Efficacy and safety of homoharringtonine plus cytarabine and aclarubicin for patients with myelodysplastic syndrome-RAEB

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Abstract. The aim of the present study was to evaluate the treatment outcome of homoharringtonine, cytarabine (AraC) and aclarubicin combination therapy as induction treatment for myelodysplastic syndromes-refractory anemia with excess blasts (MDS-RAEB). A total of 24 patients with MDS-RAEB who were aged between 18 and 66 years were treated with homoharringtonine, AraC and aclarubicin (HAA regimen). The HAA regimen consisted of homoharringtonine (2 mg/m^2 intramuscularly twice daily, days 1-3), AraC (75 mg/m^2 injected subcutaneously twice daily, days 1-7) and aclarubicin (12 mg/m^2, days 1-7). The overall response rate was 79% with a complete remission rate of 58.3% and partial remission rate of 20.7%. There was no evidence of early mortality in this group of patients. The median overall survival (OS) was 36.2 months (95% confidence interval, 24.6-47.4 months), and the estimated three year overall survival rate was 45.8%. In conclusion, HAA combination therapy is a suitable induction regimen for patients with MDS-RAEB, which may improve the outcome for *de novo* higher-risk MDS patients, particularly of those with favorable and intermediate cytogenetics.

Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem-cell disorders characterized by abnormal differentiation, morphology, and maturation of hematopoietic cells in bone marrow (1). Patients with higher-risk MDS, which include intermediate-2 and high risk of the International Prognostic Scoring System (IPSS), have a peculiar propensity to evolve into acute myeloid leukemia (AML), and often have a median survival time of <12 months (2,3). Currently, hypomethylating agents (such as azacitidine and decitabine), lenalidomide, intensive chemotherapy, and allo-hematopoietic stem cell transplantation may be therapeutic options for patients with higher-risk MDS (4). However, high-intensity chemotherapy is generally reserved for higher-risk patients, particularly young patients eligible for intensive therapy that lack a suitable stem cell donor (2,5). Unfortunately, treatment of MDS patients with chemotherapy only results in a few long-term survivors (2,5,6). Therefore, innovative approaches for the treatment of higher-risk MDS should be developed.

Homoharringtonine (HHT), a plant alkaloid isolated from the Chinese evergreen *Cephalotaxus harringtonia*, was first investigated in China and has been used for the treatment of chronic myeloid leukemia, AML, and MDS (7-12). It was reported that HHT functions as a protein synthesis inhibitor at the initiation and elongation phases of translation, and that it is a potent apoptosis inducer for hematopoietic malignancies such as AML (13-15). *In vitro* experiments have demonstrated that HHT inhibited a variety of antiapoptotic proteins including Mcl-1, xIAP and Bcl-2, downregulated Akt pathway and sensitized AML cells to TRAIL-induced apoptosis via increase of death receptor 4 (DR4) and DR5 (13,15-20). Our previous pilot study revealed that HHT combined with cytarabine (AraC) and aclarubicin (HAA regimen) resulted in a complete remission (CR) rate of 83% in the patients with *de novo* AML and an estimated 3-year overall survival rate of 53%, which is more effective than any other induction remission regimens currently available (11). Therefore, in the present study, the same regimen was used to treat patients with MDS-refractory anemia with excess blasts (RAEB). The current study presents a retrospective analysis of the outcome of the HAA regimen as induction chemotherapy in the patients with MDS-RAEB.

Patients and methods

Patients. Between January 2005 and June 2012, 24 patients with MDS-RAEB diagnosed according to the World Health Organization (WHO) classification (21) were enrolled into the presentstudy. The study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Hangzhou, China). All the patients

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Table I. Patient characteristics.

Characteristics	n (range)
Patients	24
Male/female	9/15
Median age	41 years (18-66)
FAB classification subtype	
RAEB I	9
RAEB II	15
Median WBC count	3.15x10^9/l (1.0-13.3)
Median platelet count	53.5x10^9/l (7-198)
Median hemoglobin	7.4 g/dl (3.5-11.0)
Median marrow blast %	10.75% (6-19)
Cytogenetics (%)	
Intermediate risk	18 (81.8)
High risk	4 (18.2)
Not available	2

FAB classification, French-American-British classification (22); RAEB, refractory anemia with excess blasts; WBC, white blood cells.

Table II. Complete remission analyses.

