A Rationale for the Treatment of Metastatic Neuroblastoma[1, 2]

Lawrence Helson, *M.D.*,[3] **Christiane Helson**, *R.N.*,[3] **Robert F. Peterson**, *Ph.D.*,[3] *and* **Susanta K. Das**, *Ph.D.*[3]

ABSTRACT—In developing a chemotherapeutic program for children with disseminated neuroblastoma, we established three human neuroblastoma lines in cell culture to study the effects of dibutyryl cyclic AMP, papaverine, 5-trifluoromethyl-2′-deoxyuridine, and cyclophosphamide on cell growth, biochemical behavior, and morphology. Based upon our studies, a clinical treatment program was designed. We have treated 15 patients with disseminated neuroblastoma and have established the optimum dose range and sequence of these drugs. Early results were promising; plans for continuation of clinical and experimental studies were discussed.—J Natl Cancer Inst 57: 727-729, 1976.

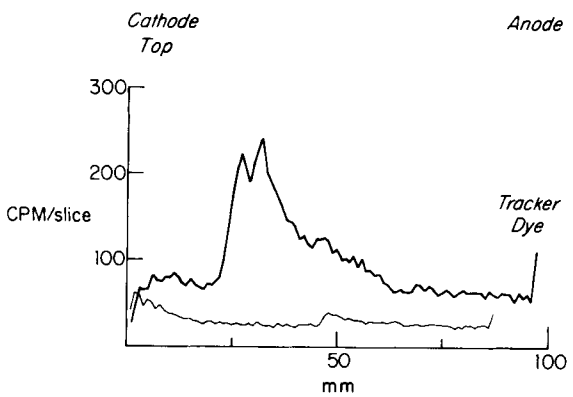
The limitations of surgery and radiotherapy are emphasized in the treatment of metastatic neuroblastoma. Reliance on systemic treatment with effective chemotherapeutic agents seems to be the optimal recourse at the present time. The variety of drugs and combinations that have become available over the last 15 years significantly increased the response rate, but the negligible cure rate remains essentially unchanged. During the period 1970–1974, a progression of observations in our laboratory and in other laboratories led to the development of a chemotherapeutic program for children with disseminated neuroblastoma. After the report of Heidrick and Ryan (*1*) that dibutyryl cyclic AMP affected the growth pattern of cells, Prasad and Hsie (*2*) found that the growth of murine neuroblastoma was similarly sensitive to dibutyryl cyclic AMP and, in addition, underwent morphologic differentiation. Prasad and Sheppard (*3*) observed that papaverine, a phosphodiesterase inhibitor, acted in a similar fashion. About this same time, we established three human neuroblastoma lines in cell culture (*4*) and studied the effect of cyclic AMP, papaverine, F3TDR, and other drugs on cell growth and morphology.

We determined that papaverine and F3TDR had inhibitory effects on growth and stimulated morphologic differentiation. The dose range in vitro was low enough to suggest that similar levels could be achieved in the sera of patients after the administration of safe amounts of these drugs. Phase 1 trials with F3TDR were already in progress; 2/6 children with metastatic neuroblastoma had a 1-A response (*5*). We instituted clinical trials with papaverine; 12-hour infusions were well tolerated and tumor response occurred but was of short duration (*6*). We concluded that papaverine and F3TDR could be used more effectively in combination with other drugs such as cyclophosphamide and vincristine. In in vitro studies, we observed that papaverine and F3TDR inhibited fucose incorporation into the neuroblastoma cell membrane (text-figs. 1, 2), thymidine and uridine transport into the tumor cells during log growth phase, and thymidine into PHA-stimulated lymphocytes (text-fig. 3).

MATERIALS AND METHODS

Obviously, papaverine should be administered separately and, when possible, after a sequence of other drugs. Accordingly, we designed a clinical treatment program called neuroblastoma-3, or N-3 (table 1), which consisted of a sequence of drug administrations incorporating these notions. A deviation from previous programs was the use of the maximum tolerated dose of cyclophosphamide, i.e., from 80 to 160 mg/kg in each course. Being aware of the side effects on normal tissues such as the heart, bone marrow, and the urinary bladder with such dosages, we tried to limit the incidence of cyclophosphamide cystitis by maintaining a urinary output of 4–6 ml/kg body weight/hour for the 8-hour period during and after cyclophosphamide infusion with the administration of a large fluid intake and fractionated doses of furosamide. The cyclophosphamide was infused at 10 a.m. as a practical measure. The 12-hour papaverine infusion is a safe administration, because its side effects (electrocardiographic T-wave flattening, tachycardia, flushing, or extrapyramidal syndrome) are of extremely brief durations, disappearing shortly after the infusion is stopped.

