administration. Since the hypothalamic-pituitary space-occupying mass lesions of LCH respond very well to 2-CDA (**2, 26, 34**), additional studies are needed to determine whether early administration of 2-CDA could reverse APHD as well as CDI.
Established ND disease is very difficult to treat. Although several reports have suggested that all-trans retinoic acid or a combination of vincristine/AraC and intravenous high dose gamma-globulin (IVIG) may have some efficacy (**35-37**), it remains unclear whether any specific type of initial systemic chemotherapy for multifocal LCH patients can limit the later occurrence of ND disease (**38**). There is an urgent need for research that can identify an innovative therapy for such cases.

4. CDI and other CNS complications experienced in the Japanese LCH study

The cases of CDI, APHD and ND disease were analyzed in the cohort of patients treated with the JLSG-96/-02 protocols from 1996 to 2009 in Japan (**39**). CDI was detected in 12.4% (43/348) of pediatric multifocal LCH patients with a median follow-up of 5.0 (range, 0.2–14.0) years, with the shortest follow-up of alive patients being 0.8 years from the initiation of treatment. Of these 43 cases, CDI was detected before LCH diagnosis in 13 cases, at the LCH diagnosis in 12 cases, during the induction/maintenance treatment in five cases, and after off therapy at a median of 21 months (range, 4–116) in the remaining 12 cases. The incidence of CDI after the initiation therapy was 5.6% in our JLSG protocols, which is lower than 9.3% by Grois et al. (5). Data indicate that our treatment protocol effectively reduces the incidence of CDI by preventing the new occurrence. Of the 43 CDI patients, APHD was noted in 30.2% (13/43), with growth hormone deficiency being observed in ten of these patients. Six patients developed ND disease. In total, chemotherapy completely resolved CDI in two patients, which suggests that early intervention with chemotherapy may be able to reverse CDI (**39**).

5. Recommendations and future trials

In practice, when LCH patients, particularly those with craniofacial bone lesions, are diagnosed, treated and followed up, they must be examined carefully for any signs suggesting that CDI is developing. In particular, inquiring about the symptoms of polydipsia/polyuria and occasional tests for plasma osmolarity with plasma AVP may help to diagnose CDI early after onset. Moreover, in young females, the presence of amenorrhea and/or morbid obesity could be a first sign of pituitary dysfunction together with CDI. Repeated brain MRI examinations are also useful for detecting the early signs of CDI and/or ND disease. Once the precise diagnosis of CDI has been made, nasal or oral DDAVP is a safe therapeutic option that effectively controls CDI. Patients with anterior pituitary dysfunction require other hormonal replacement therapies.
Effective measures that can reverse CDI or other CDI-related neurological complications, or prevent them from newly occurring, remain elusive. Studies have shown that in LCH-induced CDI cases, the CDI is already present at the start of chemotherapy in half of the cases, while the remaining half develop CDI during chemotherapy or after off therapy. Ideally, the early introduction of chemotherapy should be able to reverse pre-existing CDI and prevent the new occurrence of CDI. Unfortunately, however, therapeutic regimens for patients with LCH that consistently achieve these goals have not yet been identified. However, we recently found that IVIG may be able to prevent the progression of ND

disease in patients with LCH **(10, 35)**. Given this observation and the fact that IVIG is also effective for other CNS inflammatory diseases, we have hypothesized that pre-emptive measures that include high dose IVIG may reduce the incidence of LCH-related CNS diseases, namely CDI and its related neurological complications, if they are given early and are combined with chemotherapy. To that end, we have proposed that the initial treatment of patients with "CNS-risk"-LCH should contain a high dose (2g/kg/dose) of IVIG combined with conventional induction chemotherapy (**40**). However, precise efficacy of immunomodulatory agents such as IVIG for treating LCH, particularly for preventing the development of LCH-related CNS diseases, needs to be explored by future studies.

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ORIGINAL ARTICLE

Outcome of pediatric patients with Langerhans cell histiocytosis treated with 2 chlorodeoxyadenosine: a nationwide survey in Japan

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Abstract The aim of this study was to assess the outcome of treatment with 2-chlorodeoxyadenosine (2-CdA) in pediatric patients with Langerhans cell histiocytosis (LCH) in Japan. We retrospectively identified 17 pediatric LCH patients treated with 2-CdA. All patients were refractory or reactivated cases who had been initially treated according to the JLSG-02 protocol of the Japan LCH study group. At initiation of 2-CdA therapy, 4 patients had primary refractory multisystem (MS) disease with risk organ (RO) involvement (MS+), 9 patients had reactivated MS disease [5 MS+ and 4 without RO involvement (MS−)], and the remaining 4 patients had refractory/reactivated multifocal bone disease (MFB). Treatment with 2-CdA (4–9 mg/m^2/day) was administered on 2–5 consecutive days and repeated every 3–4 weeks for a period that ranged from 2 to 12 months. Four primary refractory patients were treated with 2-CdA combined with high dose of cytarabine. In MS+ patients, response to treatment was observed in 5 of the 9 patients. In MS−/MFB patients, 5 of the 8 patients showed response to treatment. In the patients who were primary refractory or had reactivation during initial chemotherapy, 4 of 10 patients showed good response. On the other hand, in the patients having reactivation while off therapy, 6 of 7 patients showed good response. These findings suggest that 2-CdA is effective for reactivated LCH while off therapy.

