CURRENT TOPIC

Bone growth mechanisms and the effects of cytotoxic drugs

Helen Robson

With improved survival rates for most childhood malignancies, it has become increasingly apparent that some children grow poorly, long after chemotherapy is stopped. However, there have been very few studies that have examined the mechanisms by which chemotherapy affects growth. In general, any such reports suggest that the effects of chemotherapy on growth are independent of the hypothalamic–pituitary axis, but act directly on the cartilage growth plate to reduce longitudinal bone growth. New knowledge about the mechanisms of chemotherapy actions on longitudinal bone growth is timely; in particular because the trend in paediatric oncology is for further intensification of treatment, as a result of the successful use of intensive chemotherapy. This review provides an overview of the cellular mechanisms that contribute to linear growth; based on my own studies and the limited data available from the literature, it also considers how cytotoxic chemotherapy might disrupt the normal growth process.

Growth in children treated for childhood cancers

Several groups of investigators have examined the effects of chemotherapy on the growth patterns of children treated for various cancers and have documented a decrease in skeletal growth rate during treatment with chemotherapy. However, the pattern of growth after completion of treatment has been a point of some controversy. Some investigators reported a permanent deficit,^{1–3} whereas others reported catch up growth, with only minimal loss in final height.^{4–6} Examination of the chemotherapy protocols used in these studies suggested that the dissimilar growth patterns observed were caused by the variable intensity of the chemotherapy regimens used. For example, in an Australian study by Kirk and colleagues⁷ there was no catch up growth and the long term adverse effects on growth were more severe than in children treated for acute lymphoblastic leukaemia (ALL) with UK schedules.⁸ There is no doubt that the chemotherapy protocol used by Kirk *et al* was more intense, both in dose and duration, than the UK schedule. In addition, there is some evidence to suggest that, together with the dose and intensity of treatment, the scheduling of the chemotherapy drugs is a determining factor for the long term growth potential. For example, in children

treated for ALL, final height equalled predicted adult height if continuation treatment consisted of only oral methotrexate and 6-mercaptopurine.⁹ Furthermore, in 22 children enrolled in the Medical Research Council funded randomised trial of childhood ALL treatment in the UK, post steroid recovery of osteoblast activity, in terms of alkaline phosphatase and procollagen type I C-terminal propeptide, was impaired by high dose systemic methotrexate.¹⁰ However, no detailed information exists to indicate which phases of chemotherapy contribute most to the growth deficit and by what mechanisms. Chemotherapy in association with craniospinal irradiation is associated with a greater degree of growth failure than is craniospinal irradiation alone,^{11 12} and although radiation induced growth hormone deficiency (GHD) can be treated with growth hormone replacement treatment, most patients fail to reach their midparental height.^{12–14} One explanation for this is that chemotherapy has an additional effect on growth, although it is difficult to determine exactly how large this effect might be. As far as the mechanism of growth failure is concerned, children treated with chemotherapy alone show no disturbance of growth hormone secretion,^{12 15} which suggests that these agents have a direct effect on the skeleton itself. In support of this, poor growth in children with osteosarcoma treated with combination chemotherapy followed by surgery is accompanied by disruption of the columnar arrangement of chondrocytes within the growth plate, as well as a reduction in the number of proliferative cells in each column.¹⁶

Regulation of postnatal longitudinal bone growth

Longitudinal bone growth is achieved by the complex, multistep process known as endochondral ossification, whereby the cartilaginous template of the axial and appendicular skeleton is replaced by bone.¹⁷ This process is initiated when chondrocytes at the epiphyseal growth plate are stimulated to proliferate and then proceed through stages of maturation and hypertrophy. In the region of cellular hypertrophy, the surrounding matrix and vascular tissue undergo calcification. The hypertrophic chondrocytes degenerate and give way to invading osteoblasts, and bone and bone marrow subsequently replace the calcified cartilage

Department of
Tumour Biochemistry,
Clinical Research,
Christie Hospital NHS
Trust, Wilmslow Road,
Manchester M20 4BX,
UK
H Robson

Correspondence to:
Dr Robson

at the metaphysis. In a recent elegant study,¹⁸ rabbit distal ulnar growth plates were excised, inverted, and re-implanted in their original beds. Longitudinal bone growth then occurred at the epiphyseal, not at the metaphyseal, surface of the growth plate, indicating that spatial polarity within the growth plate was maintained by intrinsic mechanisms rather than external signals.

