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Altered dipeptidyl peptidase IV and prolyl endopeptidase activities in chronic tonsillitis, tonsillar hyperplasia and adenoid hyperplasia

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ABSTRACT

Objective: To analyse peptidase activities in the removed tonsils and adenoids from patients with chronic tonsillitis, tonsillar hyperplasia and adenoid hyperplasia.
Methods: We have analyzed 48 tissue samples from patients undergoing tonsillectomy and adenoidectomy for chronic tonsillitis, tonsillar hyperplasia or adenoid hyperplasia. Tonsillectomy and adenoidectomy samples were collected and frozen for later enzyme analysis. The catalytic activity of a pool of peptidases (dipeptidyl peptidase IV, prolyl endopeptidase, aminopeptidase A, aminopeptidase N, aspartyl aminopeptidase, aminopeptidase B, neutral endopeptidase, pyroglutamyl peptidase I, puromycin-sensitive aminopeptidase and cystinyl aminopeptidase) was measured fluorometrically.
Results: The activity of prolyl endopeptidase was higher in tonsillar hyperplasia and adenoid hyperplasia than in chronic tonsillitis. On the contrary, dipeptidyl peptidase IV activity was higher in chronic tonsillitis than in hypertrophic tissues. When data were stratified by age and gender, dipeptidyl peptidase IV was also found to be more active in adult and male chronic tonsillitis tissues. Inversely, dipeptidyl peptidase IV activity was higher in tissues of females with tonsillar hyperplasia.
Conclusions: These data indicate the involvement of dipeptidyl peptidase IV and prolyl endopeptidase in the mechanisms underlying chronic tonsillitis, tonsillar hyperplasia and adenoid hyperplasia.

1. Introduction

Tonsillectomy and adenoidectomy are among the most common surgical procedures carried out in otorhinolaryngology, most tonsillectomies being performed on children to treat recurrent tonsillar infections, to reduce obstruction from enlarged tonsils or both [1].

The palatine tonsil and adenoid lymphoid tissue are the first point of contact for a wide range of antigens present in inhaled air and substances consumed, as this is their point of entry to the body, giving rise to some for the most common inflammatory processes in humans [2–4]. Following this antigenic stimulation, activation of B and T cells in the tonsil tissue largely depends on the expression of various molecules. These include a wide range of proteolytic enzymes which participate in the mechanisms that regulate inflammatory processes of the upper respiratory tract, modulating cytokine production and cell growth and breaking down the extracellular matrix [2,3,5–7].

Bioactive peptides are regulated through hydrolysis by specific peptidases. These peptide-converting enzymes were originally considered to be only involved in protein and peptide scavenging. However, many studies have demonstrated that they are involved in various physiological functions, playing a key role in cell communication processes [8]. Accordingly, changes in patterns of the expression and catalytic function of these enzymes may contribute to certain disease processes, including infectious, inflammatory and hypertrophic diseases [5,6,9–11].

Given this, it could be expected that under pathological conditions, such as recurrent tonsillitis, changes in the levels of these peptidases in the tonsillar tissue would be detected with respect to a child population with enlarged tonsils but no history of frequent tonsillitis or chronic inflammation in their tonsils. In

Abbreviations: CT, chronic tonsillitis; TH, tonsillar hyperplasia; AH, adenoid hyperplasia; DPPIV/CD26, dipeptidyl peptidase IV; PEP, prolyl endopeptidase; APA, aminopeptidase A, particulate acid aminopeptidase; APN, aminopeptidase N, membrane-bound alanine aminopeptidase I; Asp-AP, aspartyl aminopeptidase, soluble acid aminopeptidase; APB, aminopeptidase B, basic arginyl aminopeptidase; NEP, neutral endopeptidase; PGI, pyroglutamyl peptidase I; PSA, puromycin-sensitive aminopeptidase, soluble alanine aminopeptidase; CAP, cystinyl aminopeptidase; UP, units of peptidase, pmol of product/min.

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relation to this, some interesting research has been carried out concerning peptidase activity in chronic tonsillitis and in adenotonsillar hyperplasia. However, there are relatively few such studies and their scope has been restricted to a few enzymes [4].

The present prospective study was performed to evaluate the differences in peptidase activity in adenoids and tonsils removed from patients with recurrent tonsillitis and those from children with adenotonsillar hyperplasia without a history of severe throat infections. The aim of this study was to assess the activity of soluble and membrane-bound acid, basic, neutral, and omega peptidase, covering the whole spectrum of peptide-converting activity. The peptidases selected included aminopeptidases [PSA (EC 3.4.11.14), APN/CD13 (3.4.11.2), APB (3.4.11.6), Asp-AP (3.4.11.21), APA (3.4.11.7), CAP (3.4.11.3)], two serine peptidases [DPPIV/CD26 (EC 3.4.14.5) and PEP (3.4.21.26)], a metalloendopeptidase [NEP/CD10 (3.4.24.11)], and an omega peptidase [PGI (3.4.19.3)].

2. Methods

The authors declare that all the experiments carried out in this study comply with current Spanish and European Union regulations.

2.1. Tissue specimens

The series consisted of a total of 48 samples divided into the following categories.

Group AH were patients who had undergone adenoidectomy for obstructive adenoids, $n = 12$; male/female = 1/1; aged from 2 to 7 years old; $m = 4.29$ and SD = 2.1.

