Radiation Effects on Growth Are Altered in Rats by Prednisolone and Methotrexate

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ABSTRACT. CNS therapy for childhood leukemia has adverse effects upon growth and cognition. The cause of these deficits is unknown. In a rat model, we determined which agent, or combination of agents, in CNS therapy affected growth. Young Sprague-Dawley rats were exposed to cranial irradiation (1000 cGy), methotrexate (2 or 4 mg/kg, intraperitoneally), or prednisolone (18 or 36 mg/kg, intraperitoneally) alone or in two- or three-agent combinations. Matched control groups received appropriate sham radiation, intraperitoneal saline, or both. Body weight was recorded from 14 through 150 d of age. After the rats were killed at 150 d, body length was recorded and the head and left femur were removed to determine body and craniofacial proportions. Cranial irradiation alone, but not methotrexate or prednisolone alone, stunted growth permanently and altered craniofacial proportions. When these agents were combined, methotrexate and prednisolone modified the growth response to cranial irradiation. Methotrexate given before cranial irradiation prevented radiation stunting in males. This protection was lost when the dose of methotrexate was increased, when prednisolone was added to the combination, or when females were studied. The protection in males was effective against both growth and behavioral deficits. These results indicate that the physical and behavioral side effects of CNS therapy are better understood in the context of dose, sex, and interactions of the agents. (*Pediatr Res* 35: 416-423, 1994)

Abbreviations

ALL, acute lymphoblastic leukemia
i.p., intraperitoneal(ly)
P, treatment with prednisolone
GRF, growth hormone-releasing factor
M, treatment with methotrexate
X, treatment with cranial irradiation

CNS therapy for childhood ALL has well-documented adverse effects on growth and cognition. The growth deficits include short stature, increased weight for height, microcephaly, midfacial hypoplasia, and mandibular retrognathia (1-5). The cognitive deficits do not include mental retardation or hyperactivity, but significant reductions in overall IQ scores develop, along with impaired verbal and visual-spatial memory abilities, attention, organization, and motor output (6-8).

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The cause of these adverse effects has been reported to be cranial irradiation alone (9-11), or cranial irradiation interacting with chemotherapy (1, 4, 12). Cranial irradiation or methotrexate has been manipulated in CNS therapy in an attempt to prevent neurotoxic interactions (13-15). Yet, the impact of these manipulations is uncertain, considering that some cranial irradiation and methotrexate interactions may be protective rather than neurotoxic. Balsom *et al.* (16) found that preirradiation methotrexate protected against decreased intellectual function in ALL survivors, illustrating the importance of agent interactions and sequence of administration to long-term outcome.

We developed an animal model to study growth and behavioral deficits associated with exposures to cranial irradiation, methotrexate, and prednisolone, either as single agents or as combinations (17, 18). Cranial irradiation alone stunted growth without significantly altering behavior, whereas cranial irradiation combined with methotrexate and prednisolone disrupted behavior in addition to stunting growth. Further study of behavior (19) demonstrated how different agent interactions affected outcome: 1) behavioral "signatures" varied with the components of the agent combinations; 2) behavioral outcome was influenced by steroids as well as by cranial irradiation and methotrexate; 3) behavioral outcome was sex dependent; and 4) behavioral outcome varied with dose, which determined whether agents interacted antagonistically or synergistically. Therefore, not all agent

Table 1. Exposure groups

Exposure*	Abbreviation
Prednisolone—18 mg/kg	P ₁₈
Prednisolone—36 mg/kg	P ₃₆
Methotrexate—2 mg/kg	M ₂
Methotrexate—4 mg/kg	M ₄
Cranial irradiation—1000 cGy	X†
Prednisolone—18 mg/kg + methotrexate—2 mg/kg	P ₁₈ M ₂
Prednisolone—36 mg/kg + methotrexate—4 mg/kg	P ₃₆ M ₄
Methotrexate—2 mg/kg + cranial irradiation—1000 cGy	M ₂ X
Methotrexate—4 mg/kg + cranial irradiation—1000 cGy	M ₄ X
Prednisolone—18 mg/kg + cranial irradiation—1000 cGy	P ₁₈ X
Prednisolone—18 mg/kg + methotrexate—2 mg/kg + cranial irradiation—1000 cGy	P ₁₈ M ₂ X†

* Each exposure had a corresponding control group.