Endochondral ossification is an important determinant of both the rate and extent of longitudinal bone growth, with the two main contributing variables being the rate of new cell production and the average height of the hypertrophic cells.¹⁹ This growth plate activity is in turn subject to regulation by a number of factors, which might be of genetic, endocrine, paracrine, or autocrine origin, and it is the complex interactive effects of these substances on chondrocytes *in vivo* that determine the final growth response. Moreover, identical stimuli act differentially on chondrocytes according to the zone of chondrogenic maturation within the growth plate (reserve, proliferative, or hypertrophic), thus adding to the versatility and complexity of the system as a whole. These stimuli include various hormones such as growth hormone,²⁰ thyroid hormones (T_3),²¹ and parathyroid hormone/parathyroid hormone related peptide (PTH/PTHrP), as well as several growth factors and cytokines, such as insulin-like growth factor I (IGF-I), basic fibroblast like growth factor, and transforming growth factor β .²² More recently, Indian

hedgehog (Ihh), which is a member of a conserved family of secreted proteins that have key roles in embryonic patterning in many organisms, has been identified in growth plates from a number of species. These studies have demonstrated convincingly that Ihh and PTHrP target PTH/PTHrP receptor (PTHR/PTHrPR) expressing growth plate chondrocytes in a negative feedback loop to regulate the coordinated progression of chondrocyte maturation and hypertrophy (fig 1).^{23, 24} Although in most instances the precise mechanism of action of these regulatory peptides remains incompletely understood, it is generally accepted that the two main stimulatory peptides contributing to longitudinal bone growth are growth hormone and IGF-I.²⁰ According to the dual effector theory of growth hormone action,²⁵ growth hormone acts not only directly on chondrocyte progenitor cells of the growth plate to promote their differentiation, but also indirectly to increase the responsiveness of the proliferative cells undergoing clonal expansion from the stimulatory effects of IGF-I.²⁶

In vitro models of chondrogenesis

Over the past 15 years, important advances in our understanding of the mechanisms of chondrogenesis and thus longitudinal bone growth have been made using defined *in vitro* culture systems, which manipulate the maturational characteristics of isolated chondrocytes. In particular, the group led by Isaksson developed an *in vitro* agarose suspension culture system,

Figure 1 Schematic representation of the epiphyseal growth plate and adjacent bone. The actions of the major systemic hormones on growth plate chondrocyte maturation are shown on the right hand side and the effects of chemotherapeutic agents are indicated on the left of the diagram. Reserve zone chondrocytes differentiate to proliferating cells by a process that is stimulated by growth hormone (GH).²⁰ Proliferating cells undergo a process of clonal expansion, which is stimulated by insulin-like growth factor I (IGF-I) and probably inhibited by thyroid hormone (T_3), and begin to express the parathyroid hormone/parathyroid hormone related protein receptor (PTHR/PTHrPR) before commitment to hypertrophy. Terminal differentiation towards the hypertrophic phenotype is induced by T_3 and committed cells synthesize and secrete Indian hedgehog (Ihh). Ihh acts on perichondrial cells to stimulate the synthesis and release of PTHrP. PTHrP acts on uncommitted proliferating cells that express PTHrPR to inhibit cell proliferation, thereby delaying cell maturation and reducing Ihh synthesis to complete a feedback loop.²⁴ Both glucocorticoids and DNA damaging agents target reserve cells and proliferating chondrocytes. Whereas these effects are cytotoxic for the former, resulting in a loss of a proportion of the cells from these two zones, glucocorticoids reduce chondrocyte proliferation by maintaining a pool of slowly cycling or resting growth plate chondrocytes. These may re-enter the cell cycle and fulfil all or part of their chondrogenic potential when the glucocorticoids have been removed.

