320

SHORT NORMAL STATURE - PSYCHOLOGICAL RESPONSE TO GROWTH HORMONE TREATMENT. L.D.Voss, J.Mulligan, E.S.Mc Caughey, P.R.Betts, Southampton University Hospitals, Southampton, SO9 4XY, UK

We have recently shown the psychosocial status of short normal pre-pubertal children to be unimpaired provided selection bias is avoided. In order to evaluate the psychological effects of treatment in this community based group, 15 short normal children <3rd centile, age 7-8 yrs were assessed before and after 3 yrs on growth hormone (GH), together with age and sex matched normal controls (10-90th centile) and short controls (<3rd centile). No significant differences between groups were found on psychometric testing at the start. After 3 years, mean change in height SDS was significant in the treated group only (-2.44 to -1.17 SDS). However, no significant differences in the following psychometric tests were found between groups: IQ British Ability Scales (BAS) (p=0.53), Number Skills BAS (p=0.44), Reading Skills BAS (p=0.58), Culture Free Self Esteem Inventory (p=0.62), Behaviour Scales (Rutter) for Teachers (p=0.87) and for Parents (p=0.64). The reported incidence of teasing or bullying did not differ between any groups. In these well adjusted short children, while growth responded well to GH, no discernable psychological benefits could be demonstrated at this pre-pubertal age, nor, in contrast to some reports, were mood or behaviour adversely affected by the need for daily injections or perceived treatment 'failure'. Most overestimated their present height. Furthermore 90% of those on treatment, but also 50% of those untreated optimistically anticipated being average or even tall adults. This ongoing study will show whether this discrepancy between expectation and reality leads to psychological problems in future years.

321

SHORT NORMAL STATURE - NATURE OR NURTURE? (THE WESSEX GROWTH STUDY).

L.D.Voss, J.Mulligan, Southampton University Hospitals, Southampton, SO9 4XY, UK

The biological and environmental variables associated with non-organic short stature were examined in an unselected population of short school entrants. Of 14,346 5yr olds screened, 140 short (S), apparently normal children, < 3rd centile for height were identified and matched with 140 controls (C), 10th-90th centile. Mean birth weight of the two groups was significantly different (S 2845gm, C 3345gm, p<.001). A significant difference in mean mid-parental height (S 162.0cm, C 170.9cm, p<.001) was found suggesting that stature may be largely genetic. However, the importance of environmental factors was indicated by the large number of short children inappropriately small for parents. The discrepancy between height SD score at 5yrs and mid-parental height SD score was significantly greater in short children (S -1.18, C -0.23 p<.001). Skeletal maturation in many short children was delayed, but did not explain the discrepancy. Taking bone age into account, the predicted adult height of 68% of the short children still lay below target height and 10% were outside the normal range. Significantly more short children were in social classes IV and V (S 31%,C 13%,p<.001) and had unemployed fathers (S 24%, C 9%, p<.001). There was a high prevalence of asthma (S 16%, C 8%, p<.001) and eczema (S 18%, C 5%, p<.001). One in four short children was also judged to be psychosocially deprived. We conclude that biological variables are often insufficient to explain short stature: no child should be dismissed as normal without careful evaluation. Poor growth in any child may be an important pointer to an adverse but potentially remediable environment.

● 322

INCREASED CHROMOSOME FRAGILITY IN LYMPHOCYTES OF SHORT NORMAL CHILDREN TREATED WITH RECOMBINANT HUMAN GROWTH HORMONE

G.L. Spadoni, B. Tedeschi*, M.L. Sanna, P. Vernole*, D. Caporossi*, S. Cianfarani, B. Nicoletti*, B. Boscherini. Department of Pediatrics and Department of Public Health and Cell Biology*, Tor Vergata University, Rome, I-00173 Italy.

