Increased coagulation activity of the pleura after tube drainage and quinacrine instillation in malignant pleural effusion

V. Agrenius*, J. Chmielewska**, O. Widström*, M. Blombäck**

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ABSTRACT: Chronic malignant pleural effusions are usually treated with an intrapleurally administered irritant that creates an inflammatory reaction. The induced inflammation results in fibrin deposition and termination of fluid exudation.

In the present study several factors in the coagulation system in the pleural fluid were followed during treatment with tube drainage and quinacrine instillation into the pleural space. In the chronic exudative phase before treatment, both thrombin activity and fibrinopeptide A (FPA), were present at low levels. During treatment the levels increased markedly. Beta-thromboglobulin, a platelet marker, showed a similar pattern. Prothrombin, antithrombin III, prekallikrein and kallikrein inhibiting activity showed no such variations in activity.

The high thrombin activity and FPA level induced by treatment reflect an active process of fibrin formation which seems to play an important role in arresting chronic pleural exudation.


Malignant disease in serous membranes may cause chronic effusion. This is often seen in the pleura and the peritoneum, but less often in the pericardium. Malignant pleural effusion causes great discomfort and anxiety and the treatment is painful and time-consuming. A better understanding of the pathogenic mechanism is needed in order to find better methods of treatment. The presence of exudate gives a much greater possibility to analyse biologically active substances from the malignant cells and tumour environment than is possible from studies on solid tumours.

The pleurodesis method routinely used in our clinic includes thoracoscopy and chest tube drainage. Insertion of a drainage tube in itself causes inflammation which might be compared with the inflammation of a wound trauma. Pleurodesis also involves the application of an irritant substance (in our department quinacrine) which induces a still stronger inflammatory reaction, with destruction of the pleural surface [1]. The reaction is similar to that seen with other substances used for pleurodesis, such as talcum, Corynebacterium parvum and tetracycline [2-6]. The macroscopic picture is typical, with fibrin formation later followed by swelling of the pleura, and of vessels and collagen forming fibrotic tissue [7]. Fibrin is evidently the matrix substance for the formation of fibrotic tissue. For unknown reasons, this inflammatory process with formation of fibrotic tissue, leads to the cessation of fluid production despite the persistence of malignant tissue.

In a previous study, fibrinolytic activity was shown to be high in the exudative stage before treatment [8]. The activity decreased markedly after the trauma of inserting a chest tube for drainage. A moderate but significant further decrease in fibrinolysis was seen after quinacrine instillation into the pleural space. The aim of this study was to determine whether the phenomena of fibrin deposition and adhesion also reflect a change in coagulation activity.

Material and methods

Patients

Eleven patients, consecutively admitted to the Department of Thoracic Medicine, Karolinska Hospital, from February to December 1986, were investigated. There were six males and five females, aged 36-79 yrs, mean age 62 yrs. The following malignant tumours were diagnosed: breast carcinoma (4), adenocarcinomas of uncertain origin (3), mesotheliomas (2) ovarian carcinoma (1) and kidney carcinoma (1). All patients suffered from unilateral chronic malignant pleural effusion and were considered to benefit from pleurodesis treatment. In seven of the eleven cases the pleural fluid was macroscopically sanguinolent.

This study was approved by the Human Research Ethical Committee of the Karolinska Hospital and informed consent was obtained from each subject.
We followed our normal routine for pleurodesis treatment. Initially, a thoracentesis with collection of samples for routine analyses was performed. Subsequently, 500 ml of air was instilled to improve X-ray conditions and the safety of the thoracoscopy. On the following day, thoracoscopy was performed providing a clear view of the pleural surface and permitting good pleural biopsies. A drainage tube was inserted and connected for suction, which was started at -5 cm H₂O and continued at -15 cm H₂O. When the lung had expanded, as determined by X-ray, which was usually on the following day, 600 mg of quinacrine, dissolved in 20 ml of saline, was instilled into the pleural space.

