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Decreased fibrinolytic potential and morphological changes of fibrin structure in dermatitis herpetiformis☆

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ABSTRACT

Background: Recently, high prevalence of cryofibrinogenaemia has been observed in plasma of untreated dermatitis herpetiformis (DH) patients, and the pathological IgA and TG3 deposits in the papillary dermis were found to co-localize with fibrin and fibrinogen.
Objective: To study the fibrinolytic potential in plasma of untreated, dapsone and or/gluten-free diet treated DH patients as well as the in vitro effect of dapsone on the fibrinolytic profile.
Method: Plasma samples of 23 DH patients, 19 healthy subjects and 5 pemphigus vulgaris patients were investigated by a turbidimetric-clot lysis assay. Out of them 5 DH plasma samples representing different fibrinolytic parameters, and 3 healthy controls were selected for parallel fibrin clot preparation. The clot fibrin structure was examined by scanning electron microscopy (SEM), and the diameters of 900 fibrin fibres were determined in each clot.
Results: A significantly prolonged clot lysis time was detected in untreated DH patients. The turbidity values of DH plasma clots indicated an altered fibrin structure that was also confirmed by SEM: significantly thicker fibrin fibers were observed in untreated, TG3 antibody positive DH patients compared to healthy controls, whereas the fiber diameters of dapsone-treated patients were similar or thinner than the control values. In line with the structural changes of fibrin, the fibrinolytic profile of 5 DH patients under dapsone treatment approached the control values.
Conclusion: This study revealed that the fibrinolytic potential was impaired in the plasma of untreated DH patients, whereas dapsone corrected the fibrinolytic defect. These data suggest a pathogenic role for plasma-derived factors in the development of skin symptoms and add a new aspect to the long-known beneficial, symptomatic effect of dapsone in active DH.
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1. Introduction

Dermatitis herpetiformis (DH) is a chronic blistering skin disease characterized by grouped pruritic papules, vesicles above the elbows, knees and buttocks, but acral purpuras are also common findings mostly on fingers or toes [1,2]. Epidermal transglutaminase (TG3) is the major antigen of DH [3], and it forms insoluble aggregates with granular immunoglobulin A (IgA) depositions in the papillary dermis. On the other hand, very early observations evidenced an extravascular fibrinogen and fibronectin staining along the papillary IgA in DH [4–7]. A preserved activity of TG3 within the cutaneous IgA-fibrinogen complexes was also detected recently [8].

DH develops in a subpopulation of patients with underlying gluten sensitive enteropathy, in whom transglutaminase 2 (TG2)

Abbreviations: AB, antibody/antibodies; DH, dermatitis herpetiformis; ELISA, enzyme-linked immunosorbent assay; EMA, endomysial antibodies; GFD, gluten-free diet; Ig, immunoglobulin; SEM, Scanning Electron Microscopy; TG, transglutaminase TG2 tissue transglutaminase; TG3, epidermal transglutaminase; tPA, tissue plasminogen activator.

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and TG3 antibodies (AB) are typically present. The recent observation that untreated DH patients have a high prevalence of cryofibrinogenemia in plasma [9] prompted us to examine DH plasma samples as a possible source of skin deposited fibrinogen along with IgA and TG3. It has been shown previously that dapsone, the symptomatic treatment in DH, seems to decrease the amount of cryofibrinogen in vitro [10], but the exact mechanism of action is unknown.

The presence of plasma cryofibrinogen is indicating a temperature dependent pathology associated with the function of circulating fibrinogen. The clearance of cryofibrinogen aggregates is mediated probably by the same proteolytic mechanism as the resolution of intravascular fibrin clots, which are formed when plasma fibrinogen is converted to fibrin by thrombin. Fibrin monomers polymerize through non-covalent interactions and by isopeptide bond formation between the monomers. This clot formation/stabilization is a common phenomenon also in inflammation [11,12]. The major route for elimination of fibrin clots is their proteolytic degradation by plasmin formed from plasma plasminogen by tissue plasminogen activator (tPA) [13] and this route is very sensitive to a variety of biomechanical, chemical and cellular factors [14].

In this study we investigated the plasma and serum of DH patients for their capacity to form and resolve fibrin clots and observed a decreased fibrinolytic potential associated with a modified fibrin structure, as well as a reversal of the fibrinolytic abnormalities by dapsone, an effective symptomatic therapeutic agent in DH.

2. Materials and methods

2.1. Patients and controls

The diagnosis of DH was based on clinical symptoms, routine skin histology and on presence of granular IgA precipitates in the papillary dermis by direct immunofluorescence. In all DH patients the IgA type TG3 enzyme-linked immunosorbent assay (ELISA) and in all DH patients and healthy subjects the IgA type TG2 ELISA and/or the endomysial AB (EMA) tests were also performed. None of the patients and healthy subjects had a selective IgA deficiency and none of them received therapy with known impact on the haemostatic system before or at evaluation.

