Effect of methotrexate alone and in combination with vincristine on craniofacial morphology in growing rats

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Karsila S, Salmi TT, Rönning O. Effect of methotrexate alone and in combination with vincristine on craniofacial morphology in growing rats. Acta Odontol Scand 1999;57:271–276. Oslo. ISSN 0001-6357.

Craniofacial growth in young Long-Evans/Turku strain rats was studied following administration at different intervals of the antineoplastic agent methotrexate (MTX) and of a combination of two agents, MTX and vincristine (VCR). The 10-day-old rats tolerated only 1 subcutaneous injection of 0.75 mg/kg MTX, while single and repeated doses of 1.0 mg/kg MTX, or of the combination of MTX (1.0 mg/kg) and VCR (0.05 mg/kg), could be used for the 30-day-old rats. The rats were killed 20 days after the start of the experiment, i.e. at 30 or 50 days of age, respectively; their weights were recorded and a total of 12 craniofacial dimensions were measured. Owing to the relatively few significant sex differences, the dimensions recorded for the females and males were pooled. Administration of MTX alone or combined with VCR caused disturbed craniofacial growth, which was already evident following 1 single injection of MTX in the younger rats (10 to 30 days) and after repeated injections (every third day) of MTX alone or combined with VCR in the older rats (30 to 50 days). The body weights of all the medicated rats were initially retarded and catch-up growth, i.e. a return to control body weight levels, occurred by the end of the experiment only in the rats that received a single injection of MTX, but not after repeated injections. We conclude that the antineoplastic agent MTX alone or in combination with VCR administered at short intervals with pharmacologically adjusted doses has a short-term effect on the craniofacial skeleton of growing rats. □ *Adverse effect; craniofacial growth; methotrexate; rats; vincristine*

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The chemotherapeutic methotrexate (MTX) is an antineoplastic agent commonly used in childhood cancer diseases and included in most treatment protocols. Like all chemotherapeutic agents, it is generally known that MTX has adverse effects on a number of organs and their growth and development (1).

Using a rat model, Friedlaender et al. (2) noted that MTX induces osteopenia and depresses bone formation when investigated 14 days after the treatment. According to Wheeler et al. (3), it has an effect on both cortical and cancellous bone in rats, which is noticeable even 170 days after treatment. More explicitly, their results indicate that MTX reduces bone volume, bone formation, and osteoblast activity, and increases osteoclast activity as a result of which the bone mass remains decreased in the treated rats. Clinically, this could be associated with the increased bone fracture incidence seen in MTX-treated patients (4).

No consistent distortion of the craniofacial proportions of growing rats has been observed around 130 days subsequent to the administration of MTX alone or combined with prednisolone (5). On the other hand, most of the craniofacial dimensions were smaller in the medicated than in the control rats as early as 20 days after administration of the antineoplastic drugs vincristine (VCR) or doxorubicin (6). As the observation time, i.e. the period of growth after drug administration, is obviously of significance, it was decided to test the effect of the antineoplastic agent MTX in an experiment involving a short observation period. In the present study, 10-day-old rats were chosen to represent immature prepubertal animals while 30-day-old represented pubertal ones (7).

The investigation was carried out by recording the early dimensional changes in the craniofacial skeleton of young rats following single or repeated doses of MTX administered at different intervals. In addition, the effect of a combination of two drugs, MTX and VCR, was elucidated.

Material and methods

Animals and administration regime

A total of 210 inbred Long-Evans/Turku strain rats were used in the experiments and maintained at the Institute of Dentistry, University of Turku. Each experimental group consisted of 30 rats (15 males and 15 females). Two different age groups were formed, immature 10-day-old rats housed 6–8 pups per dam and pubertal 30-day-old animals housed 3 or 4 rats in each cage. The rats were assigned randomly to the experimental and the control group. They were given RM3(E)-feed (Special Diet Services Company, Witham, Essex, England), tap water ad libitum, and housed in a temperature- and light-controlled room (21–23°C, 12-h light–dark cycle). The weight and general condition of the animals were checked every 3rd day. Animal ethics approval for the study protocol was given by the district administrative board (Permit no. 719/97).

