Abstract. Background: Ghrelin is a natural growth hormone secretagogue (GHS), involved in the biology of a number of diseases such as lung cancer and prostate cancer. The aim of this study is to assess the relationship existing between ghrelin and testosterone, insulin, and PSA in prostate adenocarcinoma. Patients and Methods: A patient population and a control population were studied. The former consisted of 18 individuals, age range 50-75 years, with a primary histological diagnosis of prostate adenocarcinoma. Patients and Methods: A patient population and a control population were studied. The former consisted of 18 individuals, age range 50-75 years, with a primary histological diagnosis of prostate adenocarcinoma that were divided into two equal groups of 9 patients each. The control population consisted of 40 normogonadic healthy males aged between 23 and 77 years (average age 43). The first group was treated with oral bicalutamide with a daily dose of 150 mg, while the second group was treated with an intramuscular injection of 11.25 mg of leuprorelin every three months. Total ghrelin was measured with a radio immunological direct method using Phoenix’s ghrelin human RIA kit. Intra-assay variance was 8.2% and inter-assay variance was 11.4%. Acylated-ghrelin was measured by applying an extraction method using C18 columns followed by radio immunological dosage with antibody and peninsula tracer. Intra-assay variance was 6.1% and inter-assay variance was 8.7%. All other blood parameters were analysed at the central laboratory of the S. Orsola-Malpighi Polyclinic in Bologna. PSA and testosterone were used to assess response to treatment. The PSA monitoring was achieved with a chemio-luminescence assay method (Roche Modular analytics E 170). Free T was also measured using a direct RIA kit (Diagnostic Systems Laboratories, Inc.). Results: In the four months during which patients underwent pharmacological treatment, testosterone values varied significantly (p<0.05) in both groups. No variations (p>0.05) were found for ghrelin, acylated-ghrelin and insulin. Conclusion: It is concluded that in patients with prostate neoplasms there is no correlation between the variations of circulating levels of ghrelin and those of testosterone.

Ghrelin is a natural growth hormone secretagogue (GHS), involved in central based stimulation of GH release to achieve a regulated dietary intake and positive energy balance; moreover, it is also involved in several other processes within the human body. Recent studies have shown a major involvement of ghrelin in the biology of a number of diseases such as lung and prostate cancer. In its mature state, it appears as a sequence of 28 amino acids with a molecular weight of 3,314. It is derived from pre-proghrelin, which, in turn, consists of a sequence of 117 amino acids. In circulation, it can exist either as acylated-ghrelin, in which the serine amino acid is etherified in position 3 (Ser3) with n-octanoic acid, or as deacylated-ghrelin which is not etherified (Figure 1). The presence of n-octanoic acid gives the molecule its capacity to bind with the GHS-R-1a receptor and therefore to enhance GH expression. In prostate cancer and in other neoplastic tissues, a new preproghrelin-1a has recently been found, namely exon 3-deleted preproghrelin isoform (1).

Ghrelin is produced and expressed by specific cells, which are found mainly in the gastric mucous membrane. The ghrelin gastric cell is an epithelial endocrine component of the diffuse neuro-endocrinal system (DNES). Cells producing ghrelin have been found in the nucleus arcuatus and in a separate group of neurons near to the third ventricle. Their function seems to be connected to the regulation of energetic balance. Ghrelin is made by many tissues in the early phases of the development. Moreover cells producing ghrelin have been found in the first quarter of pregnancy in the thyroid, in...
the lung and in the pancreas of humans and rats. In rats ghrelin is also present in the pituitary gland. These data confirm the important role of ghrelin in the development. Ghrelin immunoreactive cells have been observed in well-defined endocrine tumors in the pituitary, lung, stomach, intestine, pancreas and prostate. Ghrelin is also present in breast tumor and thyroid and medullary thyroid carcinomas (2). Ghrelin affects the blood level regulation of testosterone (another crucial factor in prostate gland homeostasis) and is, in turn, affected by it (3, 4). This probably depends on a number of variables which have not yet been thoroughly established. Body composition, the distribution of body fat, insulin resistance and high insulin blood levels are deemed likely to have a crucial role (5-8).

In some neoplastic cell lines, ghrelin has displayed an antiproliferation activity while it has presented mitogenic activity in other kinds of tumor cells (1, 9).