Within one day after treatment all the patients developed a fever reaction. The drainage tube was removed when the fluid production fell below 50 ml·day⁻¹.

**Sampling**

The samples of fluid were taken at the initial thoracentesis, just before the injection of quinacrine, and 6, 24 and 48 h after instillation. On each occasion the total volume of fluid produced was measured. To prevent any further coagulation reaction, one part of the anticoagulant was added to six parts of pleural fluid. The anticoagulants employed were 10% sodium edetic acid (EDTA) for betathromboglobulin analysis; physiological saline containing 1,000 KIU·ml⁻¹ Trasylol® and 1,000 IU·ml⁻¹ heparin for fibrinopeptide A (FPA) measurements and 0.13 mol·l⁻¹ trisodium citrate for other assays. All samples were centrifuged at 2,500 g for 20 min and supernatants were aliquoted and stored at -70°C until assay.

**Materials**

Quinacrine hydrochloride was purchased from Boots Limited, Nottingham, UK. Trasylol® was purchased from Bayer, Leverkusen, FRG and heparin for fibrinopeptide A (FPA) measurements and 0.13 mol·l⁻¹ trisodium citrate for other assays. All samples were centrifuged at 2,500 g for 20 min and supernatants were aliquoted and stored at -70°C until assay. The FP A levels reached their maximum in fluid collected 6 h after quinacrine treatment, while thrombin activity and beta-thromboglobulin reached maximal levels in the entire treatment, including the insertion of drainage tube and during quinacrine instillation (figs. 1 and 2). The FPA levels reached their maximum in fluid collected 6 h after quinacrine treatment, while thrombin activity and beta-thromboglobulin reached maximal levels in pleural fluid collected at 24 h. See table 1 for a summary of the results.

**Laboratory methods**

FPA-levels were measured with Nossel's radioimmunoassay, as modified by Kockum and Frebelius [9]. The beta-thromboglobulin radioimmunoassay was performed according to the instructions of the manufacturer. The chromogenic substrate S-2238 was used for the assays of thrombin, prothrombin and antithrombin III. The latter was assayed by the method of Abildgaard et al. [10] modified for the Cobas-Bio centrifugal enzyme analyser. Prothrombin was determined by the method of Bergström and Blombäck [11]. Prekallikrein and kallikrein inhibiting activity were measured using the chromogenic substrate S-2302 by the methods of Gallimore and Freiberger [12]. The levels of prothrombin, antithrombin III, prekallikrein and kallikrein inhibiting activity are expressed in U·ml⁻¹ where 1 U is defined as the activity present in 1 ml of pooled human plasma. The amidolytic activity of pleural fluid on the thrombin-sensitive chromogenic substrate S-2238 was measured as described previously [13]. The activity is expressed as delta absorption per unit of time (min). The activity measured is mainly that of the alpha-macroglobulin-thrombin complex, which is active on the substrates but inactive on the macromolecular fibrinogen. Thus, both FPA-levels and the thrombin activity reflect recent thrombin activity [13].

**Statistical analysis**

Mean and standard deviations were used. The Friedman non-parametric two-way analysis of variance and multiple comparison on ranks of several related samples was performed as described by Theodorsson-Norheim [14].

**Results**

In all 11 patients the drainage tube was removed when fluid production fell below 50 ml·day⁻¹, which mostly occurred within 2–4 days (mean 3.6 days) after the injection of quinacrine into the pleural space. All of the patients developed a fever, of about 38°C within 24 h. The treatment was successful in all of the patients, since no further thoracentesis was needed. Indeed, these 11 patients all showed full expansion of the lung on X-ray with no further signs of pleural effusion.

Before treatment, the levels of FPA and beta-thromboglobulin in the pleural fluid at the time of thoracentesis were higher than normal plasma. Furthermore, thrombin activity, not normally present in plasma, was demonstrated in pleural fluid. Thrombin and FPA levels increased in parallel in all cases during the entire treatment, including the insertion of drainage tube and during quinacrine instillation (figs. 1 and 2). The FPA levels reached their maximum in fluid collected 6 h after quinacrine treatment, while thrombin activity and beta-thromboglobulin reached maximal levels in pleural fluid collected at 24 h. See table 1 for a summary of the results.

