2. Fentiman IS, Caleffi M, Brame K, Chaudary MA, Hayward JL. Double blind controlled trial of tamoxifen therapy for mastalgia. *Lancet* 1986, i, 287–288.
3. Love RR. Antioestrogen chemoprevention of breast cancer: critical issues and research. *Prev Med* 1991, **20**, 64–78.
4. Aitken JM, Hart DM, Anderson JB, Lindsay R, Smith DA, Speirs CF. Osteoporosis after oophorectomy for non-malignant disease in premenopausal women. *Br Med J* 1973, ii, 325–328.
5. Nachtigall LE, Nachtigall RH, Nachtigall RD, Beckman EM, Estrogen replacement therapy; a 10 year prospective study in the relationship to osteoporosis. *Obstet Gynaecol* 1979, **53**, 277–281.
6. Eriksen EF, Colvard DS, Berg NJ, *et al.* Evidence of oestrogen receptors in normal human osteoblast-like cells. *Science* 1988, **241**, 84–86.
7. Jordan C, Phelps E, Lindgren U. Effects of antioestrogens on bone in castrated and intact female rats. *Breast Cancer Res* 1987, **10**, 31–35.
8. Turner R, Wakley G, Hannon K, Bell N. Tamoxifen inhibits osteoclast mediated resorption of trabecular bone in ovarian hormone deficient rats. *Endocrinology* 1988, **122**, 1146–1150.
9. Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton LJ. Differential changes in bone mineral density of the appendicular and axial skeleton with aging. *J Clin Invest* 1981, **67**, 328–335.
10. Gotfredsen A, Christiansen C, Palshof T. The effect of tamoxifen on bone mineral content in premenopausal women with breast cancer. *Cancer* 1984, **53**, 853–857.
11. Fornander T, Rutqvist LE, Sjoberg HE, Blomqvist L, Mattsson A, Glas U. Long term adjuvant tamoxifen in early breast cancer: effect on bone mineral density in post menopausal women. *J Clin Oncol* 1990, **8**, 1019–1024.
12. Turken S, Siris E, Seldin D, Flaster E, Hyman G, Lindsay R. Effects of tamoxifen on spinal bone density in women with breast cancer. *J Natl Cancer Inst* 1989, **81**, 1086–1088.
13. Fentiman IS, Caleffi M, Rodin A, Murby B, Fogelman I. Bone mineral content of women receiving tamoxifen for mastalgia. *Br J Cancer* 1989, **60**, 262–264.
14. Love RR, Mazess RB, Tormey DC, Barden HC, Newcomb PA, Jordan CV. Bone mineral density in women with breast cancer treated with adjuvant tamoxifen for at least 2 years. *Breast Cancer Res* 1988, **12**, 297–301.
15. Dawes P, Wilson A, McKenzie A. Bone mineral content following administration of adjuvant tamoxifen to women aged between 37 and 59 years. *Br J Cancer* 1990, **62**, 34.
16. Wright C, Evans W, Mansell R, Compston J. The effect of long term tamoxifen on bone. *Clin Science* 1990, **79** (suppl. 23), 30.
17. Kalef-Ezra J, Glaros D, Klouvas G, *et al.* New evidence that tamoxifen does not induce osteoporosis: a nuclear activation analysis and absoptiometry study. *Br J Radiol* 1992, **65**, 417–420.
18. Cuzick J, Allen D, Baum M, *et al.* Long term effects of tamoxifen. *Eur J Cancer* 1992, **29**, 15–21.
19. Love RR, Mazess RB, Barden HS, *et al.* Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *N Engl J Med* 1992, **326**, 852–856.
20. Citrin DL, Ingle J, O'Fallon J, *et al.* Effect of diethylstilboestrol and tamoxifen on serum calcium and phosphate in metastatic breast cancer. *Proc Int Breast Cancer Res Conf London* 1985, abstract C-15.

