Eur J Cancer Clin Oncol, Vol. 20, No. 3, pp. 427–433, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Receptor-mediated Antiproliferative Effects of Corticosteroids in Lewis Lung Tumors*

PAUL G. BRAUNSCHWEIGER,†‡ HAN L. TING§ and LEWIS M. SCHIFFER†

†*Department of Experimental Therapeutics, AMC Cancer Research Center, 6401 West Colfax Avenue, Lakewood, CO* 80214, *U.S.A. and* §*Cancer Research Laboratories, Allegheny-Singer Research Corporation, Allegheny General Hospital, 320 East North Avenue, Pittsburgh, PA* 15212, *U.S.A.*

Abstract—*Dextran-coated charcoal competitive binding assays and Scatchard analysis revealed the presence of high-affinity, low capacity binding sites for dexamethasone in cytosol preparations from Lewis lung tumors.* In vitro *studies with live cells indicated approximately* 9000 *nuclear binding sites/cell for the ligand–receptor complex.* In vivo *inhibition of cell proliferation by dexamethasone, methylprednisolone and triamcinolone acetonide was found to be dose-dependent. Changes in the* [^{3}H]-*TdR labeling index, mitotic index and saturable cytosol receptor sites after dexamethasone treatment* in vivo *suggested a dose-dependent* G_1 *progression delay which, after cessation of dexamethasone treatments, was apparently reversible. Resumption of cell-cycle progression was characterized by synchronous progression through S-phase and correlated temporally with receptor site desaturation.* In vivo *studies indicated that the effectiveness of vincristine given after dexamethasone was highly sequence-dependent, with the most effective sequence interval being coincident with the interval of maximal S-phase cellularity. Other studies indicated sequential chemotherapy with dexamethasone, vincristine and 5-Fu could be effectively employed, following primary tumor excision, to increase animal survival.*

INTRODUCTION

PREVIOUS studies have indicated that corticosteroid hormones can exert an antiproliferative effect in receptor-positive solid tumor models by transiently blocking cell cycle progression in the G_1 phase of the cell cycle [1–4]. In RIF-1 tumors the duration of this antiproliferative effect, at the level of the clonogenic cell population, was directly related to the corticosteroid hormone receptor content prior to treatment [3]. It was also shown that the effectiveness of sequential strategies with corticosteroid hormones and chemotherapy agents were administered at intervals coincident with increased S-phase cellularity after dexamethasone treatments. Inasmuch as corticosteroid hormones have been shownto inhibit cell proliferation in fetal lung [5–7] and in a human lung tumor cell line, A 549 [8], we initiated these studies to quantitate the level of corticosteroid hormone receptors in Lewis lung tumors and to evaluate corticosteroid dose-dependent changes in tumor cell proliferation. Other studies were conducted to assess the efficacy of dexamethasone and sequential conbination chemotherapy for treatment of metastatic Lewis lung tumor.

Accepted 8 September 1983.
*Supported by Grant Ca26030 awarded by the NCI, DHEW, and grants to the AMC Cancer Research Center from the McDonnell Douglas Foundation and Steven W. Farber.
‡To whom reprint requests should be addressed.

MATERIALS AND METHODS

Animal and tumor models

Lewis lung tumor, obtained from Dr A. Bogden (Mason Research Inst., Worchester, MA), was maintained by *in vivo* passage in male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME). Stock tumors were passaged every 2 weeks by subcutaneous implantation of Lewis lung tumor fragments. Mice in experimental groups were inoculated with $5 \times 10^5 - 1 \times 10^6$ tumor cells (in 0.2 ml phosphate-buffered saline) prepared by enzymatic disaggregation of 14-day tumors with an enzyme cocktail containing 0.5 mg/ml trypsin, 0.5 mg/ml type II collagenase and 0.05 mg/ml DNase I (Sigma Chemical Company, St. Louis, MO) in phosphate-buffered saline (PBS) at room

temperature for 30 min. The resultant cell suspension was filtered through 10-ply serial gauze, washed 3 times with PBS, diluted and inoculated either subcutaneously on the flank or intramuscularly on the hind leg.