Measurements from the drainage tube performed prior to the injection of quinacrine, were intended as baseline values. However, even at this early stage the levels were considerably increased. The finding that the drainage tube itself was mainly responsible for the increase in values encouraged us to design some experiments. Thus, in three patients pleural fluid was collected from the drainage tube during three days (fig. 3). On the second and third days of treatment, all of the patients showed a decrease in the three parameters. However, following the injection of quinacrine, the levels rose again.
COAGULATION ACTIVITY IN PLEURAL INFLAMMATION

Fig. 1. — Levels of thrombin activity (△—△), fibrinopeptide A (FPA) (○—○); and beta-thromboglobulin (beta-TG) (□—□) before (pre-tube drainage) and during (post-tube drainage, 6, 24 and 48 h after) quinacrine instillation in the pleural space. Mean values are indicated. For comparison, the normal plasma levels are 0.29–20 pmol·ml⁻¹ for FPA and 11–34 ng·ml⁻¹ for beta-TG. Thrombin is not present in normal plasma. ΔA: Δ absorbance.

Fig. 2. — Individual levels are given to demonstrate the variation of thrombin activity, fibrinopeptide A (FPA) and beta-thromboglobulin levels before, and after insertion of drainage tube, and the maximum level after quinacrine instillation, at 24 h for thrombin activity and beta-thromboglobulin and at 6 h for FPA. ΔA: Δ absorbance.

Table 1. — Summary of results and statistical analyses

<table>
<thead>
<tr>
<th>Pleural fluid</th>
<th>Before tube drainage</th>
<th>After tube drainage</th>
<th>After quinacrine instillation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Thrombin activity x10⁻³</td>
<td>1.10±0.9</td>
<td>11.1±9.3*</td>
<td>16.1±7.4*</td>
</tr>
<tr>
<td>A·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPA pmol·ml⁻¹</td>
<td>120±140</td>
<td>1250±990*</td>
<td>1990±1150*</td>
</tr>
<tr>
<td>Beta-thromboglobulin ng·ml⁻¹</td>
<td>58±60</td>
<td>184±123*</td>
<td>152±108*</td>
</tr>
<tr>
<td>Prothrombin U·ml⁻¹</td>
<td>0.31±0.01</td>
<td>0.23±0.010</td>
<td>0.26±0.11</td>
</tr>
<tr>
<td>Kallikrein inhibiting activity U·ml⁻¹</td>
<td>0.43±0.17</td>
<td>0.42±0.15</td>
<td>0.35±0.11</td>
</tr>
<tr>
<td>Antithrombin III U·ml⁻¹</td>
<td>0.43±0.10</td>
<td>0.44±0.12</td>
<td>0.41±0.13</td>
</tr>
<tr>
<td>Prekallikrein U·ml⁻¹</td>
<td>0.41±0.12</td>
<td>0.40±0.13</td>
<td>0.37±0.13</td>
</tr>
</tbody>
</table>

Values are means±SD, 11 analyses on each occasion. *: levels before and after quinacrine instillation in the pleural space were significantly higher (p<0.001) than those at the time of the first thoracocentesis; **: levels after quinacrine instillation were significantly higher (p<0.01) than those before quinacrine instillation; FPA: fibrinopeptide A. A: absorbance.
Clinical Pleural only confirm the presence of platelet activation and produce or release a great number of substances, such as (PDGF), more important than is generally believed since they thrombin, fibrinogen, platelet-derived growth factor of immunological and inflammatory processes may be the platelets. The role of platelets in the mediation beta-thromboglobulin makes evident the activation of phase of malignant pleurisy. However, during treatment, it is significantly inhibited. Thus, pleurodesis treatment a further increase is observed. AA: A absorbance.

