ORIGINAL ARTICLE

The imbalance of cathepsin B-like activity in acromegalic patients – preliminary report

Jacek Daroszewski [1], Marek Bolanowski [1], Marcin Kaluzny [1], Maciej Siewinski [2]

1 Department of Endocrinology, Diabetology, and Isotope Therapy, Wroclaw Medical University, Poland
2 Department of Basic Sciences, Wroclaw Medical University, Poland

Correspondence to: Dr. Jacek Daroszewski, MD., PhD.
Department of Endocrinology, Diabetology and Isotope Therapy,
Wroclaw Medical University,
ul. Pasteura 4, 50-367 Wroclaw, Poland.
tel: +48 71-7842552; fax: +48 71-3270957; e-mail: daro48@o2.pl

Submitted: 2009-07-22 Accepted: 2009-12-15 Published online: 2010-04-28

Key words: **acromegaly; cathepsins; cysteine proteinases; cysteine peptidase inhibitor**

Neuroendocrinol Lett 2010; **31**(2):256–260 **PMID:** 20424577 NEL310210A04

Abstract

OBJECTIVE: Acromegaly is a rare disease due to growth hormone (GH) excess. Patients must be carefully follow up because of mortality and co-morbidity increased risks. Since routinely used GH and insulin-like growth factor-1(IGF-1) estimations are not always sufficient, patients require assessment of organ- or tissue-specific tests. Cysteine proteases (CP), including cathepsin B, have been tested in a number of pathologies in respect of a role in pathogenesis and potential utility in the disease activity and prognosis assessment. There is lack of data on CP activity in acromegaly.

MATERIALS AND METHODS: In present study cathepsin B-like and cysteine peptidase inhibitor (CPI) activities have been tested in 29 acromegaly patients and in 15 healthy controls. Cathepsin B activity was assayed with N-bansoyl-DL-arginine-β-naphthylamide (BANA) as substrate by the Barrett method. CPI activity was determined by measuring the inhibition of papain.

RESULTS: Serum cathepsin B activity (median: 1.38 U/ml) and CPI activity (median: 93.08 U/ml) were higher in acromegaly then in controls (0.93 U/ml and 82.55 U/ml, p=0.000017 and 0.00285, respectively). Neither cathepsin B nor CPI activity was correlated with GH or IGF-1 level. No correlation was recorded between cathepsin B and CPI activity.

CONCLUSION: It was shown for the first time that cathepsin B and CPI activities are increased in acromegaly. These findings suggest to study cathepsin system as an adjuvant parameter in the assessment of the overall acromegaly complications. Moreover, CP may be involved in pathomechanism of organ complications in acromegaly and may interfere with IGF-1 action.

Abbreviations :

CP	- cysteine peptidase/protease
CPI	- cysteine peptidase/protease inhibitor
GH	- growth hormone
IGF-1	- insulin-like growth factor-1

INTRODUCTION

Acromegaly is a rare endocrine disease due to growth hormone (GH) excess originating from somatotroph adenoma of the pituitary gland. Apart from the local mass effect from direct expansion of the tumor, metabolic and multisystemic complications caused by excessive GH and IGF-1 activity carry an increased mortality risk and reduced life expectancy. Mortality is increased between two and four times that of the general population, predominantly because of cardiovascular and respiratory symptoms, although patients must also be carefully followed up because of metabolic complications and increased risk of neoplastic diseases (Colao *et al.* 2008; Dekkers *et al.* 2008; Foltyn *et al.* 2008).

Since the routinely used GH and IGF-1 measurements are not always sufficient, patients require comprehensive assessment by organ- or tissue-specific tests for a precise estimation of overall morbidity. Cysteine peptidases/proteases (CP) are distributed ubiquitously in most tissues, where they catalyze the hydrolysis of proteins within lysosomes. Besides the well-documented functions of CP in protein catabolism, various specific physiological functions of these enzymes have been described. They are involved in precursor protein activation, antigen presentation, tissue remodeling and differentiation, as well as tumor formation, apoptosis, and oxidoreduction processes (Berdowska 2004; Koblinski *et al.* 2000; Kos *et al.* 2000). Cysteine peptidase/protease inhibitors (CPI) are regarded as potential protective factors able to prevent the enzyme activity and influence the pathomechanisms involved by CP action. CP, including cathepsin B, have been tested in a number of pathologies with respect to their role in pathogenesis and potential utility in assessing disease activity and prognosis (Berdowska 2004; Sohar *et al.* 2002; Yan *et al.* 1998).

