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BRIEF REPORT
Acute Lymphoblastic Leukemia After Temozolomide Treatment for Anaplastic Astrocytoma in a Child With a Germline *TP53* Mutation

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We present a case of a 12-year-old female with a germline *TP53* mutation who presented with anaplastic astrocytoma and subsequent acute lymphoblastic leukemia (ALL) 13 months after starting treatment with temozolomide (TMZ). The patient had no family history of malignancy except her grand father and his siblings. Although alkylating agents such as TMZ are known to induce secondary hematologic malignancy, only several cases of treatment-related acute leukemia have been reported after TMZ-alone chemotherapy for malignant gliomas. We demonstrate a rare case of TMZ-related ALL in a child with glioma possibly associated with a germline *TP53* mutation. Pediatr Blood Cancer. 2010;55:577–579. © 2010 Wiley-Liss, Inc.

Key words: alkylating agent; glioma; p53; temozolomide; treatment-related acute leukemia

INTRODUCTION

Temozolomide (TMZ) is an oral alkylating agent used in the treatment of gliomas. The main toxicity of TMZ is myelotoxicity [1], but the incidence of grade 3–4 events is less than 10%. Because of its efficacy and tolerability, TMZ has become the first choice of chemotherapy for high-grade gliomas with a prolonged survival [2]. Prior to the introduction of TMZ, alkylating agents such as carmustine (BCNU), lomustine (CCNU), nimustine (ACNU), and procarbazine had been used as a first line for the treatment of malignant gliomas. These alkylating agents and topoisomerase inhibitors (e.g., etoposide) are well known to induce treatment-related myelodysplastic syndrome (t-MDS), acute myeloid leukemia (AML), or acute lymphoblastic leukemia (ALL) in cancer patients [3–5]. The development of t-MDS/AML and ALL are related to dose, therapy duration, and patient age. Since the current TMZ therapy for gliomas has been introduced after the use of other alkylating agents and has shown a prolonged survival, treatment-related acute leukemia (t-AL) may be on the rise in patients with gliomas. However, reports of t-AL associated with TMZ are few and it remains still unclear whether TMZ has the same leukemogenic potential as other alkylating agents. Here, we present a case of ALL occurred in a child with anaplastic astrocytoma after TMZ alone chemotherapy possibly in association with a germline *TP53* mutation.

CASE REPORT

A 12-year-old female developed numbness in her right side and was admitted to another hospital. She had no other medical history, but her grand father had a gastric cancer and he had two siblings with cancer. Magnetic resonance imaging (MRI) of her brain showed bilateral diffuse infiltrating tumors with contrast enhancement. She was transferred to our hospital and underwent biopsy of the right frontal lobe, and diagnosed with anaplastic astrocytoma. She started combination treatment with TMZ and local brain radiotherapy. TMZ was administered orally at 75 mg/m^2/daily for 6 weeks. LBRT was administered simultaneously at whole dose of 60 Gy in 30 fractions of 2 Gy limited in high intensity region of the brain on T2-weighted MRI. After combined therapy, she started maintenance chemotherapy with TMZ at 150 mg/m^2/daily for 5 days. One month later, the dose of TMZ was increased to 200 mg/m^2/daily for 5 days every month and she received 8 more cycles of TMZ. Between fifth and sixth course she developed excessive myelosuppression and chemotherapy was suspended for 2 weeks. Thirty-seven days from the last dose of TMZ, the peripheral blood had 34% leukemic blasts, and the patient was referred to the department of pediatric oncology in our hospital. On admission, the white cell count was 4,100/μl, hemoglobin was 12.3 g/dl, and platelet count was 25,000/μl. The differential count showed 46.0% neutrophils, 4.0% eosinophils, 4.0% monocytes, 11.0% lymphocytes, 3% myelocytes, and 35.0% leukemic blasts. Serum LDH was 663 U/L. A bone marrow aspiration demonstrated a massive infiltration of blast cells (86%). On flow cytometry, the blasts were positive for CD10 (78%), CD19 (100%), HLA-DR (100%), CD45 (100%), TdT (85%), and CD34 (24%). Cytogenetic analysis showed no chromosomal abnormality. A diagnosis of precursor B ALL was made. The patient was treated with induction chemotherapy consisting of prednisolone, vincristine, pirarubicin, and asparaginase. Central nervous system prophylaxis was simultaneously administered with intrathecal methotrexate, cytarabine, and hydrocortisone. Consolidation therapy was started but soon suspended because of complications. Follow-up MRI examinations for brain tumor during the treatment for ALL revealed slight brain atrophy and hydrocephalus without evidence of disease recurrence. She died from pneumonia 8 months after the diagnosis of ALL. We performed DNA sequencing analyses for *TP53* of the tumor and peripheral blood samples collected during the brain biopsy. A premature stop codon (CGA to TGA at codon 213) was detected in both samples, while immunohistochemistry of the p53 protein revealed no positive staining in the tumor tissue.

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Conflict of interest: Nothing to declare.

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TABLE I. Review of the Literature of t-MDS/AML and t-ALL Associated With TMZ-Alone Chemotherapy

Case	Age/ sex	Diagnosis	Radiation (Gy)	Total TMZ dose (mg/m^2)	Latency after TMZ (mo)	Hematological malignancy	Cytogenetics	Refs.
1	40/M	GBM	60.0	3,100	4	ALL	45, XY, −7, der(9) (p12) t(9;22)	[11]
2	44/F	LGA	54.0	24,000	28	MDS	−5, −7	[10]
3	34/F	AOA	59.4	28,000	3	MDS/AML	−5, −7	[10]
4	12/F	AA	60.0	12,900	13	ALL	Normal	Present case

TMZ, temozolomide; GBM, glioblastoma multiforme; LGA, low-grade astrocytoma; AOA, anaplastic oligoastrocytoma; AA, anaplastic astrocytoma; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia.

DISCUSSION

In this report, we present a case of TMZ-related ALL. The present case is the second published report of treatment-related ALL in glioma patients treated with TMZ and the first published report of t-AL in children after TMZ treatment for gliomas. Since the effect of radiotherapy on secondary MDS/AL is considered to be limited [5], we assume that our case of ALL is mainly related to a treatment with TMZ and a germline *TP53* mutation. Although the reports of t-MDS/AML and ALL in glioma patients treated with nitrosoureas such as BCNU, CCNU, and ACNU have been accumulating [5–7], the leukemogenic activity of another alkylator TMZ has not fully evaluated yet. There are several reports of t-MDS/AML in association with TMZ, however, most of these cases were treated with TMZ after the treatment with other alkylating agents [7–10]. Only four cases of t-MDS/AML and ALL have been reported occurring after chemotherapy with TMZ alone in glioma patients (Table I) [10,11].

Secondary leukemia in cancer survivors accounts for 5–10% of all acute leukemias [12]. Although secondary MDS/AML are the most frequent entities among patients with secondary leukemia, secondary ALL represents certain numbers accounting for about 10% of all secondary leukemia [12,13]. In general, there are other possibilities to explain the development of ALL in cancer patients. One is genetic susceptibility to cancer. Heterozygous *TP53* mutation results in the Li–Fraumeni syndrome of a hereditary predisposition to cancer including astrocytic brain tumor and acute leukemia. However, de novo acute leukemia in patients with the Li–Fraumeni syndrome has been reported to account for only 20 of the 738 cancers (2.7%) in the 185 kindreds [14]; and germ-line *TP53* mutations are uncommon in patients with acute leukemia [15]. Another possibility is that the ALL occurred as a random event. Given the fact that the patient had a *TP53* germline mutation and a family history of cancer in the present case, the genetic background might have a substantial effect on cancer development. Considering of the leukemogenic activity of alkylators and the timing of the onset of ALL, TMZ might also be a primary factor in our patients. Moreover, since TMZ is known to have a toxic effect especially on lymphocytes [16], it is interesting that two out of four TMZ-related MDS/AMLs and ALLs in the reported cases presented as an ALL.