Cell kinetic assays

Cell kinetic assays were done *in vitro* as previously described [2, 9, 10]. Tritiated thymidine labeling indices ([^{3}H]-TdR LI) were determined *in vitro* on mechanically dissociated tumor tissue. Tissue fragments were minced with scissors in EMEM medium with 10% bovine serum (GIBCO, Grand Island, NY). The resultant suspension was filtered through gauze to remove large clumps and diluted to approximately 5×10^7 cells/ml. Aliquots (3 ml) were incubated at 37°C for 1 hr with 2 μCi/ml [^{3}H]-dT (20–40 Ci/mM, NEN, Boston, MA). Labeling was stopped on ice and 95% trypan-negative cell suspensions obtained by Ficoll-Hypaque gradient centrifugation. Cells were then applied to microscope slides with a cytocentrifuge (Shandon Southern, Sewickley, PA), air-dried, fixed in methanol and prepared for autoradiography. Cell suspensions prepared and labelled as above were subjected to an additional 30-min labeling interval at 37°C with [^{14}C]-dT (0.125 Ci/ml, 50 Ci/mM, NEN, Boston, MA). Slide preparations were made as above and prepared for double emulsion autoradiography [9]. For mitotic index (MI) and primer-dependent DNA polymerase labeling index (PDPI) determinations, aliquots of the minced tumor cell suspensions were applied to microscope slides with the cytocentrifuge and rapidly air-dried. Some slides were stained immediately with Wright–Giemsa stain for MI determinations, while others were used to estimate tumor growth fraction by the PDP assay [11]. This autoradiographic assay measures the simultaneous presence of DNA polymerase-α [12] and available primer template activity on individual cell nuclei by incorporation of [^{3}H]-TTP (20–40 Ci/mM, NEN, Boston, MA). The fraction of labeled nuclei in autoradiograms is defined as the PDP index.

Autoradiography was done using Kodak (Rochester, NY) NTB-2 liquid photographic emulsion. The emulsions (54°C) were applied by dipping, air-dried and the autoradiograms stored for up to 7 days in light, tight boxes at 4°C. At appropriate exposure times (determined by test sets) autoradiograms were developed with Kodak D-19 developer, washed, fixed, air-dried and stained with Harris hematoxylin. Tritiated dT LIs and PDPIs were determined by counting at least 500 cells. DNA synthesis times (Ts) were determined by assessing labeling patterns of 500 labeled cells in double emulsion autoradiograms [9]. Local background was usually less than one grain per equivalent cell area and cells with three or more grains/nucleus were considered labeled. LIs, MIs and PDPIs were expressed as a percentage of the total cell population.

Corticosteroid receptor assay

Corticosteroid receptor content of Lewis lung tumors was determined by the dextran-coated charcoal competitive binding assay as previously described [2, 3]. Tumors were resected, trimmed of connective tissue, weighed, immediately minced in 4–5 vols of ice-cold TEDG (10 mM Tris; 1.5 mM EDTA: 0.5 mM dithiothreitol, 10% glycerol, pH 7.4, at 4°C) buffer and homogenized with a polytron PT 10 ST homogenizer (Brinkman Instruments, Inc., Westbury, NY), with 5 10-sec bursts and 30-sec cooling periods. After centrifugation the homogenate was centrifuged (105,000 *g*) for 1 hr at 4°C in a Beckman L8-55 ultracentrifuge. Supernatants were diluted as needed to yield 4–6 mg protein/ml of cytosol, determined by the method of Lowry *et al.* [13]. Aliquots (75 μl) of the 105,000-*g* cytosol were incubated with various concentrations of [^{3}H]-dexamethasone (Dex; 40–50 Ci/mM, NEN, Boston, MA) with and without 200-fold molar excess of unlabeled ligand (Sigma Chemical Co.). All incubations were carried out in triplicate in V-well microtiter plates at 4°C for 4 hr. After incubation a 5-μl aliquot was withdrawn from each well to determine total radioactivity. Twenty-five microliters of the dextran-coated charcoal solution was then added to each well, mixed and incubated on ice for an additional 15 min. The plates were then centrifuged at 850 *g* and 50-μl aliquots counted in a liquid scintillation spectrometer (Packard Tricarb, Packard Inst., Chicago, IL) with internal quench correction and total activity analyzer. The difference in counts between samples incubated with and without 200-fold molar excess of unlabeled Dex was taken to be the specifically bound activity. Linear least-squares regression analysis of the binding data was used to estimate total saturable binding sites and apparent dissociation constants (K_d) by the method of Scatchard [14].