For acromegalic subjects there is no data on circulating CP levels and activity. Therefore, in the present study we aimed to determine plasma levels of cathepsin B-like activities in acromegalic patients in relation to their metabolic status.

MATERIALS AND METHODS

Subjects

Twenty-nine patients with acromegaly (16 females, 13 males) with a mean age of 46.6 ± 11.8 years and 15 healthy controls (8 females, 7 males) were included in the study. Sixteen patients had undergone pituitary surgery (two twice) and 16 had received three-field external-beam pituitary radiotherapy. Some patients were on stable replacement therapy with hydrocortisone (n=7), L-Thyroxine (n=1), and gonadal steroids (n=6) for the duration of the study. All the patients had been diagnosed according to the currently accepted criteria of a serum IGF-1 level for their particular age with failure of GH suppression to less than 1 µg/l after 75 g of oral glucose (Giustina *et al.* 2000). The characteristics of the patients and their serum IGF-1 and GH results are given in Table 1.

Cathepsin B-like activity

Activity was assayed with N-bansoyl-DL-arginine-β-naphtylamine (BANA) as substrate by the Barrett method (Barrett 1976). Five nmol/l BANA and 500 µl of serum were incubated with 0.01 M potassium phosphate buffer (pH 6.0) containing 2 nM EDTA, 1 mM l-cysteine, and 0.1% mercaptoethanol in a total volume of 1.0 ml for 12 h at 37 °C. The measurements of BANA hydrolysis were based on Barrett's method. One unit of activity hydrolyzes 1 µmol of substrate per hour under the reaction conditions used.

Cysteine peptidase/protease inhibitor (CPI)

Activity was determined by measuring the inhibition of papain. One inhibitory unit corresponds to the inhibition of serum samples for 20 min at 37 °C, 60 °C, and 80 °C and pH 6.0, 2.0, and 11.0. The temperature of the samples was then brought to 30 °C and pH 6.0 and the samples were incubated with 0.5M α-methylamine (pH 7.5, 37 °C). At this stage, CPI activity was determined in samples preincubated at 60 °C and pH 2.0.

We presume that CPI at 37 °C reflects the physiological activity of serum cysteine peptidase and applied the following procedure to determine CPI-37: 50 µl of serum was preincubated with 50 µl of water and 2.0 ml of 0.5 M methylamine in 0.1 M potassium phosphate, pH 7.5, for 2 h at 37 °C; 50 µl of 0.1% papain was then added and the samples were incubated for 10 min at 37 °C. Then 50 µl of 66 mM BANA solution was added and free β-naphtylamine was measured. Inhibitory activity was determined when the enzyme activity reached 20–80%. Minakata *et al.* described inactivation of α-methylamine by methylamine at 55 °C and pH 7.5, but found CPIs to be completely stable under these conditions (Minakata *et al* 1982).

Insulin-like growth factor-1 (IGF-I)

Serum IGF-I was measured using the RIA method (BioSource Europe, Belgium) with intra- and interassay coefficients of variation (CV) between 2 and 8%.

Growth hormone (GH)

Serum GH evaluations were performed using commercially available IRMA kits (Immunotech, France) with an intra-assay CV of 1.5%.

Statistics

Statistical analysis was made using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). Parameter distributions were assessed using Shapiro-Wilk's *W* test. The data were not normally distributed. Mann-Whitney's *U* test was used for assessing statistically significant differences for all parameters. Pearson's test was used for calculating statistically significant correlations. Differences were statistically significant when $p<0.05$.

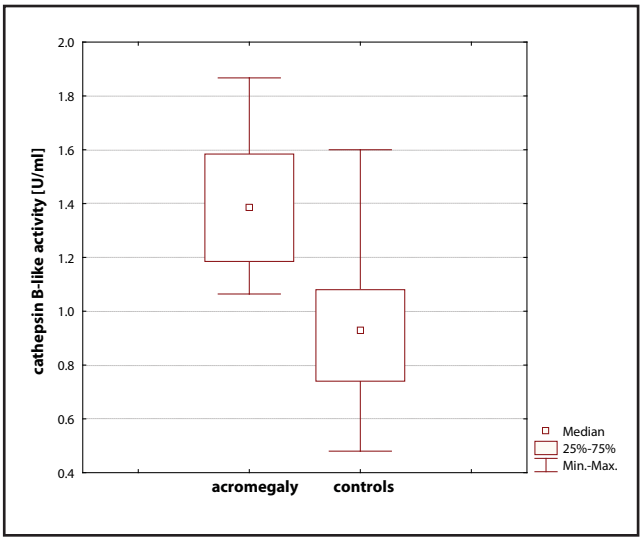

Fig. 1. Cathepsin B-like activity in patients with acromegaly and in control group.