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Insulin-like Growth Factor (IGF)-I, -II and IGF Binding Protein-2 (IGFBP-2) in the Plasma of Children with Wilms' Tumour

Walter Zumkeller, Juerg Schwander, Christopher D. Mitchell, David J. Morrell, Paul N. Schofield and Michael A. Preece

Insulin-like growth factors (IGF)-I, -II and IGF binding protein-2 (IGFBP-2) have been measured in plasma of children with Wilms' tumour. The mean levels for total serum IGF-I and -II were not significantly altered in Wilms' tumour as compared with normal control plasma. However, the chromatographic profiles for IGF-I and -II in these groups were different with regard to the presence of IGF binding proteins and high molecular weight forms of IGFs; the high molecular weight form (9–15 kD) of IGF-II was significantly reduced in Wilms' tumour. Levels of IGFBP-2 were substantially elevated in serum from Wilms' tumour patients (1025 ± 112 ng/ml compared with 416 ± 44 ng/ml in controls), and inversely correlated with the levels of high molecular weight forms of IGF-II. We suggest that IGFBP-2 measurements might be of value as a marker for monitoring this type of tumour, either as an adjunct to diagnosis or surveillance of tumour growth during therapy.
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INTRODUCTION

INSULIN-LIKE growth factors (IGF)-I and -II are growth-promoting polypeptides involved in a variety of physiological and pathological processes of the kidney [1]. In circulation, they are associated with specific binding proteins (IGFBP) which both extend their half-life and modulate their actions. The synthesis of these binding proteins is under a complex, as yet poorly understood, system of regulation [2].

The expression of IGFs depends upon the state of tissue differentiation and undergoes dynamic changes during fetal development [1]. IGF-II mRNA has been found to be highly expressed in embryonic tissues [3–5] and in a number of embryonal tumours [6–8], including nephroblastoma (Wilms' tumour, WT) [9–11], pheochromocytoma [11] and hepatoblastoma [8].

During embryonic development, IGFBP-2 expression is

Table 1. Sex, age, stage and histological features, IGFs and IGFBP-2 in patients with WT

Group	Sex	Age (years)	Stage/ histology	IGF-I (ng/ml)	IGF-II (ng/ml)	High M_r IGF-II (% of total)	IGFBP-2 (ng/ml)
WT 1	Female	0.1	V FH	193	362	(47.8)	—
WT 2	Female	0.4	II FH	—	—	—	1140
WT 3	Male	0.5	II FH	30	874	(40.2)	—
WT 4	Male	0.7	I FH	160	854	(1.3)	1360
WT 5	Female	0.8	III FH	134	1034	(1.6)	—
WT 6	Male	0.8	II MB	81	172	(29.1)	1170
WT 7	Male	0.8	I MB	90	104	(11.5)	1240
WT 8	Female	0.8	I FH	161	560	(2.3)	1170
WT 9	Male	1.1	I FH	100	174	(59.8)	580
WT 10	Male	1.4	II FH	116	208	(39.4)	410
WT 11	Female	2.3	I FH	—	—	—	1580
WT 12	Female	2.5	III FH	116	324	(12.7)	—
WT 13	Female	3.0	II FH	—	—	—	560
WT 14	Female	3.3	III UH	103	190	(46.3)	—
WT 15	Female	3.4	III FH	—	—	—	1610
WT 16	Female	3.8	V FH	45	176	(21.0)	—
WT 17	Female	5.3	III FH	164	470	(24.9)	1170
WT 18	Female	5.3	II FH	—	—	—	800
WT 19	Female	5.5	I FH	53	131	(46.6)	—
WT 20	Female	5.7	III FH	133	251	(25.5)	—
WT 21	Female	9.6	III FH	62	195	(45.1)	—
WT 22	Male	10.5	IV FH	—	—	—	540

FH/UH, favourable/unfavourable histology. MB (mesoblastic) = UH. The term unfavourable histology refers to the presence of diffuse anaplasia, rhabdoid tumour, or bone metastasising renal tumour. Tumour stage classification is in accordance with the National Wilm's Tumour Study [29]. (—), no results due to lack of material.

associated with undifferentiated embryonic stem cells, suggesting a function in early embryogenesis [12]. The cellular sites of IGFBP expression are often distinct from sites of IGF synthesis [13]. Furthermore, the IGFBP-2 gene transcript is detectable in the developing mesonephric ducts and tubules of 10-day-old rats [14; data not shown], whereas IGF-II expression is largely restricted to the metanephric blastema [5]. As yet, the roles of these peptides in organogenesis and cell differentiation, and their regulation, remains obscure.

The purpose of this study was to determine whether the IGF-I, IGF-II and IGFBP-2 levels were significantly different in the plasma of patients with WT compared with controls and to investigate their potential as tumour markers.

