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Original research

Resection of Carotid Body Tumors reduces arterial blood pressure. An underestimated neuroendocrine syndrome

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ABSTRACT

Introduction: Carotid Body Tumors (CBTs) are Paragangliomas (PGLs) located in the head and neck region which usually do not cause overt neuroendocrine symptoms and hypertension. Matrix Metalloproteinases (MMPs) have shown a strong correlation between CBTs and their clinical behavior. Aim of this study is to analyze the relationship between changes in arterial blood pressure and metalloproteinases levels after surgical resection of CBTs.
Methods: We performed a multicenter clinical study on 17 patients with benign and malignant CBTs (5 males; 12 females). Tumors were completely resected and biopsies, obtained at the time of surgery, were lysed for Western blot analysis to determine MMPs levels in tissues. An enzyme-linked immune sorbent assay (ELISA) kit was used to determine the concentration of MMPs in plasma fluid.
Blood pressure values were measured at admission and at 10 days after surgery.
Results: At the time of the admission, blood pressure values were higher in patients with CBTs respect to control patients; moreover in patients with malignant CBTs blood pressure values were higher ($P < 0.01$) respect to patients with benign CBTs. 10 days after the surgery, we documented a significant decrease ($P < 0.01$) in blood pressure values and in MMPs levels in all patients with CBTs.
Conclusion: These results suggest that, despite the CTBs are considered non-functional tumors, an "underestimated" neuroendocrine activity on arterial blood pressure may be detected.

1. Introduction

Paragangliomas (PGLs) are rare neuroendocrine tumors that arise in sympathetic and parasympathetic paraganglia and derive from neural crest cells. In 80–85% of cases, PLGs are pheochromocytomas (PCCs), whereas 15–20% are located in extra-adrenal chromaffin tissue [1]: Carotid Body Tumors (CBTs) are the most common paraganglioma (PLG) in the head and neck [2].

Usually, PGLs are benign lesions and the development of metastasis is uncommon (approximately 6%) [3,4]. CBTs have an incidence of about 1 in 30,000 [2] and are mostly benign neoplasms of the middle age [5] with a 5% of metastatic rate [6–8].

These tumors are often identified accidentally as a painless slow-growing mass and diagnosis is delayed because of subtle symptoms. Several studies have shown that hypertension, the main

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sign of neuroendocrine syndrome, is a common feature of PGLs and it can be continuous, intermittent, and often paroxysmal in nature with associated typical signs, such us palpitations, headache and diaphoresis [9–12]; nevertheless, benign as well as malignant PLGs located in the head and neck region, such us CBTs, usually do not cause neuroendocrine symptoms and/or hypertension [13–17]. These tumors causes progressive symptoms such as dysphagia, odynophagia, hoarseness of voice or cranial nerve deficits because of the strong proximity to the neck vascular, nervous and muscular structures [2,18,19]. Matrix metalloproteinases (MMPs)are zinc-dependent endopeptidases which degrade various components of the extracellular matrix (ECM) and release growth factors and cytokines that reside in the ECM [20]; ECM remodeling is regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). Through their enzymatic activity, MMPs play several roles in tumor invasion and metastasis [21] and are involved in the pathogenesis of major inflammatory [22] and vascular diseases [23–33]. Our recent experience has shown a strong correlation between PLGs (both benign and malignant neoplasms) and the expression of MMPs [34] as well as recent studies have suggested that altered expression and activity of MMPs, particularly MMP-1, -2, -3, -9, may be implicated in vascular remodeling of several cardiovascular diseases [35–38].

In this study in patients with benign and malignant CBTs we evaluated the levels of MMP-1, -2, -3, -9 in plasma and we correlated these with the values of blood pressure at admission and after surgical excision of the tumor.

2. Materials and methods

2.1. Study population and experimental protocol

We performed a multicenter clinical study, conducted between January 2013 and December 2013 (1-year-period) with prior approval from the Institutional Review Board at the University of Catanzaro, in accordance with the Declaration of Helsinki and the Guideline for Good Clinical Practice. Patients were enrolled in Department of Medical and Surgical Science of University "Magna Graecia" of Catanzaro, Department of General, Geriatric, Oncologic Surgery and Advanced Technologies of University of Naples "Federico II", Unit of Vascular and Endovascular Surgery, Department of Surgical, Anesthesiological and Radiological Sciences, University of Ferrara, Sant'Anna Hospital of Catanzaro and Unit of Vascular and Endovascular Surgery, Annunziata Hospital, Cosenza. Before the beginning of the study, all participants provided a written informed consent.