which not only allows chondrocytes to be studied in isolation, but also provides a model of chondrogenesis, such that morphological differences can be analysed with time as the cells progress towards hypertrophy.^{27, 28} In the original description of this system, Lindahl and colleagues²⁷ showed that the formation of either small or large size colonies depended on whether the cells had originated from the proliferative or stem cell layers of the growth plate, respectively. Confirmation that these colonies arose from cells of differing chondrogenic status was provided by the stimulation of the cells at different stages of maturation by growth hormone and IGF-I,²⁸ consistent with the known *in vivo* effects of these peptides. We have continued to use this model and demonstrated, by staining for markers of a hypertrophic phenotype such as alkaline phosphatase activity and proteoglycan synthesis, the eventual terminal differentiation of the cells within these colonies.²⁹

Effects of cytotoxic drugs on growth plate chondrocytes *in vitro* and the implications for longitudinal bone growth

The first detailed *in vitro* experiments were performed by Morris,³⁰ who examined the effects of seven different cytotoxic drugs on IGF-I stimulated ³⁵S-sulphate and ³H-thymidine uptake in a porcine costal cartilage bioassay. These studies indicated that cytotoxic chemotherapy might have a direct effect on the response of chondrocytes to changes in the growth hormone-IGF-I axis. Growth disturbances result when there is a disruption of the normal cellular activity of growth plate chondrocytes and/or the cells of bone. In the growth plate this might manifest as either a reduction in cell number, and hence growth plate width, or the loss of the functional integrity of the cellular matrix. In Kniest dysplasia, the impaired production of collagen type II results in the formation of imperfect collagen fibrils,³¹ leading to the disruption of the organisation of chondrocytes, and thus their closely related proliferation and biosynthesis. In the treatment of proliferating neoplastic cells, cytotoxic chemotherapy disrupts normal cell division, damages genetic material, and hence affects dividing cells. Despite the suggestion by some that epiphyseal chondrocytes are hypoxic, the growth plate is supplied by the epiphyseal artery, which passes through the reserve zone, and branches of which end in the proliferating zone.³² Consequently, one would expect chemotherapeutic agents to have a direct effect on chondrocytes from both the proliferative and reserve (which, although they have a long generation time, do undergo one to two cell doublings) zones of the growth plate. Using *in vitro* monolayer and agarose suspension culture systems of isolated rat tibial growth plate chondrocytes, we demonstrated for the first time direct effects of a variety of chemotherapeutic drugs commonly used in the treatment of childhood malignancies on the proliferative capacity of these cells.³³ Despite differing toxicities of the DNA damaging agents cisplatin, etoposide, carboplatin, and

actinomycin D in these assay systems, all drugs targeted the proliferating chondrocyte populations, causing cell loss. This then left a relatively quiescent partially matured population of chondrocytes, incapable of much further cell division and unresponsive to the stimulatory effects of growth hormone and/or IGF-I. In contrast, the purine salvage pathway inhibitors 6-mercaptopurine and 6-thioguanine had little adverse effect on proliferating or quiescent chondrocytes, but acted instead to slow their proliferation, whereas the antimetabolite methotrexate had no effect on chondrocyte proliferation and differentiation in any of our assay systems. The latter observation was surprising, given that methotrexate is extremely effective at reducing tumour cell growth in a range of paediatric cancers. However, *in vitro* studies treating human articular cartilage and human bone derived osteoblasts with lower doses of methotrexate, equivalent to those offered to patients with rheumatoid arthritis, also demonstrated the absence of major adverse effects on articular cartilage, as well as an inhibition of osteoblast proliferation, but not differentiation, with this drug.³⁴

