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Lactoferrin in Human Prostate Tissue

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Summary. Lactoferrin levels have been determined by radialimmunodiffusion in homogenates of both human benign prostatic hypertrophy obtained at open surgery from patients, some of whom had been treated with oestrogens or antiandrogens, and also in prostatic adenocarcinoma tissue removed by transurethral resection. Results show that in untreated benign prostatic hyperplasia there is a statistically lower lactoferrin level in the median compared with the lateral lobes. In patients with benign prostatic hypertrophy treated before prostatectomy with oestrogens or antiandrogens the lactoferrin concentration is decreased. In neoplastic tissue removed by open surgery, the lactoferrin level is very low. Homogenates of tissue resected from prostatic cancer patients show similarly low levels. The concentration of lactoferrin in human prostate is hormone dependent. The role of the protein is considered to be bacteriostatic.

Key words: Prostate, Lactoferrin.

INTRODUCTION

The red protein lactoferrin (Lf), first detected in milk (17), is a glycoprotein present in external secretions, neutrophilic leucocytes and some tissues of mammals. Lf resembles transferrin (Tf) in its ability to bind reversibly two atoms of Fe^{3+} with the incorporation of two molecules of bicarbonate. Both proteins have a single-chain structure of molecular weight ± 76000, and are clearly related to one another. However, Lf and Tf differ in several ways. They show no immunological cross-reaction unless denaturated. Further, the affinity of Lf is largely retained at pH values below 4.0, whereas Tf completely releases its iron under such conditions. Receptors for Lf and Tf reside on different cells, since Tf combines with reticulocyte membranes and Lf combines with the membranes of macrophages and lymphocytes (1).

Lf has been shown to be present in the prostate gland by Loisillier et al. (6) using a double immunodiffusion technique but quantitative analysis was not carried out.

The aim of the present study was to quantitatively determine the levels of Lf in several locations in benign prostatic hypertrophy (BPH) following pre-operative oestrogen/anti-androgenic treatment, and in adenocarcinomas obtained by open surgery and transurethral resection (TUR).

MATERIAL AND METHODS

Ninety prostate samples were examined. Twenty-seven right, 25 left and 11 median lobes were obtained by open surgery from patients with untreated BPH. Eight of these patients provided all 3 lobes. Six BPH patients received oestrogens or anti-androgens prior to surgery as medication for their prostatism. Two untreated BPH-patients underwent a TUR 2 years before open prostatectomy.

We had only 3 cases from whom carcinomatous tissue was removed by open surgery as it is our practice not to perform open prostatectomy when there is clinical and biochemical evidence suggesting the presence of an adenocarcinoma. In 11 other patients with prostatic cancer a TUR was done.

Normal prostates were not available. Autopsy material is unsuitable for the study of proteins, due to the proteolytic activity of prostatic tissue (19).

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The samples were transported to the laboratory in liquid nitrogen and treated immediately or stored at -76°C. Homogenates were made following Van Camp's technique (18). The total protein concentration of the homogenates was measured by the biuret method (3). The mean protein concentration is 4.76 g% for the BPH's and 2.23 g% for TUR.

Lactoferrin was determined by means of radial immunodiffusion (7). The lipoproteins were pre-precipated with dextran sulphate (10 µl of a 5% dextran sulphate solution added to 1 ml of the samples). After centrifugation, 5 min at 6000 g, the supernatant was diluted 4 times with isotonic saline and 10 µl applied in the wells of an agar-gel layer containing rabbit antiserum against human lactoferrin. A human lactoferrin standard (3.9 mg/dl) and two adequate dilutions were applied in the first three wells of twelve. We used a commercially available kit (LC-Partigen Lactoferrin, Behringwerke, Marburg, Federal Republic of Germany). After 2 days of diffusion at room temperature, the diameters of the precipitates were read with a magnifying glass. The squares of the diameters of the precipitate rings were proportional to the protein concentration.

Results are expressed in mg lactoferrin/g protein.

These measurements of lactoferrin in BPH and prostatic carcinoma are new observations and comparison with other work on the lactoferrin content of normal prostatic tissue is not possible.

RESULTS

The results obtained for 63 homogenates of tissues of untreated BPH are represented in Table 1.