During treatment there were small alterations in concentrations of other factors and inhibitors and the variation observed revealed no obvious pattern (table 1).

Discussion

Coagulation activity, as evidenced by thrombin activity and FPA-levels, was found in the pleural exudates before treatment. The levels showed considerable variation, some being higher than those seen after treatment (fig. 2). It is impossible to judge whether spontaneous cessation of fluid production would have occurred in some patients with high levels without treatment. During pleurodesis a marked increase in levels occurred. Most of this increase was achieved at the time of the insertion of the chest tube before quinacrine instillation. The increase in thrombin activity seen after the tube drainage accords with the finding that, in a certain proportion of cases, fluid production stops when no irritant is used [15].

The presence of fibrin is monitored by the interaction between the coagulation and the fibrinolytic systems [16-18]. The authors have demonstrated [8] that fibrinolysis is very active during the chronic exudative phase of malignant pleurisy. However, during treatment, it is significantly inhibited. Thus, pleurodesis treatment affects both the fibrinolytic and the coagulation systems and leads to fibrin deposition.

The increase in the concentrations of the platelet marker beta-thromboglobulin makes evident the activation of the platelets. The role of platelets in the mediation of immunological and inflammatory processes may be more important than is generally believed since they produce or release a great number of substances, such as thrombin, fibrinogen, platelet-derived growth factor (PDGF), and serotonin [19]. In this study we can only confirm the presence of platelet activation and thereby the potential of this broad range of possible activities.

In formation of distant metastases the cancer cells must form vessels and connective tissue to survive in the new environment. In experimental research on the mechanism of cancer cell formation it has been demonstrated that cancer cells produce a strongly vasoactive substance, known as, vascular permeability factor (VPF) [20]. This factor is produced mainly in the growth zone of cancer tissue and causes the neighbouring vessels to leak. The leaking plasma forms a fibrin gel because of the action of procoagulant factors from the cancer cells [21-23]. The stroma needed for support and nutrition of the cancer nodules is formed by proliferation of fibroblasts and endothelial cells in this gel. The process seems to be monitored by coagulation and fibrinolytic activity present and by cytokinins and growth factors. The formation of fluid in malignant pleural effusion may depend on VPF which causes the vessels to form the exudate. The high fibrinolytic activity may maintain this leakage. By changing the balance between the fibrinolytic and coagulation activity towards coagulation, one may stop the leakage. The permanent effect of pleurodesis treatment may reflect the destruction of a cellular source of fibrinolytic activity, such as the mesothelial cells, which are known to disappear after severe inflammation, as in cases of tuberculosis [24].

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References


Activité accrue de coagulation de la plèvre après drainage et instillation de quinacrine dans les épanchements pleuraux malignes. V. Agrenius, J. Chmielewska, O. Widström, M. Blomback. RÉSUMÉ: Les épanchements pleuraux malignes chroniques sont habituellement traités par administration intra-pleurale d'irritants provocateurs d'une réaction inflammatoire. L'inflammation induite entraîne des dépôts de fibrine et la fin de l'exsudation de liquide.

Dans la présente étude, différents facteurs du système de coagulation du liquide pleural ont été suivis au cours d'un traitement par drainage et instillation de quinacrine dans l'espace pleural. Dans la phase exsudative chronique, l'on trouve de faibles niveaux, à la fois de l'activité de la thrombine et de fibrinopeptide A (FPA). Au cours du traitement, ces niveaux augmentent de façon marquée. La bêta-thromboglobuline, un marqueur plaquettaire, se comporte de façon similaire. La prothrombine, l'antithrombine III, la prekallikrène et l'activité inhibitrice de la kallikrène, ne montrent pas de pareilles variations d'activité.

Les niveaux élevés d'activité de thrombine et de FPA au cours du traitement sont le reflet d'un processus actif de fibrinogénèse qui semble jouer un rôle important dans l'arrêt de l'exsudation pleurale chronique. *Eur Respir J.*, 1991, 4, 1135-1139.