that inhibition of the Trk receptor tyrosine kinase activity resulted in inhibition of tumor growth [23]. A phase 2 study of Lestaurtinib is under way. AZ-23 is another potential molecule that selectively inhibits TrkA/B tyrosine kinase activity [25]. In this report, we demonstrated that relatively smaller doses of compounds A and G were required for cell growth inhibition in NB cell lines, compared to necessary doses of existing anti-cancer drugs, as demonstrated by low IC_{50} values. Because the screening of the candidate molecules was performed based on the hypothetical interaction between the candidate molecules and the BDNF-binding domain rather than the tyrosine kinase domain of TrkB, the results of low-dose effect could be explained as follows: TrkB is a transmembrane protein and the BDNF-binding domain is located outside of the cellular membrane, whereas the tyrosine kinase domain is located inside the cell. Thus, it is not necessary to bring the candidate drug interaction with the BDNF domain into the inner part of the cell. Consequently, it is speculated that highly efficient molecules were identified by our in silico approach.

The candidate compounds were screened for the molecules that interact with BDNF-binding domain of TrkB. Our results showed that the molecules and BDNF may competitively bind to the domain. Therefore, we first expected that the molecules simply inhibit the cell growth without apoptosis, since BDNF triggers the signal for cell growth, survival and differentiation. In fact, the inhibitor of the intracellular kinase domain of TrkB does not induce apoptosis. However, we unexpectedly found that the candidate compounds, which are competitors to BDNF, facilitate apoptosis. We speculate that the interaction between the compounds and TrkB may induce a change in the ternary structure of the inner cellular domain of TrkB, which may participate in the regulation of apoptosis. TrkB, which initiates the survival signal during neural development, may play a conflicting role such as regulating tumor suppressive functions, which are driven by apoptosis. As TrkB is also reported to be involved in regulating tumor invasion and metastasis in some adult cancers [26–28], these candidate compounds might also be used as drugs against the other advanced cancers with expression of TrkB.

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Conflict of Interest

None declared.

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Neuroblastoma in Older Children, Adolescents and Young Adults: A Report From the International Neuroblastoma Risk Group Project

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Background. Neuroblastoma in older children and adolescents has a distinctive, indolent phenotype, but little is known about the clinical and biological characteristics that distinguish this rare subgroup. Our goal was to determine if an optimal age cut-off exists that defines indolent disease and if accepted prognostic factors and treatment approaches are applicable to older children. ***Procedure.*** Using data from the International Neuroblastoma Risk Group, among patients ≥18 months old (n = 4,027), monthly age cut-offs were tested to determine the effect of age on survival. The prognostic effect of baseline characteristics and autologous hematopoietic cell transplant (AHCT) for advanced disease was assessed within two age cohorts; ≥5 to <10 years (n = 730) and ≥10 years (n = 200). ***Results.*** Older age was prognostic of poor survival, with outcome gradually worsening with increasing age at diagnosis, without statistical evidence for an optimal age cut-off beyond 18 months. Among patients ≥5 years, factors significantly prognostic of lower event-free survival (EFS) and overall survival (OS) in multivariable analyses were INSS stage 4, *MYCN* amplification and unfavorable INPC histology classification. Among stage 4 patients, AHCT provided a significant EFS and OS benefit. Following relapse, patients in both older cohorts had prolonged OS compared to those ≥18 months to <5 years ($P < 0.0001$). ***Conclusions.*** Despite indolent disease and infrequent *MYCN* amplification, older children with advanced disease have poor survival, without evidence for a specific age cut-off. Our data suggest that AHCT may provide a survival benefit in older children with advanced disease. Novel therapeutic approaches are required to more effectively treat these patients. Pediatr Blood Cancer 2014;61: 627–635. © 2013 Wiley Periodicals, Inc.

Key words: adolescents; autologous hematopoietic cell transplant; neuroblastoma; young adults

INTRODUCTION

Neuroblastoma is a common and often lethal cancer of early childhood that accounts for 10% of pediatric cancer mortality [1]. Incidence peaks in infancy and then rapidly declines, with less than 5% of cases diagnosed in children and adolescents ≥10 years [2]. There is increasing evidence that neuroblastoma in older children and adolescents has unique biology and an indolent disease course, but ultimately dismal survival.

Clinical and biologic characteristics that are independently prognostic of outcome in neuroblastoma are currently used for risk stratification to optimally tailor therapy [3]. These include age at diagnosis, International Neuroblastoma Staging System (INSS) stage, tumor histopathology, *MYCN* amplification status and tumor DNA index [4–6]. Children ≥18 months at diagnosis have been shown to have inferior survival compared to younger children [6]. However, an age threshold that predicts more indolent disease in older patients has not been defined. In addition, due to the rarity of neuroblastoma in older patients, the impact of other baseline prognostic factors has not been tested, but the lower probability of cure suggests the importance of novel therapies [7,8].

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To better define the clinical course and biological characteristics that correlate with the unique phenotype observed in older neuroblastoma patients and to ultimately tailor therapy, we used data from the International Neuroblastoma Risk Group (INRG) database. The effect of age, INSS stage, tumor sites, histology, *MYCN* status, DNA index, chromosome 1p or 11q aberration, and initial therapy on survival outcomes was determined in older children and adolescents with neuroblastoma.

METHODS

INRG Database

The database includes 8,800 unique patients with pathologically confirmed neuroblastoma diagnosed between January 1, 1990, and December 31, 2002 at age ≤21 years. Patients with newly diagnosed neuroblastoma were enrolled on studies in North America, Europe, and Japan. In addition to diagnosis date and survival data, information on 35 potential risk factors is included in the database [3]. Of the 8,800 patients, 730 (8.3%) were ≥5 to <10 years at time of diagnosis and 200 (2.3%) were ≥10 years.

Analysis of Age

In order to identify if an optimal age threshold exists that defines an indolent phenotype in older children, the effect of age at diagnosis on survival outcomes was evaluated. The cohort of children ≥18 months at diagnosis (n = 4,027) was repeatedly divided into two groups at all possible monthly points between 18 months and 14 years, that is, 149 different cut-offs. These paired age groups (younger vs. older) were compared using Cox proportional hazards regression analyses. For each regression model, the *P*-value assessed the significance of age, and the hazard ratio (HR) quantified the size of the age effect on outcome. Within the age cut-offs with a *P*-value < 0.05, the cut-off with the maximum HR was sought for both event-free survival (EFS) and for overall survival (OS) post-relapse (Fig. 1A,B).

Analysis of Tumor Biologic Factors

MYCN amplification status was determined using standard methods, primarily fluorescence *in situ* hybridization (FISH), of each contributing cooperative group at the time of study enrollment [9–12]. Tumor DNA index was determined by flow cytometry and reported as diploid/hypodiploid (≤1.0) or hyperdiploid (>1.0). Histology was classified as favorable or unfavorable and tumor grade as differentiating or undifferentiated/poorly differentiated as defined by Shimada [13]. MKI was defined as low/intermediate versus high. Loss of heterozygosity (LOH) or aberration at chromosomes 1p or 11q was determined using FISH or polymerase chain reaction techniques.

Analysis of Clinical Factors and Treatment

The primary tumor site was classified as either adrenal or non-adrenal. Metastatic site categories were bone/bone marrow versus other sites. The INSS stage was determined at the time of study enrollment. Within INSS stage 4 patients in both diagnostic age cohorts ≥5 to <10 and ≥10 years, the effect of initial therapy (AHCT vs. no AHCT) on survival outcomes was evaluated.

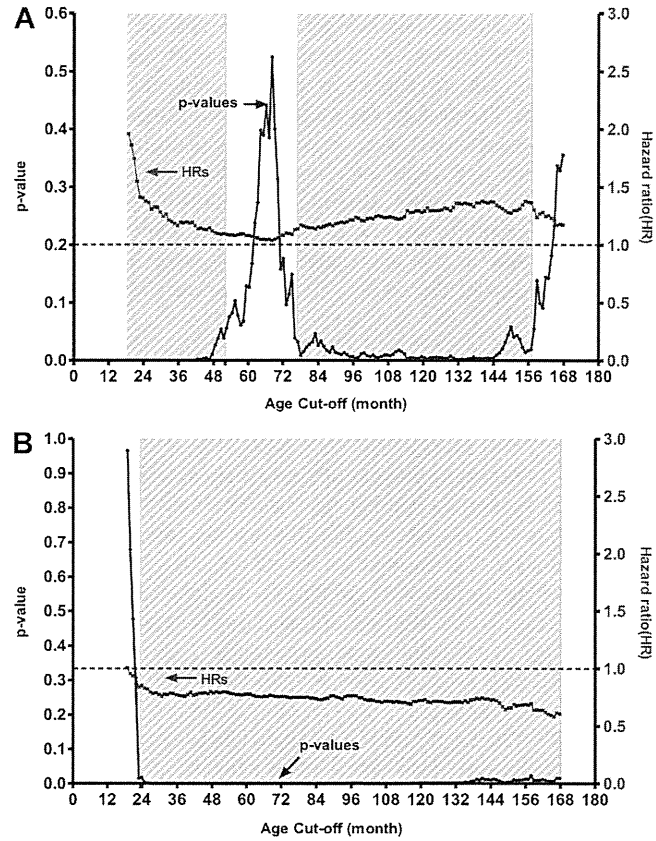

Fig. 1. Results of the 149 Cox models, one model for each age cut-off (comparing the older age group to the younger age group), within patients ≥18 months of age at diagnosis: *P*-values versus age cut-off month, and hazard ratios (HRs—increased risk of an event for older group compared to younger group) versus age-cut-off month. **A**: EFS from enrollment/diagnosis. Most of the age cut-offs have a *P*-value < 0.05. For the age cut-offs with *P* < 0.05 (within the light blue shaded area), all of the HRs are slightly larger than one, indicating that the increased risk of an event among older patients compared to younger patients is about the same no matter what age cut-off is chosen. The sharp downward slope of the far left section of the HR line and the sharp upward slope in the far right portion of the *P*-value line are artifacts of the small sample size near the edges of the lines. **B**: OS post-relapse. All of the age cut-offs have a *P*-value < 0.05. All of the HRs are slightly smaller than one, indicating prolonged survival for older patients compared to younger patients, regardless of what age cut-off is chosen. The sharp downward slope of the far left section of the *P*-value line is an artifact of the small sample size near the edge of the line.