In addition to the above chemotherapeutic drugs, glucocorticoids are now frequently used in the treatment of childhood cancers, either directly as part of the chemotherapy protocol—for example, in lymphomas and ALL, or as anti-emetics during chemotherapy.³⁵ It has long been established that the chronic use of steroids reduces linear growth^{36, 37}; however, relatively small oral doses of daily exogenous glucocorticoids are also capable of slowing growth in some children and this is variably counterbalanced by growth hormone treatment.³⁸ The growth suppressing effects of these agents are multifactorial, including a direct suppressive effect on matrix production and synthesis of local growth factors. Although a detailed discussion of these mechanisms is beyond the scope of this review, it should be mentioned that the inhibitory effects of glucocorticoids on bone growth have long been thought to be caused, at least in part, by antagonism of growth hormone action. A time and dose dependent downregulation of growth hormone receptor mRNA expression and binding capacity after dexamethasone treatment of growth plate chondrocytes, together with a reduction in the local production of IGF-I, has been demonstrated recently.³⁹ In addition, in the mouse, growth hormone administration can decrease or even prevent some of the damage to growing bones inflicted by high dose glucocorticoid treatment, such as reduced growth plate width, mineral bone content and acid, and alkaline phosphatase activity.⁴⁰ In contrast to the actions of the cytotoxic drugs, we found that the glucocorticoids, prednisolone, and dexamethasone act merely to slow or delay the growth of chondrocytes by maintaining a resting or slowly cycling population of immature cells, which remain committed to chondrogenesis.³³ After the removal of the glucocorticoids these cells would then re-enter the chondrogenic pathway and

undergo maturation (proliferation and hypertrophy), fulfilling all or at least part of their chondrogenic potential, consistent with the in vivo phenomenon of catch up growth. In support of this, we have since demonstrated that chondrocytes pretreated with the glucocorticoids are not only able to undergo clonal expansion and maturation when subcultured in suspension without the glucocorticoids, but also that their growth rate is enhanced by growth hormone and more significantly by IGF-I.²⁹ Similarly, in an in vivo study, dexamethasone was shown to slow proximal tibial growth rate, when infused for four weeks in 6 week old rabbits, compared with the contralateral vehicle treated limb.⁴¹ After dexamethasone treatment was stopped, local catch up growth was observed in the affected growth plate only, suggesting a mechanism intrinsic to the growth plate.

Based on our data we have proposed a model in which cytotoxic drugs irreversibly deplete chondrocyte cell numbers, whereas glucocorticoids have a profound but transient effect on chondrocyte proliferative capacity. However, it is also possible that, although glucocorticoid effects are transient, they might occur during important periods of rapid growth and/or be important in the timing of growth plate closure and so affect the overall growth a bone achieves. Furthermore, as already mentioned, the two main variables contributing to the rate of longitudinal bone growth are the rate of new cell production and the average height of the hypertrophic cells. Thus, suppression of the proliferation of non-stem daughter cells by glucocorticoids might reduce the numbers of hypertrophic chondrocytes arising from each stem cell division. A decrease in clone size for each stem cell division might then lead to irreversible loss of linear growth.

This work has highlighted a poorly studied area and, although our observations localise some of the effects of chemotherapy to the growth plate, many questions regarding the issue of chemotherapy induced growth impairment remain unanswered. Histological and cell kinetic studies using in vivo models of longitudinal bone growth are now essential to provide further evidence to support our data and proposed model, as well as increasing our understanding of the cellular mechanisms involved.

In conclusion, although we have direct evidence that chemotherapeutic agents affect the proliferative capacity of growth plate chondrocytes in vitro we do not know how this will translate into final achievable height. The implications for understanding growth retardation and the phenomenon of catch up growth associated with chemotherapy and/or glucocorticoid treatment are still not clear, and with progressively more intensive chemotherapy regimens, normal tissue growth is likely to be increasingly affected. These studies emphasise the need not only to understand the mechanisms by which these agents damage normal chondrocytes but also to determine how the growth potential might be maintained during treatment for childhood malignancy.