Four days of drug administration constituted a course. Three courses were administered, and the maximally tolerated dose of cyclophosphamide (160 mg/kg) was used at least once. With the other two courses, we reduced the cyclophosphamide dosage to 120 and 80 mg/kg. It should be emphasized that the large doses of drug used precluded the use of concomitant radiation therapy. After tumor response, the patient was placed



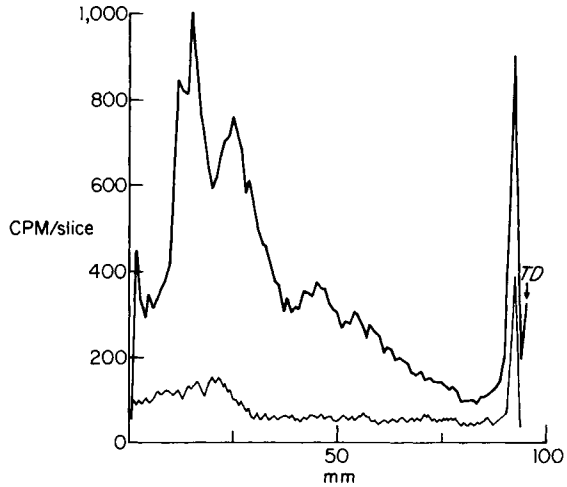
TEXT-FIGURE 1.—Ten persent SDS gels of human neuroblastoma membranes SK-N-BE, labeled with [^{3}H]fucose for 2.5 hr. *Heavy line* is the untreated control; *thin line* represents decreased labeling of cells treated with 0.04 mM of papaverine for 2.5 hr.

ABBREVIATIONS USED: F3TDR=5-trifluoromethyl-2′-deoxyuridine; PHA=phytohemagglutinin; PHA-P=protein phytohemagglutinin, polysaccharide moiety removed.

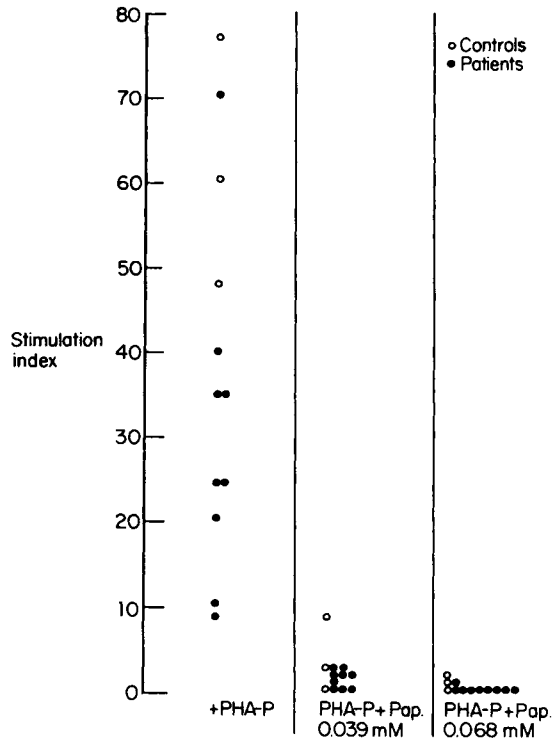
[1] Presented at the Symposium on Advances in Neuroblastoma Research, held at The Children's Hospital of Philadelphia, Philadelphia, Pa., May 30, 1975.

[2] Supported in part by Public Health Service grant CA08748 from the National Cancer Institute and by the Ann Marie O'Brien Neuroblastoma Research Fund.

[3] Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.



TEXT-FIGURE 2.—Ten percent SDS gels of human neuroblastoma membranes SK-N-SH, labeled with [^{3}H]fucose for 24 hr. *Heavy line* is the untreated control; *thin line* represents decreased labeling of cells treated with 0.01 mM of F3TDR and [^{3}H]fucose for 24 hr. TD=tracker dye.



TEXT-FIGURE 3.—Inhibition of thymidine uptake in stimulated lymphocytes by papaverine.

on a monthly maintenance schedule consisting of 2 treatment days: 60 mg cyclophosphamide/kg and 1.5 mg vincristine/M^2 on day 1 and maximally tolerated doses of F3TDR and papaverine on day 2.

RESULTS AND DISCUSSION

Fifteen patients with disseminated neuroblastoma were entered in this study. All but 2 were over the age of 2 years at diagnosis. Fourteen had significant clinical responses, i.e., distinctive subjective benefits and favorable objective changes in all measurable criteria for 1 month or more by the third course, and 10 had obvious subjective and objective tumor lysis within 2 weeks of the first course. Nine of 15 had varying degrees of resistance to cyclophosphamide, vincristine, adriamycin, and other drugs before being placed on the neuroblastoma-3 program. Eight patients had three courses and were freed of detectable disease; 3 others had one course, and all had 1-A responses.