The incidence of treatment-related AL has been reported in a large prospective study of 1,628 brain tumor patients treated with CCNU [6]. In this report, only 10.9% of the study participants were followed for more than two years, and only two cases of treatment-related AL were observed in 1,682 patients (0.12%). As the median latency between the initiation of therapy and the diagnosis of t-MDS/AML and ALL has been reported to be 31 months in brain tumor patients and to be 50–70 months in patients with other malignancies [5,17,18], the incidence of treatment-related AL may be much higher than those reported. Chamberlain and Raizer [10] have reported seven cases of t-MDS/AML during the treatment of gliomas. Of the seven patients, five patients had been treated with nitrosoureas and TMZ, and two patients with TMZ alone. These data indicate that the combination of nitrosourea and TMZ may increase the incidence of alkylator-induced MDS/AML and ALL. However, there are no data to suggest that TMZ is more likely to induce secondary hematological malignancies than nitrosoureas or to enhance the leukemogenic activity of other alkylators.

In conclusion, increasing evidence indicate that TMZ may have the same leukemogenic potential as other alkylating agents. As TMZ has only been approved in the last decade and the survival rate in glioma patients has increased, TMZ-related MDS/AML and ALL will become more frequent. Although treatment-related ALL is a relatively rare entity in secondary leukemia, it may become more problematic in patients with glioma because of the increased use of TMZ.

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Original

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Pharmacokinetic and pharmacodynamic investigation of irinotecan hydrochloride in pediatric patients with recurrent or progressive solid tumors

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Key word
CPT-11 – SN-38 – population pharmacokinetics – pharmacodynamics – pediatrics

Abstract. Objective: A multicenter Phase I/II study of Irinotecan hydrochloride (CPT-11; 40 – 45 mg/m²/dose) was conducted for the treatment of refractory pediatric solid tumors. The pharmacokinetics of CPT-11 and its metabolites were characterized using both traditional noncompartmental analysis and population pharmacokinetics using NONMEM VI; pharmacokinetic pharmacodynamic relationships of SN-38 with indices of toxicity were also evaluated. Method: 11 patients between 3 and 18 years were enrolled. Pharmacokinetic parameters and consideration of relevant covariates (performance status (PS), BSA, corrected body weight (CBW), exponent of 3/4 on weight, etc.) were evaluated. Relationships between pharmacokinetic parameters of SN-38 and percentage change from baseline in patient biochemical response data were investigated via regression analysis. Result: CPT-11 exhibited a mean clearance (CL) of 15.31 ± 5.95 (l/h) (13.06 ± 3.58 (l/hr/m²)) and $AUC_{0-\infty}$ of 3547.0 ± 1406.5 (ng × h/ml); the AUC ratio of parent CPT-11 to SN-38 was 5.0%. Based on the population pharmacokinetic analysis, decreasing PS was significantly dependent on reduction in CL of CPT-11 ($p < 0.001$). The final model for CPT-11 are as follows: CL (l/h) = 1.31 × $CBW^{0.75}$ (ωCL = 21.7%), Vss (l) = 2.66 × CBW (ωVss = 21.2%), Vc (l) = 1.13 × CBW, inter-compartment CL (l/h) = 0.257 × $CBW^{0.75}$. Percentage changes of leucocyte and neutrophil count within a first month treatment were significantly correlated with C_{max} of SN-38 ($r = 0.78$ and $r = 0.74$) and AUC_{0-2} of SN-38 ($r = 0.73$ and $r = 0.73$). Conclusion: Pharmacokinetic parameters were similar to results published in several past reports. An allometric scaling of $CBW^{0.75}$ would seem to provide a good index of dosage requirement of CPT-11 in pediatric patients.

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Introduction

Irinotecan hydrochloride (CPT-11) is an inhibitor of DNA topoisomerase I and has a broad range activity against adult and pediatric malignancies. Although CPT-11 is approved for use in a variety of tumor types in adults, treatment of tumors in pediatric has not yet been approved despite the significant antitumor activity observed in children with recurrent solid tumors [7]. CPT-11 is metabolized via carboxylesterase to active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38). SN-38 is further conjugated by UDP glucuronosyltransferase to an inactive glucuronide (SN-38G). Other CPT-11 metabolites identified include 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (APC) which was found to be only a weak inhibitor of the cell growth of KB cells in culture [19].

Several reports describing the pharmacokinetics of CPT-11 and metabolites in pediatrics have been previously published [10, 23], however the disposition in Japanese patients has not been reported. Likewise, the pharmacokinetics and pharmacodynamics of SN-38 in Japanese children was unknown. We conducted the multicenter single agent Phase I and II study (CPT-PED-05) in treatment-resistant pediatric recurrent solid tumor patients supported by the Japan Medical Association Center for Clinical Trials (JMACCT). This analysis describes the pharmacokinetics of CPT-11 and its metabolites using both non-compartmental and population-based pharmacokinetic analyses as well as the exploration of pharmacokinetic - pharmacodynamic relationships of SN-38 with indices of response in Japanese pediatric patients.

Methods

Patients

Pediatric patients between 3 and 18 years of age with recurrent or progressive pediatric solid tumors (except for malignant lymphoma) were enrolled in this study. The main eligibility criteria included: an adequate performance status (PS; Karnofsky/Lansky score ≥ 50 point), absolute neutrophils count (ANC) ≥ 1,000/mm^3, platelet count ≥ 50,000/mm^3, hemoglobin > 8.0 g/dl, AST/ALT ≤ 3 times normal values, serum bilirubin ≤ 1.5 mg/dl and serum creatinine ≤ 1.2 mg/dl. This study was approved by the Ministry of Health, Labour and Welfare of Japan and institutional review board of each participating institution. Informed written consent for this clinical trial and pharmacokinetic study was obtained from patients or parents before study entry.

Dosage and blood analysis

The starting dose of CPT-11 was 40 mg/m^2/day (dose was escalated by 5 mg/m^2/day from second course) given at 1, 2, 3, 8, 9 and 10 days, repeated every 3 weeks up to 8 weeks. CPT-11 was diluted with 100 ml of saline injection, and administered by 1 h intra-venous (i.v.) infusion once daily. During the first course only, blood samples (5 ml) were withdrawn in heparinized tubes for bioanalysis of CPT-11 and its metabolites just before infusion (0) and 1, 2, 6, 12, 24, 48 and 72 h from the start of 1 h i.v. infusion. Plasma was immediately prepared by centrifugation at 4 °C and 3,000 rpm for 10 min, after which the plasma was frozen at –20 °C until analysis.

High-performance liquid chromatography (LC-10A, Shimazu co., Japan) with a fluorescence detection (ODS-80TM column; Symmetry Shild RP18, Waters co., Japan) was performed for determination of the concentrations of CPT-11, SN-38, SN-38G and APC. A mobile phase composed of 0.1 M phosphate buffer (pH 4.0) containing 3 mM Sodium 1-heptanesulfonate : methanol (30% acetonitrile) (55 : 45) at a 45 °C column temperature, and acetonitrile : water (1 : 2) was used for SN-38 at a 25 °C column temperature. Lower limits of quantification for each analyte were as follows: CPT-11, 5.0 ng/ml; SN-38, 0.25 ng/ml; SN-38G and APC, 0.5 ng/ml. For quality control of all analytes, the mean assay precision, which was expressed as the intra-day coefficient of variation was < 10.4%, and the inter-day coefficient of variation was < 9.5%. The mean assay accuracy, which was expressed as the intra-day ratio (%) of the estimated: theoretical QC standard concentrations was 91.0 – 114.6%, and the inter-day ratio was < 92.7 – 117.4%. All standard chemical reagents were provided as a gift from Yakult Honsha Co., Ltd., Tokyo, Japan.

Pharmacokinetic Analysis

Noncompartmental methods

Individual patient plasma concentration-time curves were analyzed by standard non-compartmental methods; maximum concentration (C_{max}), time to C_{max} (t_{max}), half life ($t_{1/2}$), area under plasma concentration time curve (AUC) 0 to 24 h (AUC_{0-24}) and $AUC_{0-\infty}$ were calculated for each patient. AUC_{0-24} was estimated by means of linear trapezoidal rule, and $AUC_{0-\infty}$ was using $AUC_{0-24} + AUC_{24-\infty}$, which calculated by 24-h concentration divided by elimination rate constant value of terminal slope. Half-life was calculated from 6 to 24 h as a final semi logarithmic linear concentration curve.

