Short communication

Circulating renin-angiotensin system-regulating specific aminopeptidase activities in pre- and post- menopausal women with breast cancer treated or not with neoadyuvant chemotherapy. A two years follow up study

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ABSTRACT

We have previously described changes in several circulating renin-angiotensin system (RAS)-regulating aminopeptidase activities in pre- and postmenopausal women with breast cancer treated or not with neoadjuvant chemotherapy. Women with breast cancer presented a reduced catabolism of angiotensin II (AngII) when compared to healthy individuals, although specific enzyme activities were different between pre- and post- menopausal women. In addition, neoadjuvant chemotherapy in breast cancer patients caused changes in aminopeptidase activities leading to increased AngII catabolism independently of hormonal status. Here we extend the aminopeptidase analysis to three time points of the patient follow-up (6, 12, and 24 months). No changes occur in enzyme activities during this time period and the effects of therapy remain unaltered overtime both in pre- and in postmenopausal women.

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1. Introduction

Although renin-angiotensin system (RAS) has classically been identified in renovascular organs including the kidney and the heart and vessel walls, where its enzymatic actions and released peptides lead to blood pressure regulation and electrolyte/fluid homeostasis [1], it has been demonstrated that RAS components also participate in several steps in breast cancer. Thus, the best known RAS effector, angiotensin II (AngII), acting through its type 1 (AT_1) receptor, stimulates mitosis and cell proliferation, inhibits apoptosis and promotes angiogenesis via the up-regulation of various angiogenesis-associated genes [2]. Also, AngII has also been related to cell migration, invasiveness and metastasis [3]. However, other less known components of the RAS, including several proteolytic regulatory aminopeptidases, have also been involved. In previous reports, we had described changes in circulating RAS-regulating aminopeptidase activities in pre- and postmenopausal women with breast cancer treated or not with neoadyuvant chemotherapy prior to surgery. Our results showed differences in the metabolism of angiotensins between pre-and post- menopausal untreated women. We suggested a main role for AngII in both type of patients through different routes of the RAS cascade [4]. On the contrary, neoadjuvant therapy of paclitaxel and anthracycline would act promoting angiotensinase activities that increase AngII catabolism and therefore inhibiting tumor growth, independently of the hormonal status of the patients [5]. In the present work, we make a follow up of two years of RAS-regulating aspartyl aminopeptidase (ASAP), aminopeptidase A (APA), aminopeptidase N (APN) and aminopeptidase B (APB) activities in pre- and post- menopausal women with breast cancer who were treated or not with neoadyuvant chemotherapy previous to surgery in order to know the evolutionary pattern of RAS cascade in these patients.

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2. Materials and methods

2.1. Subjects

In the present study, we analyzed 112 women all diagnosed with ductal infiltrating carcinoma. 57 of them (20 premenopausal and 37 postmenopausal) did not receive neoadyuvant chemotherapy, whereas 55 of them (18 premenopausal and 37 postmenopausal) received neoadjuvant therapy of paclitaxel and anthracycline previous to surgery. A control group was also included, with 22 premenopausal and 37 postmenopausal healthy volunteers (controls). The clinicopathological characteristics of patients studied have already been reported [3,4]. Depending on their tumor molecular type (luminal A, luminal B, Her2/neu or triple negative), patients received adjuvant therapies after surgery that include treatments with radiotherapy, immunotherapy (Her2/neu+) and hormone therapy (tamoxifen or aromatase inhibitors). As part of their routine checkups, blood samples were take after 6, 12 and 24 months after surgery. Blood samples were obtained, allowed to cloth and centrifuged for 10 min at 3000 g to obtain the serum. Samples were rapidly frozen in liquid nitrogen and stored at −80 °C, until usage. The study was approved by the Hospital Ethics Committee and all control and patient subjects signed an informed consent form.

2.2. Renin angiotensin system-regulating aminopeptidase assays

RAS-regulating aminopeptidase activities were assayed fluorometrically using their corresponding aminoacyl-β-naphthylamide as substrates, as previously described [3,4].

2.3. Statistical analysis

To analyze the differences between groups, we have used multiple analysis of variance plus Newman-Keul's post-hoc test, using IBM SPSS V.23 software. All comparisons with p-values below 0.05 were considered significant.

