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Defibrotide Protects Endothelial Cells, but not L929 Tumour Cells, from Tumour Necrosis Factor-α-mediated Cytotoxicity

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Abstract

The effect of defibrotide on the cytotoxicity of tumour necrosis factor-α was investigated in cultured bovine pulmonary artery endothelial cells and L929 mouse tumour cells.

In endothelial cells, a 72-h incubation with tumour necrosis factor-α (1 and 10 ng mL^{-1}) reduced the number of viable cells to 63 and 51% of control, respectively. Simultaneous incubation with defibrotide (0·03–0·3 mg mL^{-1}) protected endothelial cells from tumour necrosis factor-α-mediated cytotoxicity, and increased viability in a concentration-dependent fashion to 98% of control at 1 ng mL^{-1} tumour necrosis factor-α and to 80% of control at 10 ng mL^{-1} tumour necrosis factor-α. However, under the same conditions a similar cytotoxic response to tumour necrosis factor-α in L929 tumour cells remained unaltered in the presence of defibrotide.

These findings demonstrate protection from tumour necrosis factor-α-mediated toxicity by defibrotide in endothelial cells but not in a tumour cell line. It is concluded that defibrotide might serve as a therapeutic agent to limit the vascular toxicity of tumour necrosis factor-α without affecting its antineoplastic activity.

Tumour necrosis factor-α (TNF-α) is a cytotoxic protein primarily produced by macrophages following activation by bacterial endotoxin (Old 1985). Although TNF-α promotes regression of some tumours in-vivo (Carswell et al 1975), the use of TNF-α as an antitumour therapeutic is presently limited because some of its intrinsic physiological activities are detrimental to cancer patients. Thus, TNF-α acts as a proinflammatory cytokine and is an important mediator of endotoxin-induced shock implying severe hypotension and increased procoagulant activity (Beutler et al 1985). This vascular toxicity of TNF-α has been attributed to a direct cytotoxic effect of TNF-α on the endothelium, resulting in endothelial lesions which may favour thrombus formation and vasodilation via induction of nitric oxide synthesis in the underlying smooth muscle cells (Sato et al 1986; Estrada et al 1992). Therefore, selective protection of endothelial cells without impairing the antitumour activity of TNF-α might be a useful pharmacological approach to limit vascular toxicity and thereby increase the maximal tolerated dose of TNF-α, potentially improving the therapeutic index.

Defibrotide, a single stranded polydeoxyribonucleotide isolated from bovine lung (Pescador et al 1983), is used as an antithrombotic drug but has also been shown to exert cytoprotective effects (Palmer & Goa 1993). The present study investigates the effect of defibrotide on cytotoxicity by TNF-α in cultured bovine pulmonary artery endothelial cells and L929 tumorigenic murine fibroblasts, a standard cell line for assessing the tumoricidal activity of TNF-α (Flick & Gifford 1984).

Materials and Methods

Materials

Bovine pulmonary artery endothelial cells (ATCC CCL 209) and L929 tumorigenic murine fibroblasts (ATCC CCL 1) were obtained from the American Type Culture Collection, Rockville, MD, USA. Foetal bovine serum was purchased from Gemini Bioproducts, Calabasas, CA, USA. Minimum essential medium was from Gibco Laboratories, Grand Island, NY, USA. TNF-α was a gift of Cetus Corporation, Emeryville, CA, USA. Defibrotide was a gift of Crinos, Como, Italy.

Cell culture

Endothelial and L929 cells were maintained and subcultured in minimum essential medium, supplemented with 20 or 10% foetal bovine serum, respectively. The cells were grown in a humidified incubator at 37°C and 5% CO_2.

Incubation procedure

L929 cells and endothelial cells were seeded at 2×10^4 cells/well in 96 well microtitre plates in 100 μL media containing 5% foetal bovine serum. After a 3–4 h attachment period, 100 μL media containing either no additions, TNF-α, defibrotide or TNF-α and defibrotide was added. Incubation was continued for 72 h, followed by a cytotoxicity assay.

TNF-α cytotoxicity assay

Cell cytotoxicity was measured by staining with crystal violet as previously described (Warren et al 1993). After washing with phosphate-buffered saline, cells were fixed with methanol for 5 min and then stained for 10 min with a 0·1% crystal violet solution. Following three washes with tap water, the dye was eluted with 0·1 M trisodium citrate in 50% ethanol for 1 h. Optical density at 630 nm was measured using a microtitre plate reader (Biotek EL 311 s).

Results

In endothelial cells, treatment with TNF-α (1 ng mL^{-1}) reduced the number of cells to 63% of control (Fig. 1A).

