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Secretion of Plasminogen Activators by Human Myeloid Leukemic Cells: Modulation and Therapeutic Correlations

E. L. Wilson, P. Jacobs, and E. B. Dowdle

Plasminogen activator (PA) synthesis and release are inducible cellular functions [1–7] that are subject to modulation by hormones, drugs, and other pharmacological compounds which act at a genetic level. Normal granulocytes synthesize PA [8, 9] and it is known that human cells release, plasminogen activators of two distinct immunochemical types – one similar to urokinase and the other similar to tissue activator [10–13].

In view of these facts we felt that it would be of interest to determine whether acute myeloid leukemic (AML) cells released PA and, if so, whether enzyme type and modulability could usefully be correlated with clinical or prognostic features in an given case. Since the anti-inflammatory steroid, dexamethasone, and the tumor promoter, tetradecanoyl phorbol acetate (TPA), are able to influence PA release by other cells [1, 2, 9, 14–19], these were used in the present experiments.

The results of this study show that leukemic cells secrete both types of PA and that patients with AML whose cells released only tissue plasminogen activator did not respond to combination chemotherapy [20]. Both dexamethasone and TPA could stimulate or inhibit secretion of both forms of enzyme, and PA secretion should prove a sensitive means of monitoring the responses of AML cells to biologically active compounds [21].

A. Methods

Heparinized blood samples were obtained from 18 normal and 69 patients with AML. Cells were isolated by centrifugation on a Ficoll-Hypaque cushion [20] and resuspended in RPMI containing 3% fetal calf serum to give 4×10^6 cells/ml. The appropriate concentration of TPA or dexamethasone was added and the dishes were incubated for 24 h at 37 °C in a humid atmosphere of 5% CO_2 in air. At the end of this period the medium was harvested by centrifugation and replaced with fresh medium containing compounds at the same concentration as before. At the end of the second period of incubation the medium was collected and the samples (harvest fluids) were stored at –80 °C for analysis of PA activity. PA was assayed by measuring the plasminogen-dependent release of soluble radioactive fibrin degradation peptides from insoluble ^{125}I-labeled fibrin-coated Linbro multiwell plates as previously described [22]. Molecular species of plasminogen activators were identified by electrophoretic and immunochemical procedures as previously described [10].

B. Results and Discussion

Fibrinolytic activity released by normal and leukemic cells in culture was invariably and completely plasminogen dependent [20].

I. PA Type and Response to Chemotherapy

Electrophoretic and immunochemical analyses showed that, whereas normal neutrophils invariably released only the urokinase-type of enzyme, cells from 14/69 pa-

Table 1. Correlation between clinical outcome and molecular species of plasminogen activator released by cultured cells of 69 patients with AML

Therapy	Group	Response	Nature of plasminogen activator				Totals
			TA	UK	TA and UK	Unknown	
Combination chemotherapy	A	*Assessment completed*					
		Complete remission	0	21	3	2	26
		No remission	7	5	0	1	13
		(Subtotals)	(7)	(26)	(3)	(3)	(39)
	B	*Died before assessment*	3	10	1	2	16
Palliative/ alternate therapy	C		4	7	1	2	14
Totals			14	43	5	7	69

TA, tissue activator; UK, urokinase

tients with AML secreted tissue activator, cells from 43 patients secreted the urokinase-type enzyme, and cells from five patients secreted a mixture of urokinase and the tissue-type enzymes. Cells isolated from seven patients with AML secreted too little enzyme for the activator to be identified with certainty (Table 1).

The tendency for approximately 20% of AML patients to have cells that released tissue activator was apparent in each of the three major therapeutic subdivisions in Table 1. Thus, blasts from 4/14 patients who received palliative therapy; from 3/16 patients who were treated with standard combination chemotherapy but who died before evaluation could be completed; and from 7/39 patients in whom results of therapy could be assessed released tissue activator. If one considers only the 39 patients in whom therapeutic responses could be evaluated, a satisfactory remission was induced in 83% (24/29) of patients whose cells released the urokinase-type enzyme. In contradistinction, none of the seven patients whose cells released tissue activator alone entered remission. In this limited series, therefore, there was a significant correlation ($\chi^2 = 17.8$; $P < 0.001$) between the release of tissue activator alone and a poor response to the cytotoxic regimen that was used [20].

II. Effects of Dexamethasone and TPA on PA Release

In 35/45 cases, 10^{-7} *M* dexamethasone inhibited PA secretion by at least 25%. Pronounced inhibition (greater than 75%) was observed in 26/45 cases. In 6/45 cases the steroid stimulated enzyme production (greater than 140% of control), and in 4/45 cases no effect on enzyme secretion was observed. The fact than the rate of PA release by cells from 41/45 patients with AML was modulated by 10^{-7} *M* dexamethasone implies that most AML cells possessed receptors for this steroid [21].