Systemic CL of CPT-11 was calculated by Dose/$AUC_{0-\infty}$.

Population pharmacokinetic modeling

Using the computer program NONMEM VI (GloboMax LLC, Hanover, MD, USA), simultaneous analysis of all concentration-time and patient physiologic data were performed with first-order conditional estimation (FOCE) method (double precision, Version VI) and ADVAN3/TRANS3 subroutine throughout model building process. Pharmacokinetic parameters of CPT-11 were calculated from the two compartments model included CL, inter-compartmental clearance (Q), central volume of distribution (Vc) and volume of distribution at steady-state (Vss). In the fitting process, patient body size (body surface area (BSA), height, actual body weight and corrected body weight (CBW; if actual body weight is heavier than their ideal body weight, CBW is equal to ideal body weight, otherwise CBW is actual body weight)), PS, red blood cell, albumin, bilirubin, AST, ALT, serum creatinine and CRP was used as covariates in the population model. Candidate covariates were screened in a stepwise fashion via addition to the base model.

$$CL\ (l/h) = [\theta 1 + \theta 2 \times covariate] \times \exp(\eta 1)$$
$$Vc = \theta 3 \times covariate$$
$$Vss = \theta 4 \times covariate \times \exp(\eta 2)$$
$$Q\ (l/h) = \theta 5 \times covariate$$

where θ is intercept and slope parameters. The η is random variables with a mean of zero and a variance of ω^2. Covariates were investigated on all pharmacokinetic parameters.

Exponential error models were used to describe inter-individual variability in each pharmacokinetic parameter and to define residual variability in the observed drug concentration response.

$$Cij = Cpred \times \exp(\varepsilon ij)$$

where Cij is i^{th} measured serum concentration in j^{th} individual, Cpred is estimated serum concentration (as observed for pharmacokinetic data), and εij is independently identical distributed statistical error with a mean of zero and a variance of σ^2.

An objective function value (negative value of twice log-likelihood difference: –2 l.l.d) was evaluated for comparisons among different models using the likelihood ratio test. Changes in the objective function greater than 10.828 indicate a statistically significant ($p < 0.001$) improvement in fit of data based on a chi-squared distribution with 1 degree of freedom.

Each model was evaluated using model diagnostic plots (distribution of predicted individual concentration versus observed concentration and conditional weighted residual (CWRES) versus time).

Pharmacokinetic – Pharmacodynamic analysis

The relationships between pharmacokinetic parameters of SN-38 (C_{max}, Cp72h, $AUC_{0-\infty}$) and the percentage change in patient biochemical data (myelosuppression makers (leukocyte count, ANC, platelet count, red blood cell count, hemoglobin, hematocrit, lymphocyte counts, monocyte count, basophil count, eosinophil count), AST, ALT, serum bilirubin, serum creatinine, blood urea nitrogen, CRP) were investigated using Pearson correlation analysis. Percentage change of these data was defined as a ratio of the differential between pretreatment value (pre) and nadir (within 1 month after first treatment) to pretreatment value. For instance the percentage change in ANC (%ANC) was calculated as:

$$\%ANC = [(pre\text{-}nadir)/pre] \times 100$$

Statistical analysis was performed with the Statview 5.0 software package. Descriptive statistics (mean ± standard deviation or median with range) were provided for all parameters of interest.

Results

A total of 11 patients were enrolled in the pharmacokinetic study; the demographic characteristics of these patients are listed in Table 1. The mean concentrations - time course profiles of CPT-11, SN-38, SN-38G and APC, and all concentrations of SN-38 versus post dose sampling time are shown in Figure 1. All

Table 1. Summary of patient demographic data.

	Height (cm)	Body weight (kg)	BSA (m^2)	Dose (mg/body)	Age (y)	Gender
1	127.3	23.9	0.92	36.5 (40 mg/m^2)	8	M
2	93.0	13.2	0.58	23.0 (40 mg/m^2)	3	F
3	145.5	32.9	1.15	46.0 (40 mg/m^2)	15	F
4	160.6	48.1	1.46	58.0 (40 mg/m^2)	17	M
5	140.0	45.0	1.32	59.0 (45 mg/m^2)	9	M
6	147.1	41.4	1.30	58.5 (45 mg/m^2)	11	M
7	141.9	33.3	1.15	51.5 (45 mg/m^2)	12	M
8	154.0	34.3	1.21	54.5 (45 mg/m^2)	17	F
9	102.4	14.5	0.64	29.0 (45 mg/m^2)	4	M
10	173.0	52.3	1.59	63.5 (40 mg/m^2)	15	M
11	181.4	76.9	1.97	78.5 (40 mg/m^2)	16	M
mean	142.4	37.8	1.21	46.2		

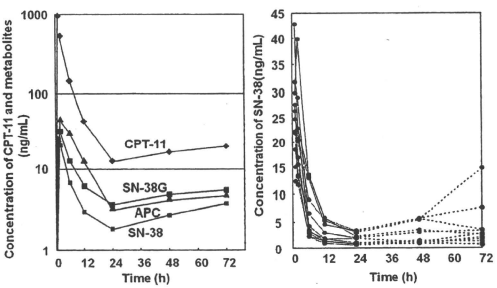

Figure 1. Mean plasma concentration of CPT-11, SN-38, APC and SN-38G based on data from 11 patients receiving 40 – 45 mg/m^2 CPT-11 (left). All the concentration-time profile of SN-38 (right).

data (88 samples) were used for pharmacokinetics and pharmacodynamics evaluation.

Pharmacokinetics

Inter-individual variability in Vc and Q were removed in an analysis due to aborting covariate step. In stepwise process, PS and body size are found to be important covariates influenced on CL of CPT-11. PS was not a significant covariate in the final model, but the effect of exacerbation of PS on increasing concentration in pediatric patients might be considered.

Pharmacokinetic parameters of CPT-11, SN-38, SN-38G and APC generated by noncompartmental analysis are summarized in Table 2. Total CL of CPT-11 normalized by BSA was 13.06 ± 3.58 (Range: 6.19 – 17.95 (l/hr/m^2), noncompartmental method). AUC ratio of SN-38 to AUC CPT-11 and additional pharmacokinetic parameters are summarized relative to several published studies in Table 3 [8, 9, 10, 14, 16, 17, 19, 22, 23, 25].

Population pharmacokinetic analysis of CPT-11 yielded the following population mean pharmacokinetic parameters: CL = 16.3 (l/hr), Vc = 37.7 (l), Vss = 79.1 (l), Q = 3.81 (l/hr). Total CL of CPT-11 with BSA as covariate and its variability were 13.5 (l/h/m^2) and ωCL = 23.3 (%), respectively.

In the model screening process, the NONMEM analysis using single covariates identified PS as having an influence on CL. The effect of body size indices ($CBW^{0.75}$, height and BSA) as covariates on CL and Vc significantly minimized the objective function value. Body size was necessary for all pharmacokinetic parameters under analysis most likely because of the wide range of patients' size. HT was best covariate effect added to base model (height > $CBW^{0.75}$ > BSA), but it's inter-individual variability was somewhat small (19.8% on CL, 17.3% on Vc) compared with past reports (34.4% on CL, 43.7% on Vc) [23]. The R value of HT and CBW0.75 in the scatter plots of predicted concentrations versus observed concentrations were almost the same; 0.861 and 0.853, respectively. $CBW^{0.75}$ was used for the final formulas for PPK parameters and is shown in Table 4.

Model diagnostic plots for the final model composed with CBW were shown in Figure 2.

