Circulatory insulin-like growth factor-I and brain volumes in relation to neurodevelopmental outcome in very preterm infants

Ingrid Hansen-Pupp¹, Holger Hövel¹, Chatarina Löfqvist², Lena Hellström-Westas³, Vineta Fellman^{1,4}, Petra S. Hüppi⁵, Ann Hellström² and David Ley¹

BACKGROUND: To evaluate the relationships between postnatal change in circulatory insulin-like growth factor-I (IGF-I) concentrations, brain volumes, and developmental outcome at 2 y of age in very preterm infants.

METHODS: IGF-I was measured weekly, and nutritional intake was calculated daily from birth until a postmenstrual age (PMA) of 35 wk. Individual β coefficients for IGF-I, IGF-I(B), representing the rate of increase in IGF-I from birth until a PMA of 35 wk were calculated. Brain magnetic resonance imaging was performed at term age, with segmentation into total brain, cerebellar, gray matter, and unmyelinated white matter volume (UWMV). Developmental outcome was evaluated using Bayley Scales of Infant Development-II.

RESULTS: Forty-nine infants, with mean gestational age (GA) of 26.0 wk, were evaluated at mean 24.6 mo corrected age. Higher IGF-I(B), UWMV, and cerebellar volume were associated with a decreased risk for a Mental Developmental Index (MDI) < 85 (odds ratio (95% confidence interval): 0.6 (0.4–0.9), 0.96 (0.94–0.99), and 0.78 (0.6–0.96), respectively). In multivariate analysis, higher IGF-I(B) and higher UWMV combined with female gender constituted the two models with the highest predictive value for MDI > 85.

CONCLUSION: A higher rate of increase in circulating IGF-I is associated with a decreased risk for subnormal MDI at 2 y of corrected age. This relationship is in part dependent on brain volume at term age.

mpaired neurodevelopment after very preterm birth remains a major concern. Combinations of risk factors interfere with growth and maturation of the immature brain during the early postnatal period (1). Decreased brain volumes and impaired developmental outcome have been observed not only after extremely preterm birth but also in moderately or late preterm infants (2–4), suggesting a deleterious effect of other factors than only gestational age (GA). Delayed maturation of white matter structures has been suggested to be a result of postnatal events rather than gestational age *per se* (5). Reduced longitudinal growth velocity and decreased nutritional intake during the first postnatal week after very preterm birth have been associated with later developmental delay (6,7).

Fetal and postnatal growth is regulated not only by nutritional intake but also by several endogenous growth factors. Insulin-like growth factor-I (IGF-I) plays a crucial role in brain development (8). Immediately after birth, circulatory IGF-I concentrations decrease rapidly, and in preterm infants, low concentrations persist during several weeks, as compared with corresponding intrauterine concentrations (9–11). In preterm infants, postnatal circulatory IGF-I concentrations have been positively associated with longitudinal growth (10,12,13).

We have previously shown that low postnatal IGF-I concentrations are related to decreased brain volumes at term age (14). As a consequence, we hypothesized that a lower rate of increase in IGF-I concentrations and lower brain volumes may be associated with impaired later neurodevelopment. We explored this by relating postnatal development of circulating IGF-I and brain growth as determined by magnetic resonance imaging at term age to neurodevelopment at 2 y of age.

RESULTS

Data at Birth and at 2 y of Age

Out of 52 eligible children, 49 were assessed at a mean (SD) corrected age of 24.6 (0.8) mo. The parents of one child declined to continue the study and two children had moved abroad. The mean (SD) GA and birth weight (BW) of the 49 evaluated children were 26.0 (1.9) wk and 889 (290) g, respectively.

The mean (SD) standard deviation score (SDS) for weight at birth was -1.0 (1.2). The mean (SD) SDS for weight, at a postmenstrual age (PMA) of 35 wk and at 2 y of age were -1.5(1.1) and -1.5 (1.2), respectively. Median (range) of Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) were 92 (50–118) and 88 (50–121). Two infants had both MDI and PDI designated as 50. Twenty-one children (43%) had a MDI < 85 and 24 children (49%) a PDI < 85.