The report, few years ago, of some growth hormone-deficient children having developed leukemia following therapy with human growth hormone, raised concern about a possible stimulatory effect of this therapy on tumor development. Since it is known that proneness to cancer is related to chromosome breakage, we decided to investigate whether recombinant human growth hormone (rhGH) therapy might increase chromosome fragility. Ten short-normal children (age: 6-11 yrs; mean height: -2.5 SD; mean growth rate: 4.1 cm/yr; mean bone age: -2.5 SD) were studied. Lymphocytes were collected at 0, 6 and 12 months of therapy, and the rate of spontaneous chromosome aberrations, the frequency of sister chromatid exchanges, the proliferative rate indices, the expression of common fragile sites induced by aphidicolin, and the sensitivity towards the radiomimetic action of bleomicin (BLM) were assessed. The mean frequency of BLM-induced chromosome aberrations increased from the pretherapy 0.11 breaks/cell (b/c) value to 0.23 b/c at 6 months of therapy ($p<0.01$), and remained at the same level (0.22 b/c) at 12 months. The frequency of damaged cells, showed a significative difference ($p<0.05$) between the pretherapy value (0.09) and the value found in the cultures performed at 6 and 12 months of therapy (0.14). An increase in spontaneous chromosome rearrangements at 6 and 12 months of therapy was also observed. These findings are supported by data obtained from the analysis of 16 short normal children already on rhGH therapy. Our data point out the need for rhGH to be given only in the strictest of indications. The opportunity of starting rhGH therapy should be carefully questioned when short stature is associated with conditions at risk for the development of tumours, for an increased chromosomal radiosensitivity, e.g. Down syndrome, or for increased chromosomal breakage, e.g. Fanconi anemia and Bloom syndrome.

323

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBPs) IN CONDITIONED MEDIUM FROM HUMAN NEUROBLASTOMA CELL LINES.

S. Bernardini*, S. Cianfarani**, R. Massoud*, M. Anniccharico-Petruzzelli***, G. Federici* and B. Boscherini**. Departments of *Biology, **Paediatrics, and ***Experimental Medicine, "Tor Vergata" University, Rome, I-00173 Italy.

A variety of cancer cell lines have been shown to produce IGFBPs, to date, however, few data exist on human neuroblastoma cell lines.The presence of IGFBPs was investigated, in basal conditions and after addition of retinoic acid (RA), in conditioned medium (CM) from two human neuroblastoma cell lines: SK-N-BE(2), a line highly sensitive to RA differentiating action, and BE(2)-M17, poorly sensitive to RA. The cells were grown in MEM Eagle modified HAMS-F12 (50/50 v/v), and FCS (12 %), and allowed to reach 80% confluency. RA (5 μM) was added for 24, 72, and 120 hours. During the last 24 hours of incubation, the cells, after two washings, were transferred into serum-free medium. Serum-free CM from the last 24 hours was collected, dyalized, and concentrated 125 times by ultrafiltration and lyophilization. The samples were assessed for total protein content (BCA) and 300 μg applied to SDS-PAGE, and analysed by western ligand blotting (WLB). Radiolabelled IGFBPs were visualized by autoradiography and quantitated by densitometry. SK-N-BE(2) CM was also incubated with human serum and then assessed by WLB for the presence of proteolytic activity against serum IGFBPs. Two major bands with an approximate M_r of about 37,5K and 25K were found in both cell lines. In SK-N-BE(2) the 37,5K band decreased considerably after 72 hr exposure to RA (50% decrease at densitometric analysis). No proteolytic activity against serum IGFBPs was found in the CM. Our results demonstrate the presence of two forms of IGFBPs in CM from two different human neuroblastoma cell lines. The secretion of 37.5K IGFBP in SK-N-BE(2) seems to be modulated by RA, suggesting a role of the IGF-IGFBP system in RA-mediated cell differentiation.

324

GH THERAPY IN NON GH DEFICIENT (NGHD) GIRLS: ITS EFFECT ON STEROID PLASMA LEVELS. R. Balducci, V. Toscano*, A. Mangiantini, F. Vaccaro, G. Municchi, B. Boscherini. Dept. of Pediatrics Univ. "Tor Vergata";*Dept. of Endocrinology Univ. "La Sapienza" Rome, Italy.