Pharmacokinetics and pharmacodynamics

Neutropenia was observed (Grade 4 (n = 0), Grade 3 (n = 2), Grade 2 (n = 4); Grade of toxicity is based on NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0) during the first course of chemotherapy. The mean of % change ANC was 50.9 ± 24.4%, and nadir ANC was observed during Day 18 to 21 in 8/11 patients. A Grade 2 of thrombocytopenia was observed in 2 patients.

Table 2. Summary of pharmacokinetic parameters by nonparametric analysis.

	CPT-11	SN-38G	APC	SN-38
C_{max} (ng/ml)	959.1 ± 357.4 (491.1 – 1,853.9)	32.1 ± 18.2 (9.2 – 74.0)	47.3 ± 20.9 (18.1 – 76.3)	26.0 ± 9.2 (13.2 – 42.8)
t_{max} (h)	1.0 ± 0 (1)	2.0 ± 0 (2)	2.0 ± 0 (2)	1.3 ± 0.5 (1 – 2)
$t_{1/2}$ (h)	5.6 ± 0.6 (4.6 – 6.5)	10.4 ± 2.8 (7.1 – 16.0)	5.7 ± 0.8 (4.8 – 7.2)	11.5 ± 2.8 (7.3 – 18.5)
AUC_{0-24} (ng × h/ml)	3,447.4 ± 1,381.0 (2126 – 7022)	243.0 ± 10.4 (105 – 461)	432.73 ± 199.0 (184 – 751)	148.7 ± 62.2 (70 – 262)
$AUC_{0-\infty}$ (ng × h/ml)	3,547.0 ± 406.5 (2,222 – 7200)	306.2 ± 156.8 (141 – 584)	462.2 ± 218.1 (193 – 814)	179.3 ± 74.1 (80 – 286)
CL (l/h)	15.31 ± 5.95 (8.19 – 20.7)			

Mean ± SD (range).

Table 3. Comparison of several studies on pharmacokinetic analysis of CPT-11.

Number of subjects	AUC ratio of SN-38/ CPT-11	CL_{CPT-11} (l/h/m^2)	$Vdss_{CPT-11}$ (l/m^2)	Dose (mg/m^2)	Age (y)	Author
32	0.16	–	–	125	> 18	Younis et al. [25]
35	0.019	–	–	200	34 – 75	Sparreboom et al. [22]
78	–	14.6	151.7	100 – 340	adult	Klein et al. [9]
40	–	14.6	136.0	145	adult	Gupta et al. [8]
74	0.028	14.4	228.2	180 – 225	38 – 81	Poujol et al. [17]
19	0.047	16.1	102.0	115 – 600	53.2	Rivory et al. [19]
34	0.049	13.0	123.0	240 – 340	31 – 80	Pitot et al. [16]
28	–	14.5	132.5	200	–	Mugishima et al. [14]
25	0.052	17.7	–	50	1 – 21	Thompson et al. [23]
16	0.06	28.2	80.4	12 – 18	1 – 21	Levy et al. [10]
mean	0.059	16.6	136.3	–	–	
This clinical trial						
11	0.050	13.5	75.8	40 – 45	3 – 17	

(Mean or range).

The percentage change of myelosuppression makers within a first month treatment were significantly correlated with C_{max} of SN-38 as illustrated in Figure 3: ANC; r = 0.78, leukocyte; r = 0.74. The relationship between the other pharmacokinetic parameters (Cp72h, $AUC_{0-\infty}$) and myelosuppression makers was not found in this study. Most sensitive myelosuppression markers, % change in leukocyte and %ANC, were also evaluated with AUC_{0-2} of SN-38 as a value of exposed to high level drug concentration, and good correlation was observed (Figure 3).

Discussion

A population pharmacokinetic model was developed for CPT-11 for the first time in Japanese pediatric patients. We have characterized the pharmacokinetics of CPT-11, SN-38, SN-38G and APC in Japanese pediatric patients with recurrent or progressive solid tumors. A relationship between dosage and individual AUC was not identified because of narrow range of dosage. Variability of $AUC_{0-\infty}$ of SN-38 was greater than $AUC_{0-\infty}$ of CPT-11 (Coefficient of variation of $AUC_{0-\infty}$

Table 4. Final model estimation of CPT-11 by population pharmacokinetic analysis.

Pharmacokinetic Parameter		Point estimation (SE)	Inter-variability (CV%)
CL_{CPT-11} (l/h) =	$\theta 1 \times CBW^{0.75}$	1.31 (0.125)	ωCL = 21.7
Vc_{CPT-11} (l) =	$\theta 2 \times CBW$	1.13 (0.114)	
Vsscpt-11 (l) =	$\theta 3 \times CBW$	2.66 (0.413)	ωVss = 21.2
Q_{CPT-11} (l/h) =	$\theta 4 \times CBW^{0.75}$	0.257 (0.0359)	
Intra-variability		ε = 24.0 (CV%)	

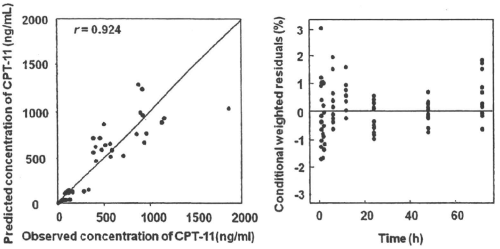

Figure 2. Distribution plots of individual prediction concentration versus observations (left). Distribution plots of conditional weighted residual versus time (right).

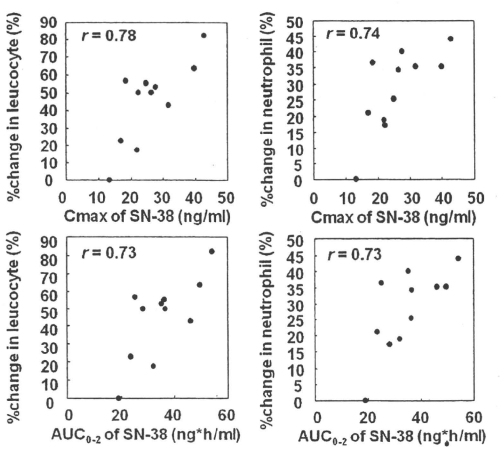

Figure 3. Relationship between myelosuppression (percentage change from baseline) and pharmacokinetic parameter of SN-38 (n = 11).

was 48.9% of SN-38 compared with 37.8% of CPT-11). Rothenberg et al. reported [20] significant correlations between dosage and AUC for CPT-11 but not for SN-38. The exposure of SN-38 is very variable due to influence of various factors including enterohepatic recirculation [25], genetic polymorphisms of UDP-glucuronosyltransferase 1A1(UGT1A1) [2] and protein binding [5]. Accumulation of SN-38 is important, because hematologic toxicity is correlated with SN-38 but not with CPT-11 [15]. In this study accumulation of SN-38 expected from observed half life ($t_{1/2}$ range: 7.1 – 16.0 h) was not predicted. However, unexpected high levels of SN-38 at 72 h was observed in 2 patients, 14.8 mg/l; 7.6 mg/l. If these elevations are related to decreasing CL, increases in the concentration of SN-38G will be likely. Rebound peaks of SN-38 have been observed at variable time points after the initial peak and consistent with enterohepatic recirculation [8]. From a pharmacodynamic view point, there was no relationship between Cp72h and myelosuppression markers.

CPT-11 is converted to its active metabolite, SN-38 in liver and intestinal tract carboxylesterase [18]. Carboxylesterase activities in several species and various tumor cells are wide range, but no significant differences were observed in human liver and colon [13]. Plasma exposure rate to SN-38 expressed by AUC ratio of SN-38 to CPT-11 was 5% in this study, consistent with previously reported values.

Actual body weight was not a good predictor for the population model during the covariate identification process. At times apparent volume of distribution and CL may be independent of actual body weight due to the influence of fat mass. In analysis of pediatric pharmacokinetics, selection of the size model is important for estimating pharmacokinetic parameters. Non-linear relationship of between weight and CL is generally recognized [1]. Clinically, BSA is superior in predicting drug requirements in pediatrics compared to body weight alone from adults, especially during infancy and childhood [6]. We considered several body size expressions as covariates on pharmacokinetic parameters including an exponent of 3/4 on weight [1]. Our results suggest that $CBW^{0.75}$ was the most appropriate covariate expression of size on CL. Our population model was the same as that

utilized in a study from the children's oncology group (the first and largest population pharmacokinetic analysis in children [23]. Allometric scaling based on corrected body weight would appear to be a useful index to calculate CPT-11 dosage.