† Exposure and matched control groups from Schunior *et al.* (18).

Fig. 1. Skull measurements. See Table 2 and Schunior *et al.* (18) for definitions of dimensions.

interactions disrupted behavior; certain combinations actually minimized behavioral deficits.

A systematic study of CNS therapy combinations that minimize neurotoxicity (growth and behavioral deficits) has not been attempted. Effects on growth and behavior have been linked in brain lesion studies in rats, where the anatomical substrate for generalized learning impairment has been shown to overlap with that of a set point for body size (20). In a study of childhood survivors of ALL, it was suggested that only children with reduced head circumference developed neuropsychologic complications (21). If behavioral deficits and stunted head growth were directly related, cranial irradiation would alter behavior whenever it stunted head size. Our prior studies of cranial irradiation in rats indicated that such a direct relationship does not occur (17, 18).

The present study explores the growth-behavior relationship in response to various CNS therapy combinations. Using the same animal model as in our behavioral studies (19), we determined whether the combinations affecting behavior also affect growth and whether sex- or dose-dependency, antagonism, or synergism influence growth as they do behavior. If the two coincide, optimal combinations that minimize both the physical and neuropsychologic deficits of CNS therapy might be possible.

MATERIALS AND METHODS

Male and female 9-d-old (day of birth = 0) pathogen-free Sprague-Dawley rats were shipped with dams from the Charles

River Laboratories (Kingston, RI), then housed 10 pups per dam. Specified nonlittermates, each sex was assigned randomly to either an experimental group or its matched controls. The rats were maintained on certified Purina Rat Chow (No. 5002, Ralston-Purina, St. Louis, MO) and tap water *ad libitum*. Light cycles were set as 12-h light (0600 to 1800 h)/dark periods. All procedures involving animals were conducted under the auspices of Forsyth's Institutional Animal Care and Use Committee.

The experimental groups are listed in Table 1. The groups treated with prednisolone (prednisolone sodium succinate in saline; Sigma Chemical Co., St. Louis, MO) received i.p. injections on postnatal d 17 and 18. Those treated with methotrexate [(+) amethopterin in saline; Sigma] received i.p. injections on d 18 only. When experimental groups received more than one treatment on d 18, the sequence of administration was always prednisolone first, methotrexate second, and cranial irradiation last, 1–3 h after injections. The doses of all three agents were selected to be clinically relevant and to avoid lethality or obvious neurotoxicity (18). Controls received sham i.p. injections of an equal volume/body weight of saline on d 17, d 18, or both to match each experimental group.

The experimental groups receiving cranial irradiation were exposed on d 18 between 0900 and 1200 h using previously described procedures (18). Rats were immobilized with pentobarbital anesthesia (25 mg/kg, i.p.). They were exposed six to eight at a time to a lateral beam of x-rays produced by a Phillips

Table 2. Modified measures

Measurement	Definition
Femoral width	Medial-lateral diameter just distal to the lateral tuberosity
Cranial length	Midline distance from anterior tip of nasal bone to the most posterior point of the interparietal/supraoccipital suture
Parietal length	Midline distance from frontal/parietal suture to interparietal/supraoccipital suture; includes the length of the parietal and intraparietal bones
Supraoccipital height	Midline distance from interparietal/supraoccipital suture to most dorsal apex of foramen magnum
Basioccipital length	Midline distance from ventral aspect of the foramen magnum to the basioccipital/sphenoid suture
Sphenoid length	Midline distance from the basioccipital/sphenoid suture to the sphenoid/pre-sphenoid suture
Palatine length	Midline distance from the most posterior point on the palatine bone to the incisive foramen
Occipital width	Widest transverse distance across supraoccipital bone including lateral sutures above the foramen magnum
Condylar width	Transverse distance between midpoints of condylar fossae