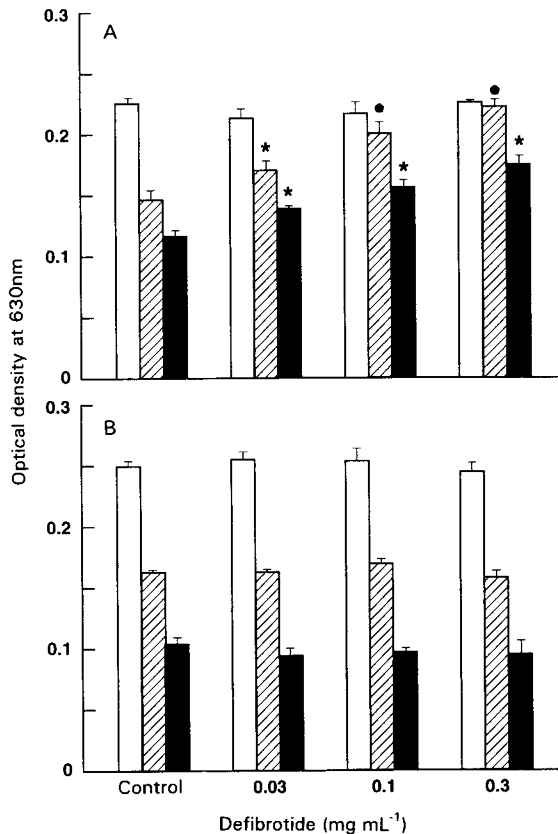

FIG. 1. Effect of defibrotide on TNF-α-induced cytotoxicity in endothelial cells (A) and L929 tumour cells (B). Incubations were carried out in the absence (□) and presence of 1 ng mL^{-1} TNF-α (▨) and 10 ng mL^{-1} TNF-α (■). $*P < 0{\cdot}05$, defibrotide vs control, two-tailed *t*-test. All data shown are mean ± s.e.m. of n = 6 observations.

Simultaneous incubation with defibrotide (0·03–0·3 mg mL^{-1}) increased viability in a concentration-dependent manner and at 0·3 mg mL^{-1} defibrotide, cytotoxicity by TNF-α (1 ng mL^{-1}) was virtually abolished (viability: 98% of control, Fig. 1A). A similar cytoprotective effect by defibrotide (0·03–0·3 mg mL^{-1}) was observed when using a higher concentration of TNF-α (10 ng mL^{-1}). Under these conditions the number of viable cells was reduced to 51% of control in the absence and to 80% in the presence of defibrotide (0·3 mg mL^{-1}) (Fig. 1A). In L929 tumour cells, incubation with TNF-α (1 and 10 ng mL^{-1}) reduced the number of viable cells to 65 and 42% of control, respectively (Fig. 1B). However, the cytotoxic response of L929 cells to TNF-α remained unaltered in the presence of defibrotide (0·03–0·3 mg mL^{-1}) (Fig. 1B). Defibrotide alone (0·03–0·3 mg mL^{-1}) had no significant effect on viability in endothelial cells or L929 cells (Fig. 1A,B).

Discussion

The present study demonstrates protection from TNF-α-mediated toxicity by the antithrombotic drug defibrotide in cultured vascular endothelial cells. Under the same conditions, the tumoricidal activity of TNF-α in L929 cells remained unaffected by defibrotide.

The endothelium protective effect of defibrotide was observed at concentrations (0·03–0·3 mg mL^{-1}) that correspond well to dosage and plasma levels of defibrotide previously described and recommended for its use in man (Coccheri et al 1988). Thus, defibrotide should be expected to provide a safe and effective defense against vascular toxicity of TNF-α, not only in-vitro but also in cancer patients, particularly since it can be administered orally and is well tolerated even at high dosage (Palmer & Goa 1993). Equally important for a potential use of defibrotide in cancer patients is the finding that within this clinically relevant concentration range of defibrotide, its protective effect against TNF-α-induced toxicity was confined to endothelial cells and did not occur in L929 tumour cells. Thus, under a supportive therapy with defibrotide, the tumoricidal activity of TNF-α can be expected to be left unimpaired and the maximal tolerated dose of TNF-α could possibly be increased to a more effective level. In addition, the observed cytoprotective effect of defibrotide may be of therapeutic relevance under conditions of increased endogenous TNF-α release, e.g. in clinical syndromes such as septic shock or cachexia (Beutler et al 1985). This is supported by a recent study showing that defibrotide actually improves the survival rate of experimental animals during septic shock (Hohlfeld et al 1992).

The cytoprotective effect of defibrotide has been attributed to its inhibitory effect on the formation of free radicals (Tettamanti et al 1992; Palmer & Goa 1993). This mechanism may also explain the decrease by defibrotide of TNF-α-dependent endothelial toxicity observed in the present study, since TNF-α has been shown to damage cells by increasing the formation of oxygen-centred radicals (Matthews et al 1987) and of the nitric oxide free radical, which has recently emerged as an autocrine, cytotoxic mediator in endothelial cells (Estrada et al 1992; Schröder et al 1992). It is possible that free radical injury is only one of a constellation of toxic effects produced by TNF-α in sensitive tumour cells (Matthews et al 1987) and that reducing their formation is, therefore, insufficient to protect them from TNF-α toxicity. In contrast, the range of toxic effects exerted by TNF-α in endothelial cells may be more limited and thus susceptible to the protective effect of defibrotide.

Together, the present study demonstrates that defibrotide at clinically relevant concentrations protects endothelial cells from TNF-α-mediated toxicity without decreasing the tumoricidal activity of TNF-α in L929 tumour cells. These results suggest that defibrotide is a possible therapeutic agent for selectively preventing TNF-α-dependent vascular toxicity during cancer therapy or under conditions of increased endogenous TNF-α release such as septic shock.

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