As demonstrated by distribution of predicted versus observed concentration and residual concentration, analyzed population means showed a goodness of fit; r = 0.924, CWRES ≤ 3%, respectively. Estimated mean patient CL (15.08 ± 5.87) was not statistically different from observed CL (15.31 ± 5.95) calculated by Dose/$AUC_{0-\infty}$ ($p < 0.9283$, F-value = 0.973). These data support the validity of the final model. Compared with previously reported pharmacokinetic parameters, CL and Vss normalized by BSA were similar, but tended to be smaller (Table 3). UGT1A1*28 is found to be associated with an increased AUC and toxicity of SN-38 in multiple races [3, 12]. However, in East Asians including Japanese, UGT1A1*6 is also associated with reduced glucuronidation of SN-38 and a high risk of severe neutropenia. UGT1A1*28 is distributed at 25.7 – 38.8% in Caucasians and 8.6 – 13.0% in Japanese. UGT1A1*6 is distributed at < 1% in Caucasians and 13.0 – 17.7% in Japanese [21]. Ethnic differences in genetic polymorphisms in UGT1A1 are well known. In future, population pharmacokinetics based on race and allele frequency will be necessary for adjusting dosage regimens to avoid severe neutropenia.

Predominant dose limiting toxicities of CPT-11 are diarrhea and myelosuppression [4]. Gastrointestinal and hematological toxicity has been associated with systemic exposure to SN-38 and biliary excretion, respectively. Several studies of pharmacokinetics and pharmacodynamics investigated % change or grade of adverse event and pharmacokinetic parameters of SN-38 [16] using multivariate regression analysis. The strongest relationship between AUC of SN-38 and neutropenia was reported [16] in the study of 240 – 320 mg/m^2 once-every-3-week schedule (maximum tolerate dose (MTD) was 320 mg/m^2). Ma et al. were not able to identify any pharmacodynamic relationship between SN-38 systemic exposure and myelosuppression at 20 – 29 mg/m^2 daily for 5 consecutive days for 2 weeks [11]. Myelosuppression in their study was minimal, except for 1 (of 24) patient that developed a Grade 3 neutropenia. These relationships will likely support the MTD. The MTD of the low dose schedule of daily × 5, every-21-days dosing was 50 mg/m^2/dose and has been published [11]. Our pharmacokinetics/pharmacodynamic analysis demonstrated a strong relationship between C_{max} of SN-38 and AUC_{0-2} of SN-38. Generally C_{max} as an exposure variable associated with cytotoxic agents administered once every 3 – 4 week cycle, while continuous administration or weekly treatment (daily × 5) is associated with steady state concentration. Occasionally, the identification of threshold concentrations represents safety or effectiveness of anticancer agents [24]. AUC_{0-2} might be such a threshold concentration, Our results suggest that C_{max} and AUC_{0-2} of SN-38 observed from the low dose continuous schedule are meaningful parameters for managing the treatment of CPT-11 in pediatric patients, but additional studies are needed to refine this correlation.

Conclusion

CL and Vss in our pediatric population were similar to past reported values, but tended to be small. In 2 patients unexpected increasing of Cp72h were observed, but no relationship to toxicity. In process of population pharmacokinetic analysis, exacerbation of PS was significantly correlated with decreasing CL. CBW was important covariates influenced on distribution volume and $CBW^{0.75}$ was on CL. Percentage change of myelosuppression makers within a first month treatment are significantly correlated with C_{max} and AUC_{0-2} of SN-38.

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ORIGINAL ARTICLE

Outcome of hepatoblastomas treated using the Japanese Study Group for Pediatric Liver Tumor (JPLT) protocol-2: report from the JPLT

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Abstract
Background In the recent years, surgical resection with pre- and/or postoperative chemotherapy has markedly improved the survival rate of hepatoblastoma patients. We herein report the results of patients treated with the current protocol of the Japanese Study Group for Pediatric Liver Tumor, JPLT-2.
Methods A total of 279 patients with malignant liver tumor were enrolled in JPLT-2. Data from 212 hepatoblastoma cases were analyzed. PRETEXT I patients were treated with primary resection followed by low doses of cisplatin–pirarubicin (tetrahydropyranyl-adriamycin). Otherwise, patients received preoperative cisplatin–pirarubicin (CITA), followed by surgery and postoperative chemotherapy. Ifosfamide, pirarubicin, etoposide, and carboplatin (ITEC) were given as a salvage treatment. High-dose chemotherapy with hematopoietic stem cell transplantation (SCT) was reserved for patients with metastatic diseases.
Results The 5-year overall survival rate (OS) in non-metastatic cases was 100% for PRETEXT I, 87.1% for PRETEXT II, 89.7% for PRETEXT III, and 78.3% for PRETEXT IV. The 5-year OS in metastatic cases was 43.9%. The outcome in non-metastatic PRETEXT IV cases was markedly improved, while the results of metastatic tumors remained poor.

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Conclusions JPLT-2 protocol achieved satisfactory survival among children with non-metastatic hepatoblastoma. New approaches are needed for patients with metastatic diseases.

Keywords Hepatoblastoma · Prognostic factors · PRETEXT · Survival

Introduction

Although hepatoblastoma is a rare malignancy in childhood, it is the most common pediatric liver tumor. National and international group studies have shown that a combination of surgical resection and cisplatin-based chemotherapy has increased the overall survival rate (OS) of hepatoblastoma patients from 30% in the early 1980s to over 70% in the 1990s [1–3]. To improve the outcome in children with liver tumors, the Japanese Study Group for Pediatric Liver Tumor (JPLT) established the first nationwide protocol for liver tumors in childhood, JPLT-1, in 1991. This study was closed in 1999 after enrolling 154 patients with liver tumors, including 145 patients with hepatoblastomas [4]. Patient results were satisfactory, with an OS of 73.4% at 6 years after diagnosis. However, the combination of cisplatin/pirarubicin failed to improve the survival rate in patients with unresectable/incompletely resected tumors. The event-free survival of patients with tumors occupying all sectors of the liver (equivalent to PRETEXT IV) and metastatic disease was less than 50%. Other group studies also have reported that treatment of unresectable and metastatic tumors remains a challenge [3, 5–7].

A refined version of the protocol, designated JPLT-2, was opened to participating institutions in 1998. Under this protocol, the PRETEXT (Pretreatment evaluation of tumor extent) staging system was adopted from SIOPEL (International Society of Paediatric Oncology Liver Tumor Study Group) as an internationally approved staging system for tumor localization. All patients other than non-metastatic PRETEXT I patients were subjected to preoperative chemotherapy. For tumors showing chemoresistance, a second-line regimen including ifosfamide was applied. For patients with metastatic disease, a protocol including high-dose chemotherapy with autologous SCT was used. Here, we report the outcomes of patients treated with the JPLT-2 protocol.

Patients and methods

Patients and eligibility

JPLT-2 was open to enrollment in 1998. Eligible patients included children younger than 15 years of age at the time of diagnosis with a previously untreated malignant liver tumor. As of December 2008, 279 cases of hepatic tumors in childhood were registered. We analyzed the treatment results of 212 hepatoblastoma cases, of which treatment was initiated according to the protocol and of which follow-up data were available (Table 1).

