

Immunopathological Events of Adverse Cutaneous Reactions to Coumarin and Heparin

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We here describe a female patient with thromboembolic disease, who exhibited allergic reactions to heparin and who developed a large necrotic area on the abdomen when coumarin treatment was instituted. On immunohistology of the necrotic lesion, tumour necrosis factor α was markedly expressed, with decreasing intensity towards the central necrotic part of the lesion. Furthermore, endothelial cell adhesion molecule expression was upregulated, particularly in the haemorrhagic zone at the periphery of the lesion. These findings suggest that the pathology of coumarin necrosis is mediated via tumour necrosis factor α -associated inflammatory events, after activation of the coagulation pathway due to an inherited or transiently induced, acquired protein C-deficiency. In view of these findings, we propose that patients be treated in the future with tumour necrosis factor α antagonists such as pentoxifylline. **Key words:** skin necrosis; protein C; TNF α ; adhesion molecules.

(Accepted July 15, 1996.)

Acta Derm Venereol (Stockh) 1997; 77: 35–38.

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Anticoagulants, such as coumarin derivatives or heparins, are efficient drugs in the prophylaxis and treatment of thromboembolic disease. Especially in the initial phase of treatment with coumarin, there is however a risk of skin necrosis which seems to result from a transient phase of hypercoagulability, due to a more rapid decrease in anticoagulatory protein C activity compared to the procoagulatory factors IX, X, and II (1). Patients with an inherited or acquired protein C-deficiency (2–4) are especially at risk. The mechanisms might include cytokines like tumour necrosis factor α (TNF α), or possibly also a direct "toxic effect" of coumarin on endothelial cells (5, 6). An immunologically mediated hypersensitivity reaction is considered unlikely, since continuation or reinstitution of the drug only exceptionally produces further lesions (7).

In contrast, heparin skin necrosis may be caused by a heparin-dependent platelet antibody, which causes platelet aggregation when heparin treatment is instituted. The subsequent thrombotic events occur in different organs, with a risk of myocardial and cerebral infarction (8, 9). Other adverse skin reactions to heparin include type IV allergic reactions after subcutaneous administration (10, 11).

In this article, we describe a patient who suffered from thromboembolic disease despite heparin prophylaxis, who later on displayed eczematous plaques due to subcutaneous heparin injections, and who furthermore developed skin necrosis after coumarin therapy. Lesions at different stages of development were studied using immunofluorescence and antibodies to TNF α , adhesion molecules, and lymphocytes. Some immunohistologic investigations were performed on a biopsy of the heparin reaction as well.

CASE REPORT

A 52-year-old, obese female patient, with a past history of three abortions and a postoperative thrombosis 27 years earlier, had undergone hysterectomy and adnectomy because of uterine carcinoma. Although low-molecular weight heparin was administered subcutaneously, she developed deep thrombosis of the left femoral vein on the 10th postoperative day. Therapy was changed to heparin-Na, 5,000 IU, given three times daily subcutaneously, but plebography showed further growth of the thrombus. Acenocoumarol was then instituted, with a starting dose of 9 mg/d p.o. On the 4th day of treatment, a painful erythematous induration appeared on the lower abdomen, which became haemorrhagic and necrotic during the subsequent days. PTT was 39.9 s, prothrombin time 17%, and platelet count 215,000/ μ l. By this time the patient was referred to our department. At the periphery of the still growing lesion on the abdomen (Fig. 1), three zones could be differentiated, namely, the incipient necrosis in the centre surrounded by a haemorrhagic and an erythematous area. Punch biopsies were taken from each of these areas. Acenocoumarol was discontinued after 6 days, and intravenous treatment with heparin (1,000–1,400 IU/h) was reinstituted. An additional thrombosis was detected in the right femoral vein at this time. Two weeks later, when heparin therapy was continued subcutaneously, infiltrated erythematous patches started to develop at sites of heparin injections. A skin biopsy was also obtained from these areas. By this time, the prothrombin time had returned to normal. Platelets ranged from 215,000 to 507,000/ μ l throughout the observation period. The highest values occurred while heparin was being administered. Protein C and protein S were 100%, and anticardiolipin antibodies were negative. Conservative treatment of the large tissue necrosis on the abdomen with topical antibiotics as well as removal of necrotic tissue was unsuccessful, and it had to be treated by wide excision.