To assess nuclear corticosteroid binding, whole-cell assays were performed [3] with single-cell suspensions prepared from solid tumors by enzymatic dissociation as described above. Viable (trypan exclusion) cells (2.5×10^6) were incubated with [^{3}H]-dexamethasone with and without 200-fold molar excess of unlabeled Dex in PBS for 1 hr at room temperature. The reaction was stopped on ice, the cells lysed in ice-cold TEDG buffer with polytron homogenization and the nuclear pellet collected by centrifugation (850 *g*). The

nuclear pellet was washed 3 times in TEDG and finally resuspended in 0.5 ml of tissue solubilizer (Soluene, NEN, Boston, MA). The nuclei were solubilized overnight and aliquots prepared for liquid scintillation counting. Specific binding was taken to be the difference in counts with and without 200-fold molar excess of unlabeled dexamethasone. The binding data were analyzed by the method of Scatchard [14] and the results were expressed as the number of dexamethasone binding sites per nucleus.

Therapeutics and therapeutic efficacy

Vincristine (Oncovin, Eli Lily, Indianapolis, IN), dexamethasone sodium phosphate (Hexadrol, Organon, West Orange, NJ), methylprednisolone (Solu-Medrol, UpJohn, Kalamazoo, MI), triamcinolone acetonide (Cenocort A-40, Central Pharmacol. Co., Seymour, IN) and 5-fluorouracil (5-fluorouracil, Roche Laboratories, Nutley, NJ) were used in these studies. All drugs were freshly prepared prior to use and administered by intraperitoneal injection. Three-dimensional caliper measurements were made at regular intervals before and after treatment. Tumor volumes were estimated from $V = l \times h \times (w/2)$ and regrowth delays were calculated by comparing the time, in days, for treated and untreated tumors to reach 4 times pretreatment size. Regrowth delays calculated for individual tumors were expressed as a multiple of the volumetric doubling time to correct for variations in tumor growth rates within and between treatment groups [2, 3]. Student's t test was used to compare group means and a P value of less than 0.05 was considered adequate justification to reject the null hypothesis.

In adjuvant chemotherapy studies, legs bearing primary Lewis lung tumors were amputated under ether anesthesia. After ligation of the femoral artery, care was taken to disarticulate the proximal end of the femur from the acetabulum so as to remove the tumor-bearing leg *in toto*. Regional nodes were also removed in an attempt to minimize the chances of local recurrence. Most surgical deaths were anesthesia-related. Local recurrence was usually less than 20% and animals that developed recurrence at the amputation site were excluded from the study. Animal survival with and without adjuvant treatments were compared and increased median survival was expressed in days.

RESULTS

Baseline cell kinetic parameters assessed in 14-day (approximately 1 cm^3) subcutaneous Lewis lung tumors ($n = 8$) were [^{3}H]-dT LI, 0.374 ± 0.021; MI, 0.0156 ± 0.0014; PDPI, 0.633 ± 0.014; and Ts, 9.5 ± 0.4 hr. Using the PDP index as an estimate of the tumor growth fraction, cell cycle times were estimated [10] to be approximately 16.2 ± 1.6 hr. Tumor volume doubling time for 1 cm^3 tumors was 1.8 days.

Using the dextran-coated charcoal competitive binding assay and Scatchard analysis, high-affinity, low-capacity binding sites for dexamethasone were detected in Lewis lung tumor cytosols. Receptor values ranged from 40 to 289 fmol/mg, with a mean of 152 ± 45 (S.E.) for a total of 35 assays. Low receptor levels (<90 fmol/mg) were most frequently associated with large necrotic tumors. Apparent K_ds, 7.69 ± 2.1 nM ($n = 35$), were not inconsistent with those seen in other corticosteroid responsive tissues. Results from studies using a whole-cell assay at ambient temperature to assess nuclear translocation and chromatin binding of the ligand–receptor complex suggested approximately 9000 binding sites per nucleus.

Figure 1 shows the effect of corticosteroid treatments on the cell proliferation of Lewis lung tumor cells. [^{3}H]-TdR LIs were determined 2 hr after the last 3 treatments given every 12 hr. Maximal proliferation inhibition with dexamethasone was noted at the 10-mg/kg dose level, with little or no added effect at 20 or 30 mg/kg. There was little or no change noted in the PDP index up to 20 mg/kg Dex. A similar level of proliferation inhibition was observed with 40 mg/kg methylprednisolone. On a mg/kg basis, triamcinolone acetonide was most effective,