Statistical Methods

For the EFS analysis, time to event was calculated from the date of study enrollment (which occurred days after diagnosis) to the first occurrence of relapse, progressive disease, second malignancy or death from any cause, or to last patient contact if no event occurred. For the OS analysis, time to death was calculated from the date of enrollment to the date of death from any cause, or to last patient contact. Time to death for OS after the first event (hereafter referred to as "OS post-relapse") was calculated from the date of relapse or progressive disease to the date of death or last follow-up. All EFS results are presented from the time of diagnosis/enrollment; similarly for OS except when specified as "OS post-relapse".

Kaplan–Meier survival curves were generated for each diagnostic age cohort, ≥18 months to <5 years, ≥5 to <10 years, and ≥10 years, and for subsets defined by baseline clinical and biologic characteristics. Survival curves were compared using the log-rank test, and P-values < 0.05 were considered statistically significant. Within patients ≥5 years old, multivariable Cox proportional hazards regression models were used to identify factors independently significantly prognostic of EFS and OS. Only factors that were significant in the univariate analysis were tested in the multivariable model; factors with $P < 0.05$ were retained in the final model.

RESULTS

Effect of Age

Of the 149 monthly age cut-offs tested, there was no statistical evidence to support an optimal age cut-off beyond 18 months, for either EFS or OS post-relapse. For EFS and OS post-relapse, all of the HR values (risk of an event for the older age group compared to the younger age group) were similar; no maximum HR value was identified (Fig. 1A,B). However, for descriptive purposes, patients were divided into the following age groups: ≥18 months to <5 years (n = 3,097) [A], ≥5 years to <10 years (n = 730) [B], and ≥10 years (n = 200) [C].

Regardless of the cut-off chosen to divide the cohort of patients ≥18 months into younger versus older patients, all HRs for EFS were greater than one, indicating that older patients had an increased risk of an event or death (Fig. 1A). However, for the analysis of OS post-relapse in patients ≥18 months old, the HRs were less than one, indicating that older patients, regardless of the age cut-off chosen, have prolonged survival compared to younger patients, suggesting a more indolent post-relapse course (Fig. 1B).

Comparing the three age groups ≥18 months, EFS (Fig. 2A) was lower for patients ≥10 years [C] compared to patients ≥18 months to <5 years [A] ($P = 0.005$) or patients ≥5 to <10 years [B] ($P = 0.01$), though EFS was no different for [A] v [B] ($P = 0.7$). As shown previously, the EFS and OS for patients <18 months was significantly better than each of the older age groups (Fig. 2A,B). EFS was lower for ≥10 years compared to ≥5 to <10 years within stages 1,2 ($P = 0.049$) and stage 4 ($P = 0.005$), but not stage 3, and not for OS within any stage (Fig. 2C–H). For those patients experiencing relapse or progression, post-relapse OS was significantly prolonged in those either ≥5 to <10 years (n = 408) or ≥10 years (n = 129) at diagnosis compared to those ≥18 months to <5 years (n = 1,615), suggestive of more indolent disease ([A] v [B] $P < 0.0001$; [A] v [C] $P < 0.0001$), though was not significantly different between the two older cohorts [B] v [C] ($P = 0.051$; Fig. 3A). Within stages 1,2, age groups had similar post-relapse OS (Fig. 3B); however, within stage 3 and within stage 4, patients ≥18 months to <5 years had worse post-relapse OS than either ≥5 to <10 years ($P = 0.006$ and $P = 0.002$, respectively) or ≥10 years ($P = 0.003$ and $P = 0.01$, respectively; Fig. 3C,D).

Patient Characteristics

There were differences in the diagnostic age cohorts when compared by baseline factors (Table I). The youngest age group (≥18 months to <5 years) had a higher proportion of patients with *MYCN* amplification, high MKI, differentiating grade and 1p LOH when compared to either of the two older age groups. Patients ≥18 months to <5 years at diagnosis also had a higher proportion of INSS stage 3 or 4 disease compared to patients ≥5 years to <10 years. Notably, there were no differences in the proportion of patients exhibiting unfavorable prognostic factors between the two older age cohorts (≥5 to <10 years vs. ≥10 years).

Event-Free and Overall Survival ≥5 to <10 Years at Diagnosis (n = 730)

Among children ≥5 to <10 years at diagnosis, 5-year EFS and OS were 39 ± 2% and 49 ± 2%, respectively. Baseline characteristics significantly prognostic of EFS and OS in univariate analyses were INSS stage, histology classification, primary tumor site, and *MYCN* amplification status (Table II). Within stage 1 or 2 patients (n = 174), histology was statistically prognostic of both EFS and OS. Chromosome 11q status and grade were prognostic of EFS and *MYCN* was prognostic of OS. Within stage 3 (n = 100), only histology was a significant prognostic indicator for both EFS and OS. Among patients with stage 4 disease (n = 432), 5-year EFS and OS were 21 ± 3% and 31 ± 3%, respectively. *MYCN* status and adrenal primary site were the only factors significantly prognostic of both EFS and OS in this group, and site of metastases in the bone or bone marrow was prognostic of EFS. Although only 18% of stage 4 patients had *MYCN* amplification, this group had particularly poor outcome.

≥10 Years at Diagnosis (n = 200)

Overall 5-year EFS and OS in this age cohort were 32 ± 4% and 46 ± 5%, respectively (Table II). Baseline characteristics that were significantly prognostic of both OS and EFS were stage, histology classification and *MYCN* status. Patients ≥10 years of age with *MYCN* amplified tumors had significantly worse EFS and OS compared to those without *MYCN* amplification (5-year EFS: 30 ± 6% vs. 15 ± 14%, $P = 0.015$; 5-year OS: 47 ± 6% vs. 0%, $P < 0.0001$). Due to small sample size, within stage 1 or 2 patients (n = 51), only histology, ploidy and primary site were evaluated and only histology was significantly prognostic of EFS. For patients with stage 3 disease (n = 32), small sample size prohibited analyses except for primary tumor site, whereby the outcome was similar for patients with adrenal tumors versus other sites. Among patients with stage 4 disease (n = 114), ploidy, metastatic site, primary site, *MYCN* status, 1p and 11q status were assessed, and only *MYCN* status was prognostic. Ten-year survival outcomes were uniformly dismal for all patients with stage 4 disease (EFS, 3 ± 3%; OS, 5 ± 5%).

Outcomes With or Without Myeloablative Consolidation

Among patients ≥5 to <10 years with stage 4 disease (n = 327), those who received consolidation therapy with AHCT (n = 121) had significantly superior EFS and OS rates compared to those who did not (5-year EFS: 30 ± 6% vs. 14 ± 3%, $P = 0.0001$; 5-year OS: 43 ± 6% vs. 23 ± 3%, $P = 0.001$; Table III). Similarly, in patients ≥10 years at diagnosis with stage 4 disease (n = 92), those who received AHCT (n = 33) also had significantly superior EFS and OS compared to those who did not 5-year EFS: 20 ± 9% v 7 ± 4%, $P = 0.03$; 5-year OS: 37 ± 13% v 18 ± 6%, $P = 0.046$.

