

SECRETORY leukocyte protease inhibitor (SLPI) is a well-known protease inhibitor. Its function is thought to be protease/protease-inhibitor balance. Free proteolytic activity, mainly pancreatic elastase, anionic trypsin and granulocytic elastase, has been demonstrated in faecal extracts from patients with ulcerative colitis. We wanted to verify that SLPI is actually secreted from normal human colonic mucosa. Also, we wanted to ascertain whether studies of SLPI secretion based on punch biopsies were dependent on biopsy area or on biopsy circumference. Normal colonic mucosa was sampled during surgery for colonic cancer. A total of 36 samples from four patients were used. Mucosa preparation was carried out using a punch biopsy technique, and samples of 3, 4 and 6 mm diameter were used. All media contained SLPI at varying concentrations. When expressed in terms of the sample area, the secretion per millimetre-squared seemed to decrease with increasing area. When calculated as secretion per circumference, secretion seemed to be constant. In conclusion, SLPI was secreted from normal human colonic mucosa. The SLPI secretion seemed dependent on the circumference of the biopsy rather than on the area of the biopsy.

Key words: Trauma, Human cell culture, Punch biopsy, Anti-leukoprotease

Secretory leukocyte protease inhibitor in punch biopsies from human colonic mucosa

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Introduction

Secretory leukocyte protease inhibitor (SLPI) is a 12 kDa non-glycosylated acid-stable antiprotease. Its amino acid sequence was elucidated in 1986.¹ Its inhibitory activity against leukocytic as well as pancreatic elastase, cathepsin G, trypsin and chymotrypsin is well known.^{1–4} It was first found in cervical secretions⁵ and has since been found on a variety of different cell surfaces. It appears in large quantities in parotid secretions⁶ as well as in bronchial secretions.^{7,8} Its role is thought to be mainly the modulation of the inflammatory process. In addition, both antibacterial and antiviral properties have been reported.^{9–12} Increased concentrations have been reported in pneumonia among the elderly.¹³

Large amounts of SLPI are swallowed^{3,14,15} and are rapidly degraded in gastric and duodenal juices.¹⁶ Recently, SLPI was demonstrated in the intestinal mucosa.¹⁷ Large amounts of free proteolytic activity, mainly pancreatic elastase and trypsin, have been found in secretions from patients with ulcerative colitis.^{18,19} SLPI secretion and regulation in response to these and other agents is thus of considerable interest. In this study, we wanted to verify that human colonic mucosa was capable of SLPI secre-

tion. For future studies, we also wanted to ascertain whether studies of SLPI based on punch biopsies were mainly dependent on biopsy area or on biopsy circumference.

Materials and methods

Mucosal culture

The procedure was approved by the local ethical committee and was performed in accordance with the Helsinki declaration. Tissue from macroscopically normal colonic mucosa was taken during surgery for the removal of colonic carcinoma from four patients with no other known bowel disease. The mean age of the patients was 64 years. The mucosa was sampled as 2–3 cm of tissue from the end of the excised material, at the end opposite to the cancer. It was placed in transport medium immediately after the blood supply was cut off, and then washed in separate test tubes containing transport medium three times, each washing lasting 2 min. All media were obtained from Gibco, Life Technologies AB (Sweden).

The transport medium consisted of 500 ml of Minimum Essential Medium (MEM) with Earle's Salts, with L-glutamine (catalogue number 31095–086),

5 ml of 0.1 mM MEM non-essential amino acids (catalogue number 11140-35), 10 ml of sodium pyruvate (catalogue number 11360-039), 5 ml of PEST (catalogue number 15140-114), and 0.5 ml of gentamycin sulphate (catalogue number 5750-037). The culture medium was the same but 10% foetal calf serum was added. In the laboratory, the mucosa was carefully dissected with scissors and samples punched out with a KAI sterile disposable biopsy punch (Stille, Sweden). Nine punch biopsies were taken from each of the patients. Biopsy diameters of 3, 4 and 6 mm were used. Three biopsies of each size were taken. A total of 36 biopsies were thus collected. After punching, the biopsies were immediately placed on the grid of a 10 mm diameter Falcon cell culture insert, with 3.0 µm of HD (high pore density membrane), and then placed in the 12-well companion plate for tissue culture (Labora, Sweden). The total well volume was 3 ml. The biopsies were incubated for 24 h in a Modular Incubator Chamber (Billrups, Rothenberg, CA, USA) at 37.4°C. The Modular Incubator Chamber was pre-ventilated for 10 min with 95% O₂ + 5% CO₂. The mucosa samples were then removed, and the medium was collected and then centrifuged at 4000 rpm for 10 min at +4°C. The supernatant was carefully collected and stored in aliquots at -70°C until analysed for the presence of SLPI by a standard enzyme-linked immunosorbent assay (ELISA) kit obtained from RD Systems (Quantikine human SLPI immunoassay). All analyses were performed in duplicate and the average was used in all calculations.

Table 1. Mean concentration of SLPI in growth medium (ng/l) after 24 h according to patient and the biopsy diameter

Patient	3 mm diameter	4 mm diameter	6 mm diameter
1	435.9	540.0	1073.8
2	429.1	324.9	541.7
3	1354.4	1575.6	2200.6
4	1466.3	2082.3	3113.3

Detailed information about specificity, accuracy and precision is given in the ELISA manual. According to this, the minimal detectable dose is typically less than 25 ng/l. At mean concentrations of 109, 696 and 2026 ng/l, the standard deviation is 8.7, 37.1 and 84.1, respectively. The analysis of variance factorial was used for statistical analysis.

Results

All media contained SLPI at varying concentrations ranging from 199 to 3258 ng/l. Media concentrations are presented in Table 1.