- Schriock EA, Schell MJ, Carter M, Hustu O, Ochs JJ. Abnormal growth patterns and adult short stature in 115 long-term survivors of childhood leukemia. *J Clin Oncol* 1991;9:400-5.
- Thun-Hohenstein L, Frisch H, Schuster E. Growth after radiotherapy and chemotherapy in children with leukemia or lymphoma. *Horm Res* 1992;37:91-5.
- Caruso-Nicoletti M, Mancuso M, Spadaro G, Dibeneditto SP, DiCataldo A, Schiliro G. Growth and growth hormone in children during and after therapy for acute lymphoblastic leukaemia. *Eur J Pediatr* 1993;152:730-3.
- Glasser DB, Duane K, Lane JM, Healey JH, Caparros-Sison B. The effect of chemotherapy on growth in the skeletally immature individual. *Clin Orthop* 1991;262:93-100.
- Holm K, Nysom K, Hertz H, Muller J. Normal final height after treatment for acute lymphoblastic leukemia without irradiation. *Acta Paediatr* 1994;83:1287-90.
- Ahmed SF, Wallace WH, Kelnar CJ. An anthropometric study of children during intensive chemotherapy for acute lymphoblastic leukaemia. *Horm Res* 1997;48:178-83.
- Kirk JA, Raghupathy P, Stevens MM, et al. Growth failure and growth hormone deficiency after treatment for acute lymphoblastic leukaemia. *Lancet* 1987;i:190-3.
- Clayton PE, Shalet SM, Morris-Jones PH, Price DA. Growth in children treated for acute lymphoblastic leukaemia. *Lancet* 1988;i:460-2.
- Mohnike K, Dorffle W, Timme J, et al. Final height and puberty in 40 patients after anti-leukaemic treatment during childhood. *Eur J Pediatr* 1997;156:272-6.
- Crofton PM, Ahmed SF, Wade JC, et al. Effects of intensive chemotherapy on bone and collagen turnover and the growth hormone axis in children with acute lymphoblastic leukemia. *J Clin Endocrinol Metab* 1998;83:3121-9.
- Olshan JS, Gubernick J, Packer RJ, et al. The effects of adjuvant chemotherapy on growth in children with medulloblastoma. *Cancer* 1992;70:2013-17.
- Ogilvy-Stuart AL, Shalet SM. Growth and puberty after GH treatment after irradiation for brain tumours. *Arch Dis Child* 1995;73:141-6.
- Shalet SM, Whitehead E, Chapman AJ, Beardwell CG. The effects of growth-hormone therapy in children with radiation-induced growth-hormone deficiency. *Acta Paediatr Scand* 1981;70:81-6.
- Lannering B, Albertsson-Wikland K. Improved growth-response to GH treatment in irradiated children. *Acta Paediatr Scand* 1989;78:562-7.
- Samuelsson BO, Marky I, Rosberg S, Albertsson Wikland K. Growth and growth hormone secretion after treatment for childhood non-Hodgkin's lymphoma. *Med Pediatr Oncol* 1997;28:27-34.
- Bar-On E, Beckwith JB, Odom LF, Eilert RE. Effect of chemotherapy on human growth plate. *J Pediatr Orthop* 1993;13:220-4.
- Hunziker EB. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. *Microsc Res Tech* 1994;28:505-19.
- Abad V, Uyeda JA, Temple HT, De Luca F, Baron J. Determinants of spatial polarity in the growth plate. *Endocrinology* 1999;140:958-62.
- Kember NF. Cell kinetics and the control of growth in long bones. *Cell Tissue Kinet* 1978;11:477.
- Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Sliotweg MC. Growth hormone and bone. *Endocr Rev* 1998;19:55-79.
- Williams GR, Robson H, Shalet SM. Thyroid hormone actions on cartilage and bone: interactions with other hormones at the epiphyseal plate and effects on linear growth. *J Endocrinol* 1998;157:391-403.
- Price JS, Oyajobi BO, Russell RGG. The cell biology of bone growth. *Eur J Clin Nutr* 1994;48:S131-49.
- Lanske B, Karaplis AC, Lee K, et al. PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science* 1996;273:663-6.
- Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science* 1996;273:613-22.
- Green H, Morikawa M, Nixon T. A dual effector theory of growth hormone action. *Differentiation* 1985;29:195.
- Oberbauer AM, Peng R. Growth hormone and IGF-1 stimulate cell function in distinct zones of the rat epiphyseal growth plate. *Connect Tissue Res* 1995;31:189-95.
- Lindahl A, Isgaard J, Nilsson A, Isaksson OGP. Growth hormone potentiates colony formation of epiphyseal chondrocytes in suspension culture. *Endocrinology* 1986;118:1843-8.
- Lindahl A, Isgaard J, Carlsson L, Isaksson OGP. Differential effects of growth hormone and insulin-like growth factor I on colony formation of epiphyseal chondrocytes in suspension culture in rats of different ages. *Endocrinology* 1987;121:1061-9.
- Robson H, Anderson E, Eden OB, Isaksson O, Shalet SM. Growth plate chondrocytes remain responsive to both IGF-I and GH following treatment with chemotherapeutic agents. *Horm Res* 1998;50(suppl 3):O33.
- Morris MJ. In vitro effects of anti-leukaemic drugs on cartilage metabolism and their effects on somatomedin production by the liver. PhD Thesis, Manchester University, 1981.
- Poole AR, Pidoux I, Riener A, et al. Kniest dysplasia is characterised by an apparent abnormal processing of the C-propeptide of type II cartilage collagen resulting in imperfect fibril assembly. *J Clin Invest* 1988;81:579-89.