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¹Department of Pediatrics, Institute of Clinical Sciences, Lund, Skane University Hospital, Lund University, Lund, Sweden; ²Department of Ophthalmology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; ³Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden; ⁴Children's Hospital, Helsinki University Central Hospital, University of Helsinki, Finland; ⁵Department of Pediatrics, Geneva University Hospital, Geneva, Switzerland. Correspondence: Ingrid Hansen-Pupp (ingrid.pupp@skane.se)

Eleven children (22%) had a MDI < 70 and seven (14%) children had a PDI < 70. None was diagnosed with cerebral palsy.

Clinical Data and Developmental Outcome at 2 y of Age

Univariate associations between clinical variables and MDI < 85 are shown in **Table 1**. A lower GA at birth, Apgar score <7 at 5 min, total steroid exposure, and ventilator treatment were associated with a subnormal MDI < 85.

Brain Volumes and Developmental Outcome at 2 y of Age

Mean (SD) values of brain volumes according to a MDI/ PDI < 85 or a MDI/PDI \ge 85 are shown in Table 2. Lower

Table 1. Contribution of clinical variables to Mental DevelopmentalIndex < 85 at 2 y of corrected age (n = 49)</td>

Variable	n (%)	OR	95% CI	P value
Gestational age (d)	_	0.95	0.9–1.0	0.043
Weight SDS at birth	_	0.5	0.5–1.3	0.505
Weight SDS at 35 gestational weeks	—	0.6	0.3–1.04	0.068
Male gender	25 (51)	0.5	0.1–1.5	0.187
Apgar score <7 (5 min)	18 (37)	3.3	1.0–11.1	0.049
Severe brain damage	5 (10)	2.2	0.3–14.3	0.639
Ventilator (days)	—	1.1	1.01–1.15	0.011
Bronchopulmonary dysplasia	38 (78)	2.4	0.6–10.5	0.311
Mean energy intake (kcal/kg/d)ª	—	1.0	0.9–1.04	0.600
Mean protein intake (g/kg/d)ª	—	0.1	0.01–1.6	0.102
Hydrocortisone treatment (mg/kg)ª	_	1.1	0.99–1.2	0.090
β-methasone treatment (mg/kg)ª	—	1.7	0.8–3.6	0.177
Total steroid exposure (estimated mg/kg) ^a	—	1.02	1.001–1.04	0.039
Maternal educational level ^b	24 (49)	0.5	0.2–1.6	0.242
Paternal educational level ^b	18 (37)	0.5	0.2–1.8	0.314

Cl, confidence interval; OR, odds ratio; SDS, standard deviation score.

^aFrom birth until 35 gestational weeks. ^bUniversity degree vs. no university degree.

Adapted from ref. (40).

cerebellar volume and lower unmyelinated white matter volume (UWMV) were associated with MDI < 85 and with PDI < 85. Lower total brain volume was associated with MDI < 85 but not with PDI < 85. No significant associations were seen between brain volumes and MDI < 70 or PDI < 70.

Insulin-Like Growth Factor-I and Clinical Data

Weekly mean IGF-I concentrations from birth until a PMA of 35 wk according to a normal and subnormal MDI are shown in **Figure 1**. Mean concentrations of IGF-I during the whole study period, from birth until a PMA of 35 wk, did not differ according to MDI categories. Mean values of IGF-I from PMA 30 to 35 wk, i.e., during the catch-up phase of growth, were higher in subjects with MDI \geq 85 (mean (SD): 34 (10 µg/l)) as compared with those with MDI < 85 (mean (SD): 27 (8 µg/l); P = 0.021).

The unstandardized β coefficient (B) for IGF-I, IGF-I(B), correlated positively with GA at birth and BW (P < 0.001), respectively, whereas no correlation was seen with SDS for BW. IGF-I(B) did not differ according to gender or Apgar score <7 at 5 min (P = 0.84 and P = 0.26, respectively).

IGF-I(B) correlated positively with SDS for weight at a PMA of 35 wk (P < 0.001). No correlation was seen between IGF-I(B) and weight SDS at 2 y of age.

