ORIGINAL ARTICLE

A Study of Angiotensin 1-7 Secretion in Cancer Patients and its Correlation to Il-17 Production and the Immune status of Patients

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ABSTRACT

In addition to the previously demonstrated anticancer activity of some human molecules, mainly represented by the pineal hormones and the endocannabinoids, angiotensin1-7 (Ang 1-7), the enzymatic product of ACE2, has recently appeared to play an important anticancer activity through both cytotoxic and anti-angiogenic effects. The functionless of the pineal gland and cannabinoid system has been shown to decline with cancer progression, by contributing to tumor dissemination itself, because of their fundamental role in the natural resistance against cancer onset and progression. In contrast, the profile of Ang 1-7 secretion in cancer still remains unknown. The aim of this preliminary study was the evaluation of Ang 1-7 blood levels in cancer patients in relation to the extension of disease and the immune status, as evaluated by determining the lymphocyte-to-monocyte ratio (LMR), and the salivary concentrations of IL-17A. The study included 20 healthy subjects as controls and 32 consecutive cancer patients affected by the most common neoplasms, 20 of them had a metastatic disease, while the other 12 patients had a locally limited disease. Ang 1-7 mean serum concentrations were significantly lower in metastatic patients than in the non-metastatic ones and in controls. On the contrary, salivary mean values of IL-17A were significantly higher in metastatic patients than in controls and in those with a locally limited disease. However, no significant correlation was seen between Ang 1-7 and IL-17A concentrations. Finally, patients with abnormally low values of LMR showed significantly higher mean levels of IL-17A than those found in controls and in non-metastatic patients. On the contrary, Ang 1-7 mean levels were lower in patients with low LMR than in those with normal LMR values, even though the difference was not statistically significant. In conclusion, the results of the present preliminary study would suggest a progressive decline in the endogenous production of Ang 1-7 with cancer progression. Then, this evidence would suggest that cancer progression may be due at least in part to a progressive decline in the endogenous production of the main natural anticancer molecules, including the pineal hormones, the endocannabinoids, and Ang 1-7, which are responsible for the natural resistance against cancer onset and growth, and whose deficiency could be corrected by a simple exogenous administration in the treatment of cancer, concomitantly to the conventional antitumor therapies.

Key words: Angiotensin 1-7, Anti-cancer immunity, Cancer, IL-17, Lymphocyte-to-monocyte ratio.

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INTRODUCTION

The discovery of cytokines has profoundly modified the interpretation of the pathogenesis of human systemic diseases, including cancer and autoimmune pathologies (1, 2). In fact, all systemic diseases have appeared to present several cytokine secretions, commonly consisting of an enhanced endogenous production of inflammatory cytokines. This evidence is not surprising, since within the group of more than 40 cytokines, most of them play an inflammatory activity, including IL-1beta, IL-6, TNF-alpha, and IL-17, while only few cytokines, consisting of IL-10, IL-27, IL-35, and IL-37 exert an anti-inflammatory action. Moreover, most cytokines have been proven to play a pro-tumoral role, whereas only IL-2 (3), IL-12 (4), and IL-37 (5) have appeared to exert a clear anticancer activity by generating an effective anticancer immune response, which is mainly characterized by an evident increase in lymphocyte count, whose decline has been proven to be associated with a negative prognosis (6). To balance the predominant pro-inflammatory pro-tumoral role of the cytokine network, the evolution of human biology has developed a neuroendocrine functional system provided by anti-inflammatory anti-tumor activity. This bio regenerative anti-inflammatory antitumor system is constituted by three fundamental neuroendocrine structures, represented by the pineal gland (7), the endocannabinoid system (8), and ACE2-angiotensin 1-7 (Ang 1-7) axis (9, 10). Moreover, these three structures have been proven to be characterized by several interactions among them. In fact, the cannabinoid agonists have been found to stimulate the release of melatonin (MLT) (11), the most investigated antitumor hormone released from the pineal gland (12), which may promote ACE2 expression rather than that of ACE, with a following enhanced production of Ang 1-7 (13). Finally, Ang 1-7 has been found to stimulate the expression of cannabinoid receptors (14), by enhancing the endocannabinoid function. However, other pineal hormones have appeared to play an important anticancer action, particularly the indole 5-methoxytryptamine (15), and the beta-carboline pinealine (16). The endocannabinoid system produces at least two cannabinoid agonists, the arachidonyl-ethanol-amide, also termed anandamide, and the 2-arachidonylglycerol (17). In addition, the endocannabinoid system may produce inhibitors of the fatty acid amide hydrolase (FAAH), the enzyme involved in cannabinoid degradation, with a consequent increase in cannabinoid endogenous content, such as the palmitoyl-ethanol-amide (PEA) (18). All molecules, including MLT and other pineal hormones, endogenous and exogenous cannabinoid agents, and Ang 1-7 display their anticancer activity through three main antitumor mechanisms (9,10,17), including direct cytotoxic antiproliferative effect, anti-angiogenic action, and immunostimulating activity, namely consisting of the inhibition of the endogenous production of anticancer pro-inflammatory cytokines, among them IL-17 would exert a predominant role, since IL-17, in addition to its direct pro-tumor action (18), may stimulate the macrophage release of other pro-inflammatory pro-tumor cytokines, including IL-6, IL-1beta, and TNF-alpha (19). Moreover, both MLT (20) and Ang 1-7 (10) have appeared to stimulate IL-2 secretion from Th1 lymphocytes, which represents at present the most clinically investigated antitumor cytokine (21,22). Therefore, the bioregenerative anticancer systems of human body may be synthesized into three neuroendocrine systems, represented by the pineal gland, the endocannabinoid system, and ACE2-Ang 1-7 axis in association with the anticancer cytokine immune response, mainly expressed by IL-2 (3), which would stimulate the secretion of the other two main antitumor cytokines, including IL-12 and IL-37 (21). Moreover, previous experimental and clinical studies had already shown that cancer progression is associated with a progressive decline in the pineal function and MLT secretion (23), and concomitantly in the endocannabinoid system (24), whose function is connected with that of the pineal gland. Finally, because of the stimulatory role of pineal- endocannabinoid axis on IL-2 secretion (20), cancer dissemination has appeared to be also characterized by a progressive decline in the endogenous production of IL-2 (21), which plays a fundamental role in the generation of an effective anticancer immunobiological response (3). On the contrary, at present there is no clinical study to evaluate the endogenous production of Ang 1-7 in cancer patients, in an attempt to establish which may the profile of Ang 1-7 secretion in patients be with locally or limited neoplastic disease. The question is particularly important, since it has been shown that hypertensive patients under chronic therapy with ACE inhibitors or AT1 receptor blockers may present a lower frequency of cancer (25), and this evidence would depend on the concomitant stimulation of ACE2 activation following ACE- angiotensin II axis inhibition, with a consequent enhanced production of Ang 1-7 (26). However, previous preliminary studies have already shown that the reduced expression of ACE2 by cancer cells is associated with a more aggressive tumor malignancy (27,28). On these bases, the present study was performed to analyse in a preliminary manner the secretion of Ang 1-7, whose anticancer properties have been well documented (9,10), in cancer patients in relation to their disease extension, the status of the anticancer immunity, and the secretion of IL-17, because of its fundamental role in cancer progression.