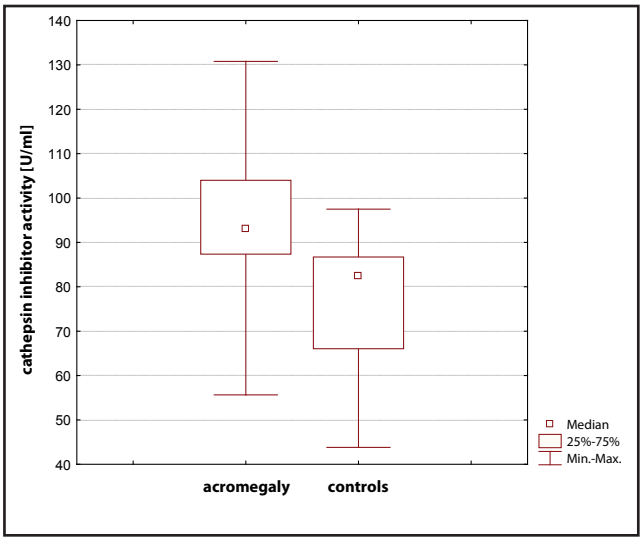

Fig. 2. Cathepsin inhibitor activity in patients with acromegaly and in control group.

RESULTS

Serum cathepsin B-like activity (median: 1.38 U/ml, Figure 1) as well as cathepsin inhibitor activity (median: 93.08 U/ml, Figure 2) were elevated in the acromegaly patients compared with the controls, who had 0.93 U/ml and 82.55 U/ml, respectively (p=0.000017 and 0.00285). Neither cathepsin B nor cathepsin inhibitor activity showed any significant correlation with GH or IGF-1 level or age of the subjects in the acromegalic and control groups. No correlation was found between cathepsin B and CPI activity (Table 2).

Tab. 1. Clinical characteristics of patients with acromegaly and their hormonal results.

Parameter	Age (years)	GH (µg/l)	IGF-1 (ng/ml)
Mean	46.6	53.8	904.0
SD	11.8	43.8	117.2
Median	46.0	39.8	919.0
25%/75% quartile	41.0/53.0	27.0/63.0	817.0/985.0
Range	24.0–78.0	10.6-225.0	686.0–1148.0

Tab. 2. Pearson's correlations coefficients between cathepsin activity, cathepsin inhibitor activity, GH, IGF-1 and age.

	GH	IGF-1	Cathepsin activity	Inhibitor activity
GH	1.0	0.25	0.02	0.2
IGF-1	0.25	1.0	0.15	0.13
Cathepsin activity	0.02	0.15	1.0	0.19
Age	0.01	0.28	–0.09	0.13

DISCUSSION

Acromegaly is a rare disease which might be considered a model of prolonged GH and IGF-1 action in a number of organs. Acromegalic patients require careful follow-up and their treatment tailored to the individual metabolic status and the stage of complications. Cathepsin B, a lysosomal cysteine protease, has been extensively investigated due to its roles in pathologies. Under normal physiological conditions the regulation of cathepsin B is well coordinated and tightly controlled at multiple levels. Under pathological conditions, this regulation can be altered at more than one level, resulting in its overproduction, redistribution, and secretion (Yan *et al* 2003).

We found increased cathepsin B-like activity in sera obtained from acromegalic patients. This observation together with the more pronounced inhibitory activity observed in our group of patients documented an imbalance in cathepsin system action. To the best of our knowledge, this is the first clinical study searching for a relationship between the GH-IGF-1 axis and cathepsin activity. Although we strongly recommend that our results be confirmed by other studies, our preliminary findings open at least two fields of future research worth discussing.

The first is the clinical utility of cathepsin system assessment in acromegalic patients. In patients suffering from acromegaly, especially those with uncontrolled or poorly controlled disease, monitoring cardiovascular function including echocardiographic and peripheral vessel Doppler evaluation, pulmonary status assessment, insulin sensitivity, control of sleep apnea, and bone studies are needed to describe the patient's general condition and the stage of organ complications. Screening for neoplasms is also indicated (Colao *et al* 2004; Bolanowski *et al.* 2006; Stelmachowska-Banas *et al.* 2009).