PATIENTS AND METHODS

Subjects

Samples were obtained from children with WT (mean age ± S.E.M., 3.1 ± 0.6 years; n = 22). Samples were taken at the Hospital for Sick Children, Great Ormond Street, London, prior to initiation of therapy. Each patient underwent routine and radiological investigations and histological examination of the surgical specimen (Table 1). Control samples were obtained from healthy children (4.5 ± 0.9 years; n = 13) in conjunction with an ongoing study at King's College, London. Permission was granted by the local ethical comittee.

Gel chromatography and radioimmunoassays

Plasma samples (250 µl) were incubated overnight with 250 µl of 2 mol/l acetic acid (pH 4.0) and chromatographed (flow rate: 0.2 ml/min) on a Sephacryl S-200 HR column (30 × 1 cm; Pharmacia, Uppsala, Sweden) equilibrated with 1 mol/l acetic acid. Fractions of 1 ml were collected and aliquots of 250 µl were lyophilised. Fractions equivalent to a kD between 0.80–0.95 (for canonical IGF-I and IGF-II) and 0.62–0.77 (for high molecular weight IGF-II, equivalent to a M_r of 9000–15 000) were analysed by specific radioimmunoassays following resuspension in 0.05 mol/l phosphate buffer (pH 7.4).

IGF-I was measured as previously described [15] using recombinant IGF-I (KabiGen AB, Stockholm) as a standard. IGF-II was measured using a monoclonal antibody to IGF-II (MAS 324, clone SI-F2) from Seralab Ltd (Crawley Down, Sussex, U.K.) and recombinant IGF-II (KabiGen AB) as a standard. Each antibody vial contained 10 µg of IgG and was reconstituted in 1 ml buffer. The initial dilution of the stock solution added to the assay was 1:6000. The biosynthetic IGFs were labelled with ^{125}I (Amersham, U.K.) using the lactoperoxidase method. The radioactive labelled peptides were diluted in order to obtain about 10 000 counts/min in 50 µl of [^{125}I]IGF-I or [^{125}I]IGF-II.

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Revised 19 May 1993; accepted 29 June 1993.

The samples were incubated overnight with specific antibodies and either [^{125}I]IGF-I or [^{125}I]IGF-II. Precipitation of the complex was achieved by adding 500 μl of polyethylene glycol (Sigma, U.K.) and centrifugation at 12000 *g* for 20 min. The supernatant was aspirated and the pellet counted in a gamma counter for 4 min. In both assays, the cross-reactivity of IGF-I or IGF-II was below 1% whereas the intra-assay variation was below 10% for both peptides. IGFBP-2 was determined in unseparated plasma samples using polyclonal antibodies raised against human IGFBP-2 as previously described [16].

Statistics

Values are expressed as means ± S.E.M. Statistical significance was assessed using Student's *t*-test.

RESULTS

The plasma IGF-I, IGF-II and IGFBP-2 levels of the individual tumour patients are listed in Table 1. The IGFBP-2 levels were significantly increased in 10 of 13 WT samples measured; two were at the extreme upper limit of normality as judged by our control group ($P < 0.0001$) (Table 2). The IGF-I levels were not significantly different in either the control or WT group. The WT group had significantly lower levels of high molecular weight IGF-II ($P < 0.0001$) as compared with the control group but the difference in total IGF-II between the two groups was of borderline significance ($P = 0.09$).

The chromatographic profiles for IGF-I and IGF-II from two typical individuals of each group are shown in Fig. 1. The profiles for IGF-I and IGF-II in these two groups were different. The differences at kD < 0.5 were probably due to different levels of assay interference by binding proteins. In addition, there was also evidence for high molecular weight forms of IGFs. Whereas only 28.4% of total IGF-II was in the high molecular weight range (9–15 kD) in the WT group, the percentage for the control group was 50.5%. There was a negative correlation between high molecular weight IGF-II and IGFBP-2 levels in the control ($P < 0.05$; $r = 0.62$) and combined WT/control group ($P < 0.001$; $r = 0.75$; $n = 20$) whereas it had marginal significance in the WT group alone. There was no correlation between IGF-I and IGF-II values in either group. No sex-related differences in peptide levels were observed and there was no apparent age-dependency of IGF and IGFBP-2 levels.