There was a trend toward a positive correlation between IGF-I(B) and mean protein intake g/kg/d from birth until a PMA of 35 wk (P = 0.05) but not with mean energy intake kcal/kg/d. Accumulative intake of hydrocortisone or β -methasone (mg/kg) and total steroid exposure (estimated mg/kg) from birth until a PMA of 35 wk correlated negatively with IGF-I(B) (P = 0.015, P = 0.002, and P = 0.001, respectively).

Insulin-Like Growth Factor-I, Brain Volumes, and Developmental Outcome at 2 y of Age

IGF-I(B) correlated positively with total brain volume, UWMV, gray matter volume, and cerebellar volume (P < 0.001, P < 0.001, P = 0.023, and P < 0.001, respectively).

Mean (SD) of IGF-I(B) was 2.1 (1.6) in infants with a MDI < 85 as compared with 3.8 (2.4) in infants with MDI \ge 85 (P = 0.009). A higher IGF-I(B), i.e., a higher rate of increase in IGF-I, was associated with a decreased risk for an MDI < 85 (OR (95% CI): 0.4–0.9). Furthermore, there was a positive relationship between IGF-I (B) and MDI as a continuous variable (P = 0.028). IGF-I(B) was neither related to PDI < 85 nor to PDI as a continuous variable.

Table 2. Mean (SD) of brain volumes (ml) at a postmenstrual age of 40 wk in children with MDI <85 (n = 21) or MDI ≥ 85 (n = 28) and PDI < 85 (n = 24) or PDI ≥85 (n = 25) at 2 y of corrected age

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	MDI <85	MDI ≥85	OR (95% CI)	P value	PDI <85	PDI≥85	OR (95% CI)	P value
TBV	374 (44)	407 (56)	0.99 (0.97–1.0)	0.045	379 (55)	406 (49)	0.99 (0.98–1.002)	0.091
GMV	190 (26)	201 (34)	0.99 (0.97–1.01)	0.306	193 (35)	201 (27)	0.99 (0.97–1.01)	0.405
UWMV	177 (21)	200 (28)	0.96 (0.94–0.99)	0.007	180 (24)	200 (27)	0.97 (0.94–0.99)	0.017
Cerebellar volume	19.5 (2.6)	22.3 (4.2)	0.78 (0.6–0.96)	0.006	19.9 (3.4)	22.3 (3.9)	0.8 (0.69–0.99)	0.027

CI, confidence interval; GMV, gray matter volume; MDI, Mental Developmental Index; OR, odds ratio; PDI, Psychomotor Developmental Index; TBV, total brain volume; UWMV, unmyelinated white matter volume.

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Multivariate analysis assessing the combination of variables with the highest prediction for a subnormal MDI is presented in **Table 3**. When cerebellar volume was entered as an independent variable, increase in IGF-I(B) remained as the sole variable with the highest predictive value for a decreased risk of MDI < 85. When UWMV was entered as an independent variable, the combination of increased UWMV and female gender had the highest predictive value for a decreased risk of MDI <85. Neither energy nor protein intake changed the contributions of either IGF-I(B) or that of UWMV and gender, for prediction of MDI.

DISCUSSION

The main findings in this study were that a higher rate of increase in circulatory concentrations in IGF-I from birth until a PMA of 35 wk in very preterm infants was associated with a decreased risk for subnormal MDI at 2 y of age. Furthermore, higher cerebellar and unmyelinated white matter volumes at term age were associated with improved developmental outcome at 2 y of age.



Figure 1. Mean (95% CI) concentrations of IGF-I according to postmenstrual age (weeks). Filled squares denote children with MDI < 85, filled circles denote children with MDI \geq 85. Error bars denote 95% CI. CI, confidence interval; IGF-I, insulin-like growth factor-I; MDI, Mental Developmental Index.

The rate of early postnatal cerebral cortical growth in very preterm infants has been related to cognitive achievement in later childhood (15). IGF-I is essential for growth and development of the human brain. Deletion of the IGF-I gene or its receptor is followed not only by pre- and postnatal growth retardation but also by microcephaly and cognitive delay (16,17).

