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Diphenylhydantoin Inhibits Cortisol-Induced Lysis of Thymocytes[1] (42368)

ARCHIE A. MACKINNEY, JR.*,[2] AND LINDA KNOBELOCH†

Hematology Laboratory, William S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, Wisconsin 53705, and †Department of Medicine, University of Wisconsin, 1600 Highland Avenue, Madison, Wisconsin 53706

Abstract. Diphenylhydantoin (DPH) shares two features with cortisol: immunosuppression and cleft palate formation. We tested the hypothesis that DPH would have effects on lymphocytes *in vitro* similar to those induced by cortisol, and the corollary that DPH would inhibit those cortisol effects. We found that DPH lysed rat thymocytes, although at higher concentrations than cortisol. When combined, DPH inhibited cortisol lysis of thymocytes. Neither drug lysed human phytohemagglutinin (PHA)-stimulated cells, but both drugs depressed DNA and RNA syntheses in PHA cells. DPH augmented cortisol inhibition of DNA and RNA syntheses in PHA cells and DNA synthesis in rat thymocytes. It had no effect on cortisol inhibition of RNA synthesis in rat thymocytes. It appears that DPH has a cortisol-like action (lysis of rat thymocytes). The actions of this drug enable us to show that cortisol lysis and the inhibition of DNA or RNA synthesis can be dissociated. These phenomena may explain some immunosuppressive effects of DPH in the human.

Diphenylhydantoin (DPH), a valuable anticonvulsant, has two features in common with cortisol: immune impairment (1–7), and induction of cleft palate (8–11). Closure of the palate is normally dependent on the programmed cell death of abutting edges of the palatal epithelium so that the underlying mesenchyme can fuse. Diphenylhydantoin or cortisol prevents the death of the adjoining epithelial surfaces, which then serve to passively block palatal fusion (10). In this bioassay system, an inactive glucocorticoid competes with DPH, suggesting a common binding site (11). The competitive binding of DPH and cortisol to the glucorticoid binding site in rat liver cytosol and thymocytes has been demonstrated by Katsumata *et al.* (12).

The second common feature of DPH and cortisol is immune impairment. One well-known immunosuppressive action of cortisol is the lysis of rat thymocytes (13). In the current study, we have investigated whether DPH has a similar action and whether it competes with cortisol to prevent death of thymocytes. We have also compared the effects of the two drugs on DNA and RNA syntheses in thymocytes and phytohemagglutinin (PHA)-stimulated human cells.

Materials and Methods. Normal, male 200-g Sprague–Dawley-derived rats were anesthetized with Nembutal ip. The thymus was removed surgically and teased apart to release the thymocytes. The cells were washed twice with RPMI 1640 and adjusted to 2.2×10^6/ml in RPMI containing 25 m*M* Hepes buffer, 2 m*M* L-glutamine, 12% heat-inactivated fetal calf serum, and 1% penicillin–streptomycin–fungizone. Cells were incubated with DPH in $NaCO_3$–NaCl buffer, pH 10.5, or with cortisol or appropriate diluents. In mixing experiments, DPH was added for 30 min before cortisol, and both drugs were left in the mixture for the duration of the incubation period. Tubes were lightly capped and incubated at 37°C for 48 hr in 5% CO_2 and air. At the end of incubation, the cells were gently resuspended, and 100-μl vols were delivered into microtiter wells. Trypan blue (25 μl of 1%) in saline was added, and 400 cells were counted after 10 min of dye exposure. The percentage lysis was equal to the control minus the test divided by the control times 100.

When [^{3}H]thymidine or [^{3}H]uridine was added to rat thymocytes, 6-hr incubations were used because of the severe depression of DNA or RNA synthesis found at 48 hr. Thymocytes were incubated with drugs for 4 hr, and then

[1] Supported by the Veterans Administration.

[2] To whom correspondence and reprint requests should be addressed.

