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Cytolethal sensitivity of human lymphoid cells to glucocorticoids and oxidised polyamines

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A series of clonally-derived glucocorticoid-sensitive and -resistant human lymphoid cell lines was used to investigate the relationship between sensitivity to the effects of oxidised polyamines and initiation of glucocorticoid-induced cytostatic and cytolethal responses. Whilst the exogenous polyamines were found to exert no effect by themselves, incubation of cells for 48 h with 10^{-4}M spermine or spermidine in the presence of serum polyamine oxidase produced severe lethal responses in all clones tested. By contrast 10^{-5}M exogenous polyamines in the presence of polyamine oxidase produced lethal effects only in glucocorticoid-sensitive clones. Spermine was more potent than spermidine. The significance of these observations is discussed.

Introduction Although glucocorticoids comprise important components of the regimen employed for treatment of human leukaemias and lymphomas, their mode of action is still poorly understood in molecular terms (1). Whilst responses are generally considered mediated through specific cytoplasmic glucocorticoid receptors (2), attempts at correlating levels of such receptors with *in vitro* cytolethal responses have largely been unsuccessful (3-10). These studies have also shown that prolonged presence of steroid is necessary (> 24 h) for induction of lethal responses *in vitro* and that a latent interval of 30-36 h (> one cell cycle) is obligatory before morphological manifestations of cell death are evident (11). Others have shown these changes are preceded by arrest of cells in the G1 phase of the cell cycle (12). This pattern of response suggests either the gradual accumulation of some toxic product or depletion of some vital trophic substance. However, they appear not to be related to deprivation of essential substrates of intermediary metabolism and are independent of changes in energy production by cells (13).

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Biologically active polyamines are now considered to play an important role in regulating growth processes in cells and intracellular levels correlate closely with cell growth rate (14). Intracellular turnover of polyamines is rapid, but whilst their synthesis is well-documentated (14), degradation is poorly understood. Work on various mammalian tissues suggests that acetylation and oxidation occurs (15-17) resulting in production of toxic low molecular weight aminoaldehydes (15). Ruminant serum contains a pyridoxal phosphate-dependent polyamine oxidase (18) which produces high molecular weight aminoaldehydes (19) that induce cytostasis of leukaemic cells (20). Cell proliferation is arrested in the G1 phase of the cell cycle in an analogous fashion to the inhibitory effects of glucocorticoids (21).

Similarities in action of glucocorticoids and oxidised polyamines in causing inhibition of cell growth led us to investigate the possibility that their mechanisms may be related.

Materials and Methods Cell culture Glucocorticoid-sensitive and -resistant clones were derived from the human lymphoid cell line CCRF-CEM (22) using limiting dilution or semi-solid agar techniques (23). They have been repeatedly recloned and no line is maintained in continuous culture for longer than 3-4 mnth. Cytolethal sensitivity to glucocorticoids was determined by exposure to 10^{-6} M dexamethasone for 72 h and viability assessed by the ability of cells to exclude trypan blue (0.5% solution). Glucocorticoid-insensitive clones were numbered: 34, 41, 44, 53, 59 and sensitive clones: 14, 18, 30, 22, 23.

Clones were grown in air in Erlenmeyer flasks at 37°C in RPMI 1640 containing morpholinopropane sulphonic acid (2.62 g/l; Hopkin & Williams Ltd., Essex, England), 10% heat-inactivated donor calf serum (Flow Labs Ltd., Irvine, Scotland, penicillin (100 IU/ml), streptomycin (100 IU/ml) and nystatin (25 IU/ml). The medium was changed twice weekly and cells were harvested for experimentation only when viability exceeded 94%.