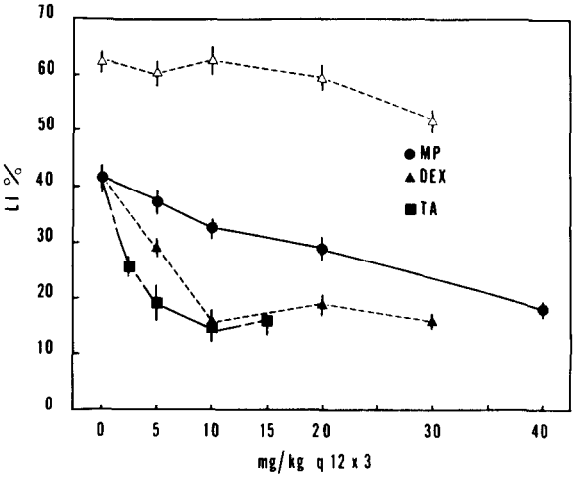

Fig. 1. The dose-dependent reduction in [^{3}H]-*dT LI* (▲,●,■) *and PDPI* (△) *labeling index (LI) by dexamethasone (Dex), methylprednisolone (MP) and triamcinolone acetonide (TA) in Lewis lung tumors. Mice were treated with Dex, MP or TA every* 12 hr *for* 3 *doses.* [^{3}H]-*dT LIs were determined* 2 hr *after the last corticosteroid treatment. Changes in PDPI were studied only after Dex treatments. Each symbol is the mean* ± *S.E.M. for* 4 *tumors.*

with maximal inhibition seen at the 5-mg/kg dose level.

Changes in the [^{3}H]-TdR LI, mitotic index and saturable receptor sites (fmol/MG cytosol protein) after 10 mg/kg Dex given every 12 hr for 3 doses are shown in Fig. 2. The receptor content prior to Dex treatment was 133 fmol/mg cytosol protein. Maximal reduction of S-phase cells was seen by 6 hr, with recovery between 12 and 18 hr. At 18 hr [^{3}H]-TdR LIs were approximately 65% and similar to the pretreatment growth fraction as estimated by PDP assay. Recovery of the mitotic index lagged behind that of [^{3}H]-TdR LIs, with maximal MIs seen at 36 hr. This response pattern would suggest that Dex blocked cell cycle progression in G_1, with progression through S-phase 12–18 hr after cessation of Dex treatments. That the apparent synchronous progression was not obvious from the MI data could be a reflection of either inadequate sampling or of variability in G_2 transit times. Also shown in Fig. 2 are the changes in saturable receptor sites after dexamethasone treatments. By 6 hr after the last dexamethasone treatment few free unsaturated binding sites were detected. Recovery to normal receptor site saturation was initiated by 12 hr after treatment and was essentially complete by 24 hr after the last dexamethasone treatment. The recovery time course for receptor site desaturation correlated temporarily with the resumption of cell cycle progression after dexamethasone treatments.

Figure 3 shows the time-dependent changes in the [^{3}H]-TdR LI for primary Lewis lung tumors after 15 mg/kg dexamethasone given every 6 hr for 4 doses. Although with this dose and schedule the antiproliferative effect lasted approximately 12 hr longer than in the 10 mg/kg study, peak [^{3}H]-TdR LIs in both studies were similar. This data would, however, suggest that in the high-dose study either the rate of entry into S-phase of progressing cells was decreased and/or the S-phase transit time prolonged with respect to that in the low-dose study.

Figure 4 shows the results from two studies to assess the relationship between the dexamethasone-induced changes in the tumor cell age distribution and chemosensitivity. Tumor-bearing mice were treated with 10 mg/kg dexamethasone every 12 hr for 3 doses, and then at various intervals thereafter with either 1 mg/kg VCR or 84 mg/kg 5-FU and the antitumor effect assessed by regrowth delay. As suggested by the kinetic data, the sensitivity to vincristine was found to be highly sequence-dependent. The best sequence interval for VCR

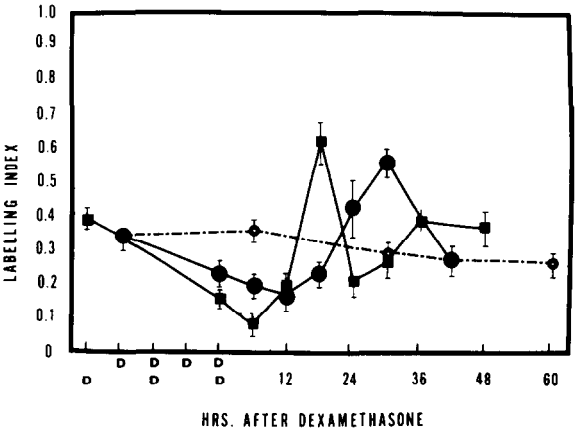

Fig. 3. The comparison between the antiproliferative effect of 15 mg/kg *dexamethasone given every* 6 hr *for* 4 *doses* (●) *and* 10 mg/fg *given every* 12 hr *for* 3 *doses* (■). ○, *Age-matched, untreated control tumors. Each symbol represents the mean* [^{3}H]-*dT labeling index* ± 1 *S.E.M. for* 4 *tumors.*