Our observations are based on weekly measurements of IGF-I obtained by blood sampling. We do not know how circulatory IGF-I concentrations interact with local IGF-I concentrations in the immature human brain. In rats, brain neuronal activity facilitates transport of systemic recombinant human IGF-I across the blood-brain barrier. This process is triggered by a protease which cleaves IGF-I from its dominating binding protein IGFBP-3, allowing free IGF-I to enter the endothelial barrier, using specific transport proteins (18). Furthermore, systemic administration of IGF-I modulates brain vessel growth and cognitive function in mice, suggesting a link between circulatory derived IGF-I and IGF-I within the brain (19,20).

IGF-I does not only promote cellular growth but has also important protective properties. Exogenous intranasal administration of IGF-I has protective effects in the developing rat brain after hypoxic or inflammatory insults, probably by interfering with antiapoptotic mechanisms (21,22). Very preterm infants are exposed to multiple hypoxic and inflammatory events during and after birth. Systemic inflammation has been related to white matter injury and impaired development in both experimental and clinical studies (23–25). We have previously shown that perinatal inflammation is associated with a concomitant decrease in IGF-I concentrations in preterm infants (9). Insufficient postnatal endogenous production of IGF-I may thus have adverse effects in the developing brain through lack of protection, as well as through reduced trophic support.

During the gestational period when premature birth may occur, the brain undergoes a complex development, which is paralleled by a rapid increase in brain volumes with the cerebellum exhibiting the highest growth rate (26). Decreased cerebellar and white matter volumes were univariately associated with a subnormal MDI and PDI, and lower UWMV contributed to a subnormal MDI in multivariate analysis. These findings are in line with other recent studies showing associations

Table 3. Best model of variables contributing to a Mental Developmental Index <85 (logistic regression backward log-likelihood ratio) with cerebellar volume and unmyelinated white matter volume evaluated in separate models

Cerebellar volume			Unmyelinated white matter volume				
Variable	β	OR (95% CI)	Р	Variable	β	OR (95% CI)	Р
IGF-I(B)	-0.48	0.62 (0.40–0.96)	0.031	UWMV	-0.046	0.96 (0.92–0.98)	0.01
				Female gender	-1.4	0.25 (0.05–1.2)	0.085

Independent variables entered into the logistic regression model: rate of increase in IGF-I (IGF-I(B)), gestational age, weight standard deviation score at birth and at a PMA of 35 wk, gender, Apgar score <7 at 5 min yes/no, severe brain damage yes/no, mean protein (g/kg/d) or energy (kcal/kg/d) from birth until a PMA of 35 wk, accumulative dose of hydrocortisone and β -methasone (mg/kg) or total steroid exposure (estimated mg/kg) from birth until a PMA of 35 wk, maternal and paternal educational level (university degree yes/no), and cerebellar volume (ml) or unmyelinated white matter volume (ml), respectively.

Cl, confidence interval; IGF-l, insulin-like growth factor-l; OR, odds ratio; PMA, postmenstrual age; UWMV, unmyelinated white matter volume. Adapted from ref. (40). between reduced brain volumes, particularly cerebellar volumes, and impaired neurodevelopment at 2 y of age (2,27,28).

Rate of increase in IGF-I during the whole study period, i.e., from birth to 35 wk PMA was related to MDI at 2 y of age. Mean values of IGF-I during the corresponding time period did not exhibit a similar relationship. Rates of change are intrinsically more sensitive but more prone to error than absolute differences. However, mean values of IGF-I differed between infants with subnormal vs. normal MDI after a PMA of 30 wk, i.e., during the phase of catchup growth. Similar temporal findings were observed when longitudinal mean IGF-I concentrations were related to differences in cerebellar volume (14). This implies that endogenous IGF-I concentrations during a defined period of catch-up growth may be of importance for brain growth, as well as later outcome.

IGF-I concentrations were not associated with PDI. In experimental studies, IGF-I has beneficial effects on neural as well as white matter development (29), implying that a positive association could be expected also with motor development The relatively low number of subjects in the cohort limited statistical power for detection of plausible associations between early postnatal events and later developmental outcome. Another limitation is that developmental follow-up was performed as early as at 2 y of corrected age, where the predictive capacity of MDI for later cognitive function has been questioned (30). It would therefore be of interest to reevaluate these children at later ages.

Gestational age at birth correlated positively with rate of increase in IGF-I, which infers that infants with the lowest GA are less capable of increasing postnatal IGF-I concentrations. This may be related to the findings that the most immature infants also experience the most pronounced growth restriction after birth (10,31).