Table 1. Administration regime of the agents injected subcutaneously. Each group consisted of 30 rats

Age at start	Group	Agent	No. of injections	Injection schedule
10 days	MTX	Methotrexate	1 × 0.75 mg/kg	10
	CO	NaCl	1 × 10.0 ml/kg	10
30 days	MTX 1	Methotrexate	1 × 1.0 mg/kg	30
	MTX 3 × 6	Methotrexate	3 × 1.0 mg/kg	30, 36, 42
	MTX 3 × 3	Methotrexate	3 × 1.0 mg/kg	30, 33, 36
	COMB	Methotrexate	3 × 1.0 mg/kg	30, 33, 36
		Vincristine	3 × 0.05 mg/kg	30, 33, 36
	CO	NaCl	1 × 10.0 ml/kg	30

The antineoplastic agents to be used were tested for their toxicity. The 10-day-old rats tolerated only one subcutaneous injection of 0.75 mg/kg MTX, as the mortality rate rose to 50% or more after higher or repeated doses, or after a combination of MTX and VCR. Female rats accepted their medicated offsprings and fed them normally until the pups were weaned at the age of 20 days. The 30-day-old rats tolerated the drugs better than younger ones. Single and repeated doses of 1.0 mg/kg MTX could be used, and also a combination of MTX (1.0 mg/kg) and VCR (0.05 mg/kg), although the weight gain of these animals was retarded subsequent to the injections.

MTX (Trexan® 25mg/ml, Orion, Finland) was diluted 1:200 and VCR (Vincrin® 1 mg/ml, Orion, Finland) 1:100 with physiological saline. A single dose of MTX (0.75 mg/kg) was injected subcutaneously into the 10-day-old rats, which were subsequently followed up to the age of 30 days. The older experimental groups received one subcutaneous injection of 1.0 mg/kg MTX at 30 days of age or one injection on either every 3rd day (30, 33, and 36 days) or every 6th day (30, 36, and 42 days). In addition, one group of 30-day-old rats (COMB) received both MTX (1.0 mg/kg) and VCR (0.05 mg/kg) injections on the 30th, 33rd, and 36th day. The rats that were 30 days old at the start of the experiment were followed up to the age of 50 days (Table 1). All the injections were given between 9 a.m. and 11 a.m. The controls were given physiological saline subcutaneously, the mean volume corresponding to that of the drug injections. Otherwise the handling between the medicated and the control rats was exactly the same.

The rats were killed by carbon dioxide asphyxia 20 days after the start of the experiment, i.e. at 30 or 50 days of age, respectively. The heads were freed of soft tissues, bleached with hydrogen peroxide, and stored in glycerol.

Biometric registrations

The following measurements were made to the nearest 0.1 mm using a digital sliding caliper (Fig. 1):

Neurocranial length (ncl)—distance from the frontonasal suture to the superior margin of the supraoccipital bone