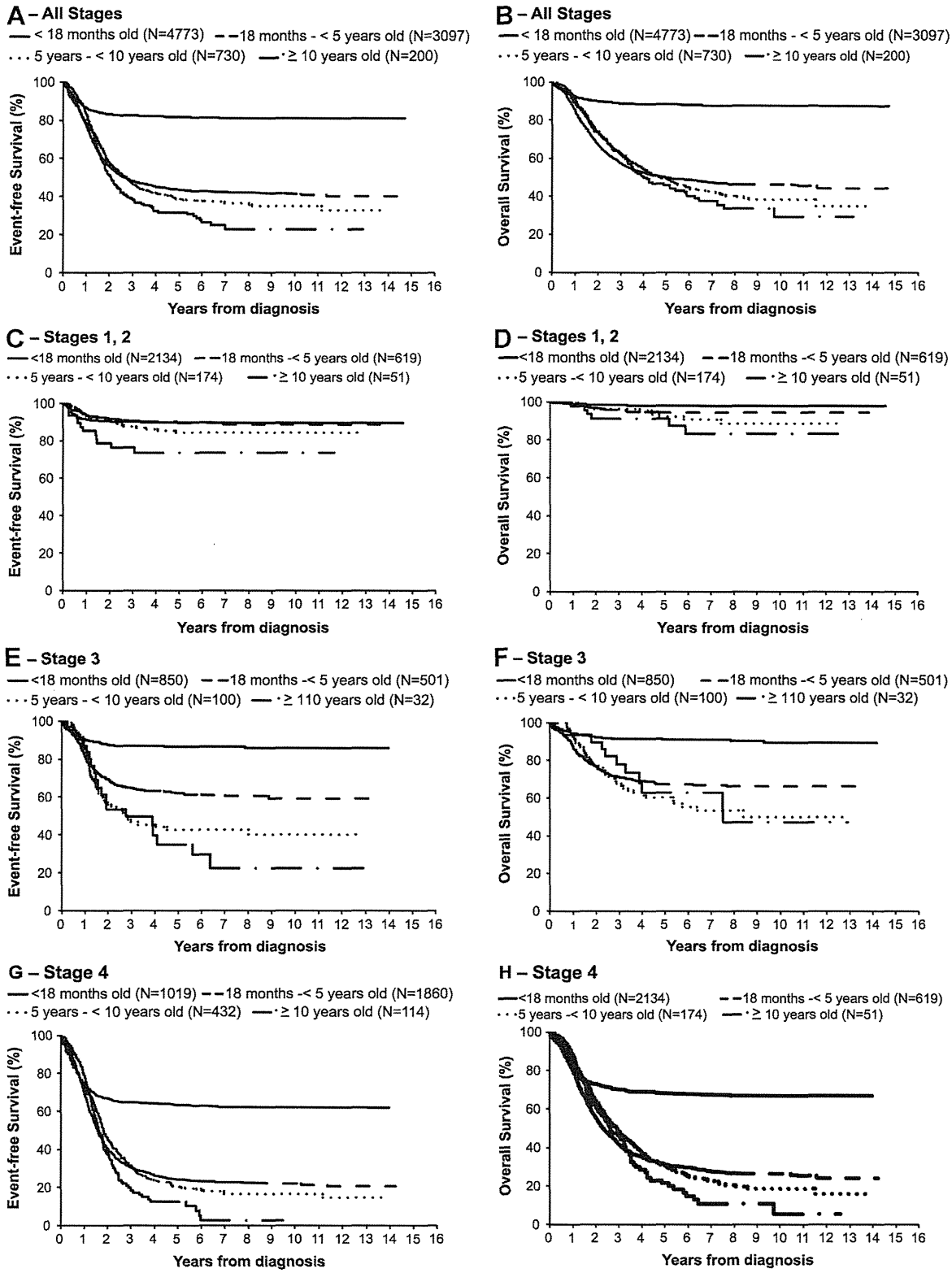

Fig. 2. Kaplan–Meier curves of EFS and OS (from diagnosis) by diagnostic age cohort. *P*-values are for the log-rank tests of all pairwise comparisons of the three age groups: (18 months to <5 years)[A], (≥5 years to <10 years)[B], (≥10 years)[C]. **A**: EFS (A v B: $P = 0.7$; A v C: $P = 0.005$; B v C: $P = 0.01$); **B**: OS (A v B: $P = 0.8$; A v C: $P = 0.5$; B v C: $P = 0.4$); **C**: EFS within Stages 1,2 (A v B: $P = 0.2$; A v C: $P = 0.001$; B v C: $P = 0.05$); **D**: OS within Stages 1,2 (A v B: $P = 0.3$; A v C: $P = 0.03$; B v C: $P = 0.3$); **E**: EFS within Stage 3 (A v B: $P = 0.005$; A v C: $P = 0.01$; B v C: $P = 0.5$); **F**: OS within Stage 3 (A v B: $P = 0.1$; A v C: $P = 1.0$; B v C: $P = 0.5$); **G**: EFS within Stage 4 (A v B: $P = 0.5$; A v C: $P = 0.03$; B v C: $P = 0.005$); and **H**: OS within Stage 4 (A v B: $P = 0.3$; A v C: $P = 0.3$; B v C: $P = 0.06$).

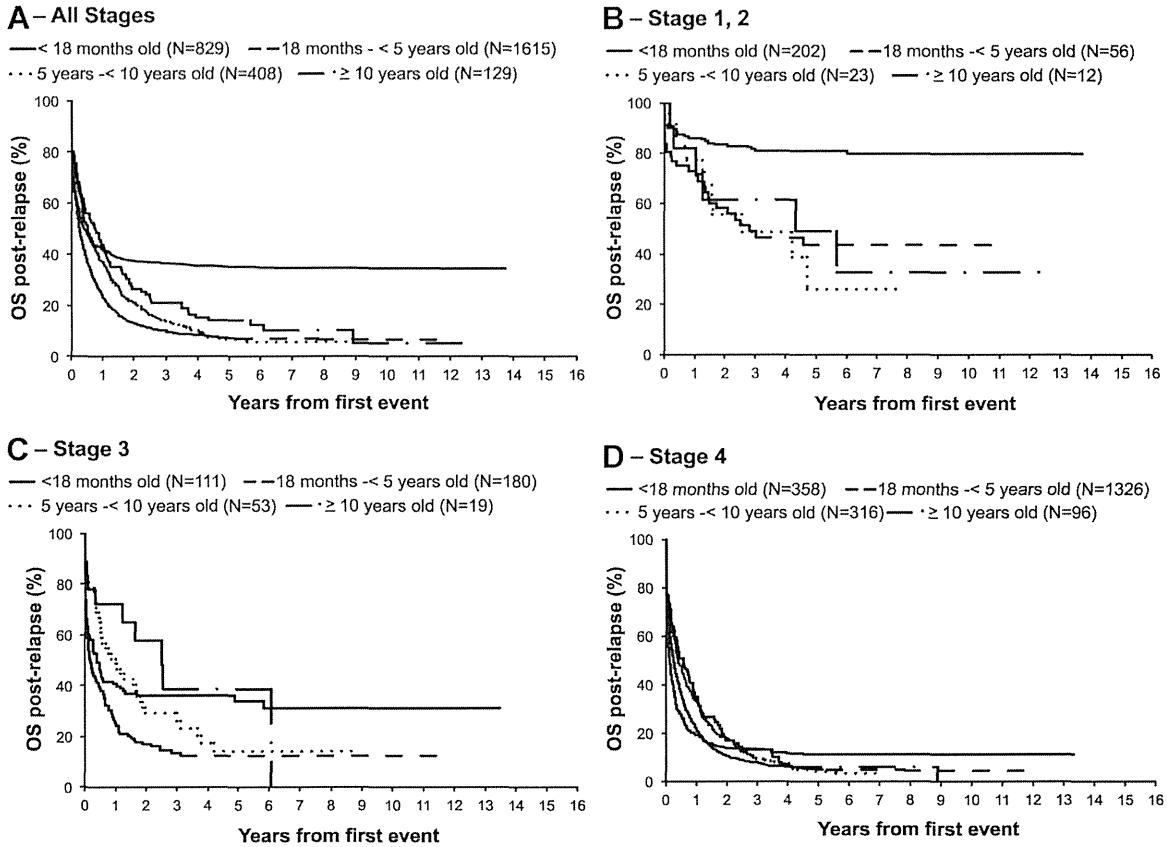

Fig. 3. Kaplan–Meier curves of OS post-relapse by diagnostic age cohort. *P*-values are for the log-rank tests of all pairwise comparisons of three age groups: (18 months to <5 years)[A], (≥5 years to <10 years)[B], (≥10 years)[C]. **A**: OS post-relapse (A v B: $P < 0.0001$; A v C: $P < 0.0001$; B v C: $P = 0.05$); **B**: OS post-relapse within Stages 1, 2 (A v B: $P = 0.8$; A v C: $P = 0.9$; B v C: $P = 0.7$); **C**: OS post-relapse within Stage 3 (A v B: $P = 0.006$; A v C: $P = 0.003$; B v C: $P = 0.1$); and **D**: OS post-relapse within Stage 4 (A v B: $P = 0.002$; A v C: $P = 0.01$; B v C: $P = 0.5$).

Multivariable Analyses of EFS and OS

Multivariable analyses were performed within patients ≥5 to <10 years and within patients ≥10 years. Results in both cohorts were similar, so without evidence for an age cut-off over 5 years, and to increase power, the cohorts were combined. For patients ≥5 years (n = 363 with known data for all factors), stage 4 (EFS: $P < 0.0001$, HR = 2.2; OS: $P < 0.0001$, HR = 3.7), *MYCN* amplification (EFS: $P = 0.03$, HR = 1.6; OS: $P < 0.0001$, HR = 2.6), and unfavorable histology (EFS: $P < 0.0001$, HR = 11.8; OS: $P = 0.002$, HR = 5.2) all carried increased risk of an event or death. Despite limited power, when the analysis was restricted to the oldest patients ≥10 years (n = 75 with known data for all factors), *MYCN* amplification (EFS: $P = 0.03$, HR = 3.2) and unfavorable histology (EFS: $P = 0.02$, HR = 11.6) carried increased risk of an event, while stage 4 (OS: $P < 0.0001$, HR = 5.3) and *MYCN* amplification (OS: $P = 0.0001$, HR = 3.8) carried increased risk of death.