Dexamethasone has generally been observed to inhibit PA synthesis by cells cultured in vitro [1, 2, 9, 18, 24], and Roblin [19] has recently suggested that synthesis of the urokinase-type enzyme is suppressed by dexamethasone whereas cellular release of tissue PA is resistant to regulation by glucocorticoids. Our observations with leukemic cells show that release of both types of PA were susceptible to either stimulation or inhibition by dexamethasone [21]. The cell type rather than the enzyme species may therefore be the determinant of dexamethasone responsiveness and, unlike other cell types studied, certain AML cells show stimulation of PA secretion in response to this glucocorticoid.

The effects of TPA on the secretion of PA by AML cells varied considerably. When added at 1 ng/ml the compound caused profound inhibition (greater than 75%) of enzyme release in 20/41 cases and stimulated in 8/41 cases. As found with dexamethasone both species of PA could be inhibited or stimulated by TPA. The effects of both TPA and dexamethasone were inhibitable by actinomycin D and hence required the transcription of new mRNA [21].

References

1. Wünschmann-Henderson B, Astrup T (1974) Inhibition by hydrocortisone of plasminogen activator production in rat tongue organ cultures. Lab Invest 30:427
2. Wigler M, Ford JP, Weinstein IB (1975) Glucocorticoid inhibition of the fibrinolytic activity of tumour cells. In: Rifkin D, Reich E, Shaw E (eds) Proteases in biological control. Cold Spring Harbor, Cold Spring Harbor, NY, p 849
3. Vassalli JD, Hamilton J, Reich E (1977) Macrophage plasminogen activator; induction by concanavalin A and phorbol myristate acetate. Cell 11:695
4. Ossowski L, Biegel D, Reich E (1979) Mammary plasminogen activator: correlation with involution, hormonal modulation and comparison between normal and neoplastic tissue. Cell 16: 929
5. Mak TW, Rutledge G, Sutherland DJA (1976) Androgen-dependent fibrinolytic activity in a murine mammary carcinoma (Shionogi SC-115 cells) in vitro. Cell 7:223
6. Wilson EL, Dowdle EB (1980) Effects of retinoids on normal and neoplastic human cells cultured in vitro. Cancer Res 40:4817
7. Wilson EL, Reich E (1978) Plasminogen activator in chick fibroblasts: induction of synthesis by retinoic acid; synergism with viral transformation and phorbol ester. Cell 15:385
8. Goldstein IM, Wünschmann B, Astrup T, Henderson ES (1971) Effects of bacterial endotoxin on the fibrinolytic activity of normal human leukocytes. Blood 37:447
9. Granelli-Piperno A, Vassalli J-D, Reich E (1977) Secretion of plasminogen activator by human polymorphonuclear leucocytes. J Exp Med 146:1693
10. Wilson EL, Becker MLB, Hoal EG, Dowdle EB (1980) Molecular species of plasminogen activators secreted by normal and neoplastic human cells. Cancer Res 40:933
11. Vetterlein D, Bell TE, Young PL, Roblin R (1980) Immunological quantitation and immunoadsorption of urokinase-like plasminogen activators secreted by human cells. J Biol Chem 255:2665
12. Markus G, Takita H, Camiolo SM, Corosanti JG, Evers JL, Hobika GH (1980) Content and characterization of plasminogen activators in human lung tumours and normal lung tissue. Cancer Res 40:841
13. Åstedt B, Wallén P, Aasted B (1979) Occurrence of both urokinase and tissue plasminogen activator in human seminal plasma. Thromb Res 16:463
14. Wigler M, Weinstein IB (1976) Tumour promoter induces plasminogen activator. Nature 259:232
15. Wilson EL, Reich E (1979) Modulation of plasminogen activator synthesis in chick embryo fibroblasts by cyclic nucleotides and phorbol myristate acetate. Cancer Res 39:1579
16. Viaje A, Slaga TJ, Wigler M, Weinstein IB (1977) Effects of anti-inflammatory agents on mouse skin tumour promotion, epidermal DNA synthesis, phorbol ester-induced cellular proliferation and production of plasminogen activator. Cancer Res 37:1530
17. Troll W, Rossman T, Katz J, Levitz M (1975) Proteinases in tumour promotion and hormone action. In: Rifkin D, Reich E, Shaw E (eds) Proteases in biological control. Cold Spring Harbor, Cold Spring Harbor, NY, p 977
18. Rifkin D (1978) Plasminogen activator synthesis by cultured human embryonic lung cells: characterization of the suppressive effect of corticosteroids. J Cell Physiol 97:421
19. Roblin R, Young PL (1980) Dexamethasone regulation of plasminogen activator in embryonic and tumour-derived human cells. Cancer Res 40:2706
20. Wilson EL, Jacobs P, Dowdle EB (to be published) The secretion of plasminogen activators by human myeloid leukemic cells in vitro. Blood (in press)
21. Wilson EL, Jacobs P, Dowdle EB (to be published) The effects of dexamethasone and tetradecanoyl phorbol acetate on plasminogen activator release by human acute myeloid leukemia cells. Blood (in press)
22. Wilson EL, Dowdle E (1978) Secretion of plasminogen activator by normal, reactive and neoplastic human tissues cultured in vitro. Int J Cancer 22:390
23. Vassalli J-D, Hamilton J, Reich E (1976) Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors and cyclic nucleotides. Cell 8:271