The measurement of GH and IGF-1 concentrations in serum remains a key element in the confirmation of a clinical suspicion of the disease as well as in the assessment of treatment effect. However, the clinical utility of IGF-1 evaluation is limited by various issues, including physiological and pathological states influencing its concentration and bioactivity as well as the lack of a robust reference range (Brooke & Drake 2007). Moreover, there is discordance between GH and IGF levels in up to 27% of patients (Holly *et al.* 1991). The variability in GH and IGF-1 assay performance, coupled with use of inappropriate conversion factor for calculating units and reference ranges, limits the applicability of international consensus criteria to local practice as well as the value of some meta-analyses (Pokrajac *et al.* 2007). Surprisingly, based on current data even a biochemical cure rate of 100% will not result in complete normalization of mortality (Dekkers *et al.* 2008). For prolonged follow-up, an assessment of the central regulation of GH and IGF-1 and their interrelations is not always sufficient (Brooke & Drake 2007; Puder *et al.* 2005). The lack of statistically significant correlation between these GH and IGF-1 serum levels was also observed in our group of patients.

Therefore, additional biochemical markers of disease activity related to peripheral tissue damage would be useful for the individualization of both therapy and follow-up. The usefulness of cathepsin measurement could be tested in an assessment of overall risk in acromegaly patients. Previously we found that the level of glycosaminoglycans (GAG) urinary excretion reflects the stage of disease and degree of organ complication in acromegalic patients (Daroszewski *et al.* 2001). This could testify in favor of the use of parameters linked with extracellular matrix metabolism in assessing chronic complications of acromegaly.

CP, including cathepsin B, have been tested as markers of severity in a number of malignancies as well as degenerative and inflammatory diseases (Berdowska 2004), and in the light of our findings this challenge may also be undertaken in acromegalic patients.

Another reason to study alterations in the cathepsin system in acromegaly is the role of CP in the pathology of malignancies and other complications of acromegaly, especially via the putative influence of these enzymes on IGF action. CP are thought to play an important role in cancer development and progression. They enable malignant cells to proliferate and disseminate by hydrolyzing extracellular matrix and basement membranes in their proximity. Increased expression and secretion of cathepsin B have been observed in various human tumors, including brain, colon, lung, and prostate (Yan *et al.* 1998; Koblinski *et al.* 2000), as well as in nonmalignant conditions such as rheumatoid arthritis (Sohar & Hammer 2002) and osteoarthritis (Baici *et al.* 1995).

Over the years, epidemiology studies have provided increasingly debated evidence that acromegaly may enhance neoplastic risk and that cancer constitutes the third leading cause of mortality in this condition (Colao *et al.* 2004; Swearingen *et al.* 1998; Orme *et al.* 1998). The recently identified relationship between the IGF system and human cancer has further persuaded many researchers that neoplasms of acromegaly may constitute an informative model to legitimize this link. The IGF family of ligands, receptors, and binding proteins is important in the regulation of cellular growth and maturation. Impairment in the function of this system may be the cause of serious metabolic and structural consequences. IGF-1 is considered an important mitogen in many types of malignancies (Khandwala *et al.* 2000).

In our group of patients, no correlation between IGF-1 and cathepsin B-like activity and cathepsin inhibitor was found. The interrelations of cathepsins and IGF-1 are not entirely understood. Studies on prostate cells have shown that alterations in the IGF-1 system action are linked to changes in their proteases (Nunn *et al.* 1997; Rajah *et al.* 1995).

Various proteinases have also been postulated to function in the limited proteolysis of insulin-like growth factor binding proteins (IGFBPs), contributing to the regulation of IGF bioavailability. The major functional site of cathepsins is intracellular and may be involved in the selected clearance of either IGFBP or IGFs via different endocytic pathways or in the general lysosomal inactivation of the IGF system (Claussen *et al.* 1997). On the other hand, IGF-1 influences CP action. It was found that IGF-1 induced cathepsin D gene expression and reporter gene activity in MCF-7 human breast cancer cells (Wang *et al.* 2000).

In conclusion, this report describes enhanced activity of one of the most potent extracellular matrix-degrading mammalian activities, cathepsin B, and increased activity of cathepsin B inhibitor in acromegaly. We suggest considering this finding as a starting point to study the cathepsin system as an adjuvant parameter in the assessment of the stage of overall acromegalic complications. Moreover, CP may be involved in the pathomechanism of organ complications in acromegaly and may interfere with IGF-1.

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