Group TH were patients who had undergone tonsillectomy for obstructive tonsillar hyperplasia without infection, $n = 12$; male/female = 1/1; aged from 2 to 7 years; $m = 4.35$ and SD = 2.2.

For Group CT, samples were palatine tonsils from patients diagnosed with chronic tonsillitis according to the following criteria: more than six episodes in the previous year and/or more than 5 in episodes per year over the previous 2 years and/or more than three episodes per year over the previous 3 years [12]; $n = 24$ and male/female = 1/1. This last group (CT) was divided into two subgroups: one of the samples taken from children, $n = 12$, aged from 3 to 8 years old; $m = 5.6$ and SD = 1.9; and the other of patients over 14 years of age, $n = 12$, aged from 15 to 36 years; $m = 23.48$ and SD = 6.3.

Fresh tissue samples were taken from surgical specimens obtained by tonsillectomy and adenoidectomy. Total tonsillectomy was performed by blunt dissection after incising the mucosa to expose the tonsillar capsule [1]. After surgery, fresh tissue samples were properly labelled and stored at −80° until the enzyme assays were performed.

The AH and TH samples were not stratified by age as all the patients were young children.

Given the ethical and legal barriers to obtaining samples of healthy tissue there were no control samples.

2.2. Sample preparation

Extracted tissue samples were homogenized in 10 mM Tris–HCl buffer at pH 7.4, for 30 s at 800 rpm using a Heidolph PZR 50 Selecta homogenizer, and ultracentrifuged in a Kontron Instruments Centrikon T-2070 centrifuge at 100,000 × g for 35 min. The resulting supernatants were used to measure soluble enzyme activities and protein concentrations. To avoid contamination with soluble enzymes, the pellets produced were washed three times by suspension in 10 mM Tris–HCl buffer at pH 7.4. The pellets were then homogenized in 10 mM Tris–HCl buffer at pH 7.4, and centrifuged at low speed (1500 × g) for 3 min to purify the samples. The supernatants thus obtained were used to determine membrane-bound enzyme activities and protein concentrations. All steps were carried out at 4 °C.

2.3. Enzyme assays

Peptidase activity was measured by incubating samples with a saturating (0.125 mM) concentration of fluorogen-derived substrates following the method described by Mantle et al. [13]. Alanine aminopeptidase activities (PSA and APN/CD13) were measured in triplicate using Ala-β-naphthylamide as a substrate. Incubations with the specific PSA inhibitor puromycin (40 μM) were performed in parallel to discriminate between the PSA and APN forms of total alanine aminopeptidase activity. DPPIV/CD26 and PEP activity were assayed using H-Gly-Pro-(-naphthylamide and Z-Gly-Pro-β-naphthylamide, respectively. NEP assay was performed by incubating samples with a saturating concentration of [D]AG(pN)PG (a Dansyl derivative).

APB (basic) and acid aminopeptidase activities (Asp-AP, APA) were quantified using Arg-β-naphthylamide, Asp-β-naphthylamide, and Glu-β-naphthylamide substrates, respectively. Omega peptidase (PGI) activity was measured fluorometrically using pGlu-β-naphthylamide as the substrate. CAP activity was analyzed using L-cystine-di-β-naphthylamide. These assays are based on the fluorescence of products generated from substrate hydrolysis by the enzyme. Reactions were started by adding 30–50 μL of sample to 1 mL of the appropriate incubation mixture depending on the enzyme and substrate analyzed as follows: PSA, APN, APB, PEP and PGI activities (50 mM sodium phosphate buffer, at pH 7.4 for PSA, APN, PEP and PGI activities and pH 6.5 for APB, and 0.125 mM aminoacyl-β-naphthylamide); Asp-AP, APA, DPPIV, CAP, and NEP activities (50 mM Tris–HCl buffer, at pH 7.4 for Asp-AP, APA, DPPIV and pH 5.9 for CAP, and 0.125 mM aminoacyl-β-naphthylamide or [D]AG(pN)PG). After 30-min incubation at 37 °C, 1 mL of 0.1 M sodium acetate buffer (pH 4.2) was added to the mixture to terminate the reaction. The product released was determined by measuring the fluorescence intensity (at 412 nm with excitation at 345 nm for β-naphthylamine, and at 562 nm with excitation at 342 nm for [D]AG) with a Shimadzu RF-540 spectrofluorophotometer. Blanks were used to determine background fluorescence. Relative fluorescence was converted into picomoles of product using a standard curve constructed with increasing concentrations of β-naphthylamine or [D]AG.

2.4. Protein measurements

Protein concentration was measured in triplicate by the Bradford method [14], using BSA (1 mg/mL) for calibration. Results were recorded as units of peptidase (UP) per milligram of protein, where 1 U of peptidase activity is the amount of enzyme required to release 1 pmol of β-naphthylamine per minute. Fluorogenic assays were linear with respect to hydrolysis time and protein content.

2.5. Statistical analysis

SPSS® software was used for statistical data analysis. Results from tonsillar (CT and TH) and adenoid tissues were compared using one-way analysis of variance (ANOVA). Post hoc comparisons were made using the Scheffé test. An unpaired Student's t-test was performed to detect differences as a function of age and gender in CT, and of gender in TH and AH. A value of $p < 0.05$ was considered statistically significant.

3. Results

Table 1 shows the peptidase activities measured in CT ($n = 24$), TH ($n = 12$) and AH samples ($n = 12$). Activity is expressed as pmol