Twenty-three DH patients, 17 males and 6 females, mean age 41 ± 13 years (range 21–74) and 12 healthy controls, 6 males and 6 females, mean age 33 ± 10 years (range 23–55) were enrolled in the turbidimetric clot-lysis assay study. Out of the total 23 DH patients the following subgroups were also selectively evaluated: a, 7/23 untreated DH patients with skin symptoms (no gluten-free diet (GFD), no dapsone treatment) b, 5/23 under dapsone medication (3/5 also under intermittent GFD) c, 11/23 under continuous GFD (Table 1). Dapsone was given to patients who wanted to get rapid improvement or received the medication in other clinics.

As a separate study 5 female pemphigus vulgaris patients (see above, Table 1) and 7 healthy subjects, 2 males and 5 females, mean age 44 ± 18 years (range 25–72 years) were examined by a turbidimetric clot-lysis assay.

All procedures have been approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (143/2010.) and were in accordance with the Helsinki Declaration. All subjects gave an informed written consent to participate in this study.

2.2. Direct immunofluorescence studies

The DIF was performed on 10 μm frozen sections of the patients' skin using fluorescein isothiocyanate (FITC) conjugated, goat antihuman complement 3 (C3), IgA, IgG and IgM AB (Dako, Glostrup, Denmark).

2.3. Serological markers

EMA were measured by indirect immunofluorescence according to the manufacturer's instructions (ImmuGlo IMMCO Diagnostics, Buffalo, NY).

TG3 and TG2 IgA AB were tested in duplicate by commercial ELISA kits. The cut-off value for the TG3 IgA ELISA (Immundiagnostik, Bensheim, Germany) was 22 AU/ml, for TG2 IgA ELISA (Orgentec Diagnostika, Mainz, Germany) was 10 AU/ml according to the manufacturer's instruction.

2.4. Turbidimetric clot-lysis assay and the in vitro effect of dapsone

This assay was described earlier [15]. Briefly: freshly, simultaneously prepared plasma and serum samples were analysed within 1 h after collection without freezing to avoid cryoprecipitation. Plasma clots were prepared with 5 μL thrombin (30 U/mL) added to a mixture of 50 μL citrated human blood plasma (collected in 3.8% sodium citrate blood collection tube) and 50 μL 0.1 μg/ml tPA (Actilyse, Boehringer Ingelheim, Germany) in 10 mM HEPES-NaOH pH 7.4 buffer containing 150 mM NaCl and 25 mM $CaCl_2$. When serum clot lysis was examined, the HEPES buffer contained also 2 mg/ml fibrinogen (human, plasminogen-depleted, Calbiochem, LaJolla, CA). The course of clot formation and dissolution was monitored by measuring the light absorbance at 340 nm (A_{340}) at 37 °C with a Zenyth 200rt microplate spectrophotometer (Anthos Labtec Instruments GmbH, Salzburg, Austria). The lysis time, defined as the time needed to reduce the turbidity of the clot to half-maximal value, was used as a quantitative parameter of the fibrinolytic activity, whereas the maximal turbidity (A_{340max}) was an indicator of the fiber size of fibrin [16]. Higher turbidity indicates thicker fiber diameters and larger clot pores [17,18].

Dapsone at 5 μg/ml [19] was applied directly to freshly prepared plasma samples of 2 untreated DH patients (2 males, 68 and 74 years old, see Patient 22 and 23 in Table 1) and 2 healthy subjects (2 males, 29 and 39 years old) for 30 min prior the clotting in the fibrinolytic assay.

2.5. Scanning electron microscope (SEM) imaging of plasma clots

Four out of 34 plasma samples were selected for SEM according to their lysis-curves (A_{340max} values), Patient 1,2,3 (P1, P2, P3) (Table 1) and a healthy subject with average control turbidity. P1, who showed the highest A_{340max}, was a TG2-TG3 AB positive, untreated DH patient, P2, who showed the lowest A_{340max}, was a TG2-TG3 AB negative, dapsone and GFD treated DH patient and P3 with medium turbidity was a TG2 AB negative-TG3 AB positive, only dapsone treated DH patient (Fig. 2A, Table 1). SEM evaluation of fibrin from further 2 untreated, seronegative DH patients (P22, P23 in Table 1) and 2 healthy subjects were done to characterize the fibrin structure before and after in vitro dapsone addition (see above).

Plasma clots were prepared as described above for the clot-lysis assay (omitting tPA from the reaction mixture). Following 30-min clotting at 37 °C, clots were fixed in 1%(v/v) glutaraldehyde in 100 mM Na-cacodylate pH 7.2 buffer for 16 h. The fixed samples were dehydrated in a series of ethanol dilutions (20–96%(v/v)), 1:1 mixture of 96%(v/v) ethanol/acetone and pure acetone followed by critical point drying with CO_2 in E3000 Critical Point Drying Apparatus (Quorum Technologies, Newhaven, UK). The specimens were mounted on adhesive carbon discs, sputter coated with gold in SC7620 Sputter Coater (Quorum Technologies, Newhaven, UK) and images were taken with SEM EVO40 (Carl Zeiss GmbH, Oberkochen, Germany).

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