Cytolethal tests Exponentially growing cells from the glucocorticoid-sensitive clone 23 and -resistant clone 53 were seeded in duplicate at 0.25 - 0.35 x 10^6 viable cells/ml in fresh medium with 10% donor calf serum. It is well-established that serum, under these conditions, contains active polyamine oxidase (18). Cells were exposed to 10^{-4} - 10^{-6}M spermine or spermidine, or 10^{-6}M dexamethasone (Sigma Chemical Co., Poole, England) in the presence or absence of the specific polyamine oxidase inhibitor, 3-hydroxybenzyloxyamine (10^{-4}M; Sandev Ltd., Essex, England). All drugs were administered in aqueous solution. Control tubes received vehicle alone. Incubation was carried out at 37°C for 72 h. Samples from each incubate were taken at 24 h intervals for determination of viable cell count by dye exclusion test. Cells from the 10 glucocorticoid-sensitive and -resistant clones were similarly incubated in the presence of 10^{-5}M spermine or 10^{-6}M dexamethasone and viability determined after 48 h. Results are expressed as percentage viable cells in the test over that in controls.

RESULTS Figure 1 shows the effects of varying the concentration of spermidine and spermine on the viable cell count of the glucocorticoid-sensitive (23) and -resistant (53) clones. Whereas 10^{-6}M polyamine had no effect on the growth of either clone, 10^{-4}M caused almost complete cell death of both by 48 h. By contrast, with 10^{-5}M polyamine a differential response was obtained. In the glucocorticoid-sensitive clone (23) 10^{-5}M polyamines produced rapid loss of viability. Spermine was more potent than spermidine in this respect. The glucocorticoid-resistant clone (53) showed no response to polyamines at this concentration. The cytolethal and cytostatic effects of 10^{-5}M and 10^{-4}M polyamine were completely abolished in both clones by the presence of the polyamine oxidase inhibitor, 3-hydroxybenzyloxyamine (Figure 2), suggesting toxicity is associated with the oxidation products of spermine and spermidine rather than the polyamines themselves. The cytolethal effect of 10^{-6}M dexamethasone is apparently unrelated to the presence of serum polyamine oxidase in the culture medium (Figure 2). The toxicity of 10^{-5}M spermine on the 10 clones with differing sensitivities to 10^{-6}M dexamethasone, is shown in Figure 3. In each case, sensitivity to glucocorticoid treatment correlated closely with that to oxidised spermine.

DISCUSSION In this study we have shown differential cytolethal responsiveness to exogenous polyamines in the presence of ruminant serum

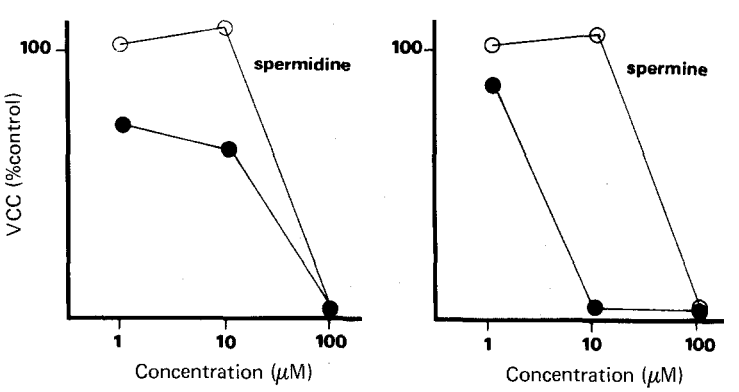

Figure 1 Effect after 48 h of varying concentrations of spermidine or spermine on growth of glucocorticoid-sensitive (●) and -resistant (○) human lymphoid cells. Results are expressed as viable cell count (VCC) compared with untreated controls.