PATIENTS AND METHODS

The study included 32 consecutive cancer patients, who were followed at Institute Biological Medicine of Milan for their neoplastic disease. Eligibility criteria were, as follows: histologically proven neoplastic disease, measurable lesions, no chronic concomitant therapy with corticosteroids or

opioids because of their immunosuppressive effects (29), no antitumor therapy for at least one month prior to study, and no therapy with ACE inhibitors or AT1 receptor blockers because of their influence on Ang 1-7 secretion (25). According to their extension of disease, patients wee subdivided into metastatic and non-metastatic patients. The results were compared to those obtained in 20 age- and sex-matched healthy subjects. A metastatic disease was present in 20 patients, while the remaining 12 patients had a locally limited disease. The immune status of patients was evaluated by determining the salivary concentrations of IL-17A, which is the most active isoform of IL-17 family (19), and the lymphocyte-to-monocyte ratio (LMR), whose decline has appeared to predict a poor prognosis and a lower survival in cancer patients (6). Ang 1-7 serum levels and salivary concentrations of IL-17A were measured by Elisa method and commercially available kits (Ang 1-7: Cloud-Clone Corp, Katy, Texas; IL-17A: Tecam Group, Barcellona, Spain). Normal values of LMR obtained in our laboratory (95% confidence limits) were greater than 2.1. The clinical characteristics of patients are reported in Table 1. Data were reported as mean +/- SE, and statistically analysed by the chi-square test, Student's t test, and coefficient of correlation, as appropriate. The study was supported by Ecohemp (Villanova del Ghebbo, Rovigo, Italy).

RESULTS

Serum mean levels of Ang 1-7 in relation to IL-17A salivary concentrations and LMR observed in cancer patients and in controls are reported in Table 2. Mean levels of Ang 1-7 observed in cancer patients were lower than those found in controls, without, however, statistically significant differences. On the contrary, by considering the extension of disease, mean values of Ang 1-7 observed in metastatic cancer patients were significantly lower than those found in both controls ($P<0.05$) and patients with locally limited disease ($P<0.01$). Salivary mean concentrations of IL-17A observed in cancer patients were significantly higher than in controls ($P<0.05$). Moreover, IL-17A mean values observed in non-metastatic patients were higher than in controls, without, however, statistically significant differences. In contrast, by considering disease extension, IL-17A mean concentrations found in metastatic patients were significantly higher than those seen in both controls ($P<0.01$) and in non-metastatic patients ($P<0.05$). However, no significant correlation occurred between Ang 1-7 and IL-17A concentrations ($r= -0.1$). Abnormally low levels of LMR lower than 2.1 occurred in 8/32 (25%), and the frequency of LMR low values was significantly greater in metastatic patients than in the non-metastatic ones (7/20(35%) vs 1/12(8%), $P<0.01$). Patients with abnormally low values of LMR showed lower Ang 1-7 mean concentrations, without, however, statistically significant differences- In contrast IL-17A concentrations found in patients with low LMR values were significantly higher than those found in both non-metastatic patients ($P<0.025$) and controls ($P<0.01$).