On the other hand, weight SDS at birth did not correlate with IGF-I(B). This suggests that although growth restricted infants have lower initial IGF-I concentrations at birth than infants with BW appropriate for GA, the rate of increase in IGF-I is similar to that of appropriate for GA infants (9,32).

Hypoxia plays a mechanistic role in the IGF-system and has been related to a decrease in IGF-I concentrations (33). In this study, Apgar score was not associated with mean IGF-I concentrations or temporal change in IGF-I. Other data on postnatal hypoxic events would have been of interest but were not evaluated in this study. Infants with bronchopulmonary dysplasia are exposed to episodes of hypoxia and bronchopulmonary dysplasia has been described as a predictive risk factor for later impairment (34). We have previously described that lower postnatal concentrations of IGF-I are associated with development of bronchopulmonary dysplasia (35). In this cohort, no association between bronchopulmonary dysplasia and developmental outcome, neither in univariate nor in multivariate analyses, could be shown.

Postnatal steroid treatment has been considered to be a risk factor for adverse neurodevelopmental outcome (36). Total steroid exposure was higher in infants with subnormal MDI. However, postnatal steroid exposure did not contribute to subnormal MDI in multivariate analysis. The absence of a significant relationship may be attributed to the relatively small study population. Improved nutritional intake during the first postnatal week has earlier been described to be associated with higher MDI in infants with extremely low BW; however, in this study, adjustments were performed only for BW but not for GA or BW small for GA in the multivariate models (7). The increase in IGF-I from birth until a PMA of 35 wk showed a tendency to correlate with mean protein intake during the corresponding time period. We could not demonstrate any contribution of nutritional intake to later neurodevelopment in multivariate models. Previously, we were not able to show any correlation between nutritional intake and brain volumes in the same cohort of infants (14). Absence of significant relationships between nutritional intake and outcome may however be due to lack of sufficient statistical power.

This study has focused on events related to postnatal growth and their impact on later developmental outcome after very preterm birth. In conclusion, we have found that a higher rate of increase in circulatory IGF-I concentrations is associated with better neurodevelopmental outcome at 2 y of age. This relationship is in part dependent on brain growth at term age.

METHODS

Study Population

Preterm infants were included between January 2005 and May 2007 in a prospective longitudinal cohort study approved by the Regional Ethical Review Board, Lund, Sweden. Inclusion criteria were a GA < 31 wk at birth, absence of major congenital anomalies, and written informed parental consent. All pregnancies were dated by ultrasound at 17–18 gestational weeks. A total of 64 infants were included with 52 infants completing the study until term age and thus eligible for follow-up. Nine infants did not survive until term age and the parents of three infants decided to leave the study.

Blood Sampling and Quantitative Analysis of IGF-I

Serum IGF-I concentrations were measured on days 3, 7, and thereafter once weekly until at least an achieved PMA of 35 wk. The blood samples were obtained from umbilical or peripheral arterial catheters and thereafter by venous puncture. After centrifugation, serum samples were stored at -80 °C until assayed. The samples were diluted 1:50, and the IGF-I concentrations were analyzed using insulin-like binding protein-blocked radioimmunoassay (Mediagnost, Tübingen, Germany) in the same assay, as described previously (37). The intra-assay coefficients of variation for IGF-I were 18, 11, and 7% at concentrations of 9, 33, and 179 µg/l. Serum IGF-I values obtained within 1 d after treatment with fresh frozen plasma were omitted from analysis (38).

Clinical Data

Weight was measured on the same day as sampling of IGF-I once weekly until a PMA of at least 35 wk. Weight SDS was calculated from a Scandinavian intrauterine growth curve based on fetal weights estimated by ultrasound (39).

Treatment with hydrocortisone (3-6 mg/kg/d) was initiated due to resistant arterial hypotension in 12 infants. β -Methasone was administered to facilitate weaning from ventilator, at a minimum postnatal age of 10–14 d (0.2 mg/kg/d) initially, with gradually tapering of dose) in 15 infants. Daily administered dose (mg/kg) of both drugs was registered, and the accumulative dose until a PMA of 35 wk was calculated. Total steroid exposure was estimated by converting β -methasone dosage into hydrocortisone equivalents (1:40). Ventilator treatment (total number of days) were registered. Bronchopulmonary dysplasia was defined as need of supplemental oxygen at a PMA of 36 wk.