Keywords Langerhans cell histiocytosis · 2-Chlorodeoxyadenosine · Reactivation

On behalf of the LCH Committee, the Japanese Pediatric Leukemia/Lymphoma Study Group

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1 Introduction

Langerhans cell histiocytosis (LCH) is defined as a clonal proliferation of phenotypically immature Langerhans cells, which contain Birbeck granules and which express CD1a antigen [1, 2]. Single-system (SS) involvement (unifocal or multifocal disease) or multisystem (MS) involvement may occur. The high frequency of disease reactivation in LCH is the main problem involved in therapy.

Several recent reports have shown that 2-chlorodeoxyadenosine (2-CdA), which is a purine substrate analog, is an effective treatment for refractory or reactivated childhood MS LCH [3, 4]. In adult LCH patients, 2-CdA has been used as a first-line therapy [5], but its efficacy in childhood LCH is unclear. In the present study, we retrospectively analyzed the case histories of children with refractory or reactivated LCH treated with 2-CdA in Japan to elucidate the efficacy and safety of 2-CdA therapy and identify the factors associated with response to this agent.

2 Materials and methods

2.1 Patients and data collection

The LCH Committee of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) sent out two questionnaires to all JPLSG affiliated hospitals. The first questionnaire requested details of the number of pediatric patients with LCH (age younger than 18 years at the time of diagnosis) who had been treated with 2-CdA during the period 2000–2006. We received replies from 157 of the 183 hospitals contacted. The second questionnaire was sent to 21 hospitals in which 2-CdA had been administered to pediatric LCH patients. This questionnaire requested information concerning diagnostic procedure, age at diagnosis, sex, site of lesion, treatment, complications and outcome. Eighteen hospitals responded to the second questionnaire. Information was collected on a total of 19 pediatric patients; 2 of them were excluded because of the lack of CD1a positivity. Thus, 17 patients with histologically confirmed LCH were analyzed.

2.2 Definition and evaluation criteria

Liver, spleen, lung and bone marrow are defined as risk organs (RO). MS disease is divided into two groups, such as MS with RO involvement (MS+) and without RO involvement (MS−).

No active disease (NAD) was defined as the disappearance of the signs and symptoms of disease with the exception of residual radiological evidence of bone lesions. Partial response (PR) was defined as a more than 50% regression of the signs or symptoms of disease without organ dysfunction or the occurrence of a new lesion. No response (NR) was defined as a less than 50% regression of the signs and symptoms of disease with or without organ dysfunction and without the occurrence of a new lesion. Progressive disease (PD) was defined as the progression of the signs or symptoms of the disease and/or the appearance of new lesions.

Reactivation was defined as reappearance or progression of the disease in any organ of the patients who achieved NAD. Refractory disease was defined as not attaining NAD/PR even after the completion of at least two consecutive induction regimens. Reactivation while on therapy indicates that disease reactivation occurred during chemotherapy. Reactivation while off therapy indicates that disease reactivation occurred after the completion of entire protocol.

2.3 Evaluation of adverse events

Adverse events were evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) version 3 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf).

2.4 Statistical analysis

Fisher's exact test was used to analyze factors influencing the attainment of NAD with 2-CdA treatment.

3 Results

3.1 Clinical presentation at diagnosis

The age at diagnosis ranged from 1 month to 9 years and 6 months. There were 13 male patients and 4 female patients. Twelve patients had MS disease (9 MS+ and 3 MS−, respectively), and 5 had SS multifocal bone disease (MFB).

3.2 Previous therapy and disease status at initiation of 2-CdA therapy

At initiation of 2-CdA therapy, all patients had refractory or reactivated disease. Thirteen patients had MS disease (9 MS+ and 4 MS−), and 4 patients had MFB disease. One patient with MFB at onset had developed the disease reactivation as MS− disease at this time. Four patients had CNS involvement: 2 with a hypothalamic-pituitary lesion (UPN 6251 and 6111), 1 with a parenchymal mass in the cerebellum and a meningeal lesion of the tentorium (UPN 3451), and 1 with a meningeal lesion of the tentorium (UPN 3601) (Table 1).