Table 1. Prevalence Clinical characteristics of 32 cancer patients.

M/F:	10/22
MEDIAN AGE:	56 years (range 40-72)
TUMOR HISTOTYPE	
- Breast cancer:	9
- Ovarian carcinoma:	6
-Endometrial adenocarcinoma:	3
-Colorectal carcinoma:	4
- Lungcancer:	3
-Prostate carcinoma:	3
- Gastriccancer:	1
-Pancreatic adenocarcinoma:	1
- Sarcoma:	2
METASTATIC DISEASE:	20/32
DOMINANT METASTASIS SITES	
- Bone:	1
- Lung:	3
- Liver:	5
- Peritoneum:	9
- Brain:	2

Table 2. Prevalence Mean serum concentrations of angiotensin 1-7 (Ang 1-7) in relation to mean salivary levels of IL-17A, and lymphocyte-to-monocyte ratio (LMR) in healthy subjects and cancer patients.

SUBJECTS	n	Ang 1-7 pg/ml	IL-17A pg/ml	LMR (X +/-SE)
HEALTHY CONTROLS	20	365 +/- 11	5.8 +/- 1.2	5.3 +/- 0.3
CANCER PATIENTS	32	329 +/- 15	16.1 +/- 2.9	3.2 +/- 0.4
NON-METASTATIC PATIENTS	12	389 +/- 13	9.7 +/- 2.3	4.2 +/- 0.2
PATIENTS-METASTATIC	20	292 +/- 16*	22.1 +/- 3.2**	2.7 +/- 0.4***
LMR VALUES				
LOW VALUES	8	316 +/- 18	14.9 +/- 2.2****	
NORMAL VALUES	24	345 +/- 12	6.8 +/- 1.6	

*P<0.01 vs non-metastatic patients, P<0.05 vs controls
**P<0.05 vs non-metastatic patients, P<0.01 vs controls
***P<0.01 vs controls, P<0.05 vs non-metastatic patients
****P<0.05 vs patients with normal LMR values

DISCUSSION

In addition to the previous demonstrated pineal and endocannabinoid deficiencies during cancer progression in humans, this preliminary study, carried out to investigate the secretion of the anticancer molecule Ang 1-7 in cancer patients, would suggest that cancer progression may be also associated with a concomitant progressive decline in the functionless of ACE2-Ang 1-7 axis, as suggested by the evidence of significantly lower Ang 1-7 blood concentrations in metastatic patients with respect to those with a locally limited disease. Obviously, the low number of patients and the different tumor histotype do not allow us to draw define conclusions about ACE2-Ang 1-7 axis in relation to each single histotype of cancer. Moreover, longitudinal studies by monitoring the biological changes in cancer patients during the clinical history of the neoplastic disease will be required to better confirm the progressive decline in the endogenous production of Ang 1-7 with cancer dissemination, and its connection with pineal failure and endocannabinoid deficiency. Because of the reciprocal stimulatory connections among MLT, cannabinoid, and Ang 1-7 secretion, each single deficiency may allow a concomitant decline in the other secretions, even though the primary deficiency could be that in the pineal function, because of its fundamental role in the regulation of most biological functions in relation to the universal rhythms, namely to the light/dark photoperiod. In addition, in agreement with the results of other authors (19), this study confirms the evidence of an abnormal endogenous production of IL-17A in patients with metastatic or locally advanced, which could play a role in tumor progression itself because of the direct pro-tumoral action of IL-17A (19). Then, the evidence of high concentrations of IL-17A, one of the most host potent inflammatory cytokines (30), in advanced cancer patients would further confirm the fundamental role of the chronic inflammatory status in promoting cancer progression, because of its association with the suppression of an effective anticancer immunity (25). The lack of an evident negative correlation between Ang 1-7 and IL-17A could depend on the different used biological fluids, as well as to the possible action of other endogenous antitumor molecules also able to inhibit IL-17 secretion. Therefore, further studies by detecting the blood concentrations of IL-17A rather than the salivary ones, will be necessary to better investigate the correlation occurring between Ang 1-7 and IL-17A, which are characterized by opposite biological effects on both cancer growth and cardiovascular system. In conclusion, because of its physiological anticancer activity in addition to its known cardio protective effects (9,10), this preliminary study would justify further clinical researches to better define the role of ACE2-Ang 1-7 axis in the pathogenesis and cure of cancer.

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