Plasma adrenomedullin levels in patients with polymyalgia rheumatica and giant cell arteritis

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ABSTRACT

Objective. *Plasma adrenomedullin (AM) levels are elevated in several inflammatory rheumatic diseases. The aims of the present study were: a) to assess whether plasma AM levels are abnormal in patients with polymyalgia rheumatica and giant cell arteritis (PMR and GCA) and b) to investigate if this parameter is related to clinical and biochemical indicators of disease activity in these patients.*

Materials and methods. *AM plasma levels were analyzed in 17 patients with PMR and GCA and in 14 healthy subjects. Twelve patients (9 PMR and 3 GCA) were studied when they had active disease before any steroid therapy and the remaining 5 patients (2 PMR and 3 GCA) were in complete clinical remission and no longer receiving steroid treatment. AM was measured by a specific radioimmunoassay.*

Results. *Plasma AM concentration was significantly higher in patients with active GCA compared to the control group ($p < 0.05$) and with patients with isolated PMR ($p < 0.05$). However, there were no significant differences between patients with active PMR and the control group. Within the PMR/GCA group with active disease, AM plasma levels were positively correlated with ESR ($r = 0.6$, $p = 0.02$), and negatively with hematocrit ($r = -0.57$, $p = 0.03$) and hemoglobin ($r = -0.55$, $p = 0.04$). No correlations were found between AM and CRP.*

Conclusions. *Plasma levels of AM are elevated in patients with active GCA and correlate with parameters that reflect the acute phase response. The differences in the secretion of AM between patients with PMR and GCA might reflect the severity of the vascular endothelial cell damage in these conditions. The role of AM in the pathogenesis of PMR and GCA needs to be assessed in a larger series of patients.*

Introduction

Human adrenomedullin (AM) is a recently identified 52-aminoacid peptide that was originally isolated from an extract of pheochromocytoma tissue (1). AM is produced not only in normal adrenal medulla but also in the lungs, kidneys, liver and cardiovascular system (heart, endothelial and vascular smooth muscle cells) (2). The major source of circulating AM in normal subjects is probably the vascular endothelium rather than the adrenal gland (3, 4). AM, acting as an autocrine, paracrine or endocrine vasorelaxing factor, may be important in the regulation of local and systemic vascular tone (5). Plasma AM levels are increased in different clinical situations, such as essential hypertension, congestive heart failure, acute myocardial infarction, liver diseases, diabetes mellitus or sepsis (6-9). Recent studies show that inflammatory cells are the major sites of AM production. Elsasser *et al*. suggested a role of AM in the development and remission of the inflammatory response (10, 11). AM production was elevated in inflammatory rheumatic diseases, specially RA in which correlated with pro-inflammatory cytokines and laboratory measures of inflammation (12). Similar findings have been also found in SLE patients (13). These observations prompted us to analyze plasma AM in two related conditions, polymyalgia rheumatica (PMR) and giant cell arteritis (GCA), because of two main reasons. First of all, GCA is a granulomatous vasculitis affecting medium and large-size arteries in elderly people (14-16), and although some controversy exists about the relationship between PMR and GCA (17), there are several lines of evidence supporting that at least a significant num-

ber of patients with PMR have subclinical vascular inflammation (18). And second, both are characterized by a brisk acute phase response related to the increased production of macrophage derived pro-inflammatory cytokines (19-21).

The aims of the present study were: a) to assess whether plasma AM levels are abnormal in patients with PMR and GCA, and b) to investigate if this parameter is related to clinical and biochemical indicators of disease activity in these patients.

Patients and methods

AM plasma levels were analyzed in 17 patients with PMR/GCA and 14 healthy subjects. Twelve patients (9 PMR and 3 GCA) were studied when they had active disease before any steroid therapy and the remaining 5 patients (2 PMR and 3 GCA) were in complete clinical remission and no longer receiving steroid treatment. PMR was diagnosed according to the criteria proposed by Chuang *et al*. (22). In patients with PMR the possibility of GCA was excluded either by a normal temporal artery biopsy or by the absence of manifestations of GCA and cure with low-dose prednisone after a long-term follow-up. All the patients with GCA had a positive temporal artery biopsy, consisting of interruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells, and fulfilled the ACR 90 classification criteria for the disease (23, 24). Remission was defined on a clinical basis as the absence of clinical symptoms and signs of the disease associated with normal laboratory values. All the patients gave informed consent.