Table 1 Characteristics and treatment outcome of LCH patients treated with 2-CdA

UPN	Sex	At 2-CdA therapy				Initial therapy	Dose of 2-CdA	Cycles	Total dose of 2-CdA (mg/m^2)	Concomitant drugs	Adverse effects[b]	Status at the end of 2-CdA	Outcome[c]
		Age	Disease type	Disease status	Lesions[a]								
4181	M	1y2m	MS	Refractory	Sk, Bs, Lu, Li, Sp, Bm	A/B1/B2	4.5 mg/m^2 × 5 days	9	202.5	HD-AraC, AraC	Neutropenia thrombocytopenia infection	NAD	20m, alive
6251	F	7m	MS	Refractory	Sk, CNS, Li, Sp, Bm	A/B1/B2	9 mg/m^2 × 3–5 days	2	72	HD-AraC, CsA, PSL	Bm suppression infection	NR	1m, died[d,e]
1031	M	1y1m	MS	Refractory	Sk, Bs, Li, Sp, Bm	A/B1	9 mg/m^2 × 3–5 days	2	72	HD-AraC	Bm suppression Aspergillus pneumonia	PD	20m, alive[d]
7041	F	2y	MS	Refractory	Sk, Li, Sp, Lu, LN	A/B1	5–6.5 mg/m^2 × 3 days	5	86	None	None	NAD	46m, alive[d]
7151	M	4y8m	MS	React on Tx	Bs, Sp, Bm	A/B1/M	5 mg/m^2 × 5 days	10	250	HD-AraC, AraC	Bm suppression	PR	9m, died[f]
3451	M	2y6m	MS	React on Tx	Bs, CNS, Lu, Li, Bm	A/M/B1/M	5 mg/m^2 × 5 days	6	150	None	Bm suppression	NR	41m, alive[d]
6221	M	1y4m7	MS	React on Tx	Sk, Bs with St	A/B2/M	4–8 mg/m^2 × 3 days	4	72	None	Neutropenia	NR	6m, alive
5301	M	3y	MS	React on Tx	Bs, DI	A/M/B1/M	5 mg/m^2 × 3 days	12	180	6-MP	None	NAD	24m, alive
4271	F	1y2m	MS	React off Tx	Sk, Li, Sp	A/B1/B2/M	5 mg/m^2 × 5 days	2	50	None	Neutropenia	PD	3m, died[e]
3601	F	1y10m	MS	React off Tx	Sk, CNS, Lu	A/B1/M	5 mg/m^2 × 5 days	9	225	None	Eosinophilia infection	NAD	18m, alive
5332	M	5y5m	MS	React off Tx	Bs, Lu	A/M	5 mg/m^2 × 3 days	5	75	None	Eosinophilia	NAD	22m, alive
5382	M	4y11m	MS	React off Tx	Sk, Bs with St	A/M/B1/M	5 mg/m^2 × 5 days	6	150	None	Thrombocytopenia	NAD	23m, alive
6111	M	7y9m	MS	React off Tx	CNS, DI	A/M/A/M	5 mg/m^2 × 3–5 days	6	150	None	Thrombocytopenia	NAD	9m, alive
6222	M	4y9m	MFB	Refractory	Bs with St	A/B2	6 mg/m^2 × 3 days	8	144	None	Neutropenia	NR	8m, alive
1061	M	2y5m	MFB	React on Tx	Bs with St	A/M	5 mg/m^2 × 5 days	8	200	None	Thrombocytopenia	NAD	7m, alive
6052	M	3y5m	MFB	React off Tx	B	A/B2/M	5 mg/m^2 × 2 days	2	20	None	Neutropenia	NAD	5m, alive[d]
5331	M	11y7m	MFB	React off Tx	Bs	A/M	5 mg/m^2 × 3 days	6	90	None	Neutropenia	NAD	25m, alive

CNS central nervous system, *Lu* lung, *DI* diabetes insipidus, *St* soft tissue, *React* reactivation, *Tx* treatment, *A* induction A, *B1* induction B1, *B2* induction B2, *M* maintenance therapy, *AraC* cytarabine, *HD-AraC* high dose cytarabine, *PSL* prednisolone, *CsA* cyclosporine A, *6-MP* 6-mercaptopurine, *Bm suppression* anemia/neutropenia/thrombocytopenia, *PD* progressive disease, *NR* no response, *NAD* no active disease excluding DI, *PR* partial response

[a] Underlining indicates risk organ

[b] Listed CTCAE grade 4 hematological events and other grade 3 events

[c] Survival in months from initiation of 2-CdA therapy

[d] Patients who received stem cell transplantation

[e] Died of LCH

[f] Died of idiopathic interstitial pneumonia

All patients in our cohort had received initial treatment according to Protocols A of Japan Langerhans Cell Histiocytosis Study Group (JLSG)-02 [6]. Use of Protocol A involves administration of vincristine (VCR) and an intermediate dose of cytarabine (AraC) and prednisolone (PSL), followed by 48 weeks of maintenance therapy with a combination of VCR/AraC/PSL, methotrexate (MTX) and vinblastine (VBL)/PSL. Patients who showed NR to Protocol A or reactivation during maintenance therapy were switched to the intensive salvage regimen, Protocol B1, which involves administration of a combination of doxorubicin (DOX), cyclophosphamide (CPA), VCR and PSL. For extremely high-risk patients who showed PD after induction A, Protocol B2 which includes continuous infusion of cyclosporine A in addition to Protocol B1 was employed. Four patients with MS+ (UPN 4181, 6251, 1031 and 7041) were refractory to both Protocol A and B1, and two patients (UPN 4181 and 6251) were refractory also to Protocol B2. One patient with MS+ (UPN 7151), who had showed PR to Protocol B1, developed PD during maintenance therapy. Three further patients with MS disease (UPN 3451, 6221 and 5301) developed reactivation of disease during maintenance chemotherapy. One of them (UPN 6221) had been resistant to Protocol A. The remaining 5 patients with MS disease developed reactivation following the completion of chemotherapy (UPN 4271, 3601, 5332, 5382 and 6111). Two of them (UPN 4271 and 3601) had been resistant to Protocol A. One patient with MFB disease and a soft tissue mass (UPN 6222) showed no response to Protocol A or B2. One patients with MFB disease developed reactivation during maintenance therapy (UPN 1061). The 2 remaining patients with MFB disease developed reactivation of disease following the completion of chemotherapy (UPN 6052 and 5331). One of them (UPN 6052) was resistant to Protocol A (Table 1).

3.3 Treatment with 2-CdA

A dose of 4–9 mg/m^2/day of 2-CdA was administered daily for 2–5 consecutive days, and this was repeated every 3–4 weeks for a total period that ranged from 2 months to 1 year. The most frequently administered dose of 2-CdA (in 7 out of 17 patients) was 5 mg/m^2 given daily for 5 consecutive days. Four patients with MS disease (UPN 4181, 6251, 1031 and 7151) were administered high dose cytarabine (HD-AraC) in addition to 2-CdA (Table 1; Fig. 1).