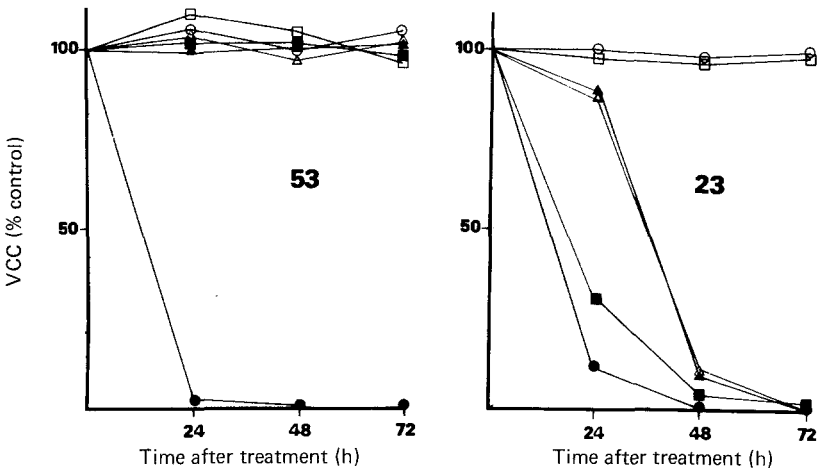

Figure 2 Effect of 10^{-4}M spermine (●), 10^{-5}M spermine (■) and 10^{-6}M dexamethasone (▲), on growth of glucocorticoid-resistant (clone 53) or -sensitive (clone 23) human lymphoid cells in the presence (open symbols) or absence (closed symbols) of polyamine oxidase inhibitor (3-hydroxybenzyl-oxyamine). Results are expressed as viable cell count (VCC) compared with untreated controls.

polyamine oxidase that correlates closely with glucocorticoid-sensitivity of cells. Our results also show the effects of exogenous polyamines are completely abolished in the presence of the serum polyamine oxidase inhibitor, 3-hydroxybenzyloxyamine, implying that oxidation products are the cytolethal agents rather than the polyamines themselves.

Reported kinetics of the cytostatic response induced by oxidised polyamines in lymphoid cells (21) are identical to those produced by

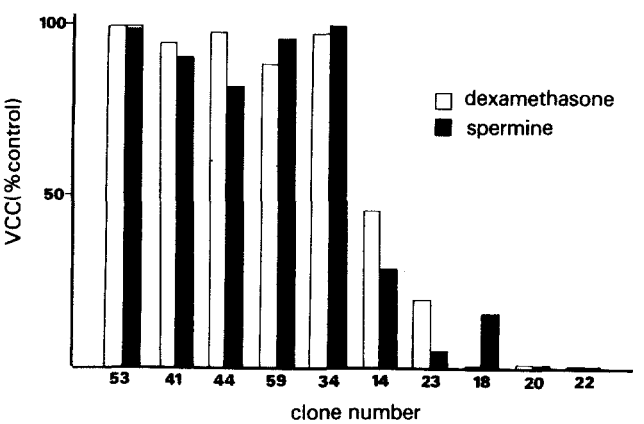

Figure 3 Effect after 48 h of 10^{-6}M dexamethasone and 10^{-5}M spermine on 10 glucocorticoid-sensitive (clones 53, 41, 44, 59, 34) and -resistant (clones 14, 23, 18, 20, 22) human lymphoid cell lines. Results are expressed as viable cell count (VCC) compared with untreated controls.

dexamethasone in glucocorticoid-sensitive human lymphoid cells (11, 12). The relationship between the actions of the two groups of compounds has still to be elucidated. One possibility is that glucocorticoids act by promoting degradation of polyamines with the generation of toxic metabolites. Polyamine oxidases have been identified in many mammalian (including lymphoid) tissues. They have been found to be flavin enzymes unrelated to the pyridoxal phosphate-dependent serum enzymes (15, 25, 26) although degradation products of both enzymes have cytotoxic properties (19, 26). It is possible that an intracellular polyamine oxidase exists in our cell lines. If this were inducible by glucocorticoids then higher concentrations of polyamine degradation products could be responsible for mediation of cytostatic/cytolethal responses.

It is also possible that the effects of the two compounds are unrelated and that their distinctive actions in glucocorticoid-sensitive and -resistant lymphoid cells reflect some other property of target cells. In this respect differences in nuclear fragility following hypotonic lysis have been shown to correlate with glucocorticoid-sensitivity of lymphoid cells. It is suggested this results from inherent differences in the stability of nuclear and other membranes in steroid-sensitive and -resistant cells related to differences in protein content of membranes (13, 27). Highly reactive aminoaldehydes or other polyamine oxidation products could initiate differential disruption by virtue of non-specific interaction with these proteins. Investigations are currently underway to distinguish between these possibilities.