DISCUSSION

We have found that plasma IGFBP-2 levels are elevated in children with WT, whereas their IGF-II levels are marginally lower than in normal controls. Although high levels of IGF-II mRNA expression have been reported in WT [9, 10], it has also been shown that elevated IGF-II mRNA expression is not a necessary event in the genesis of WT [8, 17]. Furthermore, Haselbacher *et al.* [11] found low IGF-II peptide levels in WT tissue extracts, in marked contrast to the elevated IGF-II mRNA expression levels which also included a large amount of a high molecular weight form of IGF-II. In spite of high IGF-II peptide and mRNA expression levels in pheochromocytoma, peripheral IGF-II levels in these tumour patients are not elevated, suggesting that insignificant tumour-derived IGF-II is released into the circulation, unlike the analogous situation with tumour production of catecholamines [18].

The regulation of IGFBP-2 is as yet poorly understood. WT cell lines are known to secrete significant amounts of IGF-II, including high molecular weight forms (9–15 kD), as well as a variety of IGF binding proteins [19]. This high molecular weight form of IGF-II is poorly characterised, but is present as a minor component in normal adult serum probably as a consequence of post-translational modification of the prepro-IGF-II molecule [2]. A possible explanation for the increased IGFBP-2 levels in WT might be the synthesis of different molecular forms, e.g. high molecular weight IGF or IGF analogues capable of inducing the production of IGFBP-2 by renal as well as other tissues. However, it is intriguing that the percentage of high molecular mass IGF-II (9–15 kD) was significantly lower in the WT group than in the control group. In addition, our data suggest a suppression of IGFBP-2 levels by high molecular weight IGF-II in the control group but not in the WT group, indicating a disregulation of IGFBP-2 production in this group.

IGFBP-2 was first identified as an inhibitor of IGF action by sequestration of IGFs from their receptors. Recent reports support the widespread occurrence of IGFBP-2 mRNA expression in cell lines derived from (among others) WT, colon carcinoma, breast cancer, retinoblastoma and neuroblastoma [20, 21] suggesting that expression of IGFBP-2 may be associated with malignancy; elevation of serum levels, however, has only been noted in the case of lung cancer [22]. The correlation between IGFBP-2 and malignancy strongly suggests that its role in neoplasia may either be to stimulate cell growth or invasiveness (properties not demonstrated for IGFBP-2) or alternatively to inhibit the effect of a tumour suppressing agent. IGF-II has been implicated in suppression of tumour formation [23]. Consequently, the mechanism of disregulation of IGFBP-2 is of great interest.

It is possible that IGFBP-2 expression is being induced by increased local concentrations of the cognate growth factors, but only IGF-I and IGF-I B chain (which has a decreased affinity for the binding proteins) have such an effect [24]. Patients with extrapancreatic tumour hypoglycaemia usually have both increased IGF-II and IGFBP-2 plasma levels [25].

Synthesis of proteins usually associated with a 'fetal-like' pattern of gene expression is seen in many neoplasms and may reflect the normal pattern of stem cell activity amplified to an inordinate degree by clonal selection in the tumour [26]. Enhanced production of IGFBP-2 may then reflect a normal pattern of synthesis by a population of cells in the fetal kidney or brain; *in situ* hybridisation to human fetal tissues indicates that the epithelial component of the developing kidney is one of several sites of high IGFBP-2 mRNA expression (data not shown).

IGFBP-2 levels have so far only been known to be elevated in fetal serum [27], hypopituitarism [27, 28], Laron dwarfs and

Table 2. Plasma levels (mean ± S.E.M.) of IGF-I and IGF-II (ng/ml) in patients with WT and control samples after gel chromatography. Immunoreactivity for high molecular forms of IGF-II (9–15 kD) was found in addition to the 7.5 kD form of IGF-II

	IGF-I (ng/ml)	IGF-II (ng/ml) 9 kD	< 9 kD	Total	IGFBP-2 (ng/ml)
WT	109 ± 12 (n = 16)	82 ± 21* (n = 16)	298 ± 72 (n = 16)	380 ± 74 (n = 16)	1025 ± 112* (n = 13)
HC	93 ± 10 (n = 13)	279 ± 36 (n = 13)	275 ± 30 (n = 13)	553 ± 60 (n = 13)	416 ± 44 (n = 13)

*Significantly different from control $P < 0.0001$. WT, Wilms' tumour; HC, healthy controls.