Statistical analysis (Student t-test) shows that there is no significant difference between the values found for the right and left lobes ($0.95 < P < 0.975$). However, using the same statistical test, the mean value of the 52 lateral lobes (8.28 mg/g protein, SEM 0.84) is significantly different from the mean value of the median lobes ($0.02 < P < 0.025$).

A similar result is obtained by means of the Wilcoxon test for coupled differences in 8 patients where we had the right, left and medium lobe at our disposal. The mean value of the lateral lobes was 10.18 mg/g protein. The mean value of the median lobes was 4.48 mg/g protein. Wilcoxon's test: $2\alpha \leq 0.05$.

The mean value for the Lf content in tissues from BPH-patients, treated with oestrogens or anti-androgens before prostatectomy is 4.65 mg/g protein. Oestrogen and anti-androgen treatment appear to give similar values. A verly low concentration was found in a patient who had three resections before open prostatectomy and treatment with medroxyprogesterone.

Two BPH patients had had a TUR two years before open prostatectomy. The mean value of the Lf content was 3.90 mg/g protein.

There were only 3 adenocarcinomas obtained by open surgery. The mean value of the Lf content was 2.42 mg/g protein.

In a previous publication (19) we showed that TUR material is suitable for chemical analysis. In eleven cases with carcinoma from whom tissue was removed by TUR a mean value for Lf of 2.85 mg/g protein was found.

Table 1. Lactoferrin content in 63 homogenates of untreated BPH

	n	Lf	SEM
Right lobe	27	8.38	1.27
Left lobe	25	8.18	1.19
Median lobe	11	4.05	0.65

Results in mg/g protein
n = number of cases Lf = Lactoferrin
SEM = standard error of the mean

DISCUSSION

Only Loisillier et al. (6) mention the presence of Lf in a homogenate of prostatic cancer. By means of a double immunodiffusion technique in agarose, these authors estimated the Lf concentration to be 1/600 of the total protein content of the homogenate. Studying other tissues (breast, stomach, etc.) they estimated that the Lf concentration was about 5 times higher in cancerous tissue than in normal. These results are in contradiction with ours for human prostate.

Lf is in the first instance the iron-binding protein of secretions. It is found in milk, saliva, tears, nasal secretions, gastric fluid, hepatic bile, pancreatic fluid, ascites fluid, seminal fluid, cervical mucus and in urine (9). Also Haupt and Baudner (4) observed the concentrations of Lf in secretions and noted a rather high content in human colostrum (400 mg/dl); these authors also found Lf in bronchial secretions.

More recently Lf has been found not only to be a universal component of mucosal and external secretions but several apparently unrelated cell types are able to synthesise this protein. These include neutrophilic leukocytes, acinar cells and kidney cells (8, 13). It has also been detected in the spleen (14). From the physiopathological point of view, the fact that Lf is present in neutrophils is important.

The role of Lf may be two-fold:

1. Lf secreted by mucosal and exocrine glands may protect the cellular membranes against multivalent heavy metal-cations (2).

2. It has bacteriostatic properties (11, 15).

Lysozyme, a well known bacteriolytic agent has a histological distribution quite similar to Lf. The activity of lysozyme is clearly increased in the presence of a chelating agent and it is noteworthy that Lf is one of the most powerful biological chelating agents. Masson and Heremans (9) showed that iron-poor Lf has a clear bacteriostatic effect on Staphylococcus albus, Staphylococcus aureus and Pseudomonas aeruginosa. The effect is abolished by the addition of ionized iron. In view of the high concentration of Lf in most epithelial secretions, particularly those of the respiratory and the genital tract, it is suggested that one of the functions of the protein may be to protect the mucosa from bacterial invasion. It has been shown that Lf inhibits the growth of bacteria by removing iron from the medium (10). Bluard-De Coninck et al. (1) found that Lf combines with the membrane of macrophages and lymphocytes.

In the female genital tract, Lf is present in the endometrial glands and also in cervical biopsies. Its biological purpose may again be bacteriostatic (12).

In human semen, Lf exists in relatively high concentration, originating from the seminal vesicles. It occurs both free in the seminal plasma and bound to the spermatozoal surface (5, 16).

In conclusion, the present study demonstrates the presence of the iron-binding protein Lf in human prostate. The concentration is significantly higher in the lateral lobes than in the median. In adenocarcinomatous tissue the level of Lf is decreased. Treatment with oestrogens and antiandrogens has an influence on the prostatic Lf content.

The role of Lf in the human prostate may be bacteriostatic.

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