RESULTS

Histology

In the erythematous outer zone of the lesion, there was oedema of the epidermis and of the upper dermis, with a sparse



Fig. 1. Large incipient tissue necrosis with haemorrhage on the lower abdomen of a female patient.

lymphohistiocytic infiltrate around venules. In the biopsy from the haemorrhagic area, oedema and infiltrate were markedly increased. The infiltrate contained also neutrophils and some eosinophils and extended into the subcutaneous tissue. Around venules, deposits of fibrin were present. In the specimen from the inner area which was starting to be necrotic, the epidermis was still preserved, but a marked haemorrhagic exudation in the underlying dermis was present (Fig. 2). The vessels throughout the dermis were plugged with thrombi and showed fibrinoid necrosis of the walls. Especially in the subcutaneous tissue, vessels were extremely dilated (Fig. 3). The perivascular infiltrate included neutrophils and nuclear dust. The sections from the local heparin reaction revealed a perivascular lymphocytic infiltrate in the upper dermis, with a few neutrophils and eosinophils. There were no signs of vasculitis. Some lymphocytes were found in the otherwise normal-appearing epidermis.

Immunohistology

Cryostat sections of the biopsies from the coumarin necrosis were stained with various antibodies (Table I), using the APAAP method, as described before (12). Results of immunohistochemical staining of infiltrating and endothelial cells in the different zones at the periphery of the necrosis are shown in Tables I and II. In the outer erythematous zone, staining of infiltrating and of endothelial cells in the dermis with anti-

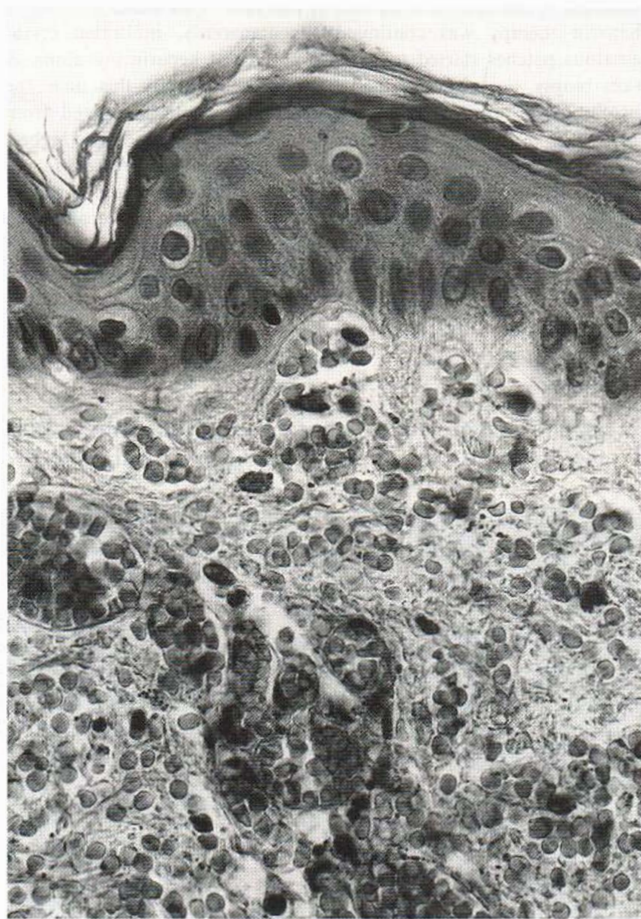


Fig. 2. Inner area of the lesion with a vast haemorrhage in the dermis and a still preserved epidermis (haematoxylin and eosin, $\times 100$).



Fig. 3. Dilated necrotic vessel in the subcutaneous tissue of the incipient necrosis with a thrombus (haematoxylin and eosin, $\times 100$).

TNF α was conspicuously increased, with a decline towards the centre of the lesion. Keratinocytes of the outer and middle zones showed marked expression, those of the inner almost necrotic zone no expression of TNF α .