Table 3. Overall growth effects of CNS therapy exposure groups*

Exposure	P ₁₈	P ₃₆	M ₂	M ₄	X	P ₁₈ M ₂	P ₃₆ M ₄	P ₁₈ X	M ₂ X	M ₄ X	P ₁₈ M ₂ X
Females	6	0	0	1	22	0	9	21	22	20	28
Males	0	0	3	0	28	0	8	16	7	24	27

*The number of significant changes ($p < 0.01$, t test or Mann-Whitney) among a total of 43 experimental-control comparisons. The 43 include body weight and length, femoral width and length, 18 craniofacial measures, and 21 body and cranial proportions.

x-ray machine operating at 250 kV peaks with 0.35-mm copper filtration. Each animal was shielded with 2-mm-thick lead placed over the body and face. The eyes, oropharyngeal mucosa, and salivary tissues were protected while the skull from behind the eyes to the midposterior neck was exposed. The calculated mid-brain dose rate was 125 cGy/min; each irradiated brain received a total of 1000 cGy $\pm$ 5% in a single fraction over 8 min. This dose approximated a biologic equivalent to the clinical dose of

2400 cGy given in 12–14 fractions (18). After pentobarbital anesthesia, control animals were sham-irradiated by similar placement in the radiation beam but with complete shielding by 6-mm-thick lead.

On d 21, all pups were weaned and housed by treatment two per cage per sex. Individual body weight was recorded in 13–24 rats per treatment per sex, three times weekly from 14 to 120 d of age and weekly from 120 to 150 d of age. Rats 150 d old were asphyxiated with CO₂ and then weighed. Body length was measured from tip of snout to base of tail. The head and left femur were removed and defleshed in a beetle colony (*Dermestes vulpinus*) as previously described (18). Eighteen craniofacial measurements (Fig. 1) and femoral width and length measurements were performed on at least 10 rats per sex for each exposure group and their matching controls. These measures have been previously defined (18), except for the modifications described in Table 2: facial and calvarial lengths were replaced with separate measures of the nasal, frontal, and parietal bone lengths; cranial length was redefined to include the entire dorsal surface; and measures revealing ventral and posterior aspects of the skulls were added (basioccipital, sphenoid, and palatine lengths, supraoccipital height, and occipital width). Each cranial measure was divided by cranial length to determine craniofacial proportions. In addition, body proportions were determined from the ratios body length/body weight, cranial length/body length, femur length/body length, and femur width/femur length. In all, a total of 43 growth parameters were considered for each exposure group and its matched control. To determine statistical significance, a t test was used for body and cranial measures and body proportions, and the Mann-Whitney test was used for cranial proportions. Statistical significance was set at the $p < 0.01$ level.

Growth evaluations were conducted on the animals described in a concurrent study of behavior (19). Spontaneous behavior was tested at 42 and 120 d of age using a computer pattern recognition system that monitored behavioral acts during a 15-min exploration of a novel environment. The computer identified multiple behavioral acts and quantified their initiations, total times, and time structures for each exposure group and its matched control.

RESULTS

With the exception of the M₂X combination in males, all treatments involving cranial irradiation (X, M₂X, M₄X, P₁₈X, and P₁₈M₂X) permanently stunted growth. In Table 3 the overall growth effects are totaled for each exposure group, and the time of significant body weight reduction posttreatment for each exposure group is illustrated in Figure 2. Mortality did not

Fig. 2. Time to recovery of normal weight after exposure. Shaded bars represent days when body weights of treated rats were significantly different from matched controls. Bars extending to 150 d indicate a permanent reduction in weight.

Fig. 3. Percent body weight reduction in X exposure groups compared with matched controls at 150 d of age. Reductions greater than 5% are statistically significant. Note that significant reductions occurred in all irradiated groups except the M₂X males.

