A Comparison of the Effects of Prednisolone and Methylprednisolone on Human Lymphoblastoid Cells

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Methylprednisolone is more effective than prednisolone in mediating long-term cytolysis and immediate inhibition of nucleoside uptake in human lymphoblastoid cells. This suggests that methylation of prednisolone makes it more potent by aiding interaction with a cellular target rather than increasing its stability.

A number of human diseases are treated with glucocorticoids. Two of their most important applications are as anti-inflammatory agents (Nacht & Garzon, 1974) and in the treatment of certain human leukaemias and lymphomas (De Vita, 1973; Simone, 1974). Two glucocorticoids commonly prescribed are prednisolone (11 β ,17,21-trihydroxypregna-1,4-diene-3,20-dione) and methylprednisolone (11 β ,17 α ,21-trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione), the sodium succinate salts of which are water-soluble.

We have examined the effects of glucocorticoids on human lymphoblastoid cells in culture as part of a study of cell death. Methylprednisolone at about 1 mM causes the lysis of human lymphoblastoid cells (Bird *et al.*, 1975) and inhibits the uptake of uridine and thymidine into these cells (Waddell *et al.*, 1976, 1977). Cytolysis is a long-term effect, requiring the continuous presence of glucocorticoid for up to 48 h for maximal effect, whereas the inhibition of uptake of uridine and thymidine is apparently instantaneous.

Methylation of prednisolone (at the 6 α position) increases the anti-inflammatory action of the hormone (Liddle, 1958), and it has been suggested that this may be due to increased half-life of the drug. It was therefore decided to compare the efficiencies of prednisolone and methylprednisolone in producing the immediate effect on inhibition of nucleoside uptake and the long-term cytolethal action.

Methods

The cell lines were derived from the lymphoid cells of patients with acute lymphoblastic leukaemia (BLA₁), chronic lymphatic leukaemia (GS₁) or Burkitt's lymphoma (JIJOYE-X). They were established in culture as described by Steel (1972). Prednisolone (Sigma, London S.W.6, U.K.) or methylprednisolone (Solumedrone; Upjohn Co., Crawley, Sussex, U.K.) was added as an aq. 1% (v/v) solution of the sodium succinate salt. Cytolysis was assessed by the entry of Nigrosine (0.25% for 10 min at 20°C)

into the cells after incubation with steroid for 48 h. Uridine uptake was measured as the total amount of [³H]uridine (The Radiochemical Centre, Amersham, Bucks., U.K.; 25.8 Ci/mmol) in whole cells after pulsing the cells with [³H]uridine for 20 min at 1 μ Ci/ml. Cells were collected on Millipore HAWP filters (pore size 0.45 μ m) and washed free of extracellular labelled uridine with 40 ml of ice-cold phosphate-buffered saline (25 mM-KH₂PO₄/100 mM-NaCl, pH 7.4). Filters were dried overnight and counted for radioactivity in 10 ml of toluene/butyl-PBD [5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole; 5 g/litre] scintillant. Efficiency corrections were made by the external-standard-ratio method, with ¹³⁷Cs as standard.

Results and Discussion

After treatment of three cell lines with 1–2 mM-steroid for 48 h, methylprednisolone was much more cytotoxic than prednisolone (Fig. 1). There was a similar but less marked effect on the growth of the cells. Methylprednisolone (1 mM) was also more potent in the inhibition of uptake of uridine into BLA₁ cells (Table 1).

It is generally accepted that glucocorticoid hormones exert their effects by binding to cytoplasmic receptor proteins, which mediate their translocation to the nucleus, where they alter gene activity (Jensen & De Sombre, 1973), but there is no evidence that this is the mechanism of action in the effects that we have observed. However, there is evidence that the cell surface may be one target for the direct action of steroids (Dell'Orco & Melnykovich, 1970; Fiskin & Melnykovich, 1971; Plagemann & Renner, 1972), and it has also been suggested that, in corticosteroid-sensitive rodent cells, the hormone causes an alteration in some undefined key membrane property (Stevens *et al.*, 1974). It seems that in our system there is a direct interaction between the steroid and the cell surface to produce inhibition of uptake of nucleosides

Fig. 1. Effect of prednisolone and methylprednisolone on cell viability

Cells from three lymphoblastoid cell lines (a, BLA₁; b, GS₁; c, JIJOYE-X) were suspended in fresh modified Eagle's medium (Gibco-Biocult, Paisley, Scotland, U.K.) buffered with NaHCO₃ and supplemented with 20% (v/v) foetal calf serum (Gibco-Biocult) at an initial concentration of 0.2×10^6 viable cells/ml. After 24 h, prednisolone or methylprednisolone was added at 0.5, 1.0, 1.25, 1.5 or 2.0 mM and the cells were left undisturbed for a further 48 h. The cultures were then assessed for the viability of the cells, as described in the text. $\square$, Prednisolone-treated; $\circ$, methylprednisolone-treated. Results are means ($\pm$ s.d.) for three experiments. Where no error bar is shown, this falls within the symbol.

Table 1. Effects of 1 mM-prednisolone and -methylprednisolone on uridine uptake into BLA₁ cells

Samples (2 ml) of BLA₁ cells (10^6 /ml) in modified Eagle's medium (Gibco-Biocult, Paisley, Scotland, U.K.) buffered with NaHCO₃ and supplemented with 20% (v/v) foetal calf serum (Gibco-Biocult) were pulsed for 20 min with [³H]uridine (1 μ Ci/ml) after preincubation with methylprednisolone (1 mM), prednisolone (1 mM) or 0.9% NaCl for 1 h. Samples were counted for radioactivity as detailed in the text. Results are means ($\pm$ 1 s.d.) for four separate experiments.

	Uptake (d.p.m./ 10^6 cells)	Inhibition (%)
Control	50400 $\pm$ 1500	—
Prednisolone	16000 $\pm$ 1100	69*
Methylprednisolone	6400 $\pm$ 100	88*

* s.d. $< \pm 1\%$

(Waddell *et al.*, 1976, 1977). Since methylprednisolone is more effective than prednisolone in producing both long-term (i.e. cytolytic) and short-term (i.e. uptake-inhibitory) effects, it is postulated that methylation may increase the potency of prednisolone by aiding its interaction with some cellular site (possibly some component of the cell surface) rather than by increasing its half-life.

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