DISCUSSION

Less than 5% of neuroblastoma is diagnosed in adolescents and adults [2,14]. Due to its rarity, there is a paucity of information regarding clinical and biologic prognostic factors and survival outcomes. Reports have been limited to data from single institution series or national cancer registries [7,8,15–19]. These reports suggest that older patients have unique biology characterized by an indolent disease course, but with ultimately dismal outcomes for high-risk patients [16,17]. The distinct phenotype of older patients may reflect underlying differences in tumor biology. Segmental chromosome aberrations, such as LOH at 11q and 3p, are associated with older age at diagnosis [7,20]. The recent identification of ATRX mutations predominantly in older patients with neuroblastoma supports inherent differences in genetic mutations based on age [21].

There has been no consensus on a diagnostic age threshold that defines a group of older patients with inferior survival, and age cohort comparisons have been arbitrary [16]. An indolent phenotype was first described for children ≥ 6 years at diagnosis who had a prolonged median survival compared to younger children [15,22]. However, children ≥10 years are the focus of published multi-center experiences [7,8,16], while adolescent cohorts have also been described, making comparisons within the published literature difficult [17–19]. Of additional concern is that historically, the comparison group of younger patients has included children aged ≥12 to <18 months, who are now recognized to have more favorable outcomes [6]. We show that increasing age correlates with inferior EFS and OS, but the effect of age is best understood as a continuum with no statistically justified optimal age cut-off for patients over 18 months that defines differences in EFS or OS. We also show that increasing age at

TABLE I. Prevalence of Baseline Clinical and Biological Characteristics in Children and Adolescents With Neuroblastoma

	Diagnostic age cohort			*P*-value		
	≥18 months to <5 years (n = 3,097)	≥5 to <10 years (n = 730)	≥10 years (n = 200)	≥18 months to <5 years vs. ≥5 to <10 years	≥18 months to <5 years vs. ≥10 years	≥5 years to <10 years vs. ≥10 years
INSS stage						
1,2	619 (21)	174 (25)	51 (26)	0.02	0.1	0.7
3,4	2,361 (79)	532 (75)	146 (74)			
Unknown	117	24	3			
Ploidy						
≤1 (diploid, hypodiploid)	515 (45)	119 (48)	27 (42)	0.4	0.6	0.4
>1 (hyperdiploid)	628 (55)	129 (52)	38 (58)			
Unknown	1,954	482	135			
Histologic Classification						
Favorable	393 (29)	89 (28)	18 (20)	0.6	0.04	0.1
Unfavorable	941 (71)	230 (72)	74 (80)			
Unknown	1,763	411	108			
MKI						
Low/intermediate	683 (77)	158 (88)	53 (90)	0.002	0.03	0.7
High	200 (23)	22 (12)	6 (10)			
Unknown	2,214	550	141			
Grade						
Undifferentiated	824 (82)	187 (88)	63 (93)	0.03	0.02	0.3
Differentiating	182 (18)	25 (12)	5 (7)			
Unknown	2,091	518	132			
Metastatic site of tumor at diagnosis						
Bone marrow or bone	1,487 (87)	340 (87)	90 (86)	1.0	0.6	0.6
Other site	216 (13)	49 (13)	16 (14)			
Unknown	1,394	341	95			
Primary tumor site						
Adrenal	1,447 (50)	344 (49)	91 (47)	0.9	0.5	0.6
Other primary site	1,472 (50)	354 (51)	103 (53)			
Unknown	178	32	6			
MYCN status						
Not amplified	1,673 (72)	483 (86)	134 (91)	<0.0001	<0.0001	0.1
Amplified	658 (28)	79 (14)	14 (9)			
Unknown	766	168	52			
1p						
No loss or no aberration	416 (61)	103 (75)	45 (80)	0.003	0.005	0.4
LOH or aberration	262 (39)	35 (25)	11 (20)			
Unknown	2,419	592	144			
11q						
No loss or aberration	263 (69)	47 (62)	24 (67)	0.2	0.7	0.6
LOH or aberration	116 (31)	29 (38)	12 (33)			
Unknown	2,718	654	164			

INSS, International Neuroblastoma Staging System; MKI, mitosis karryorhexis index; LOH, loss of heterozygosity.

diagnosis correlates with *prolonged* OS post-relapse, indicative of an indolent phenotype. Children 18 months to <5 years at diagnosis have OS post-relapse that is inferior to patients ≥5 to <10 years or ≥10 years; this pattern is similar to that described in London et al. [23] for patients of all ages, where patients <18 months at diagnosis had a 2.4 times greater risk of death post-relapse than patients ≥18 months.

The largest previous series of neuroblastoma in adolescents was unable to show statistically significant differences in the prevalence of clinical and biological baseline characteristics between older and younger diagnostic age cohorts, although *MYCN* amplification has been reported in only 11–13% of patients older than 10 years at diagnosis [8,16]. We report that 14% and 9% in children ≥5 to <10 years and ≥10 years, respectively, have *MYCN* amplified disease, which is significantly less than 28% of patients ≥18 months to <5 years ($P < 0.0001$; Table I). Older patients were also more likely to have low/intermediate MKI and undifferentiated grade. Histologic classification was a significant prognostic factor; this prognostic ability was driven by grade and/or MKI, because the patients were ≥5 years old. Tests of grade and MKI were underpowered due to missing data. Older patients were less likely to have LOH at 1p compared to children ≥18 months to

TABLE II. Baseline Characteristics and Survival Outcomes in Older Children Versus Adolescents With Neuroblastoma

	Patients diagnosed at ≥10 years (n = 200)					Patients diagnosed at ≥5 to <10 years (n = 730)				
Characteristic	N (%)	5-year EFS ± SE (%)	EFS *P*-value	5-year OS ± SE (%)	OS *P*-value	N (%)	5-year EFS ± SE (%)	EFS *P*-value	5-year OS ± SE (%)	OS *P*-value
Overall	200	32 ± 4		46 ± 5		730	39 ± 2		49 ± 2	
INSS stage										
1,2	51 (26)	74 ± 9	<0.0001[a]	91 ± 5	<0.0001[a]	174 (25)	84 ± 4	<0.0001[a]	92 ± 3	<0.0001[a]
3	32 (16)	35 ± 11		63 ± 11		100 (14)	43 ± 6		60 ± 6	
4	114 (58)	12 ± 4		22 ± 5		432 (61)	21 ± 3		31 ± 3	
Unknown	3					24				
Ploidy										
≤1 (diploid, hypodiploid)	27 (42)	42 ± 13	0.8	42 ± 13	0.9	119 (48)	47 ± 7	0.3	52 ± 7	0.2
> 1 (hyperdiploid)	38 (58)	30 ± 18		43 ± 16		129 (52)	32 ± 7		40 ± 7	
Unknown	135					480				
Histologic Classification										
Favorable	18 (20)	94 ± 8	<0.0001	94 ± 8	0.001	89 (28)	96 ± 3	<0.0001	97 ± 3	<0.0001
Unfavorable	74 (80)	27 ± 8		46 ± 9		230 (72)	32 ± 5		48 ± 5	
Unknown	108					409				
MKI[b]										
Low/intermediate	53 (90)	15 ± 7	0.9	33 ± 9	0.4	158 (88)	34 ± 5	0.9	50 ± 6	0.4
High	6 (10)	0		0		22 (12)	34 ± 12		34 ± 12	
Unknown	141					548				
Grade[b]										
Undifferentiated	63 (93)	25 ± 8	0.9	40 ± 9	0.7	187 (88)	28 ± 5	0.1	43 ± 5	0.2
Differentiating	5 (7)	0		27 ± 23		25 (12)	54 ± 12		55 ± 12	
Unknown	132					517				
Metastatic site at diagnosis										
Bone marrow or bone	90 (86)	13 ± 4	0.9	20 ± 5	0.3	340 (87)	19 ± 3	0.03	30 ± 3	0.1
Other site	15 (14)	13 ± 12		45 ± 15		49 (13)	37 ± 9		45 ± 9	
Unknown	94					338				
Primary tumor site										
Adrenal	91 (47)	32 ± 6	0.5	45 ± 7	0.6	344 (49)	28 ± 3	<0.0001	37 ± 3	<0.0001
Other primary site	103 (53)	29 ± 6		45 ± 7		354 (51)	50 ± 3		61 ± 3	
Unknown	6					32				
MYCN status										
Not Amplified	134 (91)	30 ± 6	0.01	47 ± 6	<0.0001	483 (86)	45 ± 3	<0.0001	57 ± 3	<0.0001
Amplified	14 (9)	15 ± 14		0		79 (14)	20 ± 6		27 ± 6	
Unknown	52					168				
1p										
No loss or no aberration	45 (80)	34 ± 10	1.0	47 ± 10	0.4	103 (75)	44 ± 6	0.4	58 ± 7	0.3
LOH or aberration	11 (20)	24 ± 15		54 ± 26		35 (25)	38 ± 11		50 ± 11	
Unknown	144					589				
11q										
No loss or aberration	24 (67)	28 ± 17	0.6	46 ± 15	0.8	47 (62)	45 ± 11	0.2	65 ± 10	0.2
LOH or aberration	12 (33)	0		44 ± 23		29 (38)	31 ± 15		52 ± 16	
Unknown	164					651				

EFS, event-free survival; SE, standard error; OS, overall survival; INSS, International Neuroblastoma Staging System; MKI, mitosis karryorhexis index; LOH, loss of heterozygosity. [a]Stage 1,2 versus stage 3,4. [b]17 of the 18 favorable histology patients had unreported MKI and Grade.