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REFERENCES

1. Bird, C.C. (1979) In: Proceedings of the Tenovus Workshop on Glucocorticoid Action and Leukaemia, Ed. P.A. Bell and N.M. Borthwick, p. 123-139. Cardiff, Wales, U.K. Alpha Omega Publishing Co.
2. Baxter, J.D. & Tomkins, G.M. (1971) Proc. Natl. Acad. Sci. USA, 68, 932-937
3. Burrow, H.M., Bird, C.C., Warren, J.V., Steel, C.M., Barrett, I.D. & Panesar, N.S. (1981) Diag. Histopathol. 4, 175-188

4. Barrett, I.D., Panesar, N.S., Bird, C.C., Abbott, A.C., Burrow, H.M. & Steel, C.M. (1981) Diag. Histopathol. 4, 189-198
5. Barrett, I.D., Panesar, N.S., Burrow, H.M., Bird, C.C., Cawley, J.C., Child, J.A. & Roberts, B.C. (1982) Clin. & Lab. Haematol. 4, 285-297
6. Bird, C.C., Robertson, A.M.G., Read, J. & Currie, A.R. (1977) J. Pathol. 123, 145-156
7. Crabtree, G.R., Smith, K.A. & Munck, A. (1978) Cancer Res. 38, 4268-4272
8. Homo, F., Duval, D., Harousseau, J.L., Marie, J.P. & Zittoun, R. (1980) Cancer Res. 40, 2601-2606
9. Kontula, K., Andersson, L.C., Paavonen, T., Myllya, G., Teerenhovi, L. & Vuopio, P. (1980) Int. J. Cancer 26, 177-183
10. Simonsson, B., Terenius, L. & Nilsson, K. (1982) Cancer 49, 2493-2496
11. Blewitt, R.W., Abbott, A.C. & Bird, C.C. (1983) Brit. J. Cancer 47, 477-486
12. Harmon, J.M., Norman, M.R., Fowlkes, B.J. & Thompson, E.B. (1979) J. Cell. Physiol. 98, 267-278
13. Young, D.A., Nicholson, M.L., Guyette, W.A., Giddings, S.J., Mendelsohn, S.L., Nordeen, S.K. & Lyons, R.T. (1979) In: Proceedings of the Tenovus Workshop on Glucocorticoid-Action and Leukaemia. Ed. P.A. Bell and N.M. Borthwick, p. 53-74, Cardiff, Wales, U.K. Alpha Omega Publishing Co.
14. Tabor, C.W. & Tabor, H. (1976) Ann. Rev. Biochem. 45, 285-306
15. Hollta, E. (1977) Biochem. 16, 91-100
16. Seiler, N. & Al-Therib, M.J. (1974) Biochim. Biophys. Acta. 354, 206-212
17. Seiler, N., Bolkenius, F.N. & Knodgen, B. (1980) Biochim. Biophys. Acta. 633, 181-190
18. Blaschko, H. (1962) Adv. Comp. Physiol. Biochem. 1, 67-116
19. Kimes, B. & Morris, D. (1971) Biochim. Biophys. Acta. 228, 223-234
20. Allen, J.C., Smith, C.J., Hussain, J.I., Thomas, J.M. & Gaugas, J.M. (1979) Europ. J. Biochem. 102, 153-158
21. Gaugas, J.M. & Dewey, D.L. (1978) Brit. J. Cancer 39, 548-557
22. Foley, G.E., Lazarus, H., Farbler, S., Uzman, B.G., Boone, B.A. & McCarthy, R.C. (1965) Cancer 18, 522-529
23. Hamburger, J. & Salmon, S.E. (1977) Science 197, 461-463
24. Quash, G., Keolouangkhot, T., Gazzolo, L., Ripoli, H. & Saez, A. (1979) Biochem. J. 177, 275-282
25. Seiler, N., Bolkenius, F.N., Knodgen, B. & Mamont, P. (1980) Biochim. Biophys. Acta. 615, 480-488
26. Brookes, B.R. & Klamberth, O.L. (1968) Europ. J. Biochem. 5, 178-182
27. Nicholson, M.L. & Young, D.A. (1978) Cancer Res. 38, 3673-3680