3.4 Response to 2-CdA

Four MS+ patients were treated with HD-AraC in addition to 2-CdA. Two showed response (NAD/PR:1/1) and 2 did not (NR/PD:1/1). Two patients who did not respond to 2-CdA with HD-AraC underwent hematopoietic stem cell transplantation (HSCT) and one of them survived (UPN 1031).

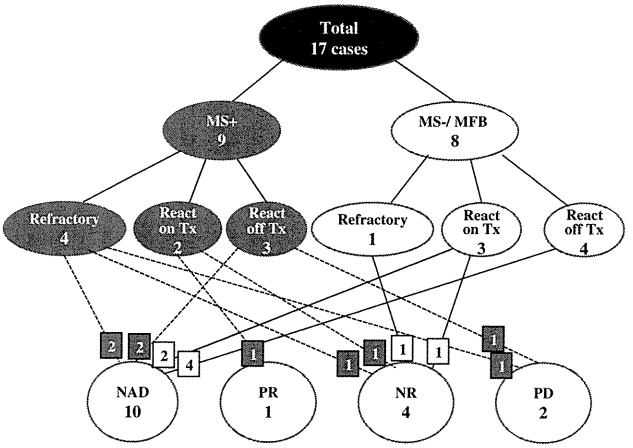

Fig. 1 Schematic representation of outcome of 2-CdA treatment in 17 cases based on disease type and time of reactivation. *MS+* multisystem disease with risk organ involvement, *MS−* multisystem disease without risk organ involvement, *MFB* multifocal bone, *React* reactivation, *NAD* no active disease, *PR* partial response, *NR* no response, *PD* progressive disease. # Both disease type and time of reactivation are described at the time of initiating 2-CdA

Of the remaining 13 patients, all but one patient (UPN 5301) were treated with 2-CdA alone (Table 1). In the 5 MS+ patients, 3 showed response (NAD/PR:3/0) and 2 did not (NR/PD:1/1). One of the 2 patients with MS+ who did not respond to 2-CdA therapy underwent HSCT and survived with NAD (UPN 3451). In the 8 MS− or MFB patients, 6 showed response (NAD/PR:6/0) and 2 did not (NR/PD:2/0). However, the 2 patients who did not respond to 2-CdA therapy survived with the disease (UPN 6221 and 6222). Collectively, 8 of 12 patients attained NAD following treatment with 2-CdA monotherapy. Of these 8 patients, six survived with NAD for a median of 20 months (range 7–25 months) from the initiation of 2-CdA therapy without any further treatment. In total, the rate of attainment of NAD was relatively high in patients who had developed reactivated disease while off therapy (6/7 vs. 4/10; $P = 0.13$).

In terms of association with the response to initial therapy, 7 of 13 patients who had been treated with induction B at onset responded to 2-CdA, including the cases treated with a combination of HD-AraC and 2-CdA (UPN 4181, 7151). On the other hand, all 4 patients who had responded to induction A initially responded to 2-CdA (UPN 1061, 5331, 5332, 6111).

3.5 Patients with CNS involvement

Four patients in our cohort had CNS involvement. Of these, 2 attained NAD: 1 patient with a meningeal lesion (UPN

3601) and 1 patient with a hypothalamic-pituitary lesion (UPN 6111). Two patients showed NR: 1 patient with a hypothalamic-pituitary lesion (UPN 6251) and 1 patient with cerebellar and meningeal lesions (UPN 3451).

3.6 Adverse events

Thirteen of the 17 patients experienced a grade 4 hematological adverse event: 10 developed neutropenia, 7 developed thrombocytopenia and 4 developed anemia. Prolonged cytopenia over 4 weeks of duration occurred in 3 of 8 patients who received more than 150 mg/m^2 of 2-CdA (UPN 7151, 6111 and 1061) (Table 1). Four of the 17 patients developed a grade 3 infection (including 1 case of pulmonary aspergillosis), 3 of whom had been treated with a combination of 2-CdA and HD-AraC. One patient with severe lymphopenia developed fatal idiopathic interstitial pneumonia following 9 courses of 2-CdA therapy (UPN 7151), although the patient underwent pneumocystis prophylaxis. Even though extensive search for clarifying pathogens including pneumocystis and fungus was conducted, none of the pathogens was identified. Two patients developed eosinophilia, with absolute eosinophil counts of more than 1,000/μl.

4 Discussion

Administration of 2-CdA is considered to be a potential therapeutic strategy in LCH, since it has been shown to cause monocytopenia and to be effective in the treatment of indolent lymphoma [7, 8]. Saven et al. [9] reported the first adult LCH patient to be successfully treated with 2-CdA, and there have been several subsequent reports of 2-CdA treatment in LCH patients [3–5, 10, 11]. The International Histiocyte Society conducted a retrospective analysis that demonstrated that 2-CdA was an effective salvage therapy in more than 50% of LCH patients who had not responded to intensive first-line therapy [10]. However, they were unable to determine the precise factors that were associated with the effectiveness of 2-CdA in the treatment of LCH. Although those retrospective analyses or case reports described a high degree of effectiveness of 2-CdA as salvage therapy for refractory/reactivated LCH, the prospective study was expected to clarify the effectiveness of this agent. The results of the LCH-S-98 study, which was a prospective phase II Histiocyte Society Study to evaluate the efficacy of 2-CdA monotherapy as salvage therapy in refractory or reactivated LCH, was published in 2009 [12]. They demonstrated that 22% of MS(+) patients had a good response (NAD and PR) while 44% progressed; 62% of patients with MS(−) or MFB disease responded and 11% progressed. These findings suggest that this agent produces higher response rate in patients with MS(−) or MFB than those with MS(+).