<5 years at diagnosis, in keeping with a lower prevalence of *MYCN* amplification.

Current risk stratification approaches use independently prognostic biologic and clinical characteristics to categorize patients into low, intermediate or high-risk groups. Among children ≥5 to <10 years and ≥10 years at diagnosis, our analysis confirms the independent prognostic significance of *MYCN* amplification, INSS stage and histology classification on both EFS and OS. LOH at chromosome 1p or 11q is now also recognized to be independently prognostic of EFS in patients with low and intermediate risk neuroblastoma [3,24]. We confirm that LOH 11q is a significant prognostic indicator of EFS in patients with stage 1 or 2 disease who are ≥5 to <10 years at diagnosis.

Previous series have reported conflicting results regarding survival outcomes in older patients with low-risk disease. Data from the Italian Neuroblastoma Registry suggests inferior OS for patients with loco-regional disease who were ≥6 years at diagnosis compared to younger children (OS 81% v 93%) [16]. Gaspar et al. [8] report an OS of 92% in six adolescents ≥10 years at diagnosis with localized disease, although EFS was inferior in older

TABLE III. Impact of High Dose Chemotherapy and Autologous Hematopoietic Cell Transplantation on Survival Outcomes in Older Children and Adolescents With INSS Stage 4 Neuroblastoma

Patient group	N	5-year EFS ± SE (%)	EFS *P*-value	5-year OS ± SE (%)	OS *P*-value
≥5 years					
AHCT	154	28 ± 5	<0.0001	42 ± 6	0.0001
No AHCT	265	13 ± 2		22 ± 3	
≥5 to <10 years					
AHCT	121	30 ± 6	0.0001	43 ± 6	0.001
No AHCT	206	14 ± 3		23 ± 3	
≥10 years					
AHCT	33	20 ± 9	0.03	37 ± 13	0.046
No AHCT	59	7 ± 4		18 ± 6	

EFS, event-free survival; SE, standard error; OS, overall survival; INSS, International Neuroblastoma Staging System; AHCT, autologous hematopoietic cell transplantation.

children. We have identified a group of patients ≥5 years at diagnosis who have favorable outcomes: patients with INSS stage 1,2 disease or favorable histology have 5-year EFS ~80% and 5-year OS >90% (Table II).

Improvements in survival for high-risk neuroblastoma patients have resulted from an intensive multi-modality approach to therapy, which includes multi-agent chemotherapy, surgery, consolidation with AHCT, radiotherapy and maintenance with isotretinoin and immunotherapy [25,26]. These therapeutic advances have not been specifically evaluated in older children and adolescents. We show that consolidation with AHCT as part of initial therapy appears to confer a survival advantage for older patients with high-risk disease. Although only 36% of stage 4 patients ≥10 years received AHCT as a component of frontline therapy, we report ultimately dismal outcomes with 10-year EFS and OS of 3 ± 3% and 5 ± 5%, respectively, indicating the desperate need to develop novel therapeutic approaches.

We report statistically significant differences in baseline characteristics for older children with neuroblastoma and identify which are prognostic of survival outcomes for the first time. Older age at diagnosis is associated with inferior EFS and prolonged survival post-relapse; however, outcome changes with increasing age-at-diagnosis are on a gradual continuum. Limitations of our study include its retrospective, observational design and the exclusion of adult patients >21 years. Although rare, reports suggest that adults with neuroblastoma have outcomes which are inferior even to older children and adolescents [2]. Our results suggest a survival advantage for older patients with stage 4 disease treated with AHCT; however, due to the observational nature of retrospective data, this may be influenced by selection bias in which only patients with good initial disease response and minimal toxicity were offered AHCT [25]. Regardless, our results support the feasibility of AHCT in this population and suggest that an intensive approach to therapy is an integral part to any curative attempt in older patients with high-risk disease. Alternative targeted therapeutic approaches should also be considered, including high-dose ^{131}I-MIBG therapy, shown to induce response in 50% of children ≥10 years with refractory neuroblastoma [27].

In conclusion, we confirm the indolent nature of neuroblastoma in older children and adolescents, who have prolonged survival following relapse (Fig. 1B). This indolent phenotype is associated with a significantly lower prevalence of high MKI, differentiating grade, *MYCN* amplification, and 1p LOH in patients ≥5 years compared to those ≥18 months to <5 years. For patients ≥5 years, the risk of an event slightly and gradually increases with increasing age at diagnosis (Fig. 1A); however, the risk of death after relapse slightly and gradually *decreases* with increasing age at diagnosis. Currently accepted prognostic factors *MYCN* amplification, INSS stage and histology are applicable to older patients. However, most older patients with indolent phenotype eventually die from disease post-relapse. Our results support the inclusion of high-dose chemotherapy and AHCT in initial therapy for children ≥5 years with stage 4 disease and highlight the desperate need for novel therapies.

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ORIGINAL ARTICLE

Efficacy of preoperative transcatheter arterial chemoembolization combined with systemic chemotherapy for treatment of unresectable hepatoblastoma in children

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Abstract
Purpose The purpose of this study was to evaluate, retrospectively, the clinical efficacy of preoperative transcatheter arterial chemoembolization (TACE) combined with systemic chemotherapy for unresectable hepatoblastoma.
Materials and methods Five boys and three girls (mean age 15.2 months) were treated with preoperative TACE combined with systemic chemotherapy for unresectable hepatoblastomas. Mean tumor diameter and mean alfa-fetoprotein (AFP) level were 11.8 cm and 549,386 ng/mL, respectively. Pretreatment, the extent of disease (PRETEXT) was: II, 1; III, 6; IV, 1. For all patients, preoperative systemic chemotherapy was administered before TACE. At each TACE, carboplatin and adriamycin mixed with iodized oil were infused into the feeding arteries. Tumor response and prognosis after treatment were evaluated.
Results TACE resulted in few Grade 1 adverse effects (AEs), without G3 or more AEs, according to CTACAE 3.0. Mean tumor shrinkage was 60.9 %, and the mean AFP decrease from initial levels was 94.8 %. In all cases TACE combined with systemic chemotherapy enabled subsequent safe and complete surgical resection. After a mean follow-up of 59 months, tumor-free survival was 75 %.
Conclusion Preoperative TACE combined with systemic chemotherapy was effective in inducing surgical resectability of unresectable hepatoblastoma.

Keywords Hepatoblastoma · TACE · Systemic chemotherapy

Introduction

Hepatoblastoma is the most common malignancy of the liver among infants and children [1–3]. It is well known that the resectability of the primary tumor is the most

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important factor determining the long-term survival of children with hepatoblastoma, and complete surgical resection of the primary tumor is absolutely vital to achieve cure [3–10]. However, 50 % of cases are unresectable at initial presentation because of local bilobar disease, portal invasion, or metastatic spread. This situation has traditionally been associated with a poor prognosis [3, 6, 7, 11]. Preoperative systemic chemotherapy is vital to reduce tumor size and control tumor spread, to convert an unresectable tumor to a resectable one, thus improving prognosis [7]. However, the associated systemic adverse effects, for example myelosuppression and cardiotoxicity, sometimes lead to delayed surgery and hence tumor regrowth and chemotherapy-related death [12]. There are also problems of drug resistance [9], and induction of a second malignancy [13, 14] by use of anticancer drugs. To reduce these disadvantages of systemic chemotherapy, transcatheter arterial chemoembolization (TACE) may be an alternative to systemic chemotherapy. TACE has the advantages of maximum drug uptake by the tumor and minimum systemic exposure to the drug. It can, furthermore, be combined with arterial embolization, to occlude feeding arteries, and thus induce ischemic tumor necrosis and prolong the dwell time of anticancer drugs in the tumor vasculature, which enhances their effect [12]. We report a series of hepatoblastoma cases that were successfully treated by preoperative TACE combined with systemic chemotherapy. The clinical efficacy of preoperative TACE combined with systemic chemotherapy, and tumor response and prognosis, were evaluated.

Materials and methods

The retrospective study was performed with the approval of our institutional review board, and written informed consent was obtained from all the patients' legal guardians.

Patients

Between March 2001 and March 2008, 11 children with hepatoblastoma were referred to our institution. Three children underwent right lobectomy without preoperative systemic chemotherapy and TACE, because their tumors were located in the right lobe only and no invasion of the portal vein and hepatic vein was revealed by preoperative contrast-enhanced computed tomography (CT). The other eight children, treated by preoperative TACE combined with systemic chemotherapy for unresectable hepatoblastoma, form the basis of this study. The patients consisted of five boys and three girls, ranging in age from 3 to 38 months (15.2 ± 4.0 months, mean ± SD). We did not perform biopsies before treatment, because of the risk of hemorrhage and invasiveness. Diagnosis was made before treatment on the basis of hypervascular hepatic tumor on contrast-enhanced CT findings and high serum α-fetoprotein (AFP) levels. Contrast-enhanced CT was performed to evaluate the tumor site on the basis of which hepatic segments were occupied by the tumor, the pretreatment extent of disease (PRETEXT) [1], the tumor size measured by the maximum diameter on cross-sectional images, and the presence or absence of distant metastasis. The tumor was considered unresectable when there was bilobar disease or inferior vena cava invasion (case 2) before treatment. The patients' characteristics before treatment are summarized in Table 1.