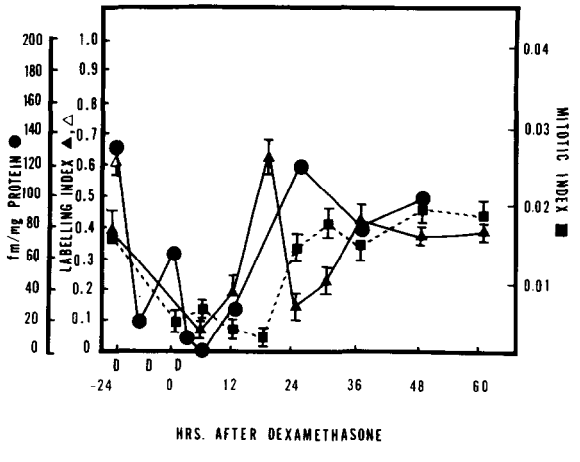

Fig. 2. Changes in the [^{3}H]-*dT LI* (▲), *MI* (■) *and saturable cytosol Dex receptors* (●) *after* 10 mg/kg *dexamethasone given every* 12 hr *for* 3 *doses. Each LI and MI point represents the mean* + 1 *S.E.M. for* 4 *tumors, while the Dex receptor data* (fmol/mg *cytosol protein) was determined from tissue pooled from at least* 2 *tumors.* △, *Pretreatment PDPI.*

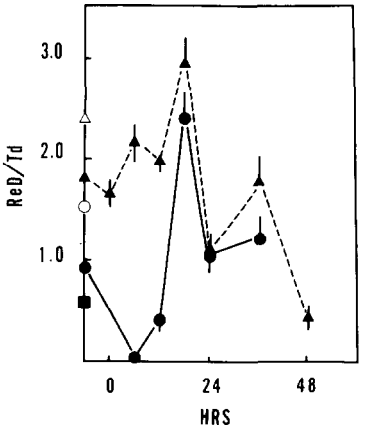

Fig. 4. Regrowth delay (ReD) as a multiple of the volume doubling time (Td) after 10 mg/kg *dexamethasone given every* 12 hr *for* 3 *doses and either* 1 mg/kg *vincristine* (●) *or* 84 mg/kg 5-*FU* (▲). ■, *Dexamethasone alone;* ○, △, *the summed effects of dexamethasone plus vincristine and* 5-*FU respectively.*

(18 hr) was coincident with the time of maximal S-phase cellularity (Fig. 2). The regrowth delay, approximately 2.4 doubling times, was about 60% longer than the summation of vincristine and dexamethasone alone treatments (1.5 Tds) and was consistent with the nearly 70% increase in [^{3}H]-TdR labeling index at 18 hr.

The antitumor effect of time-sequenced 5-FU was less sequence-dependent than for VCR; however, the best regrowth delays noted with the 18-hr sequence interval were approximately 70% longer than 5-FU alone and about 25% longer than the additive effect of Dex and 5-FU. Excepting the 6-hr sequence interval, regrowth delays observed using other sequence intervals were shorter than the predicted additive effect.

Figures 5 and 6 show the results from 2 studies to assess the effect of dexamethasone on the efficacy of adjuvant chemotherapy with VCR and 6-FU for metastatic Lewis lung tumors. In the first study (Fig. 5) tumor-bearing legs were amputated 12 days after intramuscular inoculation of 1×10^6 tumor cells. Corticosteroid receptor levels determined on 3 representative tumors from this transplant group were approximately 145 fmol/mg cytosol protein. At various intervals after the surgical procedure, mice were treated with a combination of VCR (1 mg/kg) + 5-FU (84 mg/kg) with and without dexamethasone (10 mg/kg given every 12 hr between 24 and 48 hr after surgery). Median survival times (8–10 mice per group) were determined for each sequence interval and the efficacy of the adjuvant