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[^{3}H]thymidine (1 μCi/ml, 20 Ci/mmole) was added for 2 hr prior to harvest, or [^{3}H]uridine (1 μCi/ml, 20 Ci/mmole) was added 1 hr prior to harvest. Cells were collected on glass filters, washed with saline, and extracted with 5% trichloroacetic acid for liquid scintillation counting.

Cultured human lymphocytes were studied in parallel with rat thymocytes. Heparinized normal human peripheral blood lymphocytes were separated on Ficoll gradients, washed with RMPI 1640, and cultured in RMPI 1640 with 20% autologous human serum and 1% phytohemagglutinin (PHA) for 72 hr in the presence of drugs, as described above. [^{3}H]Uridine was added 1 hr prior to harvest, or [^{3}H]thymidine 2 hr prior. The cells were collected on glass filters and processed as described above. Other cultures were tested for lysis as outlined for thymocytes at the end of 72 hr.

Data were analyzed using the Student t test for paired samples.

Results. Cortisol (10^{-7} M) induced significant rat thymocyte lysis. DPH also lysed thymocytes at concentrations above 10^{-4} M (28 μg/ml) (Fig. 1). However, when DPH was preincubated with thymocytes before cortisol, lysis was inhibited significantly at 3.6×10^{-5} M DPH (10 μg/ml, normal pharmacologic plasma level) and 3.6×10^{-7} M cortisol (high physiological plasma level), as well as at other points above and below these values, indicated by the asterisks in Fig. 2 ($P < 0.05$).

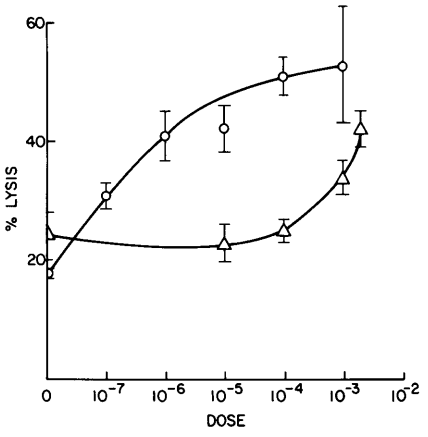

FIG. 1. Rat thymocyte lysis in the presence of cortisol (open circles) or diphenylhydantoin (open triangles).

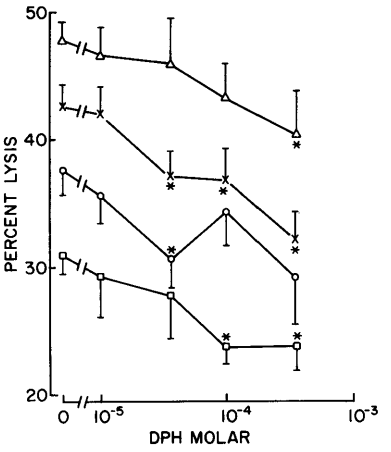

FIG. 2. Rat thymocyte lysis in the presence of cortisol [3.6×10^{-6} M (open triangles), 1×10^{-6} M (x's), 3.6×10^{-7} M (open circles), and 1×10^{-7} M (open boxes)] after the addition of various concentrations of diphenylhydantoin. Asterisks indicate values different from those at the same concentration of cortisol without diphenylhydantoin ($P < 0.05$).

In Fig. 3, the effects of cortisol and DPH on rat thymocytes and human PHA cells are compared. The rat lysis data reflect the inhibitory effect of DPH on cortisol lysis. However, lysis of PHA cells was, if anything, less in the presence of cortisol, with the effect cancelled by DPH. Cortisol inhibited the uptake of uridine or thymidine in PHA cells; DPH contributed to the inhibition in both cases. In contrast, inhibition of uridine incorporation in rat thymocytes was not augmented by DPH. Incorporation of thymidine was inhibited only at the highest concentration of DPH; cortisol alone had no statistically significant effect.

Discussion. DPH lysed rat thymocytes at concentrations greater than 10^{-4} M. It also inhibited cortisol-induced lysis at concentrations compatible with blood levels of both drugs. Since previous studies indicated that DPH competed with cortisol for the glucocorticoid binding site, we reasoned that DPH may inhibit other cortisol effects on lymphocytes. The opposite proved true.