Subtypes of receptors for ghrelin have been found even in tumors originating from tissues that physiologically did not express these receptors, as for example the breast. In some studies desacylated ghrelin, a biologically inactive peptide, unable to stimulate the GH release in vivo and in vitro, inhibited the proliferation of tumoral cells. This could explain a possible activity on receptors different from the GHS-R1a. Ghrelin, as well as its cells and receptors, are present in the prostate under normal conditions. A recent study (1) has described, for the first time, in vitro generation of ghrelin by some prostate cancer cell lines. This study has shown that the proliferation of neoplastic cells is promoted by the activation of the ERK1/2-MAPK pathway.

The activation of the ERK1/2 MAPK cascade has been shown in relation to more aggressive forms of tumors. High levels of ERK1/2 have been recorded in a number of androgen-independent, metastatic late-stage prostate cancer, suggesting that this cascade may contribute to the development and progression of prostate cancer. Ghrelin promotes cell growth through the ERK1/2 pathway without inhibiting cell apoptosis in prostate cancer cells. A preproghrelin isoform, namely exon-3-deleted preproghrelin isoform, has recently been identified in humans and mice and together with mature ghrelin, has been found to be highly expressed in vitro in prostate neoplasm. Exon-3-deleted preproghrelin isoform concentrations have been found to be higher than normal also in breast tumor, albeit only as mRNA in this case.

The aim of this study is to assess the relationship existing between ghrelin and testosterone, insulin, PSA and prostate adenocarcinoma.

Patients and Methods

This is a prospective study which took place between December 2005 and October 2007. Two populations were studied: a patient population and a control population. The former consisted of 18 individuals, age range 50-75 years, with a primary histological diagnosis of prostate adenocarcinoma, locally advanced disease referred for hormone-suppressive therapy. The recruited patients were then divided into two equal groups of 9 patients each. The first group, which was conventionally labelled Group A, was treated with oral bicalutamide with a daily dose of 150 mg, while the second group, labelled as Group B, was treated with an intramuscular injection of 11.25 mg of leuprorelin every three months. Group B was also treated with bicalutamide at a dosage of 50 mg for 15 days to avoid flare up occurrences. Both groups were examined on two occasions: the first at the beginning of treatment (T0) and the second, four months later (T1). The control population consisted of 40 normogonadic healthy males aged between 23 and 77 years (average age 43). In this group suspicion of prostate cancer was excluded by PSA blood sampling (PSA <1 ng/ml), digital rectal examination (negative for any suspect of cancer and normal volume) and familiar history of prostate cancer.

Patients were assessed both under the clinical and laboratory profile through the case history of physical examination, body mass index definition and three venous blood samples. All the patients were invited to maintain their usual diet throughout the study. The following parameters were assayed in each blood sample: PSA (ng/ml), free testosterone (pg/ml), insulin (µU/ml), total ghrelin (pg/ml) and acylated-ghrelin (pg/ml). Total ghrelin was measured with a radioimmunological direct method using Phoenix’s ghrelin human RIA kit. Intra-assay variance was 8.2% and inter-assay variance was 11.4%. Acylated-ghrelin was measured by applying an extraction method using C18 columns followed by radioimmunological dosage with antibody and peninsular tracer. Intra-assay variance was 6.1% and inter-assay variance was 8.7%. All other blood parameters were analysed at the central laboratory of the S. Orsola-Malpighi Polyclinic in Bologna. PSA and testosterone were used to assess response to treatment.

The PSA monitoring was achieved with a chemio-luminescence assay method (Roche Modular analytics E 170). Free T was also measured using a direct RIA kit (Diagnostic Systems Laboratories, Inc.).

Data were processed with Windows SPSS v.10. software for statistical analysis. To ensure a thorough exhaustive evaluation and comprehension of scientific evidence (although limited to studies published in English), a bibliographical search was conducted on PubMed.

Results

In Group A (patients treated with bicalutamide), free testosterone, increased from an average of 7.88 pg/ml (S.D.±3.37 pg/ml) at T0 to an average of 11.69 pg/ml (S.D.±4.44 pg/ml) at T1. In group B (patients treated with leuprorelin) average T0 testosterone was 9.77 pg/ml (S.D.±2.98 pg/ml) and average T1 testosterone was 1.72 pg/ml (S.D.±1.00 pg/ml). The statistical analysis conducted with a Generalized Repeated Measurement Linear Model shows that both time (p=0.026) and group (p<0.001) induce significant variations in blood levels of free testosterone. The two groups were successively and separately studied. ANOVA variance statistical analysis was performed on Group A to compare the variations between T0 free
testosterone and T1 free testosterone and barely significant changes \((p=0.05)\) were found. ANOVA variance statistical analysis was performed on Group B to compare the variations between T0 and T1 free testosterone and highly significant changes \((p<0.001)\) were found. This shows that the effect identified with the Generalized Repeated Measurement Linear Model for statistical analysis was accounted for mainly by leuprorelin-induced testosterone changes.