A number of studies have shown that GH may act directly or indirectly (via IGF1) at ovarian and adrenal levels thus increasing their steroidogenic activity. As GH therapy is now used also in short NGHD subjects, the aim of our study was to evaluate gonadal and adrenal plasma steroids in short NGHD patients treated with GH. Insulin was also measured as GH therapy in NGHD patients may cause hyperinsulinemia, and because a relationship between hyperinsulinemia and hypeandrogenism exists. We evaluated the plasma steroid pattern in 10 NGHD short girls (stature -2.8 ± 0.6 SD; age 11.9 ± 1.2 yrs) observed at the beginning of puberty. In order to improve their final height, these girls were treated with GH (Saizen 0.08-0.1 IU/Kg s.c. day for six days weekly) and, in order to delay epiphyseal closure, with GnRH analogue leuprolide (Enantone 3.75 mg every 25 days). Estradiol (E2), testosterone (T), androstenedione (A), 17α-hydroxyprogesterone (17OHP), deidroepiandrosterone sulphate (DHA-S), sex hormone binding globulin (SHBG) and insulin after OGTT were evaluated by RIA at the beginning and 6, 12, 18 months during therapy. We report the results at the beginning and at 18 months of therapy as mean ± SD. Plasma levels of E2 were lowered to prepubertal values at 6 months and remained stable at 12 and 18 months. Plasma levels of T, A, 17OHP, DHA-S did not show any significant change during the study (T: 24.7 ± 6.3 and 20 ± 11 ng/dl, A: 112 ± 29 and 121.4 ± 27.3 ng/dl, 17OHP: 70.4 ± 28 and 80 ± 18.2 ng/dl, DHA-S 102.5 ± 53.6 and 124.8 ± 74.8 ug/dl). Plasma levels of SHBG did not change (78.6 ± 25.6 and 80.2 ± 28.4 M). Insulin (uU/ml) basal levels and insulin peak after OGTT were 12.5 ± 3.5 and 78.9 ± 28.5, 10.3 ± 4.2 and 123.5 ± 60.7. **Conclusions:** our data show that in short NGHD girls, GH therapy at the dosage given, is not able to: (1) directly stimulate ovarian E2 production (2) induce any modification of plasma levels of the major androgens of ovarian and adrenal origin evaluated in this study (3) induce modification of SHBG plasma levels thus the androgen peripheral availability remains unchanged. Therefore there are no adverse side effects of GH treatment on plasma steroids in short NGHD patients.

325

IDENTIFICATION OF RESPONSIVE AND UNRESPONSIVE SHORT CHILDREN TO GROWTH HORMONE THERAPY USING A MULTIVARIATE DISCRIMINANT ANALYSIS OF PRE-TREATMENT AUXOLOGICAL DATA. A. Spagnoli, G.L. Spadoni, A.M. Pasquino*, M. Ortone*, F. Vaccaro, S. Cianfarani, B. Boscherini. Departments of Pediatrics, Tor Vergata University and La Sapienza University*, Rome, Italy.

BACKGROUND: Growth hormone (GH) has been proved to increase height velocity (HV) in some short children who are not GH deficient. OBJECTIVE: Multivariate discriminant analysis was employed to identify, using pre-treatment auxological data, the children responsive to GH. DESIGN: Open prospective study. SETTING: University hospital. SUBJECTS: Fifty-five (35 males) patients that met the following criteria were studied: birth weight >2.500 Kg; no signs of dysmorphic disease; stature below the 3rd centile for chronological age (CA); height velocity below the 25th centile for bone age (BA); no signs of puberty; growth hormone response to pharmacological stimulation >10 ng/ml; no evidence of organic disease; treatment with daily subcutaneous administrations of GH at the dosage of 12-16 UI/m^2/week. MAIN OUTCOME MEASURES: Eight pre-treatment auxological variables; HV increase after six months of therapy. A HV increase >2.5 cm/y was considered as a positive response. RESULTS: Thirty-two (58.2%) patients were responsive. Using univariate analysis, the variables found to have predictive value ($p<0.001$) were: height velocity (SD for CA) and bone age (SD). These variables were employed in the multivariate discriminant analysis. The equation obtained was: $Score=1.09X_1 - 0.83X_2 + 0.19$ (X_1=HV, X_2=BA). Using this scoring system we obtained a sensitivity of 95.7% and a specificity of 91.0% in predicting responsiveness to GH (X^2 with Yates correction=36.78, $p<0.0001$). No correlation was found between responsiveness and GH dosage. CONCLUSIONS: Discriminant analysis may permit the pre-treatment prediction of response to GH therapy in short children and therefore the identification of patients in which GH would be useless.