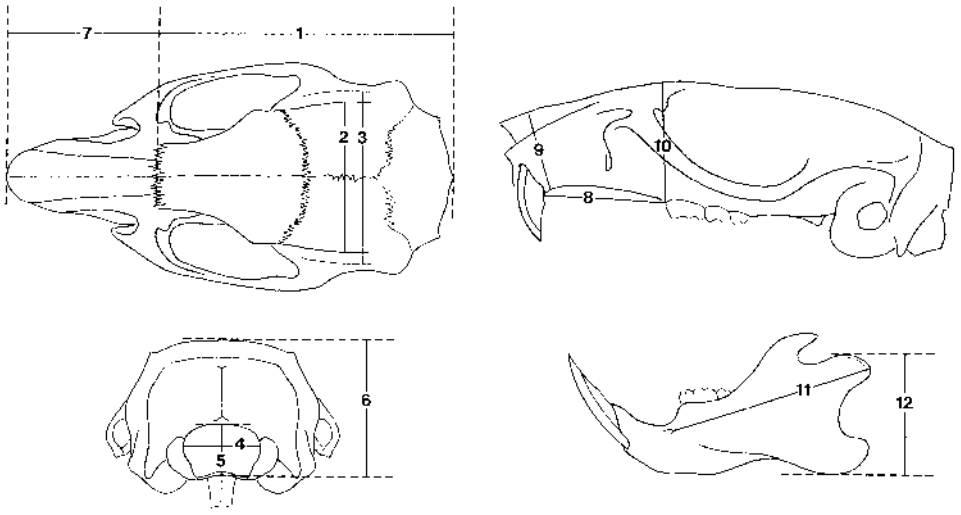

Fig. 1. Linear measurements performed on the craniofacial skeleton of the rat. 1 = neurocranial length (ncl), 2 = calvarial width (cw), 3 = neurocranial width (ncw), 4 = width of foramen magnum (fmw), 5 = height of foramen magnum (fmh), 6 = neurocranial height (nch), 7 = nasal length (nl), 8 = sagittal diastema (sd), 9 = anterior height of the snout (ash), 10 = posterior height of the snout (psh), 11 = mandibular length (ml), and 12 = mandibular height (mh).

Table 2. Measurements of skulls (mm) and weights (g) of rats given a single injection of MTX (0.75mg/kg) at 10 days of age and killed at 30 days. Controls (CO) received physiological saline

	MTX		CO		
	Mean	SEM	Mean	SEM	Significance
Neurocranial length (ncl)	22.82	0.17	23.47	0.07	***
Calvarial width (cw)	13.17	0.04	13.34	0.02	***
Neurocranial width (ncw)	14.93	0.05	15.05	0.03	**
Width of foramen magnum (fmw)	6.28	0.03	6.31	0.02	n.s.
Height of foramen magnum (fmh)	5.35	0.02	5.38	0.02	n.s.
Neurocranial height (nch)	10.04	0.05	10.24	0.03	n.s.
Nasal length (nl)	11.40	0.09	11.61	0.05	**
Sagittal diastema (sd)	8.40	0.06	8.64	0.04	n.s.
Anterior height of the snout (ash)	5.93	0.04	6.07	0.03	n.s.
Posterior height of the snout (psh)	8.19	0.05	8.38	0.03	*
Mandibular length (ml)	14.94	0.10	15.22	0.06	**
Mandibular height (mh)	8.15	0.06	8.27	0.04	n.s.
Initial weight (w1)	17.75	0.26	17.52	0.32	n.s.
Final weight (w2)	68.73	1.71	71.53	1.25	n.s.

Statistically significant difference between the groups, as determined by Student's *t*-test.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Calvarial width (cw)—longest distance between the temporal crests

Neurocranial width (ncw)—intertemporal width immediately superior to the zygomatic process

Width of the foramen magnum (fmw)—longest transversal diameter of the foramen magnum

Height of the foramen magnum (fmh)—vertical diameter of the foramen magnum

Neurocranial height (nch)—distance from the inferior surface of the basioccipital bone to the vertex

Nasal length (nl)—distance from the anterior margin of the nasal bone to the frontonasal suture

Sagittal diastema (sd)—distance from the posterior junction of the premaxilla and incisors to the anterior cementoenamel junction of the first maxillary molars

Anterior height of the snout (ash)—distance from the inferior surface of the premaxilla to the superior surface of the nasal bone

Posterior height of the snout (psh)—distance from frontomaxillary suture to the alveolar bone anterior to the first maxillary molar

Mandibular length (ml)—distance between the mental foramen and posterior aspect of the condylar process

Mandibular height (mh)—distance between the inferior border of the mandible and superior aspect of the condylar process.

The first 6 measurements represented neurocranial, the next 4 maxillary, and the last 2 mandibular dimensions, respectively. All the measuring points needed were available in skulls of both age groups.