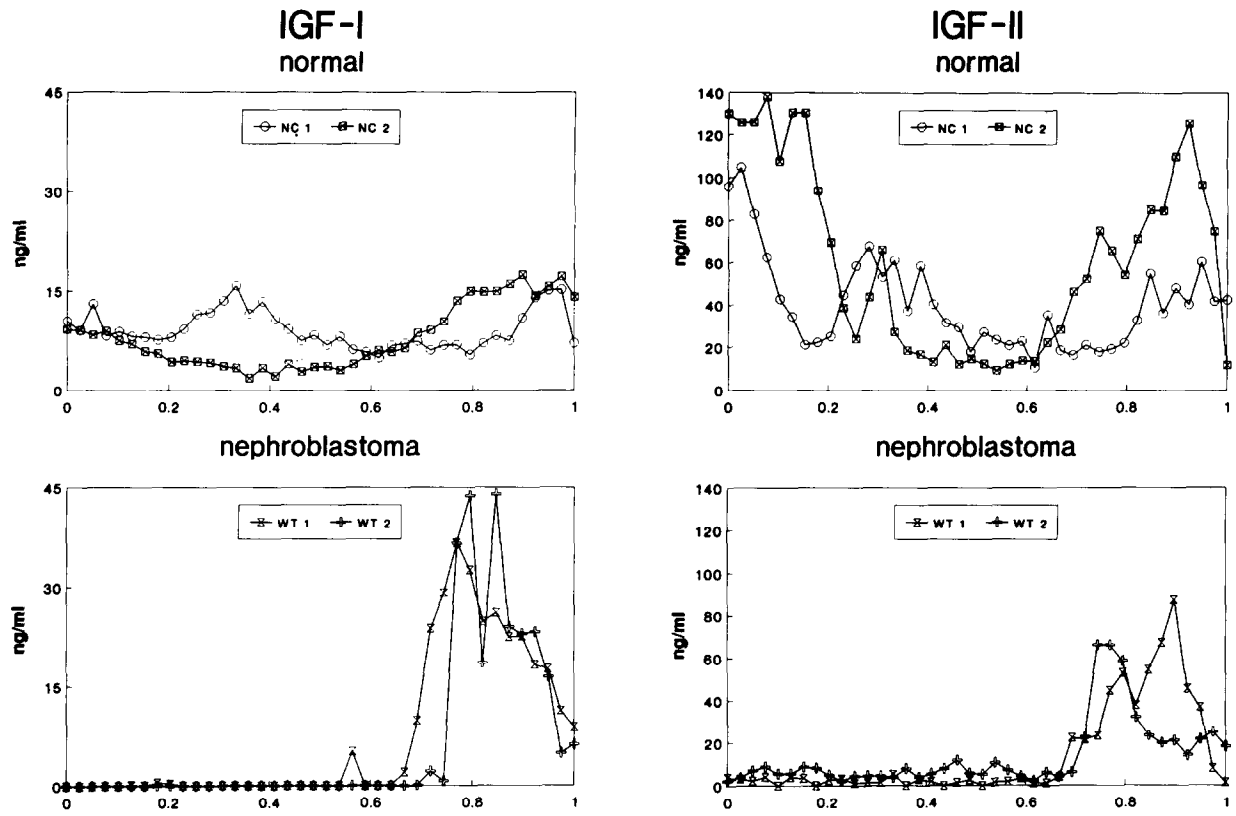

Fig. 1. Plasma IGF-I and IGF-II chromatography profiles from two children with WT as well as two healthy controls (HC) after acid gel filtration on a Sephacryl S-200 HR column. The horizontal axis represents the collected fraction numbers expressed as a proportion of the eluted volume between V_o and V_i (KD).

pygmies [28]. These conditions are readily distinguished from neoplastic processes. Therefore, our observation that IGFBP-2 levels are elevated in the plasma of patients with WT would suggest that IGFBP-2 might be used to monitor tumour growth. The recent report of elevated IGFBP-2 serum levels in patients suffering from lung cancer suggests that the monitoring of IGFBP-2 levels may be generally useful in a range of malignancies [22].