Direct immunofluorescence was performed with anti-IgG, anti-IgM, anti-IgA, and anti-C3 (Behring, Marburg, Germany), also on frozen tissue sections, and showed deposits of IgM and C3 in small- and medium-sized venules in the incipient necrosis in the centre of the lesion and in the surrounding haemorrhagic area.

Immunohistologic assessment of the heparin reaction was made with a limited panel of antibodies (# in Table I). The infiltrate in the upper dermis consisted mainly of UCHL-1-positive lymphocytes, with a predominance of CD4-positive helper cells over CD8-positive suppressor cells and a few L26-positive cells. Some UCHL-1- and CD4-positive cells were also found in the epidermis, with CD4 staining dendritic and non-dendritic cells. Most of the infiltrating cells in the dermis stained strongly with anti-LFA-1 β , and they were partly HLA-DR- and ICAM-1-positive. Individual intraepidermal dendritic and non-dendritic cells stained with anti-LFA-1 β , some also with anti-HLA-DR, and only single non-dendritic cells were reactive with anti-ICAM-1.

Patch tests

The patient displayed a strong reaction to one heparin preparation (Liquemin[®], Roche, Germany) and weaker reactions to another heparin (Heparin-Natrium Braun[®], Braun Melsungen, Germany) and to low-molecular weight heparin (Fraxiparin[®], Sanofi Winthrop, Germany), all with a maximum at 72 h. The preparations were preservative-free. An epicutaneous test with coumarin was negative.

DISCUSSION

Coumarin necrosis was first described in 1943 (13), and many theories have since been discussed regarding its aetiology. An association with a high loading dose of coumarins was suspected early on, with resulting hypocoagulation and bleeding (14). Histologic examination supported, however, thrombosis rather than bleeding as the primary event, a theory that is

Table I. Antibodies used in the study and immunoreactivity of the infiltrating cells in three distinguishable zones at the periphery of expanding coumarin necrosis

Antibody	Antigen, CD code	Outer zone erythema	Middle zone haemorrhage	Inner zone necrosis
UCHL1 # ^a	CD45RO	++	+++	+
T4 # ^a	CD4	++	+++	+
T8 # ^a	CD8	(+)-+	+	(+)
T4/T8		7/3	7/3	4/1
L26 # ^a		0	0	0
anti-TNF α ^c	TNF α	+++	++	++
MON6010 ^c	ELAM-1, CD62E	0	0	0
anti-VCAM-1 ^b	VCAM-1, CD106	+	++	(+)-+
anti-ICAM-1 # ^b	ICAM-1, CD54	++	+++	+
anti-LFA-1 β # ^d	LFA-1 β , CD18	++	+++	++
anti-HLA-DR # ^a	HLA-DR	+	+++	++

+++ = abundant positive cells/intense staining; ++ = numerous positive cells/marked staining; + = fewer positive cells/moderate staining; (+) = single positive cells/weak staining; 0 = no positive cells/no staining.

= antibodies used for both coumarin necrosis and heparin reaction.

a = DAKO, Glostrup, Denmark

b = Immunotech, Marseille, France

c = Monosan, Uden, Netherlands

d = Carl Figdor, Nijmegen

e = Genzyme, Cambridge, Mass, U.S.A.

Table II. Immunoreactivity of the endothelial cells in three distinguishable zones at the periphery of expanding coumarin necrosis

Antibody	Antigen, CD code	Outer zone erythema	Middle zone haemorrhage	Inner zone necrosis
anti-TNF α	TNF α	++	+	(+)
MON6010	ELAM-1, CD62E	++	+++	+
anti-VCAM-1	VCAM-1, CD106	(+)-+	+-++	(+)
anti-ICAM-1	ICAM-1, CD54	(+)	+-++	(+)
anti-HLA-DR	HLA-DR	(+)	++	(+)-+

+++ = abundant positive cells/intense staining; ++ = numerous positive cells/marked staining; + = fewer positive cells/moderate staining; (+) = single positive cells/weak staining; 0 = no positive cells/no staining.