Table 4. Changes in body and craniofacial proportions*

	Exposure									
	Females					Males				
	X	P ₁₈ X	M ₂ X	M ₄ X	P ₁₈ M ₂ X	X	P ₁₈ X	M ₂ X	M ₄ X	P ₁₈ M ₂ X
Body proportions										
Body length/wt			+		+	+	+		+	+
Feml/body length	+				+	+				
Femw/feml							+			
Cral/body length						+				
Cranial proportions										
Nasl/cral	+		+		+		+			
Frol/cral										
Parl/cral		+								
Pal/cral		+								
Bocc/cral	+		+		+	+				+
Sph/cral	+	+		+	+					+
Socc/cral	+	+	+	+	+	+	+		+	+
Occh/cral	+	+	+	+	+	+	+		+	
Occw/cral	+				+					
Fach/cral										
Nash/cral					+					
Biz/cral					+	+				+
Tns/cral										
Max/cral										+
Min/cral	+				+					
Conw/cral										
Manl/cral						+				

* + Signifies change from control value ($p \leq 0.01$, t test or Mann-Whitney). Feml, femoral length; Femw, femoral width; Cral, cranial length; Nasl, nasal length; Frol, frontal length; Parl, parietal length; Pal, palatine length; Bocc, basioccipital length; Sph, sphenoid length; Socc, supraoccipital height; Occh, occipital height; Occw, occipital width; Fach, facial height; Nash, nasal height; Biz, bizygomatic width; Tns, transnasal width; Max, transfrontal maximum width; Min, transfrontal minimum width; Conw, condylar width; Manl, mandibular length.

confound the growth effects of radiation; treatment-related death occurred only in three animals exposed to P₃₆M₄. Exposures without cranial irradiation (P₁₈, P₃₆, M₂, M₄, P₁₈M₂, and P₃₆M₄) did not permanently reduce body weight, shorten length, alter

body proportions, or distort craniofacial proportions in any consistent pattern. In fact, neither dose of prednisolone (P₁₈ or P₃₆) retarded body weight at any time. Methotrexate, alone (M₂ and M₄) or combined with prednisolone (P₁₈M₂ and P₃₆M₄),

Table 5. *Body proportions*

Exposure	Body weight (g)		Body length (mm)		Body length/weight		Cranial length/body length	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Females								
X*	331.5 ± 38.8	297.4 ± 18.1†	228.5 ± 4.6	215.7 ± 4.4‡	0.688 ± 0.047	0.728 ± 0.041§	0.206 ± 0.004	0.209 ± 0.003§
n	19	20	14	14	14	14	14	14
P ₁₈ X	379.0 ± 29.7	339.9 ± 39.9†	219.3 ± 6.8	210.2 ± 7.2	0.590 ± 0.042	0.614 ± 0.064	0.219 ± 0.004	0.218 ± 0.009
n	23	21	13	12	13	12	13	12
M ₂ X	354.8 ± 28.9	314.6 ± 35.9†	214.4 ± 5.0	207.1 ± 6.4	0.614 ± 0.029	0.665 ± 0.059	0.219 ± 0.005	0.218 ± 0.006
n	24	21	15	13	15	13	15	13
M ₄ X	378.7 ± 39.9	324.1 ± 40.2‡	218.6 ± 5.1	208.8 ± 5.5‡	0.576 ± 0.046	0.619 ± 0.064	0.217 ± 0.004	0.219 ± 0.007
n	23	23	14	13	14	13	14	13
P ₁₈ M ₂ X*	313.9 ± 25.2	249.9 ± 25.4‡	221.4 ± 6.1	205.8 ± 8.1‡	0.708 ± 0.041	0.830 ± 0.078‡	0.207 ± 0.005	0.211 ± 0.008
n	18	19	17	19	17	19	16	19
Males								
X*	612.8 ± 50.3	427.6 ± 62.9‡	267.2 ± 6.5	237.7 ± 9.7‡	0.444 ± 0.031	0.576 ± 0.056‡	0.190 ± 0.003	0.199 ± 0.005‡
n	20	17	13	11	13	11	12	10
P ₁₈ X	692.3 ± 80.4	574.5 ± 99.3‡	244.2 ± 5.2	239.4 ± 6.5	0.352 ± 0.035	0.401 ± 0.050§	0.210 ± 0.005	0.207 ± 0.004
n	21	22	12	12	12	12	12	12
M ₂ X	602.1 ± 49.7	576.4 ± 83.4	242.0 ± 8.3	238.4 ± 11.3	0.409 ± 0.033	0.419 ± 0.051	0.210 ± 0.007	0.207 ± 0.005
n	21	25	13	17	13	17	13	17
M ₄ X	666.7 ± 66.0	571.8 ± 103.9†	248.8 ± 6.5	234.6 ± 10.2†	0.361 ± 0.027	0.426 ± 0.059	0.206 ± 0.007	0.207 ± 0.006
n	21	21	12	12	12	12	12	12
P ₁₈ M ₂ X*	629.8 ± 77.0	454.8 ± 73.2‡	261.5 ± 6.4	235.1 ± 8.2‡	0.420 ± 0.045	0.513 ± 0.059†	0.190 ± 0.006	0.194 ± 0.005
n	13	13	13	11	13	11	13	11