Statistical analysis

The measurements of the 30- and 50-day-old control rats, 20 animals in each group, were repeated at an interval of at least 2 weeks. An estimate for random variation in 2 measurements was calculated using the formula $\sqrt{\Sigma d^2/2n}$ where d is the difference between the duplicate measurements in each case and n is the number of duplicates. This can be interpreted as estimate of measurement error. The error varied between 0.03 mm and 0.11 mm, the most repeatable (error <0.05 mm) being measurements 3–5, 8, and 10, while measurements 1 and 7 were less readily repeatable (error 0.10–0.11 mm). One measurement was discarded because of poor repeatability, whereas the error in the remainder was regarded as having an insignificant effect on the results.

Statistical comparisons between two 10-day-old groups of rats were performed with Student's *t*-test, and the differences between the groups of 30-day-old rats were determined with the one-way analysis of variance (ANOVA). To make an adjustment for multiple comparisons, the post hoc pairwise differences between groups were tested with Tukey's test. As the ANOVA revealed statistically significant sex differences only in the measurements of the anterior height of the snout and mandibular height, the dimensions recorded for the females and males were pooled. *P*-values less than 0.05 were interpreted as statistically significant.

Results

Young rats, observation period from 10 to 30 days

One injection of MTX. The length (ncl) and width (cw, ncw) of the neurocranium, the nasal and mandibular length (nl, ml), and the posterior height of the snout (sph) were significantly smaller in the experimental rats than in the controls. There was no significant difference in final weight (w2) between the groups (Table 2).

Weanling rats, observation period from 30 to 50 days

One injection of MTX (MTX 1). With the exception of the enlarged dimensions of the foramen magnum (fmw, fmh), the measurements, including the final weight (w2), did not differ significantly from those of controls (Table 3).

One injection of MTX every 6th day (MTX 3×6). All craniofacial dimensions remained within normal range, while the final weight (w2) was significantly lower than that of controls (Table 3).

One injection of MTX every 3rd day (MTX 3×3). Of the neurocranial dimensions, only the height (nch) was significantly smaller in the experimental animals, whereas the width and height of the foramen magnum (fmw, fmh) were significantly larger. All the measurements depicting the dimensions of facial structures, i.e. nasal length (nl), sagittal diastema (sd), anterior and posterior height of the snout (ash, psh), and mandibular length and height (ml, mh) were significantly smaller in the treated rats, as was also the final weight (w2).

Combination of drugs, one injection of MTX and VCR every 3rd day (COMB). The neurocranial length and height (ncl, nch) and the calvarial width (cw) were smaller in the experimental rats, while the dimensions of the foramen magnum (fmw, fmh) were greater. In the facial area the vertical dimensions, i.e. the anterior and posterior height of the snout (ash, psh) and the mandibular height (mh) were shorter than those of controls. The mandibular length (ml) was reduced, whereas the horizontal dimensions of the snout (nl, sd) remained within normal range in the experimental rats as compared with controls (Table 3).

A comparison between the medication regimens used with the older rats (30 to 50 days) revealed no significant difference between those that received a single injection (MTX 1) or 1 injection of MTX every sixth day (MTX 3×6). The rats that received one injection of MTX every 3rd day (MTX 3×3) showed significant changes in the vertical dimensions of the snout (ash, psh) and mandible (mh), for example, and in the final weight (w2) as compared with the MTX 1 group. The group that received a combination of 2 drugs, MTX and VCR (COMB), differed from the MTX 3×3 group only in the area of the neurocranium, i.e. the neurocranial length (ncl) and calvarial width (cw) were smaller (Table 3).

Discussion

Administration of MTX alone or in combination with VCR to the growing rats resulted in differential changes in the morphogenesis of the craniofacial skeleton, i.e. the changes were not uniform because some measurements were reduced, others remained virtually unchanged whereas a couple had increased. The younger animals (10 to 30 days) showed more dimensional deviations following only 1 drug injection than the older ones (30 to 50 days). The 10-day-old rats represent immature animals and their strong reaction to MTX can be understood on the basis that this drug is one of the most toxic chemotherapeutic agents when administered during pregnancy (8). Relatively low tolerance to other chemotherapeutic drugs, such as doxorubicin, VCR, and cyclophosphamide, has also been reported recently in young rats (6, 9). Tolerance of MTX appears to increase fairly rapidly with age, as no more than 1 dose of 0.75 mg/kg could be given to the present 10-day-old rats, whereas one injection of 2 mg/kg or even 4 mg/kg MTX was evidently well tolerated by Sprague-Dawley rats at 18 days of age (5). Another explanation for the different findings could, of course, lie in the variation in reactivity to drugs among different strains of the same species (10).