Maternal and paternal education was classified into three categories: completed compulsory school, upper secondary education, or university degree. Cerebral ultrasound was performed at days 1, 3, and 7; at 3 and 6 wk of age; and at term. Severe intracranial hemorrhage was defined in the presence of intraventricular hemorrhage grade III or periventricular hemorrhagic infarction. White matter damage was diagnosed in the presence of periventricular echodensities persisting for more than 7 d or periventricular cysts. Severe brain damage was defined as severe intracranial hemorrhage or white matter damage.

Nutritional Regime and Calculation of Intake

The nutritional strategy was based on individualized enteral nutrition using maternal or donor breast-milk (fortified if required) and additional parenteral nutrition starting soon after birth, as described previously (10). Minimal enteral feeding was started within 3h of age and administered every 2–3h (1–2ml/meal) with a gradual increase in volume. Previously frozen and pasteurized donor breastmilk was used until the mother could provide her own breast milk. Administered breast-milk was analyzed at day 7 and then weekly for protein (g/100 ml) and energy (kcal/100 ml) content in a 24h sample. Daily enteral and parenteral intakes of protein (g/kg/d) and energy (kcal/kg/d) were prospectively registered until a PMA of at least 35 wk.

Magnetic Resonance Imaging

Magnetic resonance imaging was performed on a 3-T Siemens Magnetom Allegra head scanner (Siemens AG, Medical Solutions, Erlangen, Germany) at term age (mean (SD): 40.1 (0.6)) gestational weeks. The protocol for image acquisition and image processing has been described in detail previously (14).

The voxels for every tissue class were summed to calculate the tissue volumes. These included total brain volume, gray matter volume, myelinated and unmyelinated white matter volume, and cerebellar volume. Myelinated white matter volume referred to only ~5% of white matter volume at term age and showed a low intraindividual correlation in repeated measures and was discarded from further analysis (14).

Follow-Up at 2 y of Corrected Age

Developmental outcome was assessed by a psychologist using the Bayley Scales of Infant Development 2nd Edition with the two different index scales, MDI and PDI. A subnormal development was defined as an index score of 1 SD below the normative mean (MDI or PDI < 85) and a developmental delay as 2 SD below the normative mean (MDI or PDI < 70). Infants with MDI or PDI scores of 50 or below were assigned a score of 50 according to the manual (40). A standardized neurological examination including weight, length, and head circumference measurements were performed (by I.H.-P. and H.H.) after the developmental assessment.

Statistical Analysis

Statistical analysis was performed using the program package IBM SPSS statistics 20 for Microsoft Windows (IBM, Armonk, NY).

To evaluate temporal changes in IGF-I concentrations, IGF-I(B) was calculated by linear regression analysis in each individual from weekly IGF-I values from birth until an achieved PMA of 35 wk.

Relationships between IGF-I(B) and categorical or continuous variables were assessed with the Mann-Whitney U-test or Spearman rank correlation coefficient. Univariate analyses between other clinical variables and categorical outcome variables were assessed using $\chi^{\scriptscriptstyle 2}$ test, Fischer's exact test, or one-sample *t*-test as appropriate. The contribution of IGF-I(B) to developmental outcome was assessed using logistic regression analysis (backward log-likelihood ratio) using MDI < 85 as a dependent variable. Independent variables entered into the multivariate analysis were IGF-I(B), GA, SDS weight at birth and at a PMA of 35 wk, gender, Apgar score < 7 at 5 min yes/no, severe brain damage yes/no, mean protein (g/kg/d) or energy (kcal/kg/d) from birth until a PMA of 35 wk, accumulative dose of hydrocortisone and β -methasone (mg/kg) from birth until a PMA of 35 wk alternatively total steroid exposure during the same time period, cerebellar volume, UWMV and maternal and paternal educational level. Due to covariation, cerebellar volume and UWMV as well as steroid intake and protein/energy intake were evaluated in separate regression models. P values < 0.05 were considered as significant.

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