* Data originally presented in Schunior *et al.* (18).† $p \leq 0.001$; *t* test.‡ $p \leq 0.0001$; *t* test.§ $p \leq 0.05$; *t* test (not considered statistically significant).|| $p \leq 0.01$; *t* test.Table 6. *Craniofacial proportions*

Exposure	Basioccipital/cranial length		Sphenoid/cranial length		Supraoccipital/cranial length	
	Control	Exposed	Control	Exposed	Control	Exposed
Females						
X	0.150 ± 0.003	0.140 ± 0.005*	0.173 ± 0.006	0.163 ± 0.006*	0.132 ± 0.003	0.123 ± 0.008†
n	14	13	14	14	14	14
P ₁₈ X	0.149 ± 0.003	0.145 ± 0.007	0.173 ± 0.007	0.163 ± 0.007†	0.134 ± 0.004	0.124 ± 0.007*
n	13	12	13	12	13	12
M ₂ X	0.157 ± 0.010	0.148 ± 0.008†	0.175 ± 0.006	0.169 ± 0.007‡	0.136 ± 0.006	0.128 ± 0.008†
n	16	13	16	13	16	13
M ₄ X	0.148 ± 0.006	0.141 ± 0.007‡	0.174 ± 0.006	0.164 ± 0.010†	0.134 ± 0.005	0.125 ± 0.006*
n	14	13	14	13	14	13
P ₁₈ M ₂ X	0.148 ± 0.005	0.137 ± 0.004§	0.172 ± 0.008	0.156 ± 0.005§	0.128 ± 0.004	0.116 ± 0.006§
n	17	19	17	19	17	19
Males						
X	0.157 ± 0.005	0.140 ± 0.006§	0.176 ± 0.010	0.165 ± 0.008‡	0.133 ± 0.006	0.121 ± 0.006*
n	13	10	13	10	13	10
P ₁₈ X	0.153 ± 0.004	0.149 ± 0.009	0.174 ± 0.006	0.169 ± 0.007	0.139 ± 0.009	0.123 ± 0.003§
n	12	12	12	12	12	12
M ₂ X	0.163 ± 0.004	0.156 ± 0.010‡	0.180 ± 0.007	0.175 ± 0.011	0.138 ± 0.007	0.128 ± 0.012‡
n	13	17	13	17	13	17
M ₄ X	0.155 ± 0.006	0.146 ± 0.010	0.179 ± 0.004	0.173 ± 0.011	0.135 ± 0.006	0.120 ± 0.010*
n	12	12	12	12	12	12
P ₁₈ M ₂ X	0.156 ± 0.004	0.140 ± 0.009*	0.176 ± 0.005	0.160 ± 0.007*	0.135 ± 0.004	0.120 ± 0.009*
n	13	13	13	13	13	13

* $p \leq 0.001$; Mann-Whitney test.† $p \leq 0.01$; Mann-Whitney test.‡ $p \leq 0.05$; Mann-Whitney test (not considered statistically significant).§ $p \leq 0.0001$; Mann-Whitney test.

suppressed the growth rate after dosing, but compensatory growth returned body weight to control levels by d 80, with female weights returning sooner than male weights.