All tumors were diagnosed as hepatoblastoma (1 well differentiated and 7 poorly differentiated) on the basis of pathological examination of resected specimens. Tumor diameter was 11.8 ± 1.2 cm (mean ± SD; 6–16 cm), and AFP level was 549,386 ± 216,091 ng/mL (mean ± SD). The pretreatment extent of disease (PRETEXT) was: II, 1; III, 6; IV, 1.

Treatment

The procedure used for managing cases of unresectable hepatoblastoma was as follows. Patients initially underwent systemic chemotherapy in accordance with the principles of the regimen of the Japanese Study Group for Pediatric Liver Tumor (JPLT-2) protocol [15]. The chemotherapy regimen consisted of repeated courses of cisplatin (CDDP), 80 mg/m^2 × 1 day, and tetrahydropyranyl-adriamycin (THP-ADR), 30 mg/m^2 × 2 days. These courses were repeated every 4 weeks until the tumor showed no response or poor response to systemic chemotherapy, as assessed by enhanced CT scan and serum AFP levels. When their cardiac function, renal function, liver function, and inflammatory reaction improved, patients underwent TACE, by use of the following procedure, irrespective of the resectability of the primary tumor. Under general anesthesia, the femoral artery was catheterized by use of the Seldinger technique. A 3-F or 4-F sheath (Supersheath; Medikit, Tokyo, Japan) was placed in the groin. Under fluoroscopic digital subtraction angiography (DSA), a 3-Fr or 4-Fr cobra-shaped catheter (Medikit) was manipulated into the celiac axis and superior mesenteric artery. Arteriography using 61 % iopamidol (Iopamiron 300; Bayer Japan, Tokyo, Japan) was performed to reveal the anatomy of the hepatic artery and portal vein, identify accessory arteries, and confirm the patency of the portal vein. Through the catheter, a 2.4-Fr (Sniper 2; Clinical Supply, Gifu, Japan) or 2.0-Fr (Mester Cath; Medikit) microcatheter was selectively introduced and directed to the artery supplying the tumor. First, carboplatin (200 mg/m^2) was injected into the feeding artery.

Table 1 Patients' characteristics

No.	Age (m)/gender	Tumor size (cm)	AFP (ng/mL)	Tumor site	Obstruction or stenosis of PV or IVC	AST/ALT (IU/L)	PRETEXT	Histology
1	15/F	15	1,839,700	MAP	RPV	196/75	III	Well differentiated
2	18/M	13	634,000	AP	IVC	27/10	II	Poorly differentiated
3	22/M	11	267,000	MAP	RPV	40/12	III	Poorly differentiated
4	11/M	6	26,895	MAP	Patent	47/13	III	Poorly differentiated
5	4/M	8.5	158,773	LMA	Main PV	35/14	III	Poorly differentiated
6	38/M	11	30,416	MAP	Patent	23/5	III	Poorly differentiated
7	3/F	16	954,300	LMAP	PV	113/15	IV	Poorly differentiated
8	10/F	14	484,000	LMA	LPV	96/35	III	Poorly differentiated
Mean	15.2	11.8	549,386			72/22	–	–

Tumor site: *L* lateral segment, *M* medial segment, *A* anterior segment, *P* posterior segment

Tumor size Max diameter of tumor, *AFP* alfa-fetoprotein, *PV* portal vein, *RPV* right portal vein, *LPV* left portal vein, *IVC* inferior vena cava, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Then, 2–5 mL iodized oil (Lipiodol; Laboratoire Guerbet, Roissy Charles de Gaulle, France), a contrast material containing 1/3 iodized oil, and 30 mg/m^2 THP-ADR were mixed by pumping 10 times by use of a three-way stopcock valve and two 5–10-mL syringes. The dosage of lipiodol was adjusted individually depending on tumor size, necrotic area assessed by enhanced CT, hepatic angiographic findings, and the clinical status of each patient. THP-ADR dispersed in lipiodol was injected into the feeding artery of the tumor until near-complete stasis of the feeding artery had occurred on angiography. The TACE procedure was performed once only. In this study, hepatoblastomas were not embolized with gelatin sponge particles.

Subsequent surgical resection was usually performed ~3 weeks after TACE, when the tumor volume appeared to have decreased sufficiently to enable safe resection by either lobectomy or extended lobectomy. In five cases, two courses of postoperative systemic chemotherapy were added. The chemotherapy regimen consisted of repeated courses of CDDP, 80 mg/m^2 × 1 day, and THP-ADR, 30 mg/m^2 × 2 days or IFO, 3.0 mg/m^2/day × 2, or THP-ADR, 30 mg/m^2/day × 2, etoposide (VP16),100 mg/m^2/day × 5, and carboplatin, 400 mg/m^2/day.

Assessment

The effects of preoperative systemic chemotherapy and TACE were evaluated on the basis of AFP level and tumor volume, calculated by use of the equation volume = 1/2 × length × (transverse diameter)^2, with follow-up CT. In addition, tumor response after systemic chemotherapy and TACE was classified into four grades in accordance with the modified Response Evaluation Criteria for Solid Tumors (mRECIST). The extent of tumor necrosis was histologically evaluated as the percentage necrotic or fibrotic area in the largest section of the surgical specimen. Adverse events related to the TACE were evaluated on the basis of the Common Terminology Criteria for Adverse Events ver. 3.0 and the SIR complication classification system. Tumor recurrence and survival were assessed by reviewing medical records and follow-up enhanced CT. Medical check and monitoring of AFP were conducted 3-monthly; enhanced CT was conducted within 1 month postoperatively, at 3 months, and then every 6 months for 2 years. After the 2nd year, enhanced CT was alternated on a 6-month basis. The follow-up periods after treatment ranged between 13 and 93 months (mean 59 months).

Results

The results of preoperative systemic chemotherapy and TACE are summarized in Table 2.

The number of preoperative systemic chemotherapy cycles ranged from 4 to 6, with a mean of 4.5. The time interval between preoperative systemic chemotherapy and TACE ranged from 16 to 37 days, with a mean value of 26.8 days. The dosage of lipiodol ranged from 2 to 5 (mean 3.9) mL.

Response to preoperative systemic chemotherapy and TACE was as follows. A marked reduction in tumor size associated with a decrease in AFP level occurred after preoperative systemic chemotherapy and TACE (Table 3). After preoperative systemic chemotherapy, compared with initial levels, mean tumor shrinkage was 44.3 % (0–71.9 %) and mean AFP decrease was 63.4 % (0–99.8 %). For all children with obstruction of the portal vein and IVC before treatment, these obstructions were improved after preoperative systemic chemotherapy. After

Table 2 Results of preoperative systemic chemotherapy and TACE

No.	CITA (kur)	Tumor size after CITA (cm)	Time interval from CITA (day)	Catheter system (Fr)	Carboplatin (mg)	ADR (mg)/ LPD (mL)	Treated vessels: infused drug ratio	Procedure time (min)
1	4	11	37	4	80	12/5	RHA: 4 LHA: 1	68
2	5	8.7	28	4	80	13/5	RHA	55
3	4	7.8	23	4	90	11/4.8	RHA	60
4	4	4.5	16	4	90	13/2	A4: 2 RHA: 1	50
5	5	9.6	28	3	60	9/3.6	PHA	55
6	4	10	28	3	100	16/5	RHA: 9 A3: 1	60
7	6	8.5	20	3	50	7.4/2.5	MHA: 9 RHA: 1	100
8	4	7.7	34	3	85	12/3	LHA: 2 MHA: 1	60
Mean	4.5	8.5	26.8	–	79.3	11.6/3.9	–	63.5

CITA preoperative systemic chemotherapy, *cisplatin* 80 mg/m^2/day, *THP-ADR* tetrahydropyranyl-adriamycin 30 mg/m^2/day × 2
CDDP carboplatin, *ADR* tetrahydropyranyl-adriamycin, *LPD* lipiodol

Table 3 Response to preoperative chemotherapy

Patient no.	Tumor shrinkage (%)		AFP decrease (%)		mRECIST		PRETEXT after CITA and TACE	Tumor necrosis (%)
	CITA	CITA + TACE	CITA	CITA + TACE	CITA	CITA + TACE		
1	32.1	51.5	23.0	99.9	PR	CR	II	99
2	52.1	69.3	86.3	97.1	PR	PR	II	80
3	38.1	50.4	81.2	90.1	PR	PR	II	70
4	71.9	81.8	88.8	98.1	PR	CR	I	90
5	0	33.3	0	82.4	PD	PR	II	15
6	21.1	46.4	31.7	93.3	PR	PR	II	85
7	67.9	78.4	97.1	97.6	PR	PR	III	80
8	70.9	76.6	99.8	99.9	PR	PR	II	85
Mean	44.3	60.9	63.4	94.8				75.5

Tumor necrosis: the extent of tumor necrosis was histologically evaluated as percentage necrotic or fibrotic area in the largest section of the surgical specimen

preoperative systemic chemotherapy followed by TACE, compared with initial levels, mean tumor shrinkage was 60.9 % (33.3–81.8 %) and the mean AFP decrease was 94.8 % (82.4–99.9 %). According to modified RECIST criteria, evaluated by enhanced CT, 25 % (2/8) of children achieved complete response and 75 % (6/8) partial response after preoperative systemic chemotherapy followed by TACE. No delay of surgery was encountered. Subsequently, tumor extirpation was performed for all cases (Fig. 1; Table 4), and the time interval between TACE and surgical resection ranged from 19 to 25 days, with a mean of 22 days. Preoperative systemic chemotherapy followed by TACE enabled subsequent safe and complete surgical resection in all cases.

