Low Anti-streptokinase IgG Concentrations Following Streptokinase-streptodornase Treatment of Leg Ulcer Patients

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We have evaluated whether neutralising anti-streptokinase IgG antibodies are produced following streptokinase-streptodornase therapy of leg ulcer patients. Serum anti-streptokinase IgG concentrations in 10 leg ulcer patients were determined before, and 1 week, 2 weeks, and 3 weeks following the treatment. We observed only a negligible increase in neutralizing anti-streptokinase IgG concentrations during the observation period, which was probably of no therapeutic significance. Key words: skin ulcer; fibrinolysis; antibody.

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Some leg ulcers are contaminated by the presence of fibrin, necrotic tissue and pus which may prevent the formation of granulation tissue and epithelialization of the ulcer. In the management of such patients, preparations containing streptokinase and streptodornase are reportedly effective in promoting enzymatic cleansing and ulcer healing (1-3). Streptokinase is a non-enzymatic protein which converts the proenzyme plasminogen to plasmin, the primary fibrin-resolving enzyme of the organism (4). Plasmin has a broad substrate specificity and can resolve tissue constituents as well (4). Streptodornase liquefies the contents of pus (1).

Streptokinase is a product of streptococci and is antigenic in humans (5). Antibodies specific for streptokinase are produced in response to streptococcal infections (5) and intravenous infusions of streptokinase (6, 7). This antibody’s production is of clinical significance because the plasminogen-activating properties of streptokinase are neutralized by its complex formation with the specific antibodies (5).

It remains to be systematically investigated whether streptokinase therapy of leg ulcer patients elicits production of significant amounts of neutralizing anti-streptokinase antibodies (8-10). If this is the case, it may explain why such therapy fails to improve the healing in some patients (1-3). Therefore, we have determined anti-streptokinase antibody concentrations in patients treated with streptokinase-streptodornase preparations.

MATERIALS AND METHODS

Patients and blood sampling

We evaluated consecutive patients with leg ulcers caused by venous insufficiency and/or arteriosclerosis referred to the Department of Dermatology, Bispebjerg University Hospital of Copenhagen. Ten patients eligible for local fibrinolytic therapy were included in the present study. They received treatment with 100,000 U SK and 25,000 U streptodornase (Varidase, product of Lederle, American Cyanamid Company, Wayne, N. J., USA). One vial of Varidase was dissolved in 5 ml isotonic saline, gently mixed with 15 g of a premixed hydrogel wound dressing (IntraSite Gel, Smith + Nephew, Hull, UK). The preparation was applied to the ulcers once daily until debridement had been obtained or the responsible physician chose another therapy. Median treatment length was 7 days (range 1-16 days).

The study was approved by the local ethics committee, and the Declaration of Helsinki was observed. All patients gave their informed consent before inclusion in the study.

We prepared serum and citrate-stabilized plasma from blood samples collected in evacuated glass tubes (Vacutest, Terumo, Leuven, Belgium) before treatment, and 1, 2 and 3 weeks following therapy. Serum and plasma aliquots were frozen below −20°C until assay. There were no drop-outs in the blood sampling procedure.

Assay

We determined serum concentrations of anti-streptokinase IgG with the use of a new optimized enzyme-linked immunosorbent assay (to be published). Unless otherwise stated, the assay and reagents were performed at room temperature. In brief, into the wells of a microtitre plate (Maxisorp, Nunc-ImmuNo Plate, Denmark) were pipetted a 165 U/ml streptokinase solution (Streptase, Behring, Germany). After 24 h of incubation, further protein-binding to the wells were blocked using phosphate-buffered saline (PBS; pH 7.2) containing 0.1% Tween and 1% human serum albumin. The plates were stored under cover for up to one week at 5°C with the blocking buffer.

After washing, we added duplicate samples of patient serum, standards and controls diluted in PBS 0.1% Tween 20. The samples were incubated for 2 h during continuous shaking and then washed, and after peroxidase-conjugated rabbit anti-human-IgG (Dako, Copenhagen, Denmark) had been added – incubated for 1 h during continuous shaking. Following an additional wash, the substrate 1,2 phenylenediamine (Dako, Copenhagen, Denmark) was added and the microplates were left in the dark for 30 min. During this time, the enzyme peroxidase oxidized the substrate 1,2 phenylenediamine. The native substrate is almost colourless, whereas the reaction product is yellow. The absorbances were determined at the maximum intensity for the reaction product, i.e. 492 nm, with the use of a photometer (TIM-20, InterMed, Denmark).

Since no international standard for anti-streptokinase IgG concentrations exists, we arbitrarily defined the concentration to be 1 U/ml in pooled serum collected from 1,000 apparently healthy blood donors. The standard curve was constructed from serum collected from a patient who had high serum anti-streptokinase IgG concentrations. Samples were assayed in one session to eliminate inter-assay variation. The variation between duplicate determinations was less than 5%.

In clot lysis time experiments modified from (10), we mixed 0.20 ml of plasma with 0.10 ml of a Varidase solution to obtain final streptokinase concentrations ranging from 250 U/ml to 2,500 U/ml and immediately clotted the mixture with 0.10 ml of a 100 NIH units/ml thrombin solution (Leo Pharmaceuticals, Copenhagen, Denmark).

Statistics

Fluctuations of serum anti-streptokinase concentrations between study periods were evaluated with the use of the Friedman chi-square test. A value of p<0.05 (two-tailed) was considered statistically significant.

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RESULTS
Serum concentrations of anti-streptokinase IgG showed a slight but statistically significant increase (Friedman $X^2 = 9.68$, df = 3, $p < 0.05$), during the 3-week observation period (Fig. 1). Inspection of the individual data revealed that the concentrations of anti-streptokinase IgG increased in all patients. In 9 out of the 10 patients, serum concentrations of anti-streptokinase IgG remained below 10.0 U/ml, whereas one patient had concentrations ranging from 25.5 U/ml before treatment to 30.0 U/ml after 3 weeks.

In the clot lysis time experiments, we observed in pooled, normal plasma obtained from 20 apparently healthy individuals that the times ranged from 55 s (Varidase concentration 2.500 U/ml) to 145 s (Varidase concentration 250 U/ml). In contrast, the clot lysis times were always above 160 s (Varidase concentrations from 250 U/ml to 2.500 U/ml) in identical experiments using the patient plasma which contained high anti-streptokinase IgG concentrations (25.5 U/ml to 30.0 U/ml).

DISCUSSION
We observed only a minor increase of serum anti-streptokinase IgG concentrations following local streptokinase-streptodornase therapy of leg ulcer patients (Fig. 1). These findings suggest that such therapy only elicits a negligible humoral immune response to the streptokinase. This response contrasts the propensity of leg ulcer patients to develop cellular immune reactions to streptokinase and a variety of locally applied medications (11, 12), and notably the marked production of anti-streptokinase IgG in the first weeks following an intravenous infusion of streptokinase (6, 7).

One of the patients had serum concentrations of anti-streptokinase IgG which significantly prolonged the clot lysis time (see Results), indicating that the streptokinase-mediated plasminogen activation and fibrin resolution were impaired. Thus, high concentrations of anti-streptokinase antibodies may explain a reduced efficacy of streptokinase therapy in some individual leg ulcer patients (1, 3).

We conclude from these observations that local streptokinase treatment of leg ulcer patients only elicits a slight production of anti-streptokinase IgG antibodies. However, the pretreatment levels of such antibodies may influence the outcome of therapy.

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