These results indicate that growth is affected primarily by exposures involving cranial irradiation. Although effects on growth centered around cranial irradiation, these effects were not

equal in all X exposure groups. Percent weight reduction varied among the X exposure groups (Fig. 3), as did significant changes in body and craniofacial proportions (Table 4). Radiation exposures tended to stunt body weight more than length, resulting in long bodies relative to weight (*i.e.* increased body length/weight ratio in Table 5). Cranial length was shortened by all X exposures

Fig. 4. Growth curves after exposure to X, M_2X , and $P_{18}M_2X$. ●, controls; ○, exposed. Shaded areas represent ages at which weights were significantly reduced from control levels. Female rats had permanently stunted growth from all three exposures. M_2 protected the male rats from permanent growth stunting induced by X alone except when prednisolone was added to the M_2X exposure. X and $P_{18}M_2X$ growth curves were previously presented in Schunior *et al.* (18).

(data not shown). Certain craniofacial proportions were altered consistently whether exposure was X alone or X combined with other exposures; basioccipital, sphenoid, and supraoccipital height tended to be reduced relative to cranial length (Table 6). Occipital height ratios demonstrated similar reductions after all X exposures (data not shown). In contrast to these similarities, ratios of femoral length to body length tended to increase with X and $P_{18}M_2X$ (18), but they were generally unaffected by the other X combinations (Table 4). Whereas X alone allowed for some head sparing (body length shortened more than cranial length) (18), X combinations reduced cranial length in proportion to body length (see cranial length/body length in Table 5). Similarly, not all X combinations proportionally increased occipital width and transfrontal minimum width in females as X alone did in our earlier study (18) (Table 4). The M_2X combination in males resulted in more normalized craniofacial proportions, different from the X pattern of craniofacial distortion. Because some effects varied among the X exposure groups while the dose of radiation remained the same, the drugs preceding the

radiation apparently modified the radiation response. This modification tended to be a protective effect; examples of the best protection occurred in M_2X males and examples of the least protection in $P_{18}M_2X$ males and females (Fig. 3; Tables 3 and 4).

Although prednisolone and methotrexate did not permanently reduce body weight in either sex, their modification of radiation-induced weight reduction was often dramatic (Fig. 4). The severe weight reduction caused by X alone was mitigated by M_2X , resulting in the male rats reaching control weight levels by d 52. However, the addition of prednisolone to this combination ($P_{18}M_2X$) resulted in a loss of protection and returned the males to the X pattern of permanent weight loss. A higher dose of methotrexate (M_4X) before the radiation also led to a loss of this protection (Fig. 3). The protection did not occur with either M_2X or M_4X in the females (Figs. 3 and 4). Therefore, the modifying effects of methotrexate and prednisolone seemed dose, sex, and drug combination dependent.

Table 7. Coincidence of growth and behavioral deficits*

	Females		Males	
	Behavior	Growth	Behavior	Growth
P ₁₈			+	
P ₃₆				
M ₂				
M ₄				
X		+		+
P ₁₈ M ₂				
P ₃₆ M ₄	+			
P ₁₈ X	+	+	+	+
M ₂ X	+	+		
M ₄ X	+	+	+	+
P ₁₈ M ₂ X		+	+	+

* + Indicates significant ($p < 0.01$) change. Behavior = significant RS values at 6 wk or 4 mo. See Mullenix *et al.* (19). Growth = significant reduction in body weight at 150 d.