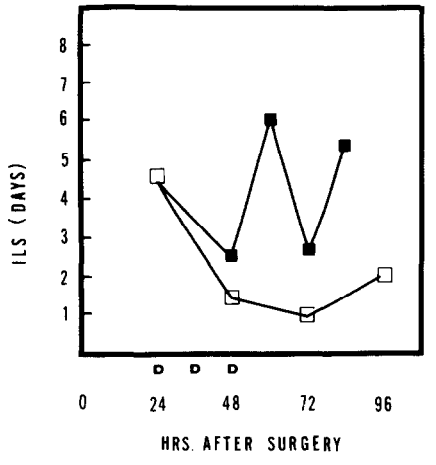

Fig. 5. The effect of adjuvant chemotherapy with VCR (1 mg/kg) and 5-FU (84 mg/kg) with (■) and without (□) dexamethasone (10 mg/kg every 12 hr for 3 doses) on animal survival time after surgical resection of 12-day, intramuscular Lewis lung tumors. In these studies Dex was given at 24, 36 and 48 hr after amputation. Increased life span (ILS) was taken to be the difference between the median survival for animals receiving adjuvant treatments and those receiving surgery alone. Each treatment group consisted of 8–10 tumor-bearing mice.

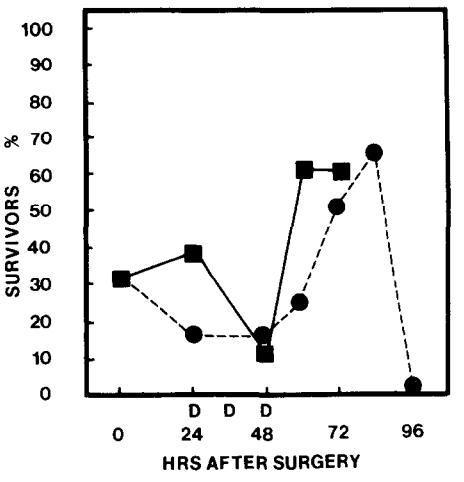

Fig. 6. The effect of adjuvant chemotherapy with the VCR, 5-FU combination, with (●) and without (■) dexamethasone, on animal survival after surgical resection of 8-day intramuscular Lewis lung tumors. Percentage survivors represents the proportion of treated animals surviving for at least 100 days after treatment. Each treatment group consisted of 8–10 tumor-bearing mice.

treatments determined by subtracting the median survival of the surgery alone group (22.4 days) from that seen after surgery and adjuvant chemotherapy. The results indicate that the VCR + 5-FU combination was most effective 24 hr after surgery and that the effectiveness of this combination decreased as adjuvant therapy was delayed. When dexamethasone was given after surgery the most effective adjuvant therapy intervals were 60 and 84 hr after surgery, i.e. 12 and 36 hr after dexamethasone treatment.

In the second experiment, tumors amputated 8 days after i.m. inoculation of 5×10^6 tumor cells showed corticosteroid receptor levels of approximately 280 fmol/mg of cytosol protein. Surgery alone resulted in approximately 35% long-term (no gross lung tumor 100 days after surgery) survivors. The VCR, 5-FU combination was most effective (60–65% long-term survivors) when sequenced 60–72 hr after surgery. When dexamethasone treatments were initiated 24 hr after surgery (10 mg/kg every 12 hr for 3 doses) the best sequence interval for the VCR, 5-FU combination was seen 72–84 hr after surgery, i.e. 24–36 hr after the last dexamethasone treatment.

DISCUSSION

Previous studies with fetal lung tissue have shown that corticosteroid hormones can accelerate the maturation of type II alveolar cells [15, 16], inhibit their proliferation [5–7] and stimulate surfactant production [17]. Corticosteroid hormone receptor levels in fetal lung tissue decrease during the neonatal period [18] and type II alveolar cells have been shown to possess

specific corticosteroid receptors [19]. It has been suggested that corticosteroids initiate the withdrawal of cells from active proliferation into a G_0 compartment for differentiation [20]. Tumor cell line A-549, derived from a human type II cell carcinoma, was shown to possess specific corticosteroid hormone receptors and to exhibit non-toxic dose-dependent proliferation inhibition after corticosteroid treatments *in vitro* [8].

The present studies in Lewis lung tumors indicate the presence of both cytoplasmic and nuclear high-affinity, low-capacity binding sites for dexamethasone. In comparison to previously studied fibrosarcomas [3] and mammary tumor [2] models, this tumor model exhibited a somewhat lower level of dexamethasone binding sites and provided an opportunity to compare response times with those seen in other higher-receptor-content tumors. Further, since the Lewis tumor model metastases to the lung, the model also provided the opportunity to evaluate the potential application of dexamethasone in an adjuvant setting.