In an earlier experiment, the same craniofacial structures were studied after the administration of VCR or doxorubicin (6). The present findings regarding the younger rats (10 to 30 days) are in line with the effect of VCR observed in rats of the same age, especially in the neurocranial area (ncl, cw, ncw), whereas MTX did not disturb facial growth as markedly.

The finding that multiple injections of MTX in 30-day-old rats had a more accentuated effect than a single dose is in accordance with our earlier unpublished observations on the effects of VCR and doxorubicin. However, the MTX 3×6 group of rats did not differ significantly from the controls, except in the final weight (w2); nor did it differ significantly from the MTX 1 group, most probably because the 6-day interval between the injections was sufficiently long to allow recovery. The growth disturbances were clear in both groups in which injections were repeated every 3rd day, i.e. in the MTX 3×3 and COMB groups.

A detailed comparison between the older rats of the present investigation (30 to 50 days) and those of the same age in an earlier unpublished report (6) reveals that the changes following a single injection of MTX, VCR, or doxorubicin were virtually the same, with the exception of the dimensions of the neurocranial area (ncl, cw, ncw), which were not affected by the MTX treatment. This was also the case following repeated doses of the agents, whereas the lengths of both jaws (nl, sd, ml) were affected only by MTX.

The dimensions of the foramen magnum (fmw, fmh) of the younger animals (10 to 30 days) remained within normal range after administration of all the chemotherapeutic drugs used in the present and earlier experiments (6). The size of the foramen magnum of the older rats was already slightly increased after a single injection but, with the exception of the longer injection intervals (MTX 3×6), the change was more pronounced following repeated doses. The foramen magnum grows mainly by cartilage-mediated separation of the 4 bone elements that constitute the occipital complex, and there is some "filling in" apposition of bone at the posterosuperior margin of the foramen in the rat (11). This apposition may have been disturbed in the present animals. Moreover, even bone resorption at the foraminal margins seems probable, since

Table 3. Measurements (mm) of skulls and weights (g) of rats given single (MTX 1) or repeated injections of methotrexate (MTX 3 × 6, MTX 3 × 3) or combination of 2 different drugs, methotrexate and vincristine (COMB) at 30 days of age and killed at 50 days. Controls (CO) received physiologic saline. The superscripts (A–E) refer to the table of comparison (see below)