1. Zumkeller W, Schofield PN. The role of insulin-like growth factors and IGF-binding proteins in the physiological and pathological processes of the kidney. *Virchows Archiv B Cell Pathol* 1992, **62**, 207–220.
2. Humbel RE. Insulin-like growth factors I and II (review). *Eur J Biochem* 1990, **190**, 445–462.
3. Lund PK, Moats-Staats BM, Hynes MA, *et al.* Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. *J Biol Chem* 1986, **261**, 14539–14544.
4. Stylianopoulou F, Efstratiadis A, Herbert J, Pintar J. Pattern of insulin-like growth factor II gene expression during rat embryogenesis. *Development* 1988, **103**, 497–506.
5. Brice AL, Cheetham JE, Bolton VN, Hill NCW, Schofield PN. Temporal changes in the expression of the insulin-like growth factor II gene associated with maturation in the human fetus. *Development* 1989, **106**, 543–554.
6. Tricoli JV, Rall LB, Karakovsis CP, *et al.* Enhanced levels of insulin-like growth factor messenger RNA in human colon carcinomas and liposarcomas. *Cancer Res* 1986, **46**, 6169–6173.
7. Daughaday WH, Emanuele MA, Brooks MH, Barbato AL, Kapadia MS, Rotwein P. Synthesis and secretion of insulin-like growth factor II by a leiomyosarcoma with associated hypoglycaemia. *N Engl J Med* 1988, **319**, 1434–1440.
8. Schofield PN, Engström W. Insulin-like growth factors in human cancer. In Schofield PN, ed. *The Insulin-like Growth Factors; Structure and Biological Functions*. Oxford, Oxford University Press, 1992, 240–257.
9. Reeve AE, Eccles MR, Wilkins, RJ, Bell GI, Millow LJ. Expression of insulin-like growth factor-II transcripts in Wilms' tumour. *Nature* 1985, **317**, 258–260.
10. Scott J, Cowell ME, Robertson ME, *et al.* Insulin-like growth factor II in Wilms' tumors and embryonic tissues. *Nature* 1985, **317**, 260–262.
11. Haselbacher GK, Irminger JC, Zapf J, Ziegler WH, Humbel RE. Insulin-like growth factor II in human adrenal pheochromocytomas and Wilms' tumors: expression at the mRNA and protein level. *Proc Natl Acad Sci USA* 1987, **84**, 1104–1106.
12. Schwander J, Mary J-L, Landwehr J, Böni M, Margot J, Binkert C. The regulation of the mRNA of an insulin-like growth factor binding protein (IBP-2) in the rat. In Drop SLS, Hintz RL, eds. *Insulin-like Growth Factor Binding Proteins*. Amsterdam, Excerpta Medica, 1989, 125–131.
13. Hill DJ, Han VKM, Clemmons DR, Camacho-Hubner C, Wang JF. Expression, distribution and biological action of insulin-like growth factor binding proteins during development. In Drop SLS, Hintz RL, eds. *Insulin-like Growth Factor Binding Proteins*. Amsterdam, Excerpta Medica, 1990, 55–61.
14. Wood TL, Streck RD, Pintar JE. Expression of the IGFBP-2 gene in post-implantation rat embryos. *Development* 1992, **114**, 59–66.
15. Morrell DJ, Dadi H, More J, *et al.* A monoclonal antibody to human insulin-like growth factor I: characterisation, use in radioimmunoassay and effect on the biological activities of the growth factor. *J Mol Endocrinol* 1989, **2**, 201–206.
16. Mary J-L, Schwander J. A radioimmunoassay for hIGFBP-2. *Program of the 2nd International Symposium on Insulin-like Growth Factors/Somatomedins*, 12–16 January 1991, San Francisco (U.S.A.), 1991, 234.
17. Little MH, Aldett G, Smith PJ. Enhanced expression of insulin-like growth factor II is not a necessary event in Wilms' tumour progression. *Carcinogenesis* 1987, **8**, 865–868.
18. Gelato MC, Vassalotti J. Insulin-like growth factor-II: possible local growth factor in pheochromocytoma. *J Clin Endocrinol Metab* 1990, **71**, 1168–1174.
19. Zumkeller W, Schofield PN, Morrell DJ, Preece MA. Secretion of insulin-like growth factors by Wilms' tumor cells. 11th Joint Meeting of British Endocrine Societies, 23–26 March 1992, Harrogate. *J Endocrinol* 1992, **132** (suppl.), abstract 151.