There were 3 deaths, 2 of which could be ascribed to marrow-suppressive effects of the drugs. In a 14-month-old infant, a second episode of varicella developed during the leukopenic phase and persisted weeks after the bone marrow recovered; the cause of death was varicella pneumonia. On autopsy, residual neuroblastoma cells were detected in the bones, lymph nodes, and liver. The second child had a prolonged history of diarrhea and was maintained with fluid and electrolyte infusions while under treatment for some months. She experienced a cardiac arrest after a particularly explosive episode of diarrhea. She had a syndrome similar to that described by Verner and Morrison (7). On autopsy, the residual tumor found was composed of cells resembling early ganglioneuroma. The third patient, a 5-year-old child, died 1 month after his third course of treatment. He experienced an unusually prolonged marrow depression, hepatotoxicity, bacterial sepsis, and *Mucor* abscesses in his intestines. His unusual sensitivity to the cumulative effects of 3 courses may have been in part due to his concomitant condition of adrenal hyperplasia and to the long-term administration of replacement steroids.

Four other children in this series received 3 courses, including 160 mg cyclophosphamide/kg in each course, within 12 weeks without significant ensuing toxicity. This dosage probably constitutes the maximum tolerated dose, and the program was redesigned to diminish the possibility of such toxicity by decreasing the cyclophosphamide dosages subsequent to the course containing 160 mg/kg. We would emphasize that these children responded to therapy before the occurrence of toxicity.

An additional observation which indicates that this particular drug combination may be both cytotoxic and differentiative are four documented conversions of metastatic and primary neuroblastomas to ganglioneuroma: One was diagnosed at autopsy, and three were found by second-look biopsies after three courses of drug. This incidence of conversion to ganglioneuroma in such a small sampling either is fortuitous or suggests that the combination of drugs, possibly F3TDR and papaverine,

TABLE 1.—*Clinical treatment program*

Drug and schedule	Dosage, mg/kg
Cyclophosphamide 10 a.m. on days 1, 2 (3 optional)	40–80
Vincristine 10 p.m. on days 1, 2	0.03
F3TDR 9 a.m. on days 3, 4	20–45
Papaverine 10 a.m. to 10 p.m. (infusion) on days 3, 4	45

may be acting on tumor cells in vivo in the same manner as they do in vitro.

Our intentions are *a*) to pursue this chemotherapy program in a larger number of patients in order to establish the optimum dose range of all four drugs and *b*) to continue experimental studies with human neuroblastoma heterotransplanted to nude mice exposed to these same drugs (*8*).

REFERENCES

(*1*) HEIDRICK ML, RYAN WL: Cyclic nucleotides on cell growth in vitro. Cancer Res 30:376–378, 1970

(*2*) PRASAD KN, HSIE AW: Morphologic differentiation of mouse neuroblastoma cells induced in vitro by dibutyryl adenosine 3′:5′-cyclic monophosphate. Nature [New Biol] 233:141–142, 1971

(*3*) PRASAD KN, SHEPPARD JR: Inhibitors of cyclic nucleotide phosphodiesterase induce morphological differentiation of mouse neuroblastoma cell culture. Exp Cell Res 73:436–440, 1972

(*4*) BIEDLER JL, HELSON L, SPENGLER BA: Human neuroblastoma cells in continuous culture: Morphology and growth, tumorigenicity, and cytogenetics. Cancer Res 33:2643–2652, 1973

(*5*) HELSON L, Yagoda A, MURPHY ML, et al: Clinical trials with 5-trifluoromethyl-2′-deoxyuridine (F3TDR). *In* Advances in Antimicrobial and Antineoplastic Chemotherapy (Hejzlar M, Semonsky M, Masak S (eds.). Munich, Urban & Schwarzenberg, 1972, pp 617–619

(*6*) HELSON L, HELSON C, LAI KB, et al: Papaverine effects of growth and function of tumor cells and lymphocytes. Proc Am Soc Clin Oncol 15:172, 1974

(*7*) VERNER JV, MORRISON AB: Non-β islet tumors and the syndrome of watery diarrhea, hypokalemia and hypochlorhydria. Clin Gastroenterol 3:595–608, 1974

(*8*) HELSON L, DAS SD, HAJDU SI: Human neuroblastoma in "nude" mice. Cancer Res 35:2594–2599, 1975