Characteristics	CR/total	CR rate (%)	P-value
Age			0.665
<50	11/18	61	
≤50	3/6	50	
Gender			0.403
Male	4/9	44	
Female	10/15	66.6	
Cytogenetic			0.1
Intermediate risk	10/16	63.6	
High risk	3/6	50	
Marrow blast count			0.678
5-9%	6/9	60	
10-19%	8/15	57.1	

CR, complete remission.

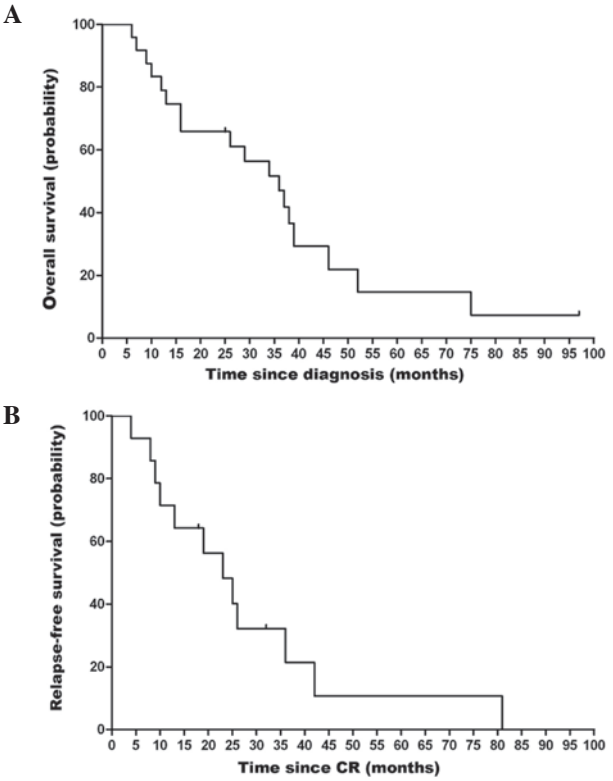

Figure 1. Kaplan Meier curves for survival analyses of the patients. (A) Overall survival of all patients treated with HAA regimen. (B) Relapse-free survival of the patients achieved complete remission.

signed informed consent prior to the enrollment. Other eligibility criteria were an Eastern Cooperative Oncology Group performance status of 0-2 and normal cardiac function (23). The clinical parameters assessed at presentation included age, sex, hemoglobin (Hb), white blood cell count (WBC), platelet count and percentage of bone marrow (BM) blasts. A cytogenetic study at diagnosis on BM was performed.

Treatment. All the enrolled patients received induction therapy: This consisted of a combination of HHT (2 mg/m^2 intramuscularly twice daily, days 1-3) with aclarubicin (12 mg/m^2, days 1-7) and AraC (75 mg/m^2 injected subcutaneously twice daily, days 1-7). The response evaluation criteria for the patients were as follows (24): i) CR was defined as normalization of blood counts with ≥1.0x10^9/l, Hb ≥110 g/dl, platelets ≥100x10^9/l, and marrow blasts ≤5% without evidence of dysplasia. ii) Partial remission (PR) was defined as CR except that marrow blasts should reduce by ≥50% compared with the pretreatment levels. iii) Bone marrow CR was defined as ≤5% marrow blasts without evidence of dysplasia, but complete recovery of blood counts was not achieved. Toxicity of induction therapy was graded according to the National Cancer Institute common toxicity criteria (10). For the patients with CR or PR, a second course was repeated using the same drugs and doses, whereas patients failing to response were offered palliative care. Postremission therapy was offered in rotation after the achievement of CR as following: HAA regimen, AraC (150 mg/m^2, days 1-7) in combination with a second drug, which including daunorubicin (45 mg/m^2, days 1-3), idarubicin (10 mg/m^2, days 1-3), etoposide (75 mg/m^2, days 1-5), mitoxantrone (10 mg/m^2, days 1-3), or aclarubicin (12 mg/m^2, days 1-5). During chemotherapy, the patients received subcutaneous injections of granulocyte colony-stimulating factor at 5 mg/kg, from the day neutrophil count was <0.5x10^9/l until the neutrophil count was >1.0x10^9/l on 3 successive days. Maintenance therapy was administered to all CR patients and continued until the patient was 3 years in remission.