This is the first nationwide survey to have assessed the outcome of 2-CdA treatment in LCH patients in Japan. In this survey, we identified 17 pediatric LCH patients who had received second-line treatment with 2-CdA following ineffective initial treatment according to the JLSG-02 protocol. NAD attained in the present study was 64.7% (11/17), which is similar to that reported in previous retrospective studies [10]. In terms of biological and clinical characteristics that may predict a favorable outcome with 2-CdA therapy, reactivation of disease that occurs while off therapy may be a factor that appears to be associated with a favorable response to treatment, although it is not statistically significant because of the small number of the current cohort. The time of reactivation is considered to be deeply associated with the sensitivity for the chemotherapeutic agents in each patient. In other words, the disease that reactivates while off therapy might maintain sensitivity even for the agents used initially, indicating that resuming the original treatment might be also effective. In this cohort, 2-CdA was used for the patients who were resistant to chemotherapeutic agents, including not only VCR, but also Ara-C, DOX and CPA, suggesting that these patients had highly chemo-resistant disease. Thus, 2-CdA is not suitable to treat such cases, if used as a single agent.

Neutropenia and/or thrombocytopenia were the most frequently observed adverse events occurring during 2 CdA treatment in our cohort. These adverse events were observed more frequently in patients who had received combination therapy with AraC. 2-CdA is known to induce prolonged cytopenia [13], although this was not a cause for discontinuation of therapy in our cohort. One patient in our cohort died of interstitial pneumonia with lymphopenia. Transient neutropenia and thrombocytopenia are relatively common adverse events. However, in our cohort, prolonged cytopenia occurred in 3 of 8 patients who received more than 150 mg/m^2 of 2-CdA. These findings suggest that further studies are indicated to determine the total amount of 2-CdA that can be administered without the development of prolonged hematological toxicity.

The treatment of patients with refractory/reactivated MS+ disease is challenging. A combination of AraC and 2-CdA has been shown to exert synergistic cytotoxicity, even in resistant cells [14]. Bernard et al. [15] have reported chemotherapy with 2-CdA and HD-AraC to be a promising treatment for patients with refractory LCH. They administered a combination of 2-CdA (9 mg/m^2/day) and AraC (1,000 mg/m^2/day) for 5 days and control of disease was achieved in all ten patients in their study. However, all patients suffered grade 4 hematological toxicity and two patients died of infection. In our cohort, treatment with 2-CdA, even when combined with HD-AraC, only induced

remission in 1 of 4 patients with refractory MS+ disease. The low response rate in our refractory cases might be attributable to the higher resistance to treatment of our patients, who had not responded to the high intensity Protocol B of the JLSG [6].

HSCT is considered to be a curative treatment for patients with refractory/reactivated MS+ disease [16, 17]. However, this procedure has a high mortality rate [16]. Before performing HSCT, it is important to control disease activity with minimum toxicity. In one patient in this survey (UPN 3451), who developed reactivated MS+ disease while maintenance therapy, partial control of the disease with minimal adverse events was achieved using 2-CdA, and the patient subsequently underwent successful HSCT.

Optimal treatment for LCH involving the central nervous system has not yet been established, and the response of LCH patients with parenchymal brain lesions to chemotherapy has been disappointing [18, 19]. Our cohort included four patients with CNS-LCH (Table 1). 2-CdA crosses the blood–brain barrier, and 2-CdA levels in the cerebrospinal fluid are 25% of those found in plasma [20]. 2-CdA would therefore be expected to be effective in LCH patients with CNS lesions, with the exception of CNS degeneration (CNS-D). Dhall et al. [21] reported that 8 of 12 patients with CNS-LCH who were treated with 2-CdA showed a complete response radiographically. Treatment with 2-CdA was also shown to be effective for CNS-LCH in our cohort. These findings suggest that 2-CdA may be the most effective agent for a CNS lesion in LCH. Further studies of larger patient samples are required to establish the efficacy of 2-CdA in CNS-LCH.

In conclusion, our results suggest that 2-CdA is a potentially useful agent in the treatment of indolent recurrent LCH or CNS-LCH. Further prospective studies are warranted to firmly establish the role of this agent in the treatment of patients with LCH.

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Nationwide Survey of Bisphosphonate Therapy for Children With Reactivated Langerhans Cell Histiocytosis in Japan

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Background. Several studies have suggested that Langerhans cell histiocytosis (LCH) is responsive to treatment with bisphosphonates (BPs). However the efficacy and safety of BPs therapy for childhood LCH is unknown. ***Procedure.*** Data on children with LCH who had received BPs therapy were collected retrospectively from hospitals participating in the Japanese Pediatric Leukemia/Lymphoma Study Group. ***Results.*** Twenty-one children with histologically proven LCH were identified. Of these, the case histories of 16 children who had been treated with pamidronate (PAM) for disease reactivation were analyzed in detail. The median post-PAM therapy follow-up period was 2.8 years (range: 0.9–9.3 years). The median age at commencement of PAM therapy was 9.4 years (range: 2.3–15.0 years). All children had one or more bone lesions but none had risk organ (RO) involvement. In the majority of the children, six courses of PAM were administered at a dose of 1.0 mg/kg/course at 4-week intervals. In 12 of the 16 children, all active lesions including lesions of the skin (n = 3) and soft tissues (n = 3) resolved. Of these children, eight children had no active disease for a median of 3.3 years post-PAM therapy (range: 1.8–9.3 years). Progression-free survival (PFS) was 56.3 ± 12.4% at 3 years. PFS was significantly higher in children with a first reactivation compared with children experiencing a second or subsequent reactivation. ***Conclusions.*** PAM may be an effective treatment for reactivated LCH with bone lesions. A prospective trial of the efficacy of PAM in recurrent pediatric LCH is warranted. Pediatr Blood Cancer. 2011;56:110–115. © 2010 Wiley-Liss, Inc.

