Renin angiotensin system-regulating aminopeptidase activities in serum of pre- and postmenopausal women with breast cancer

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ABSTRACT

Angiotensin peptides regulate vascular tone and natriohydric balance through the renin angiotensin system (RAS) and are related with the angiogenesis which plays an important role in the metastatic pathway. Estrogen influences the aminopeptidases (APs) involved in the metabolism of bioactive peptides of RAS through several pathways. We analyze RAS-regulating AP activities in serum of pre- and postmenopausal women with breast cancer to evaluate the putative value of these activities as biological markers of the development of breast cancer. We observed an increase in aminopeptidase N (APN) and aminopeptidase B (APB) activities in women with breast cancer; however, a decrease in aspartyl-aminopeptidase (AspAP) activity in premenopausal women. These results suggest a slow metabolism of angiotensin II (Ang II) to angiotensin III (Ang III) in premenopausal women and a rapid metabolism of Ang III to angiotensin IV (Ang IV) in pre- and postmenopausal women with breast cancer. An imbalance in the signals activated by Ang II may produce abnormal vascular growth with different response between pre- and postmenopausal women depending on the hormonal profile and the development of the disease.

Keywords:
Breast cancer
Renin angiotensin system
Menopause
Aminopeptidases

Introduction

Breast cancer is the most frequent spontaneous malignancy diagnoses in women in the Western world. Although breast cancer develops in women as the result of a combination of external and endogenous factors, the mechanisms responsible for the initiation and progression of the disease have not been described.

In the promotion and progression of carcinogenesis, the angiogenesis (the recruitment of new blood vessels) plays an important role as an essential component of the metastatic pathway. Tumor vessels have an aberrant response to constrictor hormones, such as angiotensins and endothelins. Angiotensins peptides regulate vascular tone and natriohydric balance through the renin angiotensin system (RAS) but also are involved in the control of cell growth and vascular permeability. Angiotensin II (Ang II) stimulates angiogenesis and tumor growth. Ang II degradation begins with the action of aspartyl-aminopeptidase (AspAP) and aminopeptidase A (APA) which remove the N-terminal aspartic (Asp) to produce angiotensin III (Ang III), a less potent vasoconstrictor peptide than Ang II. Ang III is also produced from angiotensin I (Ang I) through the production of des-Asp1-Ang I, which is further converted to Ang III by the action of angiotensin-converting enzyme (ACE). Ang III is further converted to angiotensin IV (Ang IV) by aminopeptidase B (APB) or aminopeptidase N (APN).

By other hand and on the basis of experimental, epidemiological, and clinical studies, the ovarian hormones are strongly implicated in the development of breast cancer and also it is well known that APs are affected by sex hormones. In this way, different authors have described that estrogen administration increases plasma Ang II and ACE activity. Ang III is further converted to angiotensin IV (Ang IV) by aminopeptidase B (APB) or aminopeptidase N (APN).

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Materials and methods

Experimental design

In the present study, we have analyzed 20 premenopausal and 37 postmenopausal women with breast cancer and 22 premenopausal and 37 postmenopausal healthy volunteers (controls). The study was approved by the Hospital Ethics Committee and all patients signed an informed consent form.

All breast cancers were diagnosed as ductal infiltrating carcinomas. Blood samples were obtained 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 use.

Renin angiotensin system-regulating aminopeptidase assays

AspAP was determined fluorimetrically using aspartyl-β-naphthylamide (AspNNap) as the substrate, according to the method previously described. Briefly, 10 μL of each sample was incubated in triplicate for 30 min at 37 °C with 100 μM of the substrate solution: 100 μM AspNNap, 1.3 mM ethylenediaminetetraacetic acid (EDTA) and 2 mM MnCl2 in 50 mM of phosphate buffer, pH 7.4.

APA activity was measured in the same way using glutamyl-β-naphthylamide (GluNNap) as the substrate, as previously described. 10 μL of each supernatant were incubated for 30 min at 37 °C with 100 μM of the substrate solution: 100 μM AlaNNap or 100 μM ArgNNap and 0.65 mM DTT in 50 mM of phosphate buffer, pH 7.4.

All the reactions were stopped by adding 100 μL of 0.1 M acetate buffer, pH 4.2. The amount of β-naphthylamine released as the result of the enzymatic activities was measured fluorimetrically at 412 nm emission wavelength with and excitation wavelength of 345 nm.