	MTX 1[A]		MTX 3 × 6[B]		MTX 3 × 3[C]		COMB[D]		CO[E]	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Neurocranial length (ncl)	26.12	0.10	26.09	0.14	26.01	0.16	25.40	0.10	26.33	0.13
Calvarial width (cw)	13.22	0.03	13.23	0.03	13.19	0.03	13.04	0.03	13.28	0.03
Neurocranial width (ncw)	15.43	0.04	15.50	0.04	15.41	0.04	15.40	0.04	15.51	0.04
Width of foramen magnum (fmw)	6.50	0.03	6.44	0.03	6.51	0.03	6.51	0.03	6.39	0.03
Height of foramen magnum (fmh)	5.26	0.03	5.25	0.02	5.28	0.02	5.32	0.02	5.17	0.02
Neurocranial height (nch)	11.00	0.04	10.95	0.03	10.88	0.05	10.79	0.04	11.05	0.04
Nasal length (nl)	13.89	0.11	13.89	0.08	13.62	0.08	13.86	0.06	14.00	0.08
Sagittal diastema (sd)	10.32	0.03	10.29	0.04	10.15	0.05	10.24	0.05	10.36	0.05
Anterior height of the snout (ash)	7.07	0.04	7.04	0.04	6.96	0.04	6.90	0.04	7.10	0.04
Posterior height of the snout (psh)	9.75	0.05	9.78	0.05	9.59	0.05	9.59	0.05	9.83	0.04
Mandibular length (ml)	17.60	0.05	17.64	0.06	17.49	0.08	17.39	0.08	17.80	0.07
Mandibular height (mh)	10.70	0.05	10.03	0.04	9.81	0.06	9.76	0.05	10.18	0.05
Initial weight (w1)	69.13	0.85	69.00	1.16	69.97	1.24	70.37	0.81	69.60	1.12
Final weight (w2)	151.03	3.56	147.07	3.53	139.67	3.04	140.70	3.15	156.83	4.01
	A–E	B–E	C–E	D–E	A-B	A–C	A–D	B–C	B–D	C–D
Neurocranial length (ncl)	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	***	***
Calvarial width (cw)	n.s.	n.s.	n.s.	***	n.s.	n.s.	***	n.s.	***	**
Neurocranial width (ncw)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Width of foramen magnum (fmw)	*	n.s.	**	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Height of foramen magnum (fmh)	*	n.s.	**	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Neurocranial height (nch)	n.s.	n.s.	**	***	n.s.	n.s.	***	n.s.	*	n.s.
Nasal length (nl)	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sagittal diastema (sd)	n.s.	n.s.	***	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Anterior height of the snout (ash)	n.s.	n.s.	**	***	n.s.	*	***	n.s.	**	n.s.
Posterior height of the snout (psh)	n.s.	n.s.	***	***	n.s.	*	*	**	**	n.s.
Mandibular length (ml)	n.s.	n.s.	**	***	n.s.	n.s.	n.s.	n.s.	*	n.s.
Mandibular height (mh)	n.s.	n.s.	***	***	n.s.	***	***	**	***	n.s.
Initial weight (w1)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Final weight (w2)	n.s.	**	***	***	n.s.	***	**	n.s.	n.s.	n.s.

Statistically significant difference between the groups as determined by Tukey's test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

the transversal diameter of the foramen magnum also increased. The explanation for this could lie in the increased osteoclastic activity observed following MTX administration (2, 3) and a possible increase in pressure in the brain cavity associated with decreased cranial dimensions in rats subjected to antineoplastic therapy (6).

Contrary to our finding of major changes in the craniofacial morphology of 50-day-old rats, Schunior et al. (5) did not report any marked deviations in the craniofacial frame in 150-day-old rats that had received a single injection of prednisolone at 17 days and a larger dose of MTX than in the present experiment at 18 days. As the initial lag in weight gain was similar in the 2 experiments, it appears reasonable to assume that, although MTX reportedly has a prolonged effect on bone growth (3), some catch-up growth of the craniofacial skeleton had occurred during the longer observation period in the experiment of Schunior et al. (5). It is worth noting that a definite catch-up in weight was recorded only for the present rats that received a single injection of MTX, not after repeated injections. The changes observable after a longer period are at present under investigation in our laboratory.

Decreased linear growth is a general problem during antineoplastic therapy in children, and cranial irradiation is regarded as the most important factor in long-term retarded growth. When chemotherapy is given without radiation, growth retardation is usually temporary and will be caught up later (12–14). On the other hand, growth restriction has been clearly demonstrated even in the absence of cranial irradiation involving the hypothalamic-pituitary axis (15, 16). Nevertheless, recent therapeutic strategies include intensive multiagent chemotherapy, whereas radiation is avoided or minimized whenever possible. In the present experiment, the addition of VCR to the treatment regime with MTX caused a minor accentuation of the retardation in weight gain, reduced the final weight and brought about changes in craniofacial morphogenesis. The changes were virtually the same as those recorded for MTX treatment at short administration intervals (MTX 3 × 3), however, and it is conceivable that although these chemotherapeutic agents have different mechanisms of action the manifestation of the growth disorder would be relatively similar if the doses of the agents were adjusted to be comparable.

