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Methylprednisolone Resistance of Cystic Fibrosis Lymphocytes

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Summary

In the current study, the *in vitro* viability and resistance to methylprednisolone (MP) was investigated in lymphocytes from patients with cystic fibrosis (CF) and normal controls. The addition of MP to phytohemagglutinin-stimulated lymphocyte cultures inhibits the proliferative response in a dose-dependent way. The dose response curves to MP were similar in CF cultures and controls ($P > 0.05$). Neither preincubation nor pulse exposure to MP revealed any difference between normal and CF lymphocytes.

Skin fibroblasts from patients with CF have been reported to show increased resistance to the toxic effect of dexamethasone (1–3, 5–6). This study was undertaken to investigate the suppressive effect of glucocorticoids on another cell culture system using MP on phytohemagglutinin-stimulated lymphocytes.

Abbreviations

CF, cystic fibrosis
cpm, counts per minute
MP, methylprednisolone
PHA, phytohemagglutinin

MATERIALS AND METHODS

Isolation of mononuclear cells. Peripheral blood lymphocytes were isolated from 10 CF patients aged 9–14 yr (nine males and one female and 10 age- and sex-matched controls. Informed consent was obtained from the parents. Ten milliliters heparinized venous blood was added to equal amounts of Hank's balanced salt solution. The mononuclear cells were isolated on Lymphoprep (Nyco, Norway) and washed three times in Hank's balanced salt solution. The cells were then resuspended in RPMI-1640 (Gibco, Europe), containing 10% fetal calf serum, 500 IU penicillin per ml (Leo, Denmark), and streptomycin, 333 μg/ml (Novo, Denmark). The final cell concentration was adjusted to 1×10^6 cells per ml cell suspension.

Lymphocyte cultures. Both CF and control cultures were set up *1*) with direct addition of PHA and MP and *2*) after preincubation of cultures for 24 h before PHA and MP addition. Additionally, the effect of pulse exposure of cells was studied by incubation with MP, 2.5 μg/ml cell suspension for 20 min followed by washing of the cells before *3*) direct addition of PHA and MP, and *4*) preincubation for 24 h, as above.

Incubation and harvesting. The cultures were incubated in microtiter plates (5×10^4 cells per well) with MP (Urbason, Hoechst, F.R.G.) at a final concentration range of 0–0.06–0.30–1.2–3.0–12–30 μg/ml cell suspension and PHA (Difco, Detroit, USA) 50 μg/ml cell suspension. The incubation continued for 72 h at +37°C followed by another 20 h period after [^{14}C] thymidine addition (20 nCi per well, Amersham, UK) before harvesting. The [^{14}C]thymidine uptake was measured in a liquid scintillation counter (Packard, Tri-Carb, Liquid Scintillation Spectrometer). The mean number of counts incorporated in the presence *versus* absence of steroid was expressed as the *per cent suppression* at each concentration level. Group means were compared using Student's *t* test.

RESULTS

The effect of MP addition to PHA-stimulated CF and control cultures is shown in Figure 1. MP cause a dose-dependent inhibition of radiolabeled thymidine incorporation in cell DNA. The dose response curves to MP are similar with only minor insignificant differences between CF and control cultures at any concentration level ($P > 0.05$). The PHA responses in the absence of MP were the same for CF and control cultures 8.6×10^3 cpm

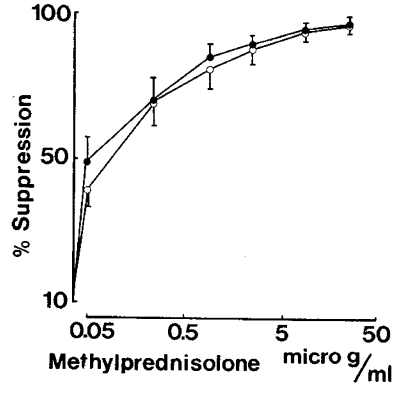

Fig. 1. Suppression of [^{14}C]thymidine uptake in phytohemagglutinin-stimulated lymphocytes by methylprednisolone. (○) Cystic Fibrosis patients and (●) controls. SEM is indicated.

Table 1. *Dose response of cystic fibrosis (CF) and control cultures to methylprednisolone (MP)*

% minus (÷) MP (μg/ml)	CF patients				Controls			
	cpm ± SD*	Pre-incubated cpm ± SD†	Pulse (MP) cpm ± SD†	Pulse (MP) pre-incubated cpm ± SD†	cpm ± SD*	Pre-incubated cpm ± SD†	Pulse (MP) cpm ± SD†	Pulse (MP) pre-incubated cpm ± SD†
0 phytohemagglutinin	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.5	0.2 ± 0.1	0.3 ± 0.3
0	8.6 ± 2.4	8.5 ± 3.2	8.4 ± 3.3	8.3 ± 3.9	8.2 ± 3.2	7.8 ± 4.3	7.2 ± 3.7	8.5 ± 3.8
0.06	5.6 ± 3.0	5.2 ± 2.2	6.0 ± 4.1	5.5 ± 3.8	4.3 ± 3.8	4.4 ± 3.2	4.0 ± 3.6	5.5 ± 3.9
1.2	2.0 ± 2.1	2.3 ± 2.5	3.2 ± 2.9	2.9 ± 2.2	1.5 ± 1.5	2.4 ± 2.1	1.2 ± 1.5	3.7 ± 3.9
12	0.6 ± 0.8	1.1 ± 1.5	1.4 ± 1.3	1.4 ± 1.3	0.9 ± 0.7	1.5 ± 1.2	0.4 ± 0.6	2.1 ± 2.8

* cpm, counts per minute and $n = 10$.
† $n = 7$.

(SD 2.4 × 10^3 cpm) and 7.2 × 10^3 cpm (SD 3.8 × 10^3 cpm), respectively [Table 1 ($P > 0.05$)]. Table 1 also showns the effect of preincubation and pulse exposure to MP. Preincubation for 24 h did not induce any difference in viability or MP dose response between CF and control cultures ($P > 0.05$). Pulse exposure to MP did not show CF cultures to be more resistant to the effect of MP even after a preincubation period ($P > 0.05$).

DISCUSSION

Epstein *et al.* (5, 6), Breslow and coworkers (1–3), and Daniel *et al.* (4) have demonstrated increased resistance of CF fibroblasts to the toxic effects of dexamethasone and other drugs of steroid structure (2, 3, 5). Accordingly, the sterol nucleus is considered important in enhanced CDF fibroblast resistance (6). The relationship to the basic defect in CF is obscure and only minor changes in the glucocoritcoid metabolites have been reported, indicating a change in the peripheral metabolism of glucocorticoids (9). Furthermore, it is of some concern that other investigators (8) have failed to reproduce these results.

In view of the clinical interest in a test system for CF, the present study was undertaken with a different synthetic steroid. This assay was chosen due to its well known response to MP (7). The results did not show either enhanced specific MP resistance or nonpsecific resistance to preincubation. The negative results may indicate that *1*) CF fibroblast resistance is not generally expressed in other cell lines such as lymphocytes, *2*) the sterol structure of the drug is less important, *3*) MP exerts different effects on different cell cultures, or *4*) the metabolism of MP depends on the cell system.

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