Pathological examination revealed massive necrosis in the excised specimens, and the percentage necrotic area within the tumor ranged from 15 to 99 %, mean 75.5 %. On microscopic examination, all resected specimens had free margins.

Complications and toxicity after TACE

For one child, extravasation of contrast from the superior adrenal artery was recognized during TACE, but it was controlled spontaneously. This complication was classified as A of the Society of Interventional Radiology (SIR) Classification System for Complications by Outcome. There was no femoral puncture complication.

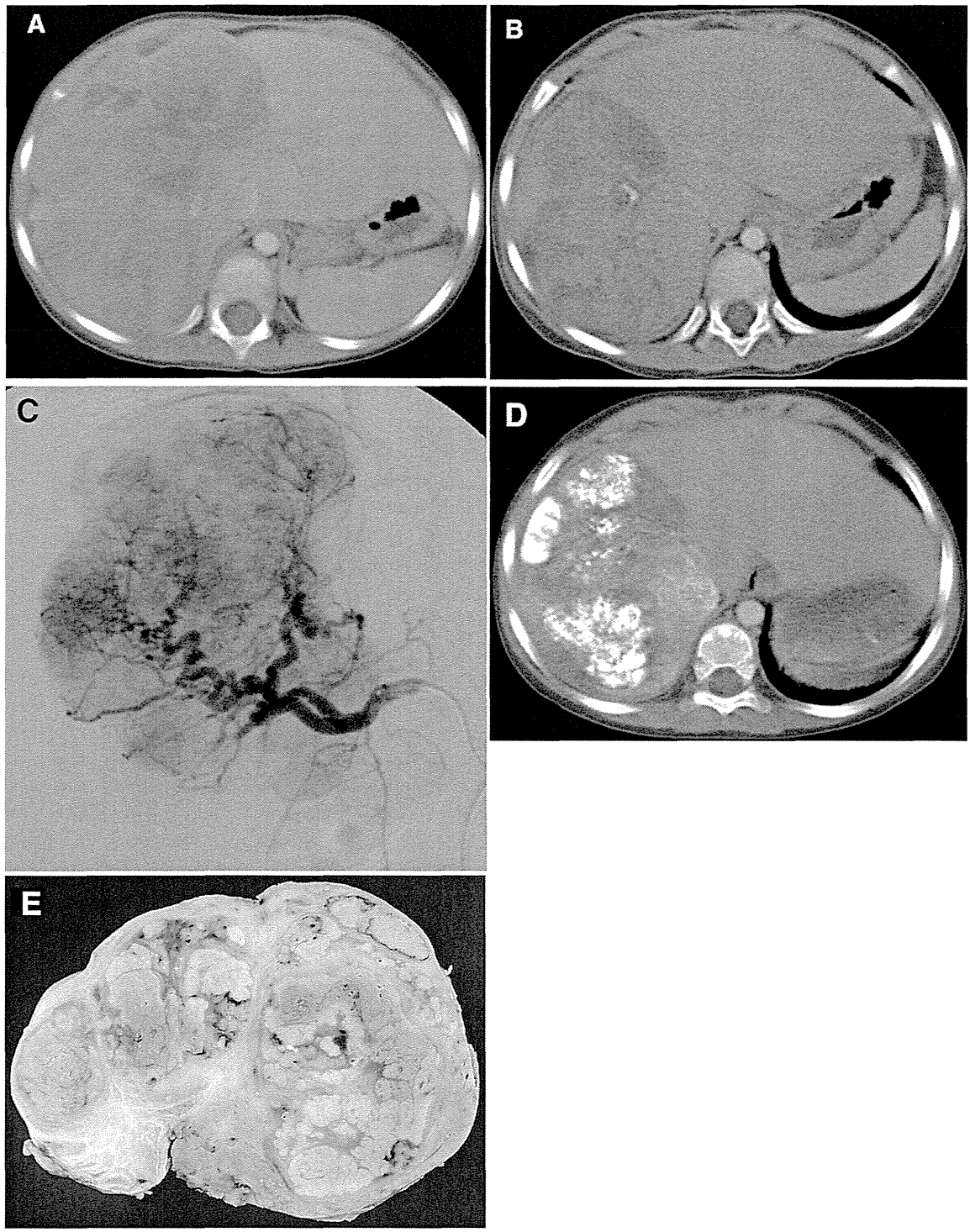

Fig. 1 38-month-old boy with poorly differentiated hepatoblastoma (case 6). Tumor size 11 × 6.4 cm, AFP 30,416 (ng/mL), PRETEXT II. **a** Enhanced CT before treatment reveals a large tumor involving almost the entire right lobe and S4. **b** Enhanced CT after CITA 4 kur. Tumor volume decreased by 21.2 %. **c** Pre-chemoembolization angiogram via the hepatic artery shows a large hypervascular lesion with abundant neovascularity. **d** After TACE, abundant lipiodol deposits appeared in the right lobe and the tumor volume decreased by 32.1 %. **e** Pathological examination of excised specimens showed the percentage necrotic area within the tumor was 85 %. Tumor shrinkage and AFP decrease were, respectively, 21.2 and 31.7 % after 4 kur CITA and 46.4 and 93.3 % after TACE combined with CITA

Table 4 Clinical courses after TACE

No.	Time between surgery and TACE (day)	Operation	Post-operative CTx (kur)	Follow-up (months)	Recurrence	Outcome
1	23	Rt. lobectomy	0	84	–	Alive
2	22	S5 + 6 segmentectomy	0	92	–	Alive
3	22	Rt. lobectomy	2	93	–	Alive
4	23	Tumor resection	0	82	–	Alive
5	25	Rt. lobectomy	2	29	–	Died[a]
6	21	Rt. lobectomy	2	64	–	Alive
7	19	S3, 4, 5, 8 segmentectomy	2	13	Intrahepatic recurrence	Died[b]
8	21	Extended Lt. lobectomy	2	16	–	Alive
	Mean: 22			Mean: 59	12.5 %	75 %

Rt. right, *Lt.* left, *CTx* systemic chemotherapy

[a] Died of a second malignancy (AML)

[b] Died after liver transplantation for intrahepatic recurrence

Grade 1 elevation of fever after TACE was observed for 62.5 % (5/8) of the children. Grade 1 abdominal pain after TACE was observed for 50 % (4/8) of the children. Grade 1 elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) after TACE was observed for 75 % of children (6/8), but no patient suffered from prolonged and severe liver dysfunction. Renal dysfunction, symptoms of myelosuppression, or signs of cardiotoxicity after TACE were not observed for any patient. No patient suffered from TACE-related major complications. No liver failure was noted after surgery and postoperative systemic chemotherapy.

Outcomes

For one patient only, liver metastases were identified in the remaining liver, 2 months after surgery. This patient died of sepsis 4 days after liver transplantation for liver metastases. Recurrence of hepatoblastoma in another patient was not recognized; however, he died of a second malignancy (acute myelogenic leukemia) 23 months after surgery. The other six patients were doing well and were free from disease at last follow-up. During the mean 59-month follow-up, tumor recurrence was 12.5 % and tumor-free survival was 75 %.

Discussion

It has been generally accepted that complete surgical removal of hepatoblastoma is essential for long-term survival [11, 16]. For most patients, however, hepatoblastoma is unresectable at presentation, so several preoperative chemotherapy, including systemic chemotherapy, or TACE protocols are currently used in different centers to increase resectability and improve long-term survival [17, 18]. For patients with unresectable or metastatic hepatoblastoma, the Pediatric Oncology Group (POG) recommended treatment strategy based on intensification of preoperative chemotherapy, depending on tumor response [19]. Recently, response to a variety of chemotherapy protocols, including CDDP and doxorubicin (DOX), has been reported to be 70–76 % [8, 20]. In our series, some patients had a marked response to preoperative systemic chemotherapy with CDDP and THP-ADR—a nearly linear decrease in AFP levels and a reduction in tumor size. For some children, however, poor response to systemic chemotherapy was recognized and reduction of tumor size was insufficient to perform complete surgical resection. Drug resistance is one of the problems of systemic chemotherapy [12]. TACE takes good advantage of the combined antitumor effects of regional chemotherapy and the tumor ischemia caused by occlusion of the feeding artery to achieve maximum reduction in tumor size. TACE can maximize drug uptake by the tumor and minimize exposure of children to drugs [12, 18]. In this study, TACE was performed after it was judged that further tumor shrinkage could not be achieved by additional systemic chemotherapy. For these poor responders to systemic chemotherapy, also, decrease in AFP levels and a reduction in tumor size was observed after TACE. To achieve sufficient tumor reduction to enable its complete resection, TACE seems to have been useful especially for poor responders to systemic chemotherapy. In this study, embolization with gelatin sponges or coils was not conducted to avoid the hepatic infarction or abscess formation related to TACE. Many previous studies on use of TACE for hepatoblastoma reported that TACE using a gelatin sponge was necessary and acceptable. In our many cases, obstruction or stenosis of the portal vein and/or IVC were recognized before treatment. After systemic chemotherapy, partial improvement of major portal vein and/or IVC obstruction was