3. Results

Fig. 1 illustrates the results obtained to specific serum aminopeptidase activities in healthy (control) premenopausal and post-

Fig. 1. Specific serum aspartyl-aminopeptidase (A, B), aminopeptidase A (C, D), aminopeptidase N (E, F) and aminopeptidase B (G, H) activities in healthy (control), premenopausal and postmenopausal women and in women diagnosed with ductal infiltrating breast cancer untreated (upper panel) or treated (lower panel) with neoadjuvant chemotherapy, before surgery and after 6, 12 and 24 months after surgery. Results are expressed in picomoles of their corresponding aminoacyl-β-naphthylamide hydrolyzed per min and per mg of protein (Mean ± SEM; *P < 0.05; **P < 0.001).

menopausal women and in women diagnosed with ductal infiltrating breast cancer treated or not with neoadjuvant chemotherapy at the time of the surgery and 6, 12 and 24 months after surgery. A significant decrease ($P < 0.05$) was found in ASAP activity levels (Fig. 1A) in premenopausal women with breast cancer at the time of surgery that remain low up to 24 months after surgery, whereas no changes were found in postmenopausal women. On the contrary, ASAP activity significantly increased ($P < 0.001$) in both pre- and post- menopausal women treated with neoadjuvant chemotherapy since surgery and up to 24 months after surgery (Fig. 1B). No changes were found in APA activity in pre- or post-menopausal women not treated with neoadjuvant chemotherapy (Fig. 1C), whereas similarly to ASAP, APA activity significantly increased ($P < 0.001$) in both pre- and post- menopausal women treated with neoadjuvant chemotherapy since surgery and up to 24 months after surgery (Fig. 1D).

Regarding APN, no changes were found in premenopausal women not treated with neoadjuvant chemotherapy over the time, whereas postmenopausal women showed significantly higher APN activity levels since surgery and up to 24 months later (Fig. 1E). On the contrary and similarly to ASAP and APA, APN activity significantly increased ($P < 0.001$) in both pre- and post- menopausal women treated with neoadjuvant chemotherapy since surgery and up to 24 months later (Fig. 1F). Regarding APB, we found similar results in pre- and post- menopausal women with breast cancer not treated with neoadjuvant chemotherapy, with significantly ($P < 0.001$) higher APB activity levels since surgery and up to 24 months later (Fig. 1G) than controls. Similarly, APB activity significantly increased ($P < 0.001$) in both pre- and post- menopausal women treated with neoadjuvant chemotherapy since surgery and up to 24 months later, although premenopausal but not postmenopausal women showed a higher level six months after surgery that returns to levels previous to surgery after 12 and up to 24 months (Fig. 1H).

4. Discussion

The renin-angiotensin system (RAS) has been involved in several of the hallmarks of cells during the multistep development of becoming a cancer cell that allows them to survive, proliferate and disseminate. In fact, RAS signaling, mainly by AngII through its AT$_1$ receptor, increases tumor cell proliferation and modulates the growth of vascular cells during angiogenesis [6]. We have previously described differences in the metabolism of angiotensins between pre- and post- menopausal women with breast cancer that suggested in both cases a predominant formation of AngII, although through different pathways involving different aminopeptidases. On the contrary, neoadjuvant treatment acts on all angiotensinases leading to AngII catabolism, minimizing its role as tumor promoting and even inhibiting tumor growth, independently of the hormonal status of patients. After a follow up of two years in these patients, here it is shown that no changes occur in ASAP, APA or APN activities. Little increased levels appear in APB activity in premenopausal but not postmenopausal women with breast cancer treated with neoadjuvant chemotherapy six months after surgery that are not maintained afterwards. These data suggest that the early events that occurred in breast tissue (including those involving RAS components) as a consequence of tumor initiation/progression and/or by the effects of the chemotherapy remain mainly unaltered over the time. Although further research is necessary to support that these early processes could be related to cancer recurrence and/or relapse that occurs in some patients, additional studies on the role of RAS components, including those lesser known, on the first steps of cancer initiation/progression might help to shed light on this hypothesis.
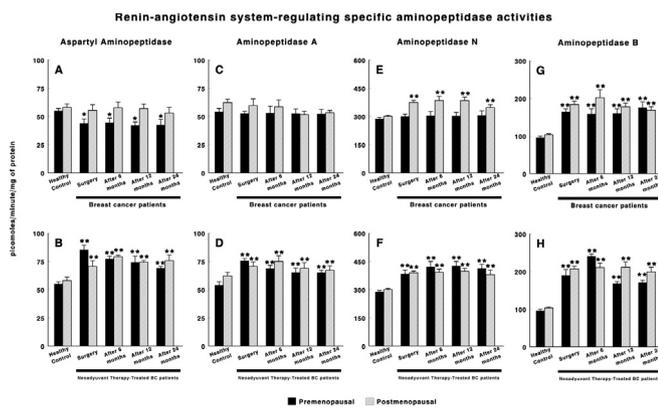
Conflict of interest statement

None to declare.

Ethical statement

This research has been approved by the Committee on Ethics in Research of the Province of Jaén, Jaén, Spain. All patients participating in the study gave their prior consent, informed and voluntary for the use of their samples.

Acknowledgments

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