The antiproliferative effects of methylprednisolone, dexamethasone and triamcinolone acetonide were shown to dose-dependent and probably mediated by a dose-dependent G_1 block in cell cycle progression. Inasmuch as changes in both receptor site saturation and cell cycle progression after dexamethasone in Lewis tumors had similar time courses, the antiproliferative effect was probably receptor-mediated. These results are, in general, not unlike previous findings in other receptor-positive solid tumor models [2, 3, 4, 21]. In the present studies maximal S-phase accumulation after 10 mg/kg Dex treatments was noted at about 18 hr, while in the higher-receptor-content C3H/HeJ mammary tumor (~200 fmol/mg) similar Dex treatments produced a 48-hr progression delay [2]. Further, the duration of the progression delay in the clonogenic cell function of RIF-1 tumors was also directly related to the corticosteroid receptor content prior to treatment.

The efficacy of sequentially administered VCR after dexamethasone was highly sequence-dependent and not inconsistent with synchronous cell cycle progression after cessation of dexamethasone treatments. In lymphoid tumor models greater than additive cytotoxicity with combinations of corticosteroids and VCR [22] was probably not kinetically based since the dexamethasone-induced G_1 block may, in these systems, be a lethal event [23] and since VCR lethality may not be restricted to S and G_2 phase cells [24].

In corticosteroid receptor-positive mammary tumor models dexamethasone treatments after partial tumor resection (debulking) delayed the onset of proliferative recovery in the residual tumor mass until after cessation of corticosteroid treatments [25]. These changes in the timing of the proliferative recovery could be exploited with adjuvant chemotherapy. In Lewis lung tumors the proliferation of the metastatic tumor cells has been shown to increase promptly after resection of the primary tumor [26]. Our studies with adjuvant combinations of VCR and 5-FU would tend to confirm these findings. Further, that dexamethasone treatments after surgery delayed the time for the most effective use of the VCR, 5-FU combination would suggest that cells in the metastatic deposits were also sensitive to the antiproliferative effects of dexamethasone.

It is uncertain why the VCR, 5-FU combination in the 12-day metastases was most effective 12 hr after dexamethasone while in 8-day metastases the combination was most effective 24–36 hr after dexamethasone. It is conceivable, however, that the differences in response are related to differences in receptor content of the metastatic tumor deposits, since we [3] previously observed that the timing for maximal S-phase accumulation of clonogenic RIF cells after 5 mg/kg dexamethasone was directly related to the receptor content at the time of treatment. The dexamethasone receptor levels in the 12-day primary tumor (145 fmol/mg) were approximately 50% of that seen in the 8-day primaries (284 fmol/mg). Thus, if the receptor content in the metastatic tumor is reflected by that of its primary, the data might suggest that, as in the RIF tumor model, corticosteroid receptor levels as well as dexamethasone dose influence the duration of the G_1 progression delay. Studies are currently underway to construct dose- and receptor-dependent response models, which may be useful for predicting solid tumor responsiveness to dexamethasone. Such response models might conceivably be of clinical utility in the design of sequential therapeutic strategies in receptor-positive tumors.

Acknowledgements—The authors gratefully acknowledge the technical contributions of Jo Harnaha, Jay Hayes, Dolores Kutzer and Frances Vibostak. We also thank Cathy Allen for preparation of the manuscript.

REFERENCES

1. BRAUNSCHWEIGER PG, STRAGAND JJ, SCHIFFER LM. Effect of methyl prednisolone on cell proliferation in C3H/HeJ spontaneous mammary tumors. *Cancer Res* 1978, **38**, 4510–4514.