Fasting venous blood samples, for general biochemical analysis and specific determinations, were obtained after 30 minutes of supine rest from an antecubital vein. Haemoglobin, hematocrit and platelet counts were measured by routine techniques. The erythrocyte sedimentation rate (ESR) was measured in the routine haematology laboratory by the Westergren method, reading taken at 1 hour and C reactive protein (CRP) was measured by immunoturbidimetry (Behring Nephelometer Analyzer II, Behring Diagnostics, Marburg, Germany).

Blood for AM determination was obtained using a chilled syringe and immediately transfered into a polypropylene chilled tube containing EDTA (1mg/ml of blood) and aprotinin (500 KIU/ml of blood). Plasma was withdraw immediately, frozen and stored at -80º until assayed. The method for adrenomedullin determination has been described in detail elsewere (25). Briefly, AM was measured by a specific radioimmunoassay with a kit supplied by Phoenix Pharmaceuticals Inc, (Mountain View CA USA) following the manufacturer´s instructions after extraction through the Sep-Pak C-18 column supplied by the manufacturer (Lida Manufacturing Corp, Kenosha WI, USA). This assay does not show any cross-reactivity with human AM (13-52), human CGPR, ET-1, atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide. The effective range of the standard curve was between 10 and 1280 pg/ml. Sensitivity was 10 pg/ml. Interassay and intraassay coefficients of variation were 15% and 10% respectively. Data are expressed as mean±sd and median (range) in the case of a non parametric variable.

Statistical analyses were performed with the SPSS Software (Statistical Package for the Social Sciences Software, Chicago, IL, USA) for Windows system. All the tests were two-tailed and the level of significance was set at the $p < 0.05$. CRP shows non parametric distribution using Kolmogorov-Smirnov test to evaluation. For normally distributed variables, analysis of variance was performed to evaluate the differences between each group. If differences were found, the Tukey's test was used for comparison between the different groups and the control group. In the same way, Kruskal-Wallis test and Mann-Witney test with Bonferroni adjustment was used to estimate differences for non- parametric variables. Correlations were done with the Pearson or Spearman test as appropriate, controlling for age.

Results

Age was significantly different between the two study groups (75 ± 7 years in the PMR/GCA group vs 56 ± 18 years in the control group, $p = 0.002$). However, AM plasma levels did not correlate with age in healthy controls or in PMR/GCA patients.

Plasma AM concentration was significantly higher in patients with GCA (66.7 ± 28.9 pg/ml) compared to the

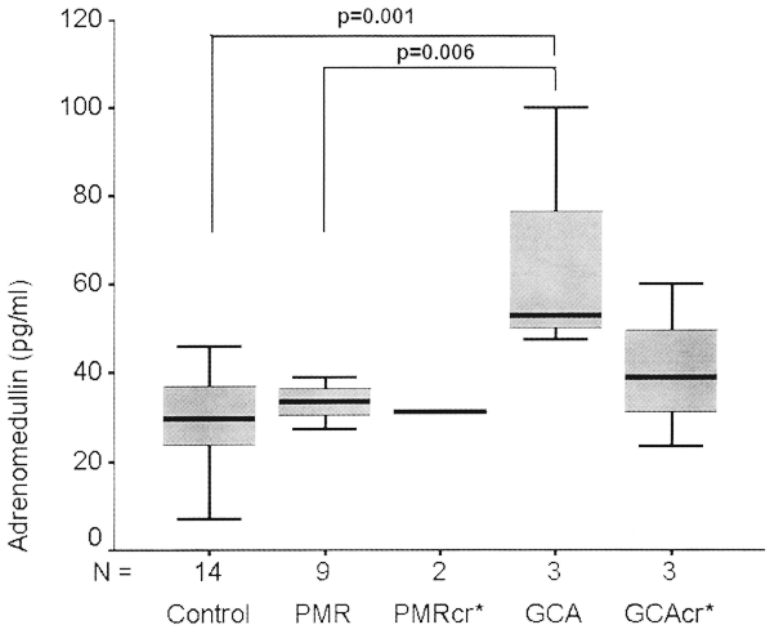

Fig. 1. AM plasma levels in the different study groups (cr* clinical remission).

