Inhibition of Human Leukocyte Mitosis by Prednisolone \textit{in Vitro}\textsuperscript{*}

\textbf{PETER C. NOWELL}

\textit{(Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania)}

\textbf{SUMMARY}

The water-soluble glucocorticoid, prednisolone 21-phosphate, has been shown to inhibit mitotic activity of normal human leukocytes in short-term culture, with a roughly linear relationship between the log dose of steroid and the percentage decrease in mitosis. Over a concentration range of \(0.002 \, \mu g/ml\) to \(10 \, \mu g/ml\), the mitotic index decreased to 25 per cent of control values. Further studies indicate that most of this inhibitory effect of the steroid operates during the first 4 hours in tissue culture, although the effect is not demonstrable until the 2d and 3d day, when mitosis begins. The findings indicate that prednisolone does not significantly inhibit mitosis directly, in these cultures, but rather inhibits the conversion of partially differentiated circulating leukocytes to a state capable of mitosis. This action occurs at the same time as that of phytohemagglutinin, which initiates mitosis in these cultures by stimulating the conversion process.

The involution of lymphoid tissues in response to treatment with adrenal cortical steroids is apparently the result of a dual mechanism: death of mature lymphocytes and inhibition of mitosis in immature lymphocytes \cite{3}. The latter phenomenon, mitotic inhibition by cortisone and related compounds, has been demonstrated both \textit{in vivo} and \textit{in vitro} with various cell systems \cite{8}, but such studies on lymphoid cells have not clearly demonstrated whether the steroid was operating by slowing down cells already moving through a mitotic cycle, or by preventing activation of mitotically inactive cells \textit{(see references in \cite{3} and \cite{8})}.

A technic has recently been described in which partially differentiated monocytes and large lymphocytes from normal human peripheral blood are converted to a state capable of mitotic activity in culture by the plant extract, phytohemagglutinin \cite{6}. This system provides a means of investigating, with normal human lymphoid cells, various aspects of mitotic inhibition by adrenal cortical hormones. For this purpose, the water-soluble glucocorticoid, prednisolone 21-phosphate,\textsuperscript{1} was used.

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\textbf{MATERIALS AND METHODS}

Leukocytes were separated by means of phytohemagglutinin from the peripheral blood of healthy donors and were grown in culture for 3 days according to the technic described by Moorhead \textit{et al.} \cite{4}. Each standard culture consisted of 10 million cells in 8 ml. of medium (3 ml. autologous plasma, 5 ml. TC-199). Colchicine was added 5 hours prior to termination of the cultures. The cells were harvested, pretreated, fixed, air-dried, and stained as previously described \cite{4}. The mitotic index of each culture was determined by counting the number of metaphases in 2000 cells. Prednisolone 21-phosphate,\textsuperscript{1} dissolved in distilled water, was added to the cultures at the time of planting, with an equal volume of water added to the controls. Final concentration of steroid in the cultures varied from \(0.002 \, \mu g/ml\) to \(10 \, \mu g/ml\).

In some experiments, the following modifications of the technic were employed:

1. Standard cultures were terminated at 2 days and at 4 days, instead of at 3 days, to test the effect of prednisolone \((0.5 \, \mu g/ml)\) over these time periods.

2. Cultures were planted without prednisolone.

\textsuperscript{1}The prednisolone 21-phosphate was supplied by Dr. Richard T. Smith, Merck, Sharp and Dohme, West Point, Pennsylvania. Its glucocorticoid activity is 4-5 times that of hydrocortisone.
had prednisolone added after 1 day or after 2 days, and then were terminated, as usual, on the 3d. These experiments tested the effect of having prednisolone (0.5 μg/ml and 10 μg/ml) present only for the last 24 or 48 hours of the culture period.

3. Cultures were made of leukocytes which had been separated from whole blood without the use of phytohemagglutinin (PHA). These PHA-free cultures were treated with prednisolone (0.5 μg/ml) at the time of planting, and then with PHA 1 hour later. Control cultures for this experiment were treated with PHA first and then with prednisolone 1 hour later. All were terminated routinely on the 3d day to determine the effect on mitotic activity of exposure to prednisolone before PHA.

From five to fifteen cultures were tested under each experimental condition, with a control culture of the same donor’s leukocytes running concurrently in each case. Mitotic indices in the control cultures ranged from 21 to 138 metaphases per 1000 cells (mean = 57) in the 3- and 4-day cultures, and from 4 to 26 per 1000 (mean = 10) in the 2-day cultures. The low 2-day values are consistent with previous evidence (5, 6) that mitotic activity in these peripheral blood cultures is minimal before the 3d day.

In a number of representative control cultures and prednisolone-treated cultures (0.5 μg/ml for 3 days and 0.5 μg/ml for final 24 hours), the total number of cells remaining at the time of termination was determined, as well as the relative percentages of large mononuclear cells and small lymphocytes. In addition, small glass slides were placed in these cultures (5, 6), and, after being stained with Giemsa, these preparations were used to compare cell morphology in the treated and untreated cultures.