Key words: bisphosphonate; bone lesion; Langerhans cell histiocytosis; reactivation

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare histiocytic disease that is characterized by uncontrolled clonal proliferation of CD1a-positive dendritic Langerhans cells (LCs). This occurs most commonly in bone tissue, but may also occur in the skin and in various other organs. Its clinical manifestation and course vary from the development of a solitary self-healing lesion to fatal multi-organ disease involving a risk organ (RO) such as the liver, spleen, lung, or hematopoietic system [1]. Although the survival rate for patients without RO involvement is close to 100% [1], recurrence is common and occurs most frequently in bone [2]. Reactivations can increase the risk for permanent consequences, such as orthopedic abnormalities, diabetes insipidus (DI), and neurological impairments [2,3]. The treatment of bone LCH involves curettage or biopsy for single bone lesions, and chemotherapy or indomethacin for multiple bone lesions or reactivated bone disease [4]. There is some evidence to suggest that prolonged low dose chemotherapy may reduce the likelihood of disease reactivation [4]. However, multiple reactivation occurs in some patients despite chemotherapy and prolonged chemotherapy with etoposide or antimetabolites may induce secondary hematological malignancies in patients with LCH [5,6].

Although the pathogenesis of LCH remains obscure, many types of immune cells other than LCs are present in LCH lesions, including lymphocytes, macrophages, eosinophils, and multi-nucleated giant cells (MGCs). The MGCs in bone, skin, and lymph node lesions express characteristic osteoclast markers such as tartrate-resistant acid phosphate, vitronectin receptor, cathepsin K, and matrix metalloproteinase-9 [7]. We previously reported that patients with LCH have high serum levels of the soluble receptor activator of NF-κB ligand (RANKL), a cytokine which induces the differentiation of pre-osteoclasts into osteoclasts and the activation of osteoclasts [8]. Since the osteoclast-like MGCs in LCH express

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various matrix-degrading enzymes involved in tissue destruction, the targeting of these cells in LCH lesions in bone and other tissues may represent a valid therapeutic approach [7].

Bisphosphonates (BPs) are pyrophosphate analogs that inhibit the recruitment of osteoclasts and reduce their activity and longevity. BPs are widely used in the treatment of a variety of bone diseases, including osteogenesis imperfecta (OI), osteoporosis, Paget's disease, and the osteolytic lesions of multiple myeloma and other malignancies [9,10]. The results of several studies have suggested that BPs may also be effective in LCH, although most of these studies have described single adult LCH cases [11–17]. To assess the efficacy and safety of BPs therapy in children with LCH, we conducted a retrospective nationwide survey in Japan.

MATERIALS AND METHODS

Data Collection

The LCH committee of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) sent out a questionnaire to all JPLSG-affiliated hospitals in the summer of 2008. This questionnaire enquired whether these hospitals had administered BPs therapy to any children with LCH (age younger than 18 years at the time of diagnosis). Replies were received from 157 of the 183 hospitals. Fourteen hospitals had administered BPs therapy to a total of 24 children with LCH. These hospitals were sent a second questionnaire requesting details of the following: (i) diagnostic procedure, (ii) age at diagnosis, (iii) sex, (iv) site(s) of the lesion(s), (v) treatment, (vi) complications, and (vii) outcome. Twelve hospitals responded to the second questionnaire and 21 children with histologically proven LCH who had been treated with various BP preparations were identified. Of these, the case histories of 16 children who had been treated with intravenous pamidronate (PAM) for disease reactivation were analyzed in detail.

Evaluation Criteria and Definitions

No active disease (NAD) was defined as the disappearance of all signs and symptoms of disease with the exception of DI, central nervous system degeneration (CNS-D), or residual radiological findings of bone lesions showing regression or stabilization. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter (LD) for all bone or mass lesions taking as reference the baseline sum LD evaluated by radiological findings or at least a 50% decrease in area of skin lesion without organ dysfunction or the occurrence of a new lesion. No response (NR) was defined as more than 70% residual in the sum of the LD for all bone or mass lesions evaluated by radiological findings or more than 50% residual in area of skin lesion with or without organ dysfunction. The evaluation of radiological findings was done by a radiologist at each institute. Progression-free survival (PFS) was defined as continuing NAD following the commencement of PAM therapy. Reactivation was defined as the reappearance of signs and/or symptoms of disease activity following a period of NAD. Adverse effects were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) [18].

Statistical Analysis

Fisher's exact test was used to analyze factors with an influence on the attainment of continuous NAD post-PAM therapy. PFS was estimated using Kaplan–Meier analysis, and is expressed as rates ± standard error. The log-rank test was used to compare the factors affecting PFS. P values of less than 0.05 were considered statistically significant.

RESULTS

Of the 16 children with reactivated disease who had received PAM-therapy, 10 were males and 6 were females (Table I). The median age at disease onset was 3.1 years (range: 0.4–14.1 years). Ten children had single system disease (two of the skin and eight of bone). Six children had MS disease including bone lesion(s) (three without RO involvement and three with RO involvement of the hematopoietic system, spleen, and lung, respectively). All but one of the children had received initial systemic chemotherapy; for the majority of the children, treatment had been administered in accordance with the JLSG-96/02 protocol [19].