Although the shape and size of the craniofacial

structures differ between humans and rats, growth mechanisms are the same or similar. Thus, on the basis of our results, it seems likely that some typical changes in the facial frame, including malocclusion, may be clinically evident if catch-up growth does not occur.

Acknowledgement.—The authors thank Hans Helenius, MSc, for guidance in statistical methodology and Timo Kohtala for data processing. The study was supported by the Cancer Society of Southwest Finland and the Finnish Dental Society.

References

1. Balis FM, Holcenberg JS, Poplack DG. General principles of chemotherapy. In: Pizzo PA, Poplack DG, editors. Principles and practice of pediatric oncology, 3rd ed. Philadelphia: Lippincott-Raven; 1997. p. 215–72.
2. Friedlaender GE, Tross RB, Doganis AC, Kirkwood JM, Baron R. Effects of chemotherapeutic agents on bone. I. Short-term methotrexate and doxorubicin (adriamycin) treatment in a rat model. J Bone Joint Surg 1984;66A:602–7.
3. Wheeler DL, Vander Griend RA, Wronski TJ, Miller GJ, Keith EE, Graves JE. The short- and long-term effects of methotrexate on the rat skeleton. Bone 1995;16:215–21.
4. Atkinson SA, Fraher L, Gundberg CM, Andrew M, Pai M, Barr RD. Mineral homeostasis and bone mass in children treated for acute lymphoblastic leukemia. J Pediatr 1989;114:793–800.
5. Schunior A, Mullenix PJ, Zengel AE, Landy H, Howes A, Tarbell NJ. Radiation effects of growth are altered in rats by prednisolone and methotrexate. Pediatr Res 1994;35:416–23.
6. Karsila S, Salmi TT, Helenius H, Rönning O. Craniofacial growth of immature rats following administration of VCR and doxorubicin. Manuscript, 1998.
7. Knorr DW, Vanha-Perttula T, Lippsett MB. Structure and function of rat testis through pubescence. Endocrinology 1970; 86:1298–304.
8. Sorosky JI, Sood AK, Buekers TE. The use of chemotherapeutic agents during pregnancy. Obstet Gynecol Clin North Am 1997;24:591–9.
9. Näsman M, Hammarström L. Influence of the antineoplastic agent cyclophosphamide on dental development in rat molars. Acta Odontol Scand 1996;54:287–94.
10. Pohjanvirta R, Unkila M, Tuomisto J. Comparative acute lethality of 2,3,7,8-tetracholorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentacholorodibenzo-p-dioxin and 1,2,3,4,7,8-hexachohlorodibenzo-p-dioxin in the most TCDD-susceptible and the most TCDD-resistant rat strain. Pharmacol Toxicol 1993; 73:52–6.
11. Baer MJ. Patterns of growth of the skull as revealed by vital staining. Human Biol 1954;26:80–126.
12. Katz JA, Pollock BH, Jacaruso D, Morad A. Final attained height in patients successfully treated for childhood acute lymphoblastic leukemia. J Pediatr 1993;123:546–52.
13. Moell C, Marky I, Hovi L, Kristinsson J, Rix M, Moe PJ, et al. Cerebral irradiation causes blunted pubertal growth in girls treated for acute leukemia. Med Pediatr Oncol 1994;22:375–9.
14. Van der Does-VandenBerg A, Vaan GAM, de Weerden JF, van Hählen K, Weel-Sipman M, van Veerman AJP. Late effects among long-term survivors of childhood acute leukemia in The Netherlands: a Dutch childhood leukemia study group report. Pediatr Res 1995;38:802–7.
15. Ogilvy-Stuart AL, Shalet SM. Effect of chemotherapy on growth. Acta Paediatr Suppl 1995;411:52–6.
16. Ilveskoski I, Saarinen UM, Wiklund T, Sipilä I, Mäkipernaa A, Perkkiö M, et al. Growth impairment and growth hormone therapy in children treated for malignant brain tumors. Eur J Pediatr 1997;156:764–9.

Received for publication 28 May 1999
Accepted 1 September 1999