DPH augmented the cortisol inhibition of thymidine uptake but had no effect on uridine uptake in rat thymocytes. We studied PHA-stimulated normal human cells and found that DPH augmented the cortisol inhibition of both thymidine and uridine uptakes. However,

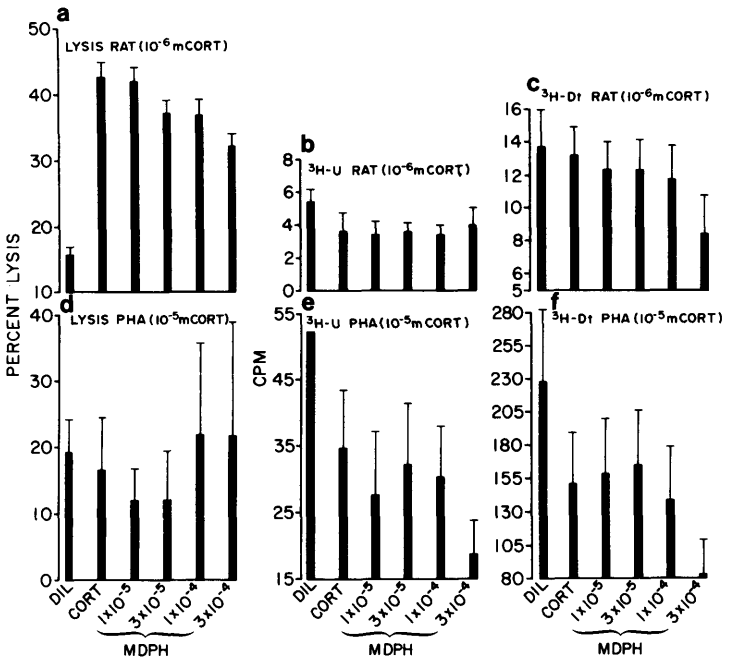

FIG. 3. Effect of diphenylhydantoin and cortisol on rat thymocytes (a–c) or human PHA-stimulated peripheral lymphocytes (d–f). Bars indicate the standard error of the mean.

cortisol did not cause lysis of PHA cells. Thus, the lysis experiments were not corroborated by other measures of cell metabolism.

The mechanism of rat thymocyte lysis has been investigated previously in some detail. After glucocorticoid attaches to the nucleus, nuclear genes are activated near the site where mouse mammary tumor virus is incorporated into the genome (13). Glucose transport is inhibited within 20 min and protein synthesis by 60 min (14). In the first hour, there is a decline in RNA synthesis (15), followed by cell lysis. Lysis is accompanied by release of endonuclease (16), although it is not certain whether this is a cause (17) or an effect (18) of cell death. Our studies showed that DPH blocked cortisol lysis while enhancing inhibition of thymidine uptake. This suggests that the inhibition of DNA synthesis and lysis are not necessarily coordinated events.

The PHA-stimulated human cell model is based on a different glucocorticoid effect. Dexamethasone added to these cells at the beginning of the culture incubation blocks the release of endogenous T-cell growth factor (TCGF), although the cells remain sensitive to exogenous TCGF (19). Lysis is not a feature of this system, and thymidine uptake is a much more sensitive endpoint for cortisol activity. Our studies indicated that cortisol and DPH have additive effects on DNA synthesis but virtually no lytic effect, further supporting the concept that DNA synthesis and lysis are dissociable phenomena.

Both DPH and cortisol are immunosuppressive. In humans, DPH decreases IgA (6), decreases peripheral blood lymphocytes (5), and suppresses delayed hypersensitivity (7). Cortisol has similar effects on humoral and cellular immunity. We interpret the *in vitro* data to indicate that DPH augments some glucocorticoid effects but inhibits others. Further studies are required to determine how these two compounds interact *in vivo* to suppress immunity and how this interaction can be modified.

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