Secretion of SLPI expressed as nanograms per litre per unit area (mm²)

The mean calculated secretion per unit area was 130.4 ng/l per mm² in the 3 mm diameter samples (range, 29.3-266.1 ng/l), in the 4 mm diameter sam-

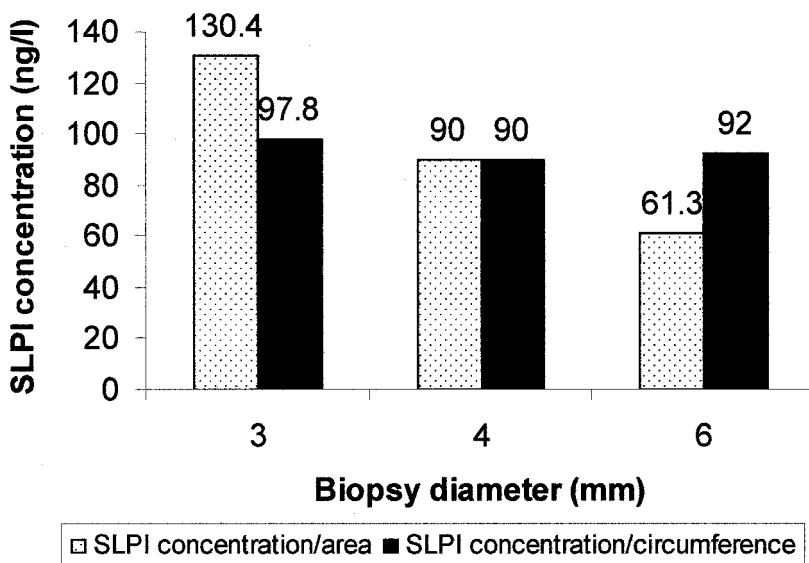


FIG. 1. SLPI calculated as mean concentration per area (light shading). In the 3 mm diameter samples the mean concentration was 130.4 ng/l per mm² (SD = 83.4), in the 4 mm samples it was 90 ng/l per mm² (SD = 62.6), and in the 6 mm diameter samples it was 61.3 ng/l per mm² (SD = 38.3). When calculated as mean concentration per circumference (dark shading), the concentration in the 3 mm diameter samples was 97.8 ng/l per mm (SD = 62.6), in the 4 mm samples it was 90 ng/l per mm (SD = 92.6), and in the 6 mm diameter sample group it was 92 ng/l per mm (SD = 57.5). There was no significant difference in SLPI concentration between sample groups when calculated as concentration per circumference. SLPI secretion decreased with increasing sample area.

ples the mean secretion was 90.0 ng/l per mm² (range, 15.8–200.9 ng/l), and in the 6 mm diameter samples it was 61.3 ng/l per mm² (range, 12.3–115.3 ng/l). There was no significant difference in the secretion per mm² between the 3 mm diameter samples and the 4 mm diameter samples ($p > 0.13$). No significant difference in secretion per mm² was shown between the 4 mm diameter samples and the 6 mm diameter samples ($p > 0.28$). There was, however, a significant difference in the secretion between the 3 mm diameter samples and the 6 mm samples ($p = 0.0126$). The secretion seemed to decrease with increasing area (see Fig. 1).

Secretion of SLPI expressed as nanograms per litre per circumference of the sample (mm)

The calculated secretion per litre per circumference in the 3 mm diameter samples was 97.8 ng (range, 22.0–199.6 ng), in the 4 mm diameter samples the secretion was 90.6 ng (range, 24.7–200.9 ng), and in the 6 mm diameter samples it was 92.0 ng (range, 24.9–172.9 ng). There was no significant difference in secretion calculated as secretion per circumference between any of the sample groups ($p > 0.47$) (see Fig. 1).

Discussion

SLPI is a protease secreted by a variety of mucosal cells. Interest in the phenomenon has mainly been directed towards the upper respiratory tract. Its role is thought to be mainly downregulation of the inflammatory process. The production of SLPI has also been demonstrated in the large and small intestines.¹⁷ In this study, we verified that SLPI is also found in the medium from cultured normal human colonic mucosa. The SLPI concentration after 24 h of incubation ranged from 199 to 3258 ng/l. Interpersonal differences in initial SLPI cell content might be an explanation for variation between patients with the same biopsy size. During surgery, the tissue is subject to considerable trauma, both direct cell trauma and indirect trauma, e.g. due to decreased blood supply. Further trauma is induced during punching as direct cell trauma. Wounded cells might also interact with cells in their close vicinity. When expressing the total cell production of SLPI in terms of the sample area, there was a significant difference in secretion between the 3 mm samples and the 6 mm samples. The secretion per mm² seemed to decrease with increasing area. This might possibly be explained by decreased metabolism in the centre of the biopsies where cells are mainly in contact with the medium from above and below. Cells located on the borders

of the biopsies are in contact with the medium also on almost all sides, which could lead to a somewhat higher metabolism. Another plausible explanation might be secretion from cells caused directly or indirectly by the punching procedure. In either case, expressing the secretion as a function of the biopsy circumference would be a possible way to determine whether the cells located on the border were the most active secreting cells in the biopsies.

No significant difference was observed between any of the samples when secretion was expressed as secretion per circumference. In this study, the secretion therefore seemed to be influenced mainly by the circumference of the biopsies (Fig. 1). This could be explained both by decreased metabolism in the middle of the biopsy as well as direct physical injury caused by the punching procedure. We hence conclude that SLPI is not only produced in normal human colonic mucosa as indicated by earlier studies, but is also actually secreted. In this study, secretion seems to take place mainly from cells located on the edges of the biopsies rather than from all the cells cultured.

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