The median post-PAM therapy follow-up period was 2.8 years (range: 0.9–9.3 years). The median age at the commencement of PAM therapy was 9.4 years (range: 2.3–15.0 years) (Table I). Prior to the commencement of PAM therapy, 6 of the children had multiple disease reactivations, and 5 children had been receiving chemotherapy and 11 had completed chemotherapy. Two of the children (UPN 7141 and 7142) have been reported previously [14]. Prior to PAM therapy, these two children had received oral etidronate therapy for 15–18 months and had shown PR. The remaining children had received PAM therapy immediately following disease reactivation. At commencement of PAM therapy, ten children had only bone lesion(s), six of whom had soft tissue mass associated with the bone lesion, and five had bone pain. The remaining six had multisystem involvement including bone and skin lesions (n = 3), DI (n = 3), CNS-D (n = 2), and soft tissue (n = 1). None of the children had RO involvement. PAM was administered intravenously at a median dose of 1.0 mg/kg/course. Four children had received 1.0–1.25 mg/kg/day daily for 3 days per course (UPN 4123, 4122, 4121, and 7091). Twelve children had received six courses of PAM administered at 4-week intervals. Four children had received more than 10 courses of PAM administered at 4- to 8-week intervals (UPN 4123, 4122, 4121, and 5081); three of these children are still receiving this therapy at the time of writing. In addition to PAM, nine children received meloxicam (MC) daily at a dose of 0.2 mg/kg. Along with PAM therapy, three children received continuous cytoreductive agents (methotrexate, vinblastine, and 6-mercaptopurine) which had been prescribed prior to the disease reactivation that led to PAM therapy. Three children experienced mild adverse effects in response to PAM therapy including pyrexia, fatigue, gastrointestinal symptoms, and hypocalcemia, which were rated as grades 1–2 according to the CTCAE. One child (UPN 5081) with cranial bone lesions, an orbital soft tissue mass, DI, and CNS-D, developed blurred vision secondary to uvenitis after 11 courses of PAM therapy without an acute phase reaction. The child's vision improved following the discontinuation of PAM therapy and the administration of immunosuppressive therapy (dexamethasone and a calcineurin inhibitor). At cessation of therapy, 12 of the 16 children (75%) had attained NAD and radiological reossification and normalization, including 3 children with skin lesions and 3 patients who had

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TABLE I. Characteristics and Outcome of Children With Reactivated LCH Who Received Treatment With Pamidronate

UPN	Gender	At commencement of PAM therapy			Concomitant drugs	Adverse effects	Response to PAM therapy	Reactivation after NAD	Subsequent treatment	Survival post-PAM therapy
		Age	Status	Lesions						
7144	F	9y6m	1st Re*2	B, Sk	MC	None	NAD after 2 courses	1.4y in Sk	PSL	2.8y+, NAD
3533	F	7y7m	2nd Re*2	Bs with St, CNS-D	MTX*4	None	Bs: PR, St: NR	NE	MTX	0.9y+, NAD
7143	M	15y0m	1st Re	B	MC	None	NAD after 2 courses	None	None	7.3y +, NAD
7145	M	14y9m	1st Re	Bs	MC	None	NAD after 2 courses	None	None	3.8y+, NAD
4123	M	14y0m	1st Re	B*3	None	None	NAD after 6 courses	None	None	1.8y+, NAD
3091	F	13y7m	11th Re	B*3	MC	Fever, fatigue, hypo Ca	NAD after 2 courses	1.0y in B	ZOL	1.2y+, NAD
3532	F	11y6m	4th Re*2	B with St	VBL*4	Fever, vomiting, diarrhea	New B lesion at 0.2y, St: NAD	NE	AraC/VCR/PSL, MTX, VBL	1.0y+, NAD
4122	M	10y0m	5th Re*2	B*3	6MP*4	None	NAD after 4 courses	None	None	2.8y+, NAD
5081	M	10y0m	5th Re	Bs*3 with St, DI, CNS-D	MC	Uvenitis	Bs: NR, St: NR	NE	VBL/DEX/CSA, 2CdA	5.7y+, NAD
7161	M	4y8m	1st Re	B	MC	Hypo Ca	NAD after 2 course	None	None	2.6y+, NAD
3121	M	8y8m	3rd Re*2	Bs*3 with St, St, DI	MC	None	NAD after 5 courses	3.3y in St	PAM	5.2y+, NAD
7141*1	F	3y0m	1st Re	Bs, Sk, DI	None	None	NAD after 2 courses	None	None	9.3y+, NAD
7142*1	F	2y3m	1st Re	Bs, Sk	MC	None	NAD after 3 courses	None	None	6.7y+, NAD
4121	M	8y0m	1st Re	B with St	DEX	None	NAD after 4 courses	None	None	2.7y+, NAD
7181	M	2y10m	1st Re	Bs	MC, PSL	None	Bs: NR, new Sk lesion at 0.5y	NE	PAM, VCR/PSL	6.8y+, NAD
7091	M	2y10m	1st Re	Bs with St	None	None	NAD after 2 courses	1.0y in B	PSL	2.7y+, NAD

SS, single system; MS, multi-system; Sk, skin; B, single bone; Bs, multiple bones; St, soft tissue; LN, lymph node; DI, diabetes insipidus; He, hematopoietic system; Sp, spleen; Thy, thymus; Lu, lung; PAM, pamidronate; Re, reactivation; CNS-D, central nerve system degeneration; MC, meloxicam; MTX, methotrexate; IVIG, intravenous immunoglobulin; DEX, dexamethasone; VBL, vinblastine; PSL, prednisolone; 6MP, 6-mercaptopurine; hypo Ca, hypocalcaemia; NAD, no active disease (apart from posterior pituitary lesion and CNS-D); PR, partial response; NR, no response; NE, non-evaluable; ZOL, zoledronate; AraC, cytarabine; VCR, vincristine; CSA, cyclosporine A; 2CdA, cladribine; *1 treated with etidronate for 15–18 months before receiving pamidronate (Ref. [14]); *2 reactivation on chemotherapy; *3 accompanied by bone pain; *4 administered continuously prior to the disease reactivation that led to PAM therapy.