Proteins were quantified also in triplicate by the method of Bradford, using bovine serum albumin (BSA) as standard. Specific soluble and membrane-bound APN, APB, AspAP and APA activities were expressed as nanomoles of Ala-, Arg-, Asp- and Glu-β-naphthylamide hydrolyzed per min per mg of protein, by using a standard curve prepared with the latter compound under corresponding assay conditions.

Statistical analysis

To analyze the differences between healthy pre- and postmenopausal, and pre- and postmenopausal women diagnosed with ductal infiltrating carcinoma, we have used unpaired Newman-
Keuls. All comparisons with p-values below 0.05 were considered significant.

Results

The Fig. 1 illustrates the results obtained to specific serum AspAP and APA activities in healthy (control) premenopausal and postmenopausal women and in women diagnosed with ductal infiltrating breast cancer. Specific serum APN and APB activities in healthy (control) premenopausal and postmenopausal women and in women diagnosed with ductal infiltrating breast cancer are shown in Fig. 2.

In postmenopausal women with breast cancer a significant increase (p < 0.001) was found in serum APN (Fig. 2A) and APB (Fig. 2B) activities. Also, in premenopausal women with breast cancer a significant increase (p < 0.001) was observed in APB (Fig. 2B). On the contrary, a significant (p < 0.05) decrease was found in AspAP activity (Fig. 1A) in premenopausal women with breast cancer. However, no differences were found between groups in APA activity (Fig. 1B).

Discussion

In the present work, we described changes in serum RAS-regulating AP activities in pre- and postmenopausal women with breast cancer. The analysis of angiotensinase activities may show the changes in their corresponding peptide substrates in the RAS, and hence reflects the changes in the function in which these peptides are involved.

Our results showed differences in the metabolism of angiotensin between pre- and postmenopausal women. In fact, in premenopausal women with breast cancer we observed a decrease in AspAP and an increase in APB activity. These data may suggest a predominant effect of Ang II. By contrast, in postmenopausal women with breast cancer we observed an increase in APB and APN activities. These results also suggested a main role for Ang II (Fig. 3).

Ang II is a multifunctional bioactive octapeptide of RAS, and stimulates the growth of solid tumor cells, including gastric cancer, breast cancer, ovarian cancer, and pancreatic cancer cells through specific G-protein-coupled AT1-R. Therefore, many researchers have described that Ang II/AT1-R might be involved in cancer, and Ang II might also be a potent mitogen for cancer cells. On the other hand, Ang II acts as a functional regulator of angiogenesis. The growth and metastasis depend on angiogenesis to connect the growing tumor to a blood supply. Ang II has been shown to be a potent angiogenic factor by inducing endothelial proliferation and increasing the expression of the angiogenic factors and angiotropes in human endothelial cells.

Our results show that in pre- and postmenopausal women, the changes observed in RAS-regulating AP activities lead to a putative increase in Ang II. However, these changes are different in each experimental growth. It may be possible because the different hormonal profile in pre- and postmenopausal women with breast cancer suggests a neuroendocrine misregulation.

In previous studies, we suggested that estradiol and progesterone influence RAS-regulating activities at different levels of the hypothalamus-pituitary-adrenal axis in female mice. In the same way, in human, several studies have also shown that sex hormones affect the RAS: thus, plasma renin activity is higher in men that in aged-matched women, and the plasma renin activity is higher in postmenopausal women that in premenopausal women or postmenopausal women with hormone replacement therapy. As it is known, the breast is an organ subjected to different hormonal influences. The physiology of breast depends on an endocrinology balance. In particular, estrogens play a key role in breast cancer development. In fact, in postmenopausal women with elevated plasma estrogens have a high risk of breast cancer. On the other hand, estrogen deficiency has been associated with an increase of ACE synthesis and activity and upregulation of the AT1-R.

Conclusion

Our present results show changes in serum angiotensinase activities from pre- and postmenopausal women with breast cancer. An imbalance in the signals activated by Ang II may produce abnormal vascular growth with different response between pre- and postmenopausal women depending on the hormonal profile and the development of the disease. Therefore, considerable attention may be focused on the development of RAS blockade therapy as a new strategy for breast cancer treatment.

Conflict of interest

None declared.

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References


