance ($p=0,06$).

Vascular outcome. We obtained higher values for CRP in the group of patients, who presented major vascular events – recurrent stroke or vascular death.

EFFECTS OF THERAPY WITH STATINS VERSUS OMEGA-3 FATTY ACIDS

The mode of how therapy with statins versus that with omega-3 fatty acids influenced the dynamic evolution of oxidative stress and inflammatory markers. We highlight an antioxidant and anti-inflammatory higher effect in the lot treated with statin – which presented a more evident improvement of oxidative and inflammatory markers levels during the study treatment.

Side effects of therapy - hepatic cytolysis and myocytolysis in lot Statin versus lot Omega-3. There were no significant differences regarding the side effects of the two therapeutic classes in patients with ischemic stroke, after 2 months of treatment.

GENERAL CONCLUSIONS

1. Levels of all oxidative stress markers, except uric acid, were significantly lower and those of CRP significantly higher in patients with ischemic stroke comparing with controls.
2. It is described a “look in the mirror” dynamic evolution curve for the oxidative and inflammatory markers, with a maximal modified value (decreased respectively increased) immediately after the stroke onset, with a further trend to recovery, but still incompletely 2 months post-ictus.
3. We didn't find a significant correlation between the clinical status and oxidative markers, but we found one between NIHSS score and levels of CRP at the onset of stroke.
4. Even if we didn't detected a straight association between the inflammatory status and oxidative stress markers, this correlation is suggested by the similar evolution profile of these 2 types of markers
5. Serum level of albumin, uric acid and TAS were insignificant lower and those of CRP significant higher in patients with territorial infarcts.
6. Values of TAS and albumin presented prognostic value regarding the short-term and long-term evolution, while serum CRP levels influenced the vascular prognostic, regarding major vascular events one year after stroke.
7. We highlight antioxidant and anti-inflammatory effects more evident for statins, comparing with omega-3 fatty acids, with an equivalent safety profile for both therapies.