RESULTS

The control cultures, after 3 days, were similar to those described previously (5, 6). Approximately half of the original inoculum remained, consisting of small lymphocytes (25 per cent), large mononuclear cells resembling blasts (75 per cent), and an occasional granulocyte. The number of cells and the percentage of small lymphocytes in prednisolone-treated cultures was the same as in the controls. However, in the cultures exposed to prednisolone for 3 days, fewer of the large mononuclear cells had the “blast” appearance characteristic of mitotic activity in these cultures, and more had the “macrophage” morphology typically associated with differentiation and degeneration (5).

The inhibitory effect on leukocyte mitosis, in standard 3-day cultures, of concentrations of prednisolone varying from .002 μg/ml to 10 μg/ml is demonstrated in Chart 1. There was a roughly linear relationship between log dose of the steroid and decrease in mitotic activity. The minimum concentration, .002 μg/ml, had no measurable effect, while the maximum concentration, 10 μg/ml, reduced mitotic activity by nearly 75 per cent.

The result of delayed addition of prednisolone to 3-day cultures is presented in Chart 2. Prednisolone treatment during only the last 24 hours of culture had no effect on mitosis at the 0.5 μg/ml level, and only a slight effect at the 10 μg/ml level. Prednisolone acting for the last 48 hours (i.e., added after 1 day of culture) had greater effect at both the 0.5 μg/ml and 10 μg/ml levels than did prednisolone acting for only the final 24 hours, but the effect was minor compared with that of similar doses acting from the time the cultures were planted.

The effect of prednisolone (0.5 μg/ml) in standard cultures terminated at 2 days and at 4 days is given in Chart 3. As shown, mitotic inhibition in both instances was the same as in standard 3-day cultures treated with the same concentration of steroid.

The effect of adding prednisolone (0.5 μg/ml) to cultures containing no phytohemagglutinin and then adding phytohemagglutinin 1 hour later is also shown in Chart 3. These cultures were terminated at 3 days and showed the same mitotic inhibition as cultures which contained phytohemagglutinin before the addition of prednisolone.

DISCUSSION

The results indicate that the water-soluble glucocorticoid, prednisolone 21-phosphate, inhibits
mitotic activity in primary cultures of normal human leukocytes. A concentration of prednisolone (0.02 \( \mu g/ml \)) comparable in glucocorticoid activity to normal levels of free cortisol in human plasma (0.08 \( \mu g/ml \)) (3), caused approximately 20 per cent reduction in mitotic activity in the leukocyte cultures, and greater concentrations produced a further decrease. The roughly linear relationship between log dose of prednisolone and per cent decrease in mitosis is consistent with previous studies of mitotic inhibition (7) and other physiological effects of steroids. The action of prednisolone appears to involve specifically the cell division mechanisms, since there was no evidence of increased cell destruction in the cultures.

The present findings further indicate that prednisolone exerts most of its inhibitory effect during the 1st day in culture. Prednisolone added after the first 24 hours had only minor effects, even at the 10 \( \mu g/ml \) level, and, since 2-day cultures treated with prednisolone from the outset showed mitotic inhibition, the relative lack of inhibition in 3-day cultures treated only over the last 48 hours was not due simply to insufficient exposure time.

During the first 24–36 hours in culture, the monocytes and large lymphocytes in the inoculum are changing over to a mitotically active state. Synthesis of DNA and mitotic activity in the cultures is minimal during this period (5, 6). Thus, it appears that prednisolone, acting during the first 24 hours, is not inhibiting cells moving through a mitotic cycle, but rather is preventing the conversion of quiescent cells to a mitotically active state.

It is also during this first 24 hours in culture that phytohemagglutinin, the plant extract which initiates mitosis in these cultures, seems to act (6). Prednisolone apparently inhibits the conversion process which phytohemagglutinin stimulates. There does not, however, appear to be direct antagonism between the two compounds (i.e., competition for the same binding site), since the action of the steroid was the same whether the leukocytes were exposed to it shortly before or shortly after the addition of phytohemagglutinin. Nor does the effect of prednisolone seem to be simply a brief delay of the conversion process initiated by phytohemagglutinin, since steroid-treated cultures examined at 4 days showed the same decrease in mitotic activity as those terminated at 3 days.

How prednisolone acts to produce this inhibitory effect is not clear. It has been postulated that mitotic inhibition by cortisone and related compounds results from alterations in glucose metabolism (1), but such studies have involved cells already mitotically active, exposed to steroid for only a few hours. Swann (8) has suggested that such a phenomenon is fundamentally different from the effect of a hormone on the conversion of partially differentiated cells to a mitotically active state, the situation which seems to be present here. In this regard it is of interest that, in our culture
system, treatment with prednisolone phosphate of leukocytes already mitotically active at the time of planting (i.e., rat bone marrow, human leukemic cells) has, thus far, failed to produce any consistent effect on mitosis.²

Not only is the metabolic action of the glucocorticoids still in dispute, but also their site of action in the cell. There is recent evidence that prednisolone may act on the cell membrane rather than within the cell [see references in (2)]. This is of particular interest, since phytohemagglutinin also may act at the cell surface (6). Obviously, further investigation is indicated. Although the present culture system is an artificial one, in the sense that mitosis is initiated by a nonphysiological mechanism, phytohemagglutinin, it appears that these cultures can be used to explore basic questions of cell division and hormone action on cells. Ultimately, it may be possible to relate such studies to the still undiscovered mechanisms controlling leukocyte production in the body.

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REFERENCES


² Unpublished data.
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