2. BRAUNSCHWEIGER PG, SCHIFFER LM. Antiproliferative effects of corticosteroids in C3H/HeJ mammary tumors and implication for sequential combination chemotherapy. *Cancer Res* 1981, **41**, 3324–3330.
3. BRAUNSCHWEIGER PG, TING HL, SCHIFFER LM. Receptor-dependent antiproliferative effects of corticosteroids in radiation-induced fibrosarcomas and implications for sequential therapy. *Cancer Res* 1982, **42**, 1686–1691.
4. BRAUNSCHWEIGER PG, TING HL, SCHIFFER LM. Corticosteroid hormone receptor levels in murine solid tumor models correlate with the kinetic response to dexamethasone *in vivo*. *Proc AACR* 1982, **23**, 208.
5. KIKKAWA Y, MOTOYAMA EK, COOK CD. The ultrastructure of the lungs of lambs. *Am J Pathol* 1965, **47**, 877–903.
6. CARSON SH, TAEUSCH HW, AVERY ME. Inhibition of lung cell division after hydrocortisone injection into fetal rabbits. *J Appl Physiol* 1973, **36**, 660–663.
7. KAUFFMAN SL. Proliferation, growth and differentiation of pulmonary epithelium in fetal mouse lung exposed transplacentally to dexamethasone. *Lab Invest* 1977, **37**, 497–501.
8. JONES KL, ANDERSON NS, ADDISON J. Glucocorticoid induced growth inhibition of cells from human lung alveolar cell carcinoma. *Cancer Res* 1978, **38**, 1688–1693.
9. BRAUNSCHWEIGER PG, POULAKOS L, SCHIFFER LM. *In vitro* labeling and gold activation autoradiography for determination of labeling index and DNA synthesis time of solid tumors. *Cancer Res* 1976, **36**, 1748–1753.
10. BRAUNSCHWEIGER PG, POULAKOS L, SCHIFFER LM. Cell kinetics *in vivo* and *in vitro* for C3H/He spontaneous mammary tumors. *JNCI* 1977, **59**, 1197–1204.
11. SCHIFFER LM, MARKOF AM, NELSON JSR. Estimation of tumor growth fraction in murine tumors by the primer-available DNA-dependent DNA polymerase assay. *Cancer Res* 1976, **36**, 2415–2418.
12. BRAUNSCHWEIGER PG, SCHIFFER LM. Nuclear DNA polymerase- and replicative potential in mammalian cells. *Eur J Cancer* 1977, **13**, 775–779.
13. LOWRY OH, ROSEBROUGH NK, FARR AL, RANDALL RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
14. SCATCHARD G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949, **51**, 660–672.
15. KIKKAWA Y, KARBARA M, MOTOYAMA EK, ORZALESI MM, COOK CD. Morphologic development of fetal lung and its acceleration with cortisol. *Am J Pathol* 1971, **64**, 423–442.
16. KAUFFMAN SL. Acceleration of canalicular development in lungs of fetal mice exposed transplacentally to dexamethasone. *Lab Invest* 1977, **36**, 395–401.
17. DELEMOS RA, SHERMETA DW, KNETSON JH, KORTAS R, AVERY ME. Acceleration of appearance of pulmonary surfactant in the fetal lamb by administration of corticosteroids. *Am Rev Resp Dis* 1970, **102**, 459–461.
18. GIANNOPOULOS G. Variations in the levels of cytoplasmic glucocorticoid receptors in lungs of various species, at different developmental stages. *Endocrinology* 1974, **94**, 450–453.
19. BALLARD PL, MASON RJ, DOUGLAS WHJ. Glucocorticoid binding by isolated lung cells. *Endocrinology* 1978, **102**, 1570–1574.
20. KAUFFMAN SL. Cell proliferation in the mammalian lung. *Int Rev Exp Pathol* 1980, **22**, 131–191.
21. BRAUNSCHWEIGER PG, STRAGAND JJ, SCHIFFER LM. Effect of methyl prednisolone on cell proliferation in C3H/HeJ spontaneous mammary tumors. *Cancer Res* 1978, **38**, 4510–4514.
22. ROSNER F, HIRSHANT Y, GRENWALD HW, DIETRICH M. *In vitro* combination chemotherapy demonstrating potentiation of vincristine cytotoxicity by prednisolone. *Cancer Res* 1975, **35**, 700–705.
23. HARMON JM, NORMAN MR, FOWLKES BJ, THOMPSON EB. Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. *J Cell Physiol* 1979, **98**, 267–277.
24. KRISHAN A, FREI E. Morphological basis for the cytologic effect of vinblastine and vincristine on cultured human leukemia lymphoblasts. *Cancer Res* 1975, **35**, 497–501.
25. BRAUNSCHWEIGER PG, SCHIFFER LM, BETANCOURT S. Tumor cell proliferation and sequential chemotherapy after partial tumor resection in C3H/HeJ mammary tumors. *Breast Cancer Res Treat* 1983, **2**, 323–329.
26. SIMPSON-HERRON L, SANDFORD AH, HOLMQUIST JP. Effects of surgery on the cell kinetics of residual tumor. *Cancer Treat Rep* 1976, **60**, 1749–1760.