Table 1 Characteristics of tumors at diagnosis

Characteristics	No.
Registered cases in JPLT-2 (1999–2008; $n = 279$)	
Hepatoblastoma	231
Hepatocellular carcinoma	13
Fibrolamellar carcinoma	2
Others	5
Unknown	28
Analyzed hepatoblastomas with follow up data ($n = 212$)	
Gender	
Male	132
Female	80
Age (mo)	
Median	17
Range	0–177
AFP (ng/ml)	
Range	28–12,700,000
No. of patients<100	1
PRETEXT	
I	16
II	64
III	83
IV	49
Metastatic	35
Ruptured	18
Extrahepatic	4
Portal invasion	8
Venous invasion	8

Clinical staging

The tumors were evaluated using state-of-the-art imaging, including computed tomography; clinical staging was based on PRETEXT (pretreatment extent of tumor) as applied in SIOPEL [12]. M (metastatic), E (extrahepatic invasion), P (main portal invasion), V (invasion of all three hepatic veins or the vena cava), and R (rupture at diagnosis) were added as annotations to PRETEXT staging. Of the 212 hepatoblastomas, 16 were PRETEXT I tumors, 64 PRETEXT II tumors, 83 PRETEXT III tumors, and 49 PRETEXT IV tumors. There were 35 cases with distant metastasis (M), 18 with primary rupture of the tumor (R), 8 with portal vein involvement (P), 8 with hepatic vein involvement (V), and 4 with extrahepatic diseases within the abdomen (E).

Treatment

An outline of the JPLT-2 protocol is shown in Fig. 1. Briefly, PRETEXT I tumors were primarily resected, and PRETEXT II–IV cases were treated with preoperative chemotherapy. At least two courses of a combination of 80 mg/m^2 cisplatin on day 1, followed by 30 mg/m^2 pirarubicin on days 2 and 3, which was designated CITA, was repeated preoperatively. CITA was allowed to be substituted with transarterial chemoembolization using 30 mg/m^2 pirarubicin and 200 mg/m^2 carboplatin (CATA-L) at the discretion of the physician. CITA was repeated until surgical resection became feasible. When CITA (CATA-L) failed to induce PR (as defined below), a combination of 3 g/m^2 ifosfamide on days 1 and 2, 400 mg/m^2 carboplatin on day 3, 30 mg/m^2 pirarubicin on days 4 and 5, and 100 mg/m^2 etoposide on days 1–5 (ITEC) was given until the tumor became resectable.

Postoperative chemotherapy was given to all cases. PRETEXT I and II tumors were treated with four courses of half-dose CITA (low-CITA). PRETEXT III, IV, and metastatic cases were treated with two courses of CITA. Patients who required salvage with ITEC preoperatively were treated with two courses of ITEC. If complete response was not obtained at this point, two additional courses were added. Metastatic cases were treated with high-dose chemotherapy using autologous hematopoietic stem cell rescue. The preconditioning regimen consisted of 200 mg/m^2 etoposide and 400 mg/m^2 carboplatin on days 6, 5, 4, 3, and 90 mg/m^2 melphalan on days 3 and 2. High-dose chemotherapy was allowed to be substituted with two courses of CITA if all metastatic lesions were eradicated in an early stage of preoperative chemotherapy without progression of disease thereafter. The JPLT-2 protocol was approved by the ethics committees or institutional review boards of the participating institutions. Written informed consent for treatment was obtained in all cases by local physicians.

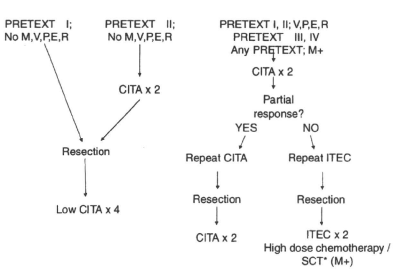

Fig. 1 Outline of the JPLT-2 protocol. Preoperative CITA was allowed to be substituted by transarterial chemoembolization (CATA-L). *SCT* autologous hematopoietic stem cell transplantation

Evaluation of response

The response to preoperative treatment was based on computed tomography or magnetic resonance imaging. Complete response (CR) was defined as the complete disappearance of any evidence of disease on imaging and normal AFP. Partial response (PR) was at least a 50% reduction in the sum of the products of the maximum perpendicular diameters of all measurable lesions. Stable disease (SD) was defined as a decrease of less than 50% or an increase of less than 25% in the sum of the products of the maximum perpendicular diameters of all measurable lesions. Progressive disease (PD) was defined as the appearance of a new lesion or an increase of over 25% in the sum of the products of the maximum perpendicular diameters of all measurable lesions. OS was defined as the time interval between the date of diagnosis and the date of death (as a result of any cause) or the date of the last follow up. Event-free survival (EFS) was defined as the time interval from the date of diagnosis to the date of progression, the date of relapse, the date of death, the date of diagnosis of a second malignant neoplasm, or the date of the last follow up, whichever occurred first.

Statistical analysis

Survival curves were constructed according to the methods of Kaplan and Meier, and comparisons of survival curves were performed with a log-rank test.

Results

Treatment outcome

The OS for the 212 hepatoblastoma cases was 82.4% at 3 years, and 80.9% at 5 years. The EFS was 69.9% at 3 years, and 62.4% at 5 years. The 5-year OS of non-metastatic PRETEXT I, II, III, and IV cases was 100, 87.1, 89.7, and 71.2%, respectively (Fig. 2a), and the 5-year EFS was 78.3, 76.2, 72.2, and 68.3%, respectively (Fig. 2b). The outcome of patients with metastatic diseases was poor, with a 5-year OS of 43.9% (EFS 20.8%).

Impact of pretreatment factors on patient prognosis

Univariate analysis of each factor was performed by comparing the survival curves of each variable using log-rank test (Table 2). Patients under 12 months of age had an excellent prognosis, with a 5-year OS of 91.5% (EFS

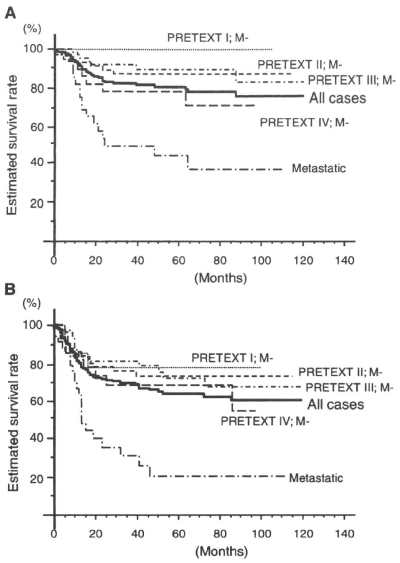

Fig. 2 Clinical stage by PRETEXT and metastasis and overall survival. **a** Overall survival of all cases, non-metastatic PRETEXT I, II, III, IV, and metastatic tumors. **b** Event-free survival

79.7%). The OS of patients ≥12 months of age was 75.8% (EFS 64.3%), which was significantly lower than that of the younger group of patients ($P = 0.0078$). PRETEXT stratification (without V, P, E, M annotations) was also significantly related to risk of death ($P = 0.0043$). Among the V, P, E, M annotations, presence of metastatic disease at diagnosis most significantly predicted an unfavorable outcome when compared to patients without metastasis. 5-year OS was 43.9% (EFS 31.3%) in the M+ group, and 88.6% (EFS 71.3%) in the M− group ($P < 0.0001$). Vein invasion also was significantly related to overall survival. 5-year OS was 66.7% (EFS 66.7%) in the V+ group, and 81.3% (EFS 63.7%) in the V− group. SIOPEL risk grouping by PRETEXT, V, P, E, M, R, and AFP had a significant impact on survival. 5-year OS of the standard risk group (PRETEXT I–III, no V, P, E, M, R, and AFP over 100 ng/dl) was 89.3%, and that of high-risk group (PRETEXT IV, any of V, P, E, M, R, or AFP under 100 ng/dl) was 64.3% ($P < 0.0001$). There was only one case with AFP lower than 100 ng/dl in the analyzed group, and this case was alive and doing well at last contact.