- 32 Shapiro IM, Mansfield KD, Evans SM, Lord EM, Koch CJ. Chondrocytes in the endochondral growth cartilage are not hypoxic. *Am J Physiol* 1997;272:C1134-43.
- 33 Robson H, Anderson E, Eden OB, Isaksson O, Shalet SM. Chemotherapeutic agents used in the treatment of childhood malignancies have direct effects on growth plate chondrocyte proliferation. *J Endocrinol* 1998;157:225-35.
- 34 Van der Veen MJ, Scheven BAA, Van Roy JLAM, Damen CA, Lafeber FPJG, Bijlsma JWJ. In vitro effects of methotrexate on human articular cartilage and bone derived osteoblasts. *Br J Rheumatol* 1996;35:342-9.
- 35 Coleman RE. Glucocorticoids in cancer therapy. *Biotherapy* 1992;4:37-44.
- 36 VanMetre TE, Jr, Pinkerton HL, Jr. Growth suppression in asthmatic children receiving prolonged therapy with prednisone or methyl-prednisone. *J Allergy* 1959;30:103-13.
- 37 Falliers CJ, Szentivanyi J, Jorgensen JR, Vukantz SC. Childhood asthma and steroid therapy have influences on growth. *Am J Dis Child* 1963;105:127-37.
- 38 Allen DB. Growth suppression by glucocorticoid therapy. *Endocrinol Metab Clin North Am* 1996;25:699-717.
- 39 Jux C, Leiber K, Hugel U, et al. Dexamethasone impairs growth hormone (GH)-stimulated growth by suppression of local insulin-like growth factor (IGF)-I production and expression of GH- and IGF-I-receptor in cultured rat chondrocytes. *Endocrinology* 1998;139:3296-305.
- 40 Altman A, Hochberg Z, Silbermann M. Interactions between growth hormone and dexamethasone in skeletal growth and bone structure of the young mouse. *Calcif Tissue Int* 1992;51:298-304.
- 41 Baron J, Oerter Klein K, Colli MJ, et al. Catch-up growth after glucocorticoid excess: a mechanism intrinsic to the growth plate. *Endocrinology* 1994;135:1367-71.