Statistical analysis. Fisher's exact test was used in order to detect the factors that influenced the CR rate. Time-to-event analysis was performed according to the Kaplan-Meier method, and the log-rank test was applied to assess

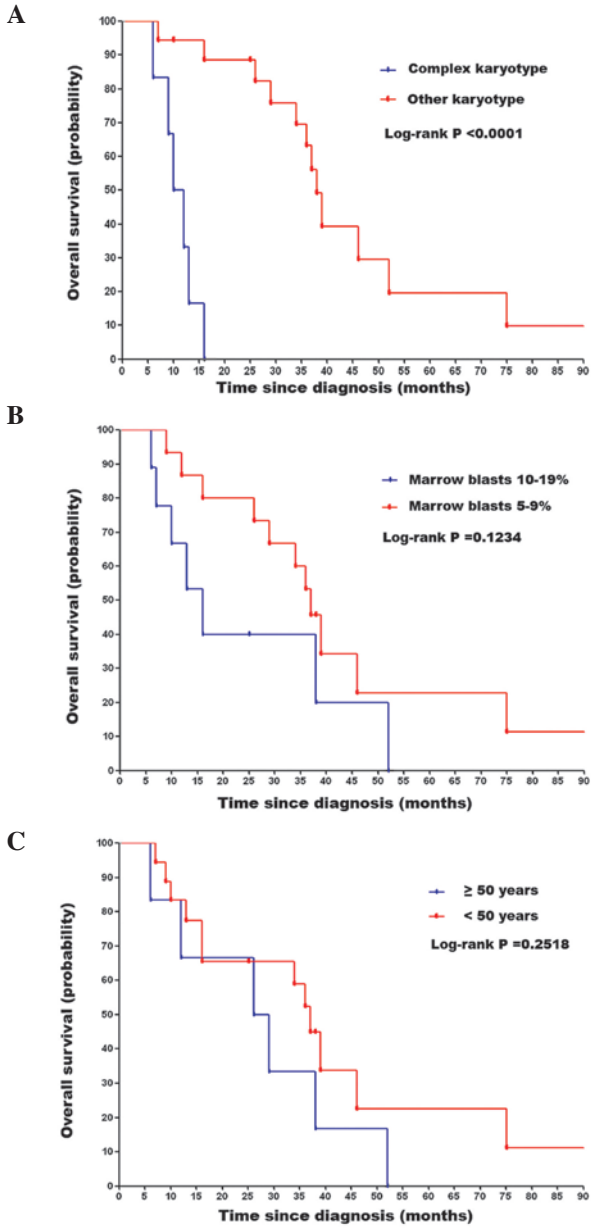

Figure 2. Kaplan Meier curves for overall survival analyses of the patients with different characteristics. (A) Complex abnormal karyotypes vs. other karyotypes [normal, -7, -8, -Y and del(20q)]. (B) High (10-19%) vs. low (5-9%) marrow blasts. (C) High (≥50 years) vs. low age (<50 years).

differences between subgroups. All statistical analysis was performed using SPSS 10.0 statistical software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a stastically significant difference.

Results

Patient characteristics. The baseline characteristics for 24 patients enrolled in the present study are summarized in Table I. The median age of this population was 41 years (range 18-66 years); 6 patients were >50 years and 18 patients were <50 years. According to the WHO classification, 9 patients had a diagnosis of MDS-RAEB I and 15 had RAEB II. Of the 22 patients who had cytogenetic studies performed, 5 patients (22.7%) had intermediate-1, 13 patients (59.1%) had intermediate-2, and 4 patients (18.2%) had high risk MDS, according to the IPSS (2,13).

Response and treatment outcome. A total of 14/24 patients (58.3%) achieved CR (including bone marrow CR) after the first course of induction treatment, and PR was observed in 5 patients (20.7%) for an overall response rate of 79%. Univariate analysis of factors influencing CR demonstrated that the age, gender, marrow blast count, and cytogenetic abnormalities of patient had no significant influence on CR rate (Table II).

Fig. 1A shows the overall survival (OS) curve for the 24 patients. The median OS time was 36.2 months [95% confidence interval (CI), 24.6-47.4 months], and the estimated 3 year OS rate was 45.8%. Univariate analysis of the factors that influenced OS showed a negative impact of complex karyotype (P<0.0001; Fig. 2A). Whereas, marrow blast count and age had no significant impacts on survival (Fig. 2B and C). Fig. 1B shows the relapse-free survival (RFS) curve for the 14 patients who achieved CR. The median RFS for these patients was 27.7 months (95% CI, 12.9-33.1 months).