20. Reeve JG, Kirby LB, Brinkman A, Hughes SA, Schwander J, Bleehen NM. Insulin-like growth factor-binding protein gene expression and protein production by human tumour cell lines. *Int J Cancer* 1992, **51**, 818–821.
21. Macaulay VM. Insulin-like growth factors and cancer. *Br J Cancer* 1992, **65**, 311–320.
22. Reeve JG, Payne JA, Bleehen NM. Production of immunoreactive insulin-like growth factor I (IGF-I) and IGF-I-binding proteins by human lung tumours. *Br J Cancer* 1990, **61**, 727–731.
23. Schofield PN, Lee A, Hill DJ, Cheetham JE, James D, Stewart C. Tumour suppression associated with expression of human insulin-like growth factor II. *Br J Cancer* 1991, **63**, 687–692.
24. Cohick VS, Clemmons DR. Regulation of insulin-like growth factor binding protein synthesis and secretion in a bovine epithelial cell line. *Endocrinology* 1991, **129**, 1347–1354.
25. Zapf J. Schmid C, Guler HP, *et al.* Regulation of binding proteins for insulin-like growth factors (IGF) in humans. *J Clin Invest* 1990, **86**, 952–961.
26. Pierce GB, Speers WC. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res* 1988, **48**, 1996–2004.
27. McCusker RH, Cohick WS, Busby WH, Clemmons DR. Evaluation of the developmental and nutritional changes in porcine insulin-like growth factor-binding protein-1 and -2 serum levels by immunoassay. *Endocrinology* 1991, **129**, 2631–2638.
28. Hardouin S, Gourmelen M, Noguiez P, *et al.* Molecular forms of serum insulin-like growth factor (IGF) binding proteins in man: relationships with growth hormone and IGFs and physiological significance. *J Clin Endocrinol Metab* 1989, **69**, 1291–1301.
29. D'Angio GJ, Breslow N, Beckwith JB, *et al.* Treatment of Wilms' tumour; results of the third national Wilms' tumour study. *Cancer* 1989, **64**, 349–360.

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Reconstitution of Recombinant Interleukin-2 (rIL-2): a Comparative Study of Various rIL-2 Muteins

Lodewijk Th. Vlasveld, Jos H. Beijnen, Johan J. Sein, Elaine M. Rankin, Cornelis J.M. Melief and Annemarie Hekman

In a previous clinical study using a continuous infusion schedule of recombinant interleukin-2 (rIL-2) we noted a nearly complete loss of activity of EuroCetus rIL-2 when dissolved in 10 ml saline and infused at a very low rate through a plastic infusion device. In the present study, we demonstrated that the loss resulted from a concentration-dependent precipitation of rIL-2 in saline and adherence of the protein to the tubing material. These phenomena were not noted for four other rIL-2 muteins tested [Glaxo, Hoffmann-LaRoche, Amgen (2 muteins)]. EuroCetus rIL-2 was found to be completely soluble in water and 5% glucose.
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INTRODUCTION

THE BIOLOGICAL effects of recombinant interleukin-2 (rIL-2) depend on a variety of factors such as the dosage, schedule and route of administration [1]. Recently, it was demonstrated that the mode of administration of EuroCetus rIL-2 may dramatically influence its bioavailability [2–5]. Emphasis has been put on the addition of albumin to the solution to prevent adherence of rIL-2 to the tubing material [2, 4, 5]. We previously reported a nearly complete loss of bioactivity of EuroCetus rIL-2 when dissolved in a small volume of saline and infused at a low rate [3]. In this present study, we examined the cause and the extent of the loss of bioactivity of various rIL-2 muteins dissolved in 10 ml of saline and pumped slowly (0.5 ml/h) through a long infusion device and studied the effect of the addition of albumin. In addition, the solubility of EuroCetus rIL-2 in saline, glucose and water was tested.

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MATERIALS AND METHODS

Interleukin-2

The rIL-2 muteins provided as lyophilised powder were reconstituted in 1.2 ml sterile water (EuroCetus) or 0.9% saline (Hoffmann–LaRoche, Glaxo) according to the manufacturer's instructions. Both Amgen rIL-2 muteins were provided dissolved in 5% glucose. Before pumping, the muteins were diluted to the required rIL-2 concentration in 10 ml of the solvent to be tested.

Infusion device

The central venous access (Vascuport®) consisting of a titanium portal with a silicone membrane and a polyurethane