In Group A, total ghrelin (GRT) varied from an average of 613.33 pg/ml (S.D.±102.96) at T0 to an average of 615.56 pg/ml (S.D.±149.59 pg/ml) at T1. In Group B, average T0 GRT was 671.11 pg/ml (S.D.±267.79 pg/ml) and average T1 GRT was 771.11 pg/ml (S.D.±308.24 pg/ml) (Figure 2). The statistical analysis showed that time \((p=0.089)\) and group \((p=0.102)\) did not induce significant variations in blood levels of GRT. ANOVA variance statistical analysis was performed on Groups A and B to compare the variations between T0 and T1 GRT. No significant changes were found for Group A \((p=0.971)\) or Group B \((p=0.473)\).

Average T0 GRT in the patient population (GRT0) was 642.22 pg/ml (S.D.±199.04 pg/ml), while that in the control population (GRTC) was 1,023.65 pg/ml (S.D.±415.54 pg/ml). T-test performed between the average GRT0 and and the average GRTC showed no significant difference between the two parameters \((p=0.61)\) (Figure 3). In Group A, acylated-ghrelin (GRA) ranged from an average of 123.44 pg/ml (S.D.±68.72) at T0 to an average of 93.89 pg/ml (S.D.±43.98 pg/ml) at T1. In Group B, average T0 GRA was
ANOV A statistical analysis showed homogeneous values significant variations in blood levels of acylated-ghrelin. The statistical analysis showed that neither time (p=0.795) nor group (p=0.053) induced significant variations in blood levels of acylated-ghrelin. ANOVA statistical analysis showed homogeneous values between the two groups both at the beginning and at the end of the study (p=0.568). T-test performed on the average values of both groups, taken separately, established that there was no significant variation between T0 and T1, thus confirming a basic consistency of values both in time and between the groups.

In Group A, insulin (I) ranged from an average of 8.44 μU/mL (S.D.±4.19 μU/ml) at T0 to an average of 7.56 μU/mL (S.D.±4.77 μU/ml) at T1. In Group B average T0 insulin was 11.11 μU/mL (S.D.±6.57 μU/ml) and average T1 insulin was 9.56 μU/mL (S.D.±3.00 μU/ml). The statistical analysis showed that neither time (p=0.300) nor group (p=0.774) induce significant variations in blood levels of insulin.

Discussion

In the four months during which patients underwent pharmacological treatment, testosterone values varied significantly (p<0.05) in both groups. In Group A bicalutamide brought about an increase in the average testosterone concentration which rose from 7.88 pg/ml (S.D.±3.27 pg/ml) at T0 to 11.69 pg/ml (S.D.±4.44 pg/ml) at T1 (p=0.05). The drug used in Group A is a pure anti-androgen which acts by competitively binding testosterone receptors so as to create a state of relative androgenic deficit. This entails an increase in the synthesis of gonadotropins and testosterone. In Group B, leuprolrelin induced a decrease of testosterone from an average of 9.77 pg/ml (S.D.±2.98 pg/ml) at T0 to an average of 1.72 pg/ml (S.D.±1.00 pg/ml) at T1 (p=0.001). This alteration of the androgenic profile in the two groups did not bring about any change in the values of GRT. Between T0 and T1 there were no significant variations (p>0.05) of GRT in the two groups either linked to time or to treatment. This finding is in contrast with previous studies on ghrelin. In the two groups no variations were found for insulin levels (p>0.05). Previous studies (10) indicated insulin as a possible mediator between testosterone and ghrelin variations. No variations (p>0.05) were found for acylated-ghrelin, the other natural GHS studied.

This study has yielded a clearcut difference in the behavior of testosterone against the behavior of total ghrelin, acylghrelin and insulin. Average GRT in the patient population at T0 was 642.22 pg/ml (S.D.±19.04 pg/ml) whereas average GRT in the control population was 1,023.65 pg/ml (S.D.±415.54 pg/ml). T-test statistical analysis of average GRT in both populations established that there was no significant difference (p=0.61).

As a conclusion, it can be claimed that in patients with prostate neoplasms there is no correlation between the variations of circulating levels of ghrelin and those of testosterone. Some of the findings could also open the way for further studies about a possible correlation, not significant at the moment because of the restricted size of the sample, between more advanced and/or non-differentiated neoplasms and ghrelin levels.

References


