SUMMARY

The biochemical effects of 6-α-methylprednisolone-21-succinate, a synthetic corticosteroid hormone, were further investigated on a long-term culture of human glioma cells. Following treatment with this corticosteroid, cellular mitosis was suppressed within 24 hr in proportion to the dose and duration of exposure. Incorporation of tritiated thymidine into glial tumor cells was inhibited over 60% after a 2-hr exposure to the drug; however, less effect on uridine (25% inhibition) and leucine (5% inhibition) incorporation was observed. The primary site of action of this corticosteroid might involve the inhibition of DNA synthesis.

INTRODUCTION

The synthetic corticosteroids dexamethasone and MPS have been widely used in the treatment of patients with brain tumors. Although clinical improvement has been usually attributed to the ability of the corticosteroid to diminish cerebral edema associated with intracranial neoplasms, there is experimental evidence for a more direct antitumor effect.

Corticosteroids in high doses have inhibited the growth of transplantable glia and nonglial tumors, including cerebral melanomas in mice (11), s.c. carried murine ependymomas, and N-nitrosomethylurea-induced rat gliomas after s.c. (18) and intracerebral implantations (8). Growth inhibition and cytotoxicity have been assessed in cell cultures derived from human glioblastomas after exposure to synthetic corticosteroids (12). MPS was more injurious to glioma cells in vitro than dexamethasone and was cytolytic in doses of 400 μg/ml. These findings on neural tumor cells paralleled similar observations of corticosteroid effects on cells of lymphatic and fibroblastic origin (5, 6). The mechanisms underlying corticosteroid cytotoxicity and inhibition of human glioma cell growth in vitro have been explored in the following studies on the effects of MPS on cellular mitosis and protein and nucleic acid synthesis.

MATERIALS AND METHODS

Cell Lines and Culture Medium. A long-term cell culture of cloned glioma cells propagated from a patient with a glioblastoma was used in the present study. This glial tumor cell line was grown at 37° in Falcon plastic dishes containing Eagle's basal medium plus 10% fetal calf serum. The tumor clone showed the characteristic features of malignant glioma cells including higher enzymatic activities of acid phosphatase, lactate dehydrogenase, and glucose 6-phosphate dehydrogenase and greater sensitivity to concanavalin A and bovine enterovirus infection (4, 17). This cell line also contained specific glial fibrillary acidic proteins.

Drugs and Radioactive Precursors. MPS (Solumedrol, Upjohn Co., Kalamazoo, Mich.) and epicortisol (11α-hydroxy cortisol, Schwarz/Mann, Orangeburg, N. Y.) were used as the steroid sources. VCR ( Oncovin, Eli Lilly and Co., Indianapolis, Ind.) was used for the mitotic inhibitor. Thymidine-methyl-3H (specific activity, 2 Ci/m mole), uridine-5-3H (specific activity, 24.6 Ci/m mole), and leucine-4,5-3H (specific activity, 5 Ci/m mole) were obtained from New England Nuclear, Boston, Mass.

Measurement of Mitotic Inhibition. VCR was used as a mitotic blocking agent to provide the means of distinguishing between those events that occurred before and after metaphase. Stock cultures of tumor cells were trypsinized and approximately 10⁴ tumor cells were suspended in 1 ml Eagle's basal medium plus 10% fetal calf serum. An 0.02-ml (200 cells) amount of this cell suspension was inoculated in each well of the microtest plate as described previously (3). After overnight incubation at 37° in 5% CO₂, the cells were exposed to a medium containing different concentrations: MPS, 50 to 400 μg/ml; VCR, 0.001 μg/ml; or both agents in combination. Epicortisol was dissolved in dimethyl sulfoxide prior to experiments. The final concentration of dimethyl sulfoxide in the medium was 0.25%. After different periods of exposure to the drug, control and treated cells were fixed with methanol and stained with Giemsa. The percentage of mitotic cells was determined by counting at least 2000 cells in 12 well cultures.

Measurement of Radioactive Incorporation. The incorporation of thymidine-3H, uridine-3H, or leucine-3H into acid-insoluble materials was used as a measure of DNA, RNA, or protein synthesis. For these experiments, trypsin-dissociated cell suspension was adjusted to 5 × 10⁴ cells/ml, and 2 ml of the cell suspension were plated in Falcon plastic dishes (35 mm). After overnight incubation, cells were exposed to the drug for different periods. Control and treated cells were pulse labeled with thymidine-3H, uridine-
3H, or leucine-3H, for 1 hr. The radioactive materials were removed from the dishes, and they were washed 3 times with Puck's saline A, 3 times with cold 10% trichloroacetic acid, and twice with ethanol:ether (3:1, v/v). The cells in the dishes were digested with 0.2 N NaOH overnight. An aliquot was placed in each scintillation vial filled with 10 ml of Insta-gel emulsifier for the aqueous sample (Packard Instrument Co., Downer's Grove, Ill.). Radioactivity was then measured in a Packard Tri-Carb Model 3375 scintillation spectrophotometer. All experiments were done in duplicate.