Table 2 Pretreatment prognosis factors and prognosis

Variable	No. of patients	5-year OS (%)	Chi-square	Degrees of freedom	*P* value
Age					
<365 days	66	91.5	7.075	1	0.0078
≥365 days	146	75.8			
PRETEXT grouping					
I	16	93.8	13.161	3	0.0043
II	64	84.4			
III	83	86.1			
IV	49	63.2			
Metastasis					
Yes	35	43.9	33.163	1	<0.0001
No	177	87.8			
Portal invasion					
Yes	8	57.1	3.346	1	0.0673
No	204	81.8			
Venous invasion					
Yes	7	66.7	4.748	1	0.0293
No	205	81.3			
Extrahepatic					
Yes	4	75.0	0.289	1	0.5910
No	208	81.0			
Rupture					
Yes	18	88.9	0.380	1	0.5376
No	194	80.2			
SIOPEL risk group					
Standard	133	89.3	15.728	1	<0.0001
High	79	64.3			

Preoperative chemotherapy and surgical resection

Primary resection was carried out in 27 cases, and the remaining 185 cases received preoperative chemotherapy according to the protocol (Fig. 3). Forty courses of CITA in 28 patients were substituted with CATA-L. Early death was encountered in three cases. A total of 121 cases (65% of all cases treated with preoperative chemotherapy) was successfully treated with partial hepatectomy after repeated courses of CITA and/or CATA-L (range 1–8 courses; median 3 courses). Among the localized tumors that were treated with preoperative chemotherapy, 43 of 46 PRETEXT II cases (93.5%), 51 of 73 PRETEXT III cases (69.9%), and 10 of 30 PRETEXT IV cases (33.3%) were successfully resected after preoperative chemotherapy (Table 3). One case was treated with total hepatectomy with liver transplantation (LTx) after two courses of CITA. In 60 cases, PR was not obtained after CITA and/or CATA-L and the tumor remained unresectable. ITEC was used in 56 of these cases. The other four cases were further treated with other regimens. Among the 56 cases treated with ITEC, 39

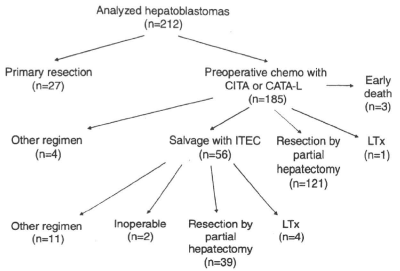

Fig. 3 Preoperative chemotherapy and surgical treatment. *LTx* total hepatectomy with liver transplantation

Table 3 Resectability after CITA/CATA-L as preoperative chemotherapy

PRETEXT category	No.	No. resected after CITA (%)[a]
PRETEXT I; M–	1	1 (100%)
PRETEXT II; M–	47	43 (93.5%)
PRETEXT III; M–	73	51 (69.9%)
PRETEXT IV; M–	30	10 (33.3%)
Metastatic	35	16 (45.7%)

[a] Excludes total hepatectomy with liver transplantation

were resected after usage of ITEC. Four of these cases received LTx after ITEC, while two remained inoperable and progressed without definitive surgery. Eleven were subjected to further chemotherapy with other agents.

Surgical resection and outcome

Microscopic total resection of the primary liver tumor was obtained in 135 of the 212 patients with partial hepatectomy (microscopic resectability rate, 63%). Resection including microscopically positive margin was obtained in 174 cases (82%). The 3-year OS of the completely resected and microscopic residual group was 87.7% (EFS 49.8%), whereas that of macroscopic disease and unresectable cases was 55.8% (EFS 73.8%) (Chi-square 20.422, $P < 0.001$). Total hepatectomy with LTx was selected as a primary surgical approach in only six cases. There were three cases in which LTx was performed for relapsed/residual tumors.

Treatment of metastatic cases

Metastatic cases were initially treated by CITA with or without conversion to ITEC, followed by surgical resection. Complete response (disappearance on imaging) of metastatic lesions by chemotherapy alone was obtained in 15 of 35 (42.8%) metastatic cases. Partial response of metastasis was obtained in ten cases, and in six cases, lesions were unchanged. Data on response in other cases were missing. Resection of the primary tumor was possible in 25 cases. High-dose chemotherapy was performed in 14 cases, of which 4 were alive at last contact, and 10 died. These included two deaths related to adverse effects of high-dose chemotherapy.

Toxicity

Toxicity as a result of the JPLT-2 protocol was assessable in 196 cases. Grade 3 or 4 neutropenia was observed in 177 cases (90.3%). Septic episodes supported by evidence of bacteremia were noted in 10.2% of patients (18 of 196 assessable cases). Renal toxicity with decreased creatinine clearance (<50 ml/min/m^2) was observed in 5.1% of cases (7 of 136 cases evaluated). Ototoxicity was evident in 7 of 84 cases (8.3%) evaluated either by audiometry or ABR, typically showing loss of high pitch audibility. Cardiotoxicity was identified in 5.5% of patients (8 of 146 cases assessed on cardiac ultrasound), although none of the patients had clinical symptoms. In this series, there were seven chemotherapy-related deaths, including four as a result of high-dose chemotherapy with stem cell support. Second malignancy was encountered in three cases (acute leukemia), and two died.

Discussion

Cisplatin-based pre- and postoperative chemotherapy has contributed substantially to the marked improvement in the prognosis of patients with hepatoblastoma [1–3]. Nevertheless, complete resection of the primary tumor is crucial for cure [8–10]. The outcome of patients with unresectable and/or metastatic tumors remains poor [3, 5–7], which compels us to identify new therapeutic approaches for this subgroup of tumors. The JPLT-2 protocol includes two approaches designed to improve the outcome of hepatoblastoma, particularly unresectable and/or metastatic cases. A salvage regimen, designated ITEC (ifosfamide, pirarubicin, etoposide, and carboplatin), was administered for tumors that did not show at least partial response to the first-line regimen CITA (cisplatin and pirarubicin). For tumors with metastases, the standard protocol includes high-dose chemotherapy with hematopoietic SCT for postoperative consolidation.

The present study demonstrates that the treatment results in cases using the JPLT-2 protocol are satisfactory for localized tumors. We believe that the combined 91.7% 3-year and 90.6% 5-year OS for non-metastatic PRETEXT

I–III tumors are comparable to the results of other multicenter studies [1–3, 11]. When compared with the JPLT-1 results, there are marked improvements in the survival of non-metastatic PRETEXT III patients (JPLT-2 92.0 vs. JPLT-1 77.8% 3-year OS; JPLT-2 81.6 vs. JPLT-1 67.5% 3-year EFS), and non-metastatic PRETEXT IV patients (JPLT-2 78.3 vs. JPLT-1 50.3% 3-year OS; JPLT-2 82.3 vs. JPLT-1 47.1% 3-year EFS). On the other hand, there was no apparent improvement in the treatment results of metastatic cases, with a 3-year OS of 44.2% (64.8% at 3 years and 32.4% at 6 years in JPLT-1).

JPLT-2 adopted the PRETEXT system for clinical staging and treatment stratification. The value of PRETEXT classification for patient stratification has been demonstrated previously [12–14]. SIOPEL currently uses PRETEXT, the presence of extrahepatic diseases, and AFP levels to stratify patients into standard risk and high-risk groups. In Children's Oncology Group (COG), the presence of metastasis has been the single criteria for inclusion in the highest risk category, Stage IV [14]. Thus, using PRETEXT, we categorized metastatic diseases as a distinct group of patients and assigned patients for specified treatment protocols. Meyers et al. [14] empathized in their retrospective COG study that the presence of metastatic disease (COG Stage IV) is associated with a high risk of death, regardless of the extent of liver involvement, as quantified by PRETEXT. Our results support this finding. PRETEXT classification had a significant impact on the patient outcome; however, the presence of metastatic diseases on diagnosis was the strongest predictor of poor prognosis, regardless of SIOPEL classification.