Toxicity. Mortality was not associated with treatment with the HAA regimen as an induction therapy. The most common toxicities for cycle 1 were myelosuppression, which occurred in all patients. The nadirs of the neutrophil count and platelet were $0.2x10^9$/l (range 0.1-$0.35x10^9$/l) and $4x10^9$/l (range 2-$13x10^9$/l), respectively. The median times to recovery of neutrophil (≥$1.0x10^9$/l) and recovery of the platelets (≥$20x10^9$/l) were 24.5 days (95% CI, 19.9-29.2 days) and 27.2 days (95% CI, 18.5-35.9 days), respectively. The most common nonhematological toxicities were nausea, emesis, diarrhea and constipation, which were mild with grades I-II.

Discussion

Higher-risk MDS represents a significant therapeutic treatment challenge for any current chemotherapeutics because of poor responses to combination chemotherapy and shorter survival (25). Previous large studies using various induction regimens containing AraC in various combinations with idarubicin, fludarabine, topotecan, and cyclophosphamide demonstrated CR rates of ~40-60% for higher-risk MDS, median OS rate 10.4-13 months, and treatment-related early death 10-20% (2,3,26,27). In the present study, an HAA regimen was implemented for MDS-RAEB in adults who were aged 18-66 years. A total of 17/22 patients (77.3%) were scored as higher-risk by IPSS (28). The HAA regimen produced a CR rate of 58.3%, and a median OS of 36.2 months. The median RFS time of the patients who achieved CR was 27.7 months. This compares favorably with the outcomes of traditional intensive chemotherapies reported previously (26,27,29). The patient selection appears to be likely reason for this, since a high proportion of younger patients was included. Previously, Fenaux *et al* (30) reported that RAEB and predominantly RAEB in transformation (RAEBt) patients treated with an anthracycline-AraC regimen whose CR exceeded 2 years were young with normal karyotypes. Consistent with this result, in the present study, patients with other karyotypes [normal, -7, -8, -Y and del(20q)], had an improved OS compared with those with complex karyotypes. However, it is worth noting that no

statistical difference in OS was observed in comparison of high age (≥50 years) vs. low age (<50 years), in the present study.

For patients with higher-risk MDS, HAA regimen was found to be effective in inducing durable remissions although the number of patients in the current study was too small to make any definitive conclusions. HHT has also been administered to adults with MDS (7,12,31-33). Daver *et al* (31) reported that the CR rate was 11% in patients with intermediate and high risk MDS, who received HHT alone. In addition, combination therapy of HHT and low-dose AraC has been demonstrated to be an effective strategy for advanced MDS and RAEBt and CR was observed in 46.9% (32); however, the median OS was shorter (18.2 months). Encouraging results from an open-label, randomized, controlled phase III study using an HAA regimen as an induction therapy in *de novo* AML patients aged 14-59 years have been reported (34). In that study, 150/206 patients (73%) received HAA regimen achieved CR versus 125/205 (61%) in the patients who were treated with daunorubicin and AraC (DA regimen); 3-year event-free survival was 35.4% versus 23.1% (P=0.0023) (34). These differences appeared to be mainly a consequence of improved outcome in the patients with favorable and intermediate cytogenetics. Moreover, our recent study (35) showed *in vitro* synergy between HHT and aclarubicin. This combination therapy could synergistically induce the apoptosis of $CD34^+/CD38^-$ primary AML cells. The probable mechanism of synergy arises from the inhibition of PI3K/Akt and WNT/β-catnin signaling pathways, which may be the reason for the clinical benefit of HAA regimen in treating AML and higher-risk MDS.

Despite the presence of myelosuppresion in all of the patients in the present study, which resulted in accompanying risks of infectious complications, the induction mortality was notably low (0 by day 30) compared with previous reports in patients with AML who received an HAA regimen (4% to 5.8% by day 30) (11,34). One of the major issues concerned with HHT is cardiovascular complications. A previous study showed that a high dose HHT (5-6 mg/m^2/day) was associated with severe hypotension and cardiovascular collapse (36). The studies using HAA regimen in AML demonstrated that only 2% of patients had toxic cardiac effects (11,34). Collectively, these results support the safety of HAA regimen.

Altogether, the data from the present study indicates that the HAA regimen may improve the outcome of *de novo* higher-risk MDS patients, particularly of those with favorable and intermediate cytogenetics. However, this conclusion was obtained from the comparison with historical controls. Thus, a prospective controlled study is needed to confirm these results.

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