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# ENHANCEMENT OF NITROSOUREA CYTOTOXICITY IN VITRO USING HYDROCORTISONE

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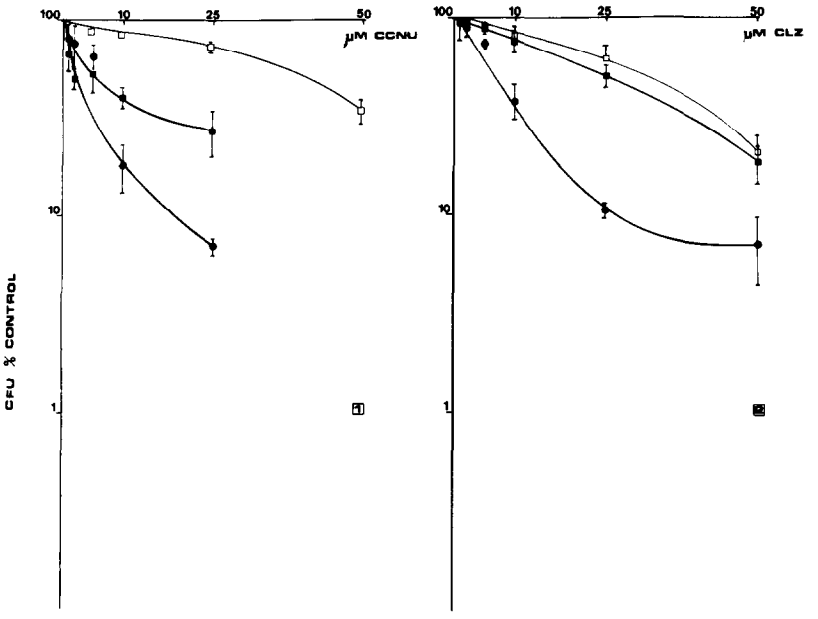
Both corticosteroids and alkylating agents are widely used in combination modality for the treatment of a number of neoplastic diseases (1). Usually, these combinations are designed in an empiric fashion, with little consideration of possible molecular mechanisms. Previous data have suggested that corticosteroids (2) and other transcriptional modifiers, such as sodium butyrate (3), have the potential to modify nuclear structural architecture. Concomitant with steroid-mediated stimulation of transcription was an increased alkylation of extended, presumed transcriptionally active, regions of chromatin. This increased drug binding in the specific chromatin subfraction did not result in an overall increase in total nuclear alkylation. This present communication has correlated the previous molecular events of steroid stimulation with a synergistic reduction in cell survival as measured by colony-forming ability.

## Methodology

HeLa S3 were maintained at 37° in Eagles minimum essential medium (Spinners) supplemented with 10% fetal calf serum (M.A. Bioproducts, Walkerville, Md.). Some log phase cultures ($4 \times 10^5$ cells/ml) received 10μM hydrocortisone-21-sodium succinate (HC) (Sigma, St. Louis, Mo.) prior to treatment with various concentrations of either 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (chlorozotocin; CLZ) or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). Soft agar colony forming assays were performed in quadruplicate in 15 x 60mm petri dishes; 0.3ml samples of control or drug treated cultures (corrected to $2 \times 10^5$ cells/ml) were diluted into 19.7ml of complete medium and Noble agar (Difco Scientific, Detroit, Mich.), final concentration 0.3%. After mixing, the plates were allowed to solidify at room temperature for 30 minutes and transferred to a humidified incubator at 37°C under 5% $CO_2$. Colonies of greater than 32 cells (five divisions or more) were scored as viable after 10 days. Control cultures pretreated with HC gave identical CFU values to those receiving no steroid. Plating efficiencies of approximately 30% were achieved.

## Results and Discussion

Figures 1 and 2 represent the colony forming potential of HeLa cells following treatment with various concentrations of CLZ or CCNU, with or without HC treatment. On a molar basis, CCNU was more cytotoxic than CLZ, with all cells dead at 50μM, irrespective of steroid pretreatment. The combination of HC pretreatment and CCNU decreased survival and colony forming potential from 40% to 18% of control at 10μM and 27% to 7% at 25μM. The combination of CLZ and HC pretreatment produced enhanced cytotoxicity over CLZ alone at all concentrations of drug, the greatest increase occurring at 25μM (51 vs 10% survival). Simultaneous administration of 10μM HC with either nitrosourea resulted in an enhanced cell survival with CCNU and did not affect the cytotoxicity of CLZ. Thus, the scheduling of the steroid was critical to cytotoxicity, with synergistic effects occurring only following HC priming. Our previous observations (2) have suggested that it is possible to direct both CLZ and CCNU alkylation and carbamoylation into transcriptionally active chromatin (sensitive to both DNase I and DNase II). This could be linked to a disaggregation




FIGURES 1 and 2

The effect of CCNU (1) or CLZ (2) ± HC on HeLa S3 cell survival. Points represent the mean of four colony forming assays (± S.D.) expressed as percent of control. Where administered, HC was at 10μM. Nitrosoureas were present for 2 hr. either following HC or with no HC pretreatment.

■ CCNU or CLZ no HC
● CCNU or CLZ with 22 hr., 10μM HC pretreatment
□ CCNU or CLZ with concomitant 10μM HC treatment

and overt relaxation of chromatin superstructure within the nucleus. This relaxation resulted in nuclear swelling, presumably mediated through nuclear matrix steroid receptors (4), which could function by altering the dynamic structural properties of the non-histone acidic protein scaffold of the nucleus (5). These HeLa cells possessed HC receptors at 8 fmol/μg DNA. Both transcriptional chromatin (2,3) and the nuclear matrix (5) are preferential targets for nitrosourea alkylation and carbamoylation. Therefore, present data on corticosteroid/nitrosourea synergism suggest that one or both of these nuclear targets may be important in determining the site-specific cytotoxic properties of nuclear reactant drugs such as nitrosoureas. In addition, the possibility of manipulating the nuclear structure with "transcriptional modifiers" such as corticosteroids provides a molecular rationale for their inclusion in combined modality drug regimens, although maximal efficacy of such combinations may depend upon their correct scheduling.

REFERENCES

1. R. W. Talley, Corticosteroids: In Cancer Medicine (eds. J. F. Holland and E. Frei) p. 1411. Philadelphia, Lea and Febiger (1973).
2. K. D. Tew, P. S. Schein, D. J. Lindner, A. L. Wang and M. E. Smulson, Cancer Res. 40, 3697 (1980).
3. K. D. Tew, S. Sudhakar, P. S. Schein and M. E. Smulson, Cancer Res. 38, 3371 (1978).
4. E. R. Barrack and D. S. Coffey, J. Biol Chem. 255, 7265 (1980).
5. K. D. Tew, Nitrosoureas Current Status and New Developments (eds. A. Prestayko, S. Crooke, L. Baker, S. Carter and P. Schein) p. 197 Academic Press, N.Y. (1981).