endorsed by our present findings and confirmed by physiological considerations of coagulation. The vessels in the evolving necrosis were plugged with thrombi and already showed beginning destruction of the walls. Deposits of IgM and C3 in small- and medium-sized venules were detected by immunofluorescence, in agreement with some cases of coumarin necrosis reported so far in the literature (15). In addition, there was increased staining with anti-TNF α in the outer erythematous area, with possibly related endothelial activation involving leukocyte-endothelial cell interaction in the middle zone of the lesion, indicating that vascular factors play a role. A direct pathogenetic role of TNF α is likely, since it produces procoagulatory effects in cultured human endothelial cells and after infusion also in vivo, resulting in decreased protein C activation (16, 17). Furthermore, TNF α can induce ELAM-1 expression on endothelial cells, which was strongly enhanced in the haemorrhagic zone of the lesion. ELAM-1 contributes to adhesion of neutrophils to endothelial cells and to transendothelial migration of neutrophils. Activation of LFA-1 on leukocytes, as also noted in the lesion, can intensify binding of neutrophils to the endothelial cells via ICAM-1. Another endothelial adhesion molecule detected in the lesion, namely VCAM-1, plays an additional role in this process. These alterations of immunologic properties of endothelial cells might

well promote thrombosis. Whether or not their expression represents a primary event in coumarin necrosis cannot currently be determined with certainty. In patients who developed coumarin necrosis under long-term anticoagulation therapy (15), infections, via mediation of TNF α , have also been considered to be important. Drugs other than coumarin could play an additional role in this scenario. According to Wankmüller et al. (18), about one quarter of patients suffered from an infection at the time of necrosis, and more than one third received antibiotic treatment. In animal studies, coumarins have been shown to exert a direct influence on vessel walls, with endothelial proliferation, degeneration of the media, and rupture of capillaries (6), probably also mediated by cytokines.

Vascular factors could explain some peculiarities of coumarin necrosis, which affects mostly postmenopausal obese or younger women with a relative estrogen deficiency after childbirth or ovariectomy (18). The significance of vessel-protective properties of estrogens in coumarin-necrosis has, however, not yet been proven. Moreover, there is no explanation for the predilection sites of necrosis, like hips, buttocks, thighs and female breasts, all areas that are rich in subcutaneous fat. Pressure, low temperature or relative hypoxemia might be predisposing factors.

In our patient, it is remarkable that in spite of heparin therapy, she continued to develop progressively new thromboses. There was no evidence of heparin-induced platelet antibodies with consecutive thrombocytopenia. In this situation, not only necrosis of cutaneous tissue may occur, but also thrombotic events in different organs, with the risk of myocardial and cerebral infarction (8, 9). In contrast, thrombosis in coumarin necrosis is limited to skin and subcutaneous tissue. In our patient, skin necrosis was undoubtedly caused by coumarin, considering the succession of medication. Ellbrück et al. (19) have described a patient similar to our case who not only developed coumarin necrosis, but who also presented with a generalized drug eruption after intravenous heparin and with local reactions to different subcutaneous heparins. Most probably, this represents a fortuitous coincidence, since delayed-type hypersensitivity reactions, especially to subcutaneously injected heparins, are not rare. Notably, this complication also affects mostly middle-aged, obese women, so that hormonal influences have been considered (10). Our histologic and immunohistologic findings fit well to those described in the literature, although epidermal involvement was more pronounced in some of the reported cases, with spongiosis and vesiculation (10, 11). These results support the interpretation that a delayed type hypersensitivity is the essential immunological event in this reaction.

The major culprit in coumarin skin necrosis seems to be hereditary or relative protein C-deficiency during the initial phase of coumarin therapy. For many years, treatment consisted mainly of nonspecific measures like intravenous heparin or fibrinolytic therapy. Recently, Schramm et al. (20) administered a purified protein C concentrate to a patient with coumarin necrosis, with prompt relief of pain and disappearance of erythema as well as rapid healing of the central necrosis. Since there is increased expression of TNF α on endothelial and infiltrating cells, resulting in upregulation of proinflammatory adhesion molecules, additional or alternative treatment with pentoxifylline as a TNF α antagonist might prove useful in preventing development and progression in early stages of necrosis. It remains, however, to be clarified which specific mechanisms confine coumarin necrosis to the skin and to certain body regions.

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