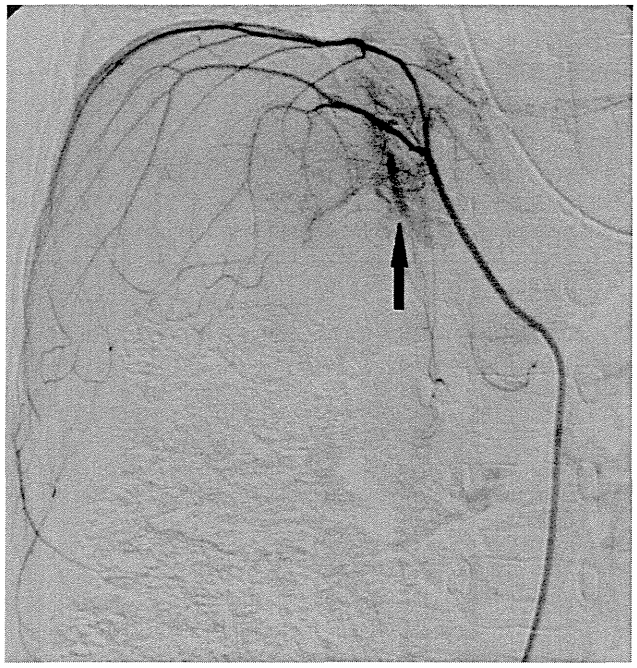

Fig. 2 15-month-old girl with well differentiated hepatoblastoma (case 1). Tumor size 15 cm, AFP 1,839,700 (ng/mL), PRETEXT III. Right inferior phrenic angiogram reveals extravasation of contrast from superior adrenal artery during TACE (*arrow*); it was controlled spontaneously, however

achieved but obstruction of the second or third portal branches occasionally persisted. Therefore, TACE without a gelatin sponge was used in this study [10–12, 17, 18]. Despite this, however, sufficient tumor shrinkage (mean 60.9 %) and AFP decrease (mean 94.8 %) were achieved to enable complete surgical resection after preoperative systemic chemotherapy followed by TACE. Mean tumor shrinkage in this series compares favorably to other reports of systemic chemotherapy alone or repeated TACE alone [18, 21], but the mean AFP decrease in this study was slightly better than in reports for repeated TACE alone [18]. In this study, TACE combined with systemic chemotherapy also enabled subsequent safe and complete surgical resection for all patients, and all resected specimens had free margins on microscopic examination. The histomorphology of hepatoblastoma also determines the response to treatment, thereby affecting outcome [27]. Though the poorly differentiated subtype was present in seven of the eight patients, pathological examination revealed moderate tumor necrosis (mean 75.5 %). In one child (case 5), tumor necrosis was fair compared with the other children. In almost all previous studies reporting TACE for hepatoblastoma, use of embolic agents resulted in surgical resectability with a low incidence of severe complications [5–8, 12, 18]. Because poor responders to TACE without use of an embolic agent are sometimes recognized, embolic agent might be necessary.

In this study, there were no severe complications after TACE. One reason might be that patency of the portal vein was achieved after preoperative systemic chemotherapy. Major portal vein obstruction is known to be a risk factor for liver infarction after TACE [24]. However, major complications related to use of chemoembolic agents, for example acute liver failure, liver infarction, liver abscess, tumor rupture, or pulmonary embolism, must be considered [22–26]. For one patient, iatrogenic dissection or perforation of the superior adrenal artery was recognized during TACE (Fig. 2), but extravasation of contrast from the superior adrenal artery could not be recognized without embolization on selective angiography after TACE. The risk of complications related to the manipulation of a catheter or guidewire, for example iatrogenic dissection or perforation of the celiac artery and its branches, could be higher for children than for adults because of the smaller diameter of each vessel. Needless to say, skillful and careful handling of the smaller devices under fluoroscopic control is the only way to avoid this risk.

Tumor-free survival (75 %) in this series compares favorably with other reports [12, 19]. The reasons might be that our children had no distant metastasis and our combination of systemic chemotherapy and TACE might be able to control not only the main tumor but also nonvisualized micrometastasis. For one patient, only, with PRETEXT IV (case 7), however, although the tumor responded well to preoperative chemotherapy, and complete surgical resection was performed successfully, the tumor recurred locally 2 months after surgical resection. This may be because preexisting micrometastasis was not controlled by our combined therapy. We agree that liver transplantation offers a chance of curing even patients with PRETEXT IV [28]. In one other patient (case 5), no recurrence was seen during follow-up, but he died 29 months postoperatively as a consequence of secondary malignancy. Although rare, we have to recognize that a second malignancy induced by the extremely high dosage of anticancer drugs is a major problem [14].

One limitation of this study is that the long-term effect of preoperative TACE combined with systemic chemotherapy on fertility or perinatal outcome is unclear and must be further evaluated by use of randomized, controlled trials.

In conclusion, the results of this study suggest that preoperative TACE combined with systemic chemotherapy is effective in inducing surgical resectability of unresectable hepatoblastomas, with a low incidence of complications. These results are encouraging, but we cannot draw firm conclusions because of the small number of patients studied.

Conflict of interest We have no direct or indirect financial interest in the products under investigation or the subject matter discussed in this manuscript.

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Prenatal administration of neuropeptide bombesin promotes lung development in a rat model of nitrofen-induced congenital diaphragmatic hernia

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ABSTRACT

Background/purpose: Fetal medical treatment to improve lung hypoplasia in congenital diaphragmatic hernia (CDH) has yet to be established. The neuropeptide bombesin (BBS) might play an important role in lung development. The present study aims to determine whether prenatally administered BBS could be useful to promote fetal lung development in a rat model of nitrofen-induced CDH.

Methods: Pregnant rats were administered with nitrofen (100 mg) on gestation day 9.5 (E9.5). BBS (50 mg/kg/day) was then daily infused intraperitoneally from E14, and fetal lungs were harvested on E21. The expression of PCNA was assessed by both immunohistochemical staining and RT-PCR to determine the amount of cell proliferation. Lung maturity was assessed as the expression of TTF-1, a marker of alveolar epithelial cell type II.

Results: The lung-body-weight ratio was significantly increased in CDH/BBS(+) compared with CDH/BBS(−) ($p < 0.05$). The number of cells stained positive for PCNA and TTF-1 was significantly decreased in CDH/BBS (+) compared with CDH/BBS(−) ($p < 0.01$). The TTF-1 mRNA expression levels were significantly decreased in CDH/BBS(+) compared with CDH/BBS(−) ($p < 0.05$).

Conclusions: Prenatally administered BBS promotes lung development in a rat model of nitrofen-induced CDH. Neuropeptide BBS could help to rescue lung hypoplasia in fetal CDH.

The treatment of newborn babies with congenital diaphragmatic hernia (CDH) remains challenging for pediatric surgeons [1]. Recently, we have introduced new therapeutic approaches in addition to a gentle ventilation strategy and achieved a survival rate of more than 90% in cases of isolated CDH [2]. However, it continues to be impossible to rescue babies with extreme pulmonary hypoplasia. In order to solve this problem, several surgical fetal therapies, including tracheal occlusion, have been developed to promote fetal lung growth [3]. However, the risk of premature birth remains a serious problem with no improvements.

Neuropeptide bombesin (BBS) is a 14-amino acid peptide originally identified in skin of the frog *Bombina bombina*[4]. Its mammalian homologue, which has been identified as gastrin-releasing peptide (GRP), and BBS are referred to collectively as "bombesin-like peptides" (BLPs). The receptors of BBS are known to be widely distributed in the central nervous system and gut [5]. The authors have previously noticed the concept of the brain–gut axis and reported that BBS maintained intestinal mucosal structures and exhibited an immunomodulatory effect in transplanted intestinal allografts while preserving the graft microcirculation and preventing ischemic reperfusion injury [6–10]. In addition, this multipotent neuropeptide has been reported to promote the growth and maturation of the developing fetal lung in both humans and nonhuman primates [11–13]. It was also reported that the highest level of bombesin-like peptide occurred in mid-gestation human fetal lung [14]. However, there have been no reports that exogenously administered BBS could promote growth and maturity of immature lung in pathological condition like immature lungs in CDH.

To evaluate lung maturity, immunohistochemical staining against proliferating cell nuclear antigen(PCNA) has been widely used and reported that PCNA-positive cells in the fetal lung decrease during the late stage of pregnancy in rats [15]. TTF-1 is known as a marker of alveolar epithelial cells type II (AECs-II) and is considered to play an important role in stem cell production in the alveolar epithelium [16]. The differentiation from AECs-II into alveolar epithelial cells type I (AECs-I) should be one of the key processes in lung development in late gestation and the number of TTF-1-positive cells was reported to increase in immature lungs. TTF-1 should be appropriate to evaluate lung maturity in addition to PCNA.

The aim of this study was to investigate whether BBS promotes lung growth and maturity in a rat model of nitrofen-induced CDH.

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