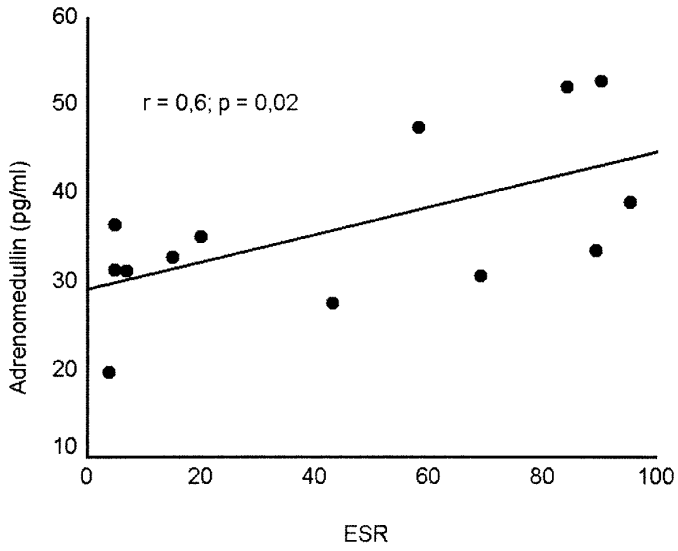

Fig. 2. Correlation between AM and ESR in the group with active disease.

control group (29.3 ± 9.8 pg/ml) (p = 0.001) and with patients with isolated PMR (34 8.7 pg/ml) (p = 0.006) (Fig. 1). These differences remained significant when controlling for age. However, there were no significant differences between patients with isolated active PMR (34 ± 8.7 pg/ml) and the control group (29.3 ± 9.8 pg/ml) (P = 0.9). Neither the control group nor the group with active PMR have statistically significant differences with the PMR/-GCA group in clinical remission (31.2 ± 0.07 pg/ml). Although the differences did not reach statistically significance, probably due to the small number of patients (n = 3), AM plasma levels in patients with GCA before steroid therapy (66.7 ± 28.9 pg/ml) had a tendency to decrease after steroid treatment (40.8 ± 18.3 pg/ml).

CRP levels and ESR were likewise significantly higher in patients with GCA (4.60 ± 1.96 and 62 ± 26.2 respectively) and in patients with isolated PMR (3.81 ± 3.49 and 47.1 ± 37.5 respectively) compared to the control group (0.27 ± 0.38 and 7.8 ± 2.3 respectively). Median and range of CRP were 0.09 (0.019-1.3) in the control group, 2.90 (0.3-10.8) in isolated PMR group, and 4.30 (2.8-6.7) in CGA group.

Within the PMR/GCA group with active disease, AM plasma levels were positively correlated with ESR (r = 0.6, p = 0.02) (Fig. 2), and negatively with hemoglobin (r = -0.55, p = 0.04). No correlates were found between AM and CRP levels in this group (r = 0.41; p = 0.18).

Discussion

In this preliminary study we demonstrate for the first time that plasma levels of AM are elevated in patients with active GCA, and that AM levels correlate with markers of inflammatory activity.

It has been reported that experimental (10, 26) and clinical situations (8, 27, 28) associated with inflammation induce high circulating levels of AM. Furthermore, plasma AM levels also reflect the degree of endothelial injury in patients with atherosclerotic disease (29), and may be involved in reparatory vessel endothelium mechanisms, specially in chronic disease.

In our patients, the cells responsible for the circulating AM may be both the macrophages/monocytes and the endothelial cells, since all of them are able to release AM when challenged by an inflammatory stimuli. As pro-inflammatory cytokines stimulate AM secretion, the elevation of AM in our patient population may be secondary to the increase in such cytokines, both at local or systemic level. However, the higher levels of AM in the GCA group suggest that the local inflammation of the arterial vessel tree may be the most important source of AM. Furthermore, the normal levels of AM in the patients with GCA in remission suggest that upon correction of the vascular inflammatory process AM secretion is normalized.

It has been suggested that exercise and glucocorticoids stimulate the secretion of AM (30). However, our data, in agreement with other inflammatory rheumatic conditions such as SLE (13), do not support this hypothesis. Indeed, patients who were in remission after steroid therapy tended to have lower levels of plasma AM. In patients with active GCA we found a significant correlation between AM and ESR but not between AM and CRP. This is not surprising since discrepant results have been reported regarding the significance of ESR and CRP in GCA patients (21).

Our study has two main limitations. First, the difference of ages between the control and PMR/GCA groups. However, it is known that AM levels do not change with age (13). We consider that the lack of influence of age on AM levels, both in our study and in the literature, suggests that the increased levels of AM in the GCA group are not due to higher age. The second limitation is the small number of patients studied, specially in the GCA group. Although we consider that it would be necessary to study a higher number of patients, the results in the GCA group are consistent.

Whether AM represents only a non-specific mechanism in response to the inflammatory process and endothelial cell damage or whether it is involved in the pathophysiology of GCA and PMR, needs to be further evaluated.

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