In the present study, all tumors of any stage except PRETEXT I were initially subjected to a combination chemotherapy of CITA. Pirarubicin is an anthracyclin derivative with less cardiotoxicity [15]. CITA is almost identical to PLADO, which was the previous standard regimen for standard risk tumors in SIOPEL [11]. Among non-metastatic cases, the resectability rate with CITA alone was 100% in PRETEXT I, 93.5% in PRETEXT II, and 69.9% in PRETEXT III. According to a recent report by SIOPEL, complete resection was performed in 95.2 and 93.8% of patients in the standard risk after treatment with cisplatin monotherapy, or PLADO, respectively [11]. Compared with this result, the resectability of PRETEXT III patients in our series appears to be low. This may be because the PRETEXT III patients in our study included those with E, P, V, and R factors, which may affect resectability. These patients are stratified as high risk in SIOPEL and are subjected to intensified induction chemotherapy. Furthermore, under the JPLT-2 protocol, treatment for patients that could not obtain PR by CITA was converted to ITEC. Therefore, there may have been some cases that were already resectable after CITA, but were further subjected to ITEC to increase the safety of hepatectomy.

Despite the high resectability in PRETEXT II cases, 11 standard risk cases (without E, P, V, or R factors) encountered some type of event, and 6 died. Among these cases, six underwent low-CITA after surgery, suggesting that the dose intensity of low-CITA may be insufficient as adjuvant therapy for hepatoblastoma.

In contrast to standard risk patients, of whom over 90% achieve long-term survival, the treatment of patients with unresectable disease and patients with metastatic disease remains a challenge. In the present study, the 3- and 5-year OS of non-metastatic PRETEXT IV cases was 78.3 and 71.2%, respectively. The outcome of this group of patients was markedly better when compared with our previous study in which 3-year OS was 50.3%. The survival rate is comparable to the recent successful SIOPEL-3 study for high-risk patients using alternating cycles of cisplatin and carboplatin plus doxorubicin, in which the 3-year OS in non-metastatic patients was 78% [16]. One of the current standard approaches for unresectable primary hepatoblastoma tumors is total hepatectomy with LTx [8, 17–21]. However, in Japan, LTx for hepatoblastoma was not covered by insurance until recently. Within the 212 analyzed cases, LTx was performed as the primary surgical resection in only six cases, including one case treated with LTx after receiving agents off the protocol. Among the 30 non-metastatic PRETEXT IV cases, there was only 1 case that was treated with primary LTx. Three cases received rescue LTx, of which the disease was cured in only one case. Therefore, it is unlikely that LTx substantially contributed to the improved survival of the non-metastatic PRETEXT IV patients in our series. ITEC was used as a second-line treatment in 18 of the 30 cases, and 14 cases received partial hepatectomy thereafter. CATA-L (transarterial chemoembolization = TACE) was used in eight cases. Although we cannot draw any conclusions about which of these truly contributed to the improvement of survival of the patients from our data, we may speculate that some of the patients in this group may benefit from conservative approaches, such as intensified systemic chemotherapy or TACE.

The results of patients with metastatic diseases remained poor. Approximately one-third of this group was treated with high-dose chemotherapy with autologous hematopoietic SCT. However, the results were unsatisfactory, and included two toxicity-related deaths. The effects of high-dose chemotherapy with SCT for high risk and refractory/recurrent hepatoblastoma are unknown, as only a small number of cases have been reported in the literature [22–27]. The details of the clinical courses of patients treated with SCT in the present study require further validation to clarify the effects of SCT in hepatoblastoma. The

SIOPEL-3 high-risk study recently reported a 62% 3-year OS and 56% 3-year EFS among patients with metastatic diseases treated with alternative cycles of cisplatin and carboplatin plus doxorubicin. This result stands out among the recent multicenter studies [3, 4, 7]. The strategy in SIOPEL-3 is to give intensified chemotherapy from the early phases of treatment, which is in contrast to JPLT-2, in which chemotherapy is intensified only in those that show resistance to CITA. It is well known that hepatoblastomas tend to become chemoresistant after four to five courses of chemotherapy [28]. Thus, a possible approach for metastatic diseases may be to escalate the initial intensity of treatment and control the metastatic sites in the early phases of treatment.

Another therapeutic approach for high-risk hepatoblastoma is to identify new agents with activity against hepatoblastoma. Among these, several investigators have reported the successful use of irinotecan in small numbers of children with refractory or relapsed hepatoblastomas [29–33]. Patients with high-risk disease will receive irinotecan in the current COG trial AHEP0731 [33]. Application of irinotecan may be another candidate of treatment strategies for metastatic diseases in the future JPLT studies.

In conclusion, JPLT-2 protocol achieved satisfactory survival among children with non-metastatic hepatoblastoma. However, there were considerable numbers of events and deaths among PRETEXT II patients without extrahepatic involvement. The survival of these patients may be further improved by giving full-dose CITA, rather than low-CITA, as adjuvant therapy. The outcomes for metastatic tumors were unsatisfactory, despite the intensified salvage regimen and SCT.

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Conflict of interest The authors declare that they have no conflicts of interest.

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Genome-Wide Analysis of Allelic Imbalances Reveals 4q Deletions as a Poor Prognostic Factor and *MDM4* Amplification at 1q32.1 in Hepatoblastoma

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In a single-nucleotide polymorphism array-based analysis of 56 hepatoblastoma (HB) tumors, allelic imbalances were detected in 37 tumors (66%). Chromosome gains were found in 1q (28 tumors), 2q (24), 6p (8), 8q (8), 17q (6), and 20pq (10), and losses in 1p (6), 4q (9), and 16q (4). Fine mapping delineated the shortest overlapping region (SOR) of gains at 1q32.1 (1.3 Mb) and 2q24.2-q24.3 (4.8 Mb), and losses at 4q34.3-q35.2 (8.7 Mb) and 4q32.3 (1.6 Mb). Uniparental disomy of 11pter-11p15.4 (*IGF2*) and loss of 11pter-p14.1 were found in 11 and 2 tumors, respectively. Expression of *HTATIP2* (11p15.1) was absent in 9 of 20 tumors. Amplification was identified in four tumors at 1q32.1, where the candidate oncogene *MDM4* is located. In the 4q32.3-SRO, *ANXA10S*, a variant of the candidate tumor suppressor *ANXA10*, showed no expression in 19 of 24 tumors. Sequence analysis of *ANXA10S* identified a missense mutation (E36K, c.106G>A) in a HB cell line. Multivariate analysis revealed that both 4q deletion and *RASSF1A* methylation (relative risks: 4.21 and 7.55, respectively) are independent prognostic factors. Our results indicate that allelic imbalances and gene expression patterns provide possible diagnostic and prognostic markers, as well as therapeutic targets in a subset of HB.

INTRODUCTION

Hepatoblastoma (HB) is a rare malignant neoplasm of the liver, with an incidence of 0.5–1.5 per million children (Perilongo and Shafford, 1999). Significant progress in clinical outcome has been achieved in the past 20 years because of advances in chemotherapy and surgical procedures; however, the mortality rate remains 20–30% and treatment results in patients in advanced stages who are refractory to standard preoperative chemotherapy regimens are unsatisfactory (Fuchs et al., 2002). To improve the outcome of these patients, innovative treatment and potent prognostic markers for better therapy planning are needed.

The molecular mechanism involved in the development and progression of HB includes overexpression of insulin-like growth factor-II (*IGF2*) (Li et al., 1998; Honda et al., 2008a), downregulation of *RASSF1A* by promoter hypermethylation (Sugawara et al., 2007; Honda et al., 2008b), and alterations of genes in the Wnt signaling pathway, most notably a high incidence of *CTNNB1* (catenin, beta 1) mutations (Koch et al., 1999).

Cytogenetic and metaphase comparative genomic hybridization (CGH) analyses of HB have revealed frequent occurrence of gains of chromosomes or chromosome arms 1q, 2q, 8q, and 20, and the loss of 4q (Weber et al., 2000; Kumon et al., 2001). More recently, a single-nucleotide polymorphism (SNP) microarray-based analysis of 17 HB samples confirmed these chromosomal gains and losses and identified uniparental disomy (UPD) of 11p (Suzuki et al., 2008).

To narrow down the regions of chromosomal loss or gain detected by previous studies, and to find previously unidentified genetic and epigenetic alterations, we analyzed 56 HB tumors using SNP arrays that can detect both chromosomal aberrations and UPD.

This study demonstrated the shortest overlapping region (SOR) of 1q gain/amplification in 1q32.1 (1.3 Mb) and that of 2q gain in

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