DISCUSSION

Cranial irradiation was responsible for a severely depressed growth trajectory not ameliorated by catch-up growth. It altered craniofacial proportions primarily posterior and ventral to the brain case. Prednisolone and methotrexate modified the growth response to cranial irradiation, despite their causing only short-term growth deficits and rare changes in body and craniofacial proportions on their own. The pattern of radiation stunting was best prevented when methotrexate preceded cranial irradiation (M₂X) in male rats. The M₂X males recovered from a short-term growth deficit to attain adult control weight and length. Increasing the methotrexate dose within the combination (M₂X to M₄X) or adding prednisolone (M₂X to P₁₈M₂X) led to the loss of protection in these males, returning the radiation pattern of stunted growth. Thus, similar to effects on behavior (19), effects on growth varied with dose, sex, and agent interactions.

Table 7 summarizes the growth-behavior relationship for all exposure groups. It shows that when treatment involved only cranial irradiation, growth deficits alone occurred. When treatment involved cranial irradiation combined with prednisolone, methotrexate, or both, growth and behavioral deficits coincided except for M₂X in males and P₁₈M₂X in females. P₁₈M₂X in females prevented behavioral but not growth deficits, whereas M₂X in males prevented both. These data suggest that, when attention is paid to dose, sex, and agent interactions, a combination can be selected that protects against both growth and behavioral deficits.

Several studies in the literature provide insight as to the potential cause of the growth and behavioral deficits observed here. The severe effect of cranial irradiation on catch-up growth in rats has been attributed to two defects: a disrupted link between catch-up growth control and growth hormone secretion, and a maladjustment of the central control for body size (22–24). High-dose glucocorticoid treatment also has been reported to reset target body size (25). This central control of body size may be located in the dorsomedial hypothalamic nuclei (20, 26, 27). Bilateral lesions of dorsomedial hypothalamic nuclei in rats stunt growth and produce growth curves similar to those in rats neonatally exposed to just cranial irradiation (20). Moreover, the coincidence of growth and behavioral deficits when cranial irradiation, methotrexate, and prednisolone interact suggests involvement of hypothalamic neuropeptides. GRF and somatostatin affect both growth and behavior. The control of GRF and somatostatin over growth hormone secretory pattern has been well established (28, 29), and hyperactivity in response to GRF and hypoactivity in response to somatostatin have been reported (30). Other brain areas besides the hypothalamus cannot be ignored, however, inasmuch as bilateral lesions of the median raphe, ventrolateral thalamus, substantia nigra, and globus pallidus also stunt growth as well as impair learning (20, 31). Thus,

various brain regions including the hypothalamus, hypothalamic neuropeptides, and growth hormone secretion should be evaluated in future studies of the cause of neurotoxicity by CNS therapy in animals.

The agent combinations examined here in rats are simpler than those used in the treatment of childhood ALL, where many chemotherapeutic agents are used, further increasing potential for agent interactions. Despite this difficulty, there are indications that our findings in animals might relate to the clinical situation. Clinical studies usually attribute effects on growth and craniofacial development to cranial irradiation (5, 9–11), although corticosteroids and chemotherapy are thought to be contributory (1, 32, 33). Clearly, it is not understood how radiation schedules and chemotherapy protocols interact to cause growth failure and growth hormone deficiency (3, 34). The growth hormone requirements of children treated for ALL are still controversial (12). Studies of growth hormone responses in both monkeys (35) and humans (36) have indicated that the hypothalamus is more sensitive than the pituitary to cranial irradiation. Therefore, hypothalamic GRF might be important to children with radiation-induced growth hormone deficiency, as has been suggested by some investigators (12, 34). Clinical studies, similar to studies in animals, find both growth and behavioral effects associated with hypothalamic neuropeptides; GRF and somatostatin have been shown to improve mental performance by modifying attention and memory function (37–39). Abnormal growth hormone responses to GRF were found in prepubertal children with attention-deficit hyperactivity (40).

These parallels between clinical and animal observations warrant additional study of the potential for "optimal" combinations of CNS therapy agents. These combinations must protect against both growth and behavioral deficits without compromising efficacy. As this study indicates, consideration of dose, sex, and agent interactions would be critical to any such investigation.

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