RESULTS

Mitotic Inhibition by MPS. MPS in concentrations of 200 and 400 µg/ml decreased the number of mitotic cells to near 0 levels within 8 hr of exposure but had only mild effects in concentrations of 50 µg/ml (Chart 1). Mitotic figures steadily increased after treatment with VCR (0.001 µg/ml), affecting 70% of the cell population after a 24-hr incubation. However, combined therapy with these 2 agents decreased the percentage of cells in mitosis in proportion to the increase in the dose of the steroid. At 400 µg/ml, MPS completely prevented mitotic activity in the cultures of glial tumor cells. In separate experiments, epicortisol at 400 µg/ml did not cause any mitotic changes at 24 hr or growth inhibition after 6 days of incubation with the same glial tumor cells.

Effect on Uptake of Protein and Nucleic Acid Precursors. MPS suppressed the uptake of thymidine-3H over 60% after a 2-hr exposure at 400 µg/ml, indicating its marked interference with DNA synthesis (Chart 2). Longer treatment with MPS, to 6 hr, produced only a slightly greater inhibition. RNA synthesis evaluated from uridine-3H incorporation was suppressed 25 to 40% after 2- to 6-hr exposure, respectively, to MPS. This corticosteroid had little effect on leucine-3H estimation of intracellular protein synthesis, decreasing some 20% from the control only after 4 to 6 hr of incubation. The suppression of DNA and RNA synthesis by MPS was then similarly evaluated with radioactive thymidine and uridine with respect to dose, after a constant 6-hr exposure to the corticosteroid (Chart 3). Thymidine-3H incorporation decreased linearly in dose-related fashion as the MPS concentrations increased from 50 to 400 µg/ml. Radioactive uridine incorporation decreased with higher doses of MPS to 200 µg/ml and leveled off to 400 µg/ml.

DISCUSSION

The demonstration of suppression and arrest of human glial tumor cell division by MPS in vitro further elaborate the nature of the inhibitory effect of this corticosteroid on the growth of neural tumor under experimental conditions. A similar suppressing effect of corticosteroid and adrenal hormones on mitotic activity has been previously described in cells of various other types (1, 2, 7, 16). The action of corticosteroids on mammalian cells depends upon a number of variables including cell type, concentration of drug, and
doses clearly indicate that the action of MPS on glial tumor-specific cytotoxic effects that are unrelated to corticosteroid concentrations, however, corticosteroids may cause non-specific conditions of growth. Not all corticosteroids have a specific effect on cell division. The degree of mitotic inhibition was closely related to the concentration of drug. At 400 \( \mu \text{g/ml} \), the drug had no effect on the cultures. The degree of mitotic inhibition was closely related to the concentration of drug. At 400 \( \mu \text{g/ml} \), the drug had no effect on the cultures.

The effect of MPS was prompt since mitotic inhibition was observed within 24 hr after addition of corticosteroid to the cultures. The degree of mitotic inhibition was closely related to the concentration of drug. At 400 \( \mu \text{g/ml} \), inhibition was noted as early as 8 hr after incubation with the drug. These findings confirm our previous observations that the growth inhibition occurring after 1 to 3 days was dose dependent (12). MPS antagonized the mitotic arrest caused by VCR, which suggests that this corticosteroid may prevent those cells in cycle from reaching the mitotic phase. Thus, MPS may have a specific inhibitory effect on certain phases of the cell cycle, not unlike other inhibitors of DNA synthesis such as methotrexate, 5-fluorouracil, cytosine arabinoside, etc.

The radioisotope experiments provide evidence for possible causal mechanisms of the mitotic inhibition observed. MPS inhibition of DNA synthesis appeared more profound than its effect on RNA and protein synthesis, and the degree of inhibition was closely related to the concentration of drug. These biochemical findings are in agreement with other studies on fibroblast cultures (14), Ehrlich ascites cells (10), and liver cells in vivo (9, 15) demonstrating that DNA synthesis was inhibited preferentially by hydrocortisone. Although a specific locus for the inhibition of DNA synthesis by MPS has not been defined, MPS may involve an interference with DNA synthesis at S phase or at a point earlier than S phase with subsequent impairment of chromosome replication and arrest of cell division.

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REFERENCES


Chart 3. The effect of concentrations of MPS on DNA and RNA synthesis. The human glioma cells were exposed to the different concentrations of MPS at 37° for 6 hr. The cells were then labeled with thymidine-3H (1 \( \mu \text{Ci/ml} \)) or uridine-3H (0.5 \( \mu \text{Ci/ml} \)) for 1 hr.
Effect of Corticosteroid on Protein and Nucleic Acid Synthesis in Human Glial Tumor Cells

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