The patients with diagnosis of CBTs were enrolled at the time of our previous study [34].

2.2. Experimental protocol

We performed a retrospective analysis of data from medical records of 14 previously enrolled patients with CBTs [34]. Moreover, we performed also a prospective study in patients enrolled in 2013.

As previously described [34], patients eligible for the study were of both sexes, from 20 up to 70 years, with diagnosis of benign and malignant vascular tumors. Tumors were completely resected according to the recent surgical technique and anatomo-pathological examination of operative sample was performed. Biopsies obtained at the time of surgery were lysed for Western blot analysis in agreement with our previous studies [13–14,17,19], anti-MMPs monoclonal antibodies were used and results were expressed as arbitrary units in agreement with our previous papers [23,25,26,29,31]. All experiments were performed in triplicate. In agreement with our previous papers [23,25,26,29,31], an enzyme-linked immune sorbent assay (ELISA) kit was used to determine the concentration of MMPs in plasma fluid. A commercially available sandwich ELISA kit with an MMPs monoclonal antibody was used to determine plasma MMP-s levels. According to recent classification [39], in all patients the valuation of blood pressure before surgical excision and 10 days later was performed (Table 2).

A further group of healthy volunteer patients (Group II) of both sexes were enrolled and represented the control group. As previously described [34], in these patients we take the blood samples in order to evaluate through the ELISA test the MMPs values and we also evaluated the blood pressure values. For both Groups I and II medical history was recorded, and clinical examination, laboratory findings, duplex ultrasonography were performed.

2.3. Statistical analysis

All data are expressed as mean ± standard error medium (SEM). Student's t test was performed in order to analyze the difference between each group with their control. Anova test was used to evaluate the difference between the groups. Differences identified by ANOVA were pinpointed by unpaired Student's t test. The test of Pearson was used to evaluate the correlation between plasma MMPs levels and blood pressure values. The threshold of statistical significance was set at $^{*}P < 0.05$. SPSS (SPSS Inc., Chicago, USA) software was used for statistical analyses. We defined this study as exploratory, therefore we did not determine a power calculation. In this light, these results could only be labeled as exploratory.

3. Results

In the present multicenter study we enrolled 3 patients (1 male and 2 females) with carotid benign paraganglioma, while data from 14 patients (4 males and 10 females; 11 patients with benign paraganglioma and 3 patients with malignant one) enrolled in a previous study were analyzed. Moreover, 3 health patients were enrolled in control group (1 male and 2 females).

At the time of the admission, blood pressure values were higher in patients with CBTs respect to control patients (data not shown); moreover in patients with malignant CBTs (numbers 15–17) blood pressure values were higher ($P < 0.01$) respect to patients with benign CBTs (numbers 1–14).

ELISA findings performed on blood samples take at the time of admission, revealed significantly higher levels ($P < 0.01$) of MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9 in patients with paraganglioma respect to control patients (data not shown). Moreover, patients with malignant CBTs (patient numbers 15–17) showed significantly higher levels ($P < 0.01$) of MMP-1, MMP-2 and MMP-3, respect to patients with benign CBTs (patient numbers 1–14) (Table 1). No significant differences were documented in plasma values of MMP-7 and MMP-13 between patients with malignant (numbers 15–17) and patients with benign ones (patient numbers 1–14) (Table 1).

An increased expression ($P < 0.01$) of MMP-1, MMP-2 and MMP-3, but not in MMP-7, MMP-8 and MMP-9, were recorded through western blot in patients with malignant paraganglioma respect to patients with benign one (data not shown). Finally, blood pressure evaluation and Elisa test performed 10 days after the surgery, documented a significant decrease ($P < 0.01$) in blood pressure values and in MMPs levels, without differences between patients with malignant CBTs (numbers 15–17) and patients with benign one (numbers 1–14) (Table 2). Finally using Pearson's test we documented a significant correlation ($P < 0.01$) between blood pressure (systolic, diastolic and differential) and MMPs, particularly with MMP1.