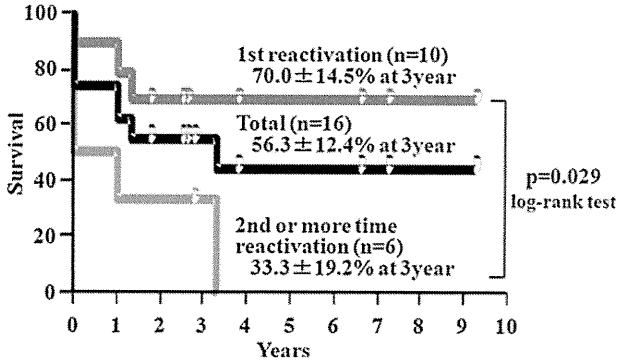

Fig. 1. Progression-free survival (PFS) post-PAM therapy. The overall PFS at 3 years was 56.3 ± 12.4%. PFS was significantly higher in children with a first reactivation compared with children experiencing a second or subsequent reactivation (70.0 ± 14.5% vs. 33.3 ± 19.2% at 3 years, $P = 0.029$).

had soft tissue masses at the commencement of PAM therapy. The median number of courses of PAM therapy in children attaining NAD was 2. Although the bone lesions of one patient showed a PR, the accompanying soft tissue masses showed NR. PAM therapy did not affect the bone lesions of three children; two of them developed a new bone lesion and a new skin lesion, respectively. Of the 12 children who had attained NAD, 8 have had NAD and complete resolution of radiographic findings in bone for a median of 3.3 years (range: 1.8–9.3 years) since the commencement of PAM therapy. The remaining four children experienced disease reactivation in bone (n = 2), skin (n = 1), and soft tissue (n = 1). These reactivated children were treated with prednisolone (PSL) (n = 2), zoledronate (ZOL) (n = 1), or PAM (n = 1), which again resulted in the complete disappearance of the lesions. The overall PFS at 3 years was 56.3 ± 12.4% (Fig. 1).

The ratio of maintaining NAD was significantly higher in children receiving PAM therapy at the first reactivation off chemotherapy compared to other patients (7/9 vs. 1/7, $P = 0.041$). PFS was significantly higher in children with a first reactivation than in children with a second or subsequent reactivation (70.0 ± 14.5% vs. 33.3 ± 19.2% at 3 years, $P = 0.029$) (Fig. 1). There was no significant difference in PFS between children who developed reactivation while off chemotherapy and those on chemotherapy (63.6 ± 14.5% vs. 40.0 ± 21.9% at 3 years, $P = 0.139$). Other factors, such as gender, age, type of disease, dose of PAM, number of PAM courses, and prescription of concomitant medication, also did not affect the PFS of children receiving PAM therapy.

DISCUSSION

Osteoclast-like MGCs in LCH lesions are a potential therapeutic target since they express the various matrix-degrading enzymes that mediate tissue destruction. In the present study, we demonstrated that intravenous PAM therapy appears to have considerable responses for 16 children with LCH. All 16 children had reactivated disease with bone lesion(s), and 6 had MS disease involving non-RO sites. In 12 of the 16 children, NAD after PAM therapy was observed for skin and soft tissue lesions as well as for bone lesions. Eight of the 16 children have had NAD for a median of 3.3 years since the cessation of PAM therapy.

Seven reports of BPs therapy for LCH have been published to date, and these studies have included a total of 14 patients, all of whom had bone lesion(s) [11–17]. Only three of these patients were children, and two of these were included in the present study. Eleven of the 14 patients had also presented with lesions in sites other than bone, including in the pituitary, skin, lung, and CNS. The preparation and dosage of the BPs administered to these 14 patients varied. In four patients, PAM was administered in 2–11 courses at a dose of 90–270 mg/course at 1–2 months intervals. With the exception of one case of renal failure, no serious adverse effects were reported. In most of the fourteen patients, BPs had been administered in order to relieve bone pain, and this was successful in all cases. Recalcification was also reported in some cases. However, these studies evaluated neither the response of LCH lesions in sites other than in bone, nor the long-term outcome of BP therapy.

The most widely used nitrogen-containing BP in children is PAM, and the most extensively investigated childhood disease for which PAM is prescribed is OI [20]. The most commonly used PAM protocol for OI is the administration of 1.0 mg/kg/day for 3 days every 4 months (i.e., an annual dose of 9 mg/kg) over a period of several years. In most of the LCH children in the present study, PAM was administered at a dose of around 1 mg/kg every month for 6 months. It may be possible to extend the duration of BPs therapy for LCH; in the present study, although PFS was not significantly higher in patients who received more courses of PAM because of the short period of follow-up and the small number of cases.

Hypocalcemia and acute phase reaction are the most common adverse events following the intravenous administration of BPs, and both resolve with supportive care [9,10]. Of the 16 children in the present study, 2 had hypocalcemia and 1 had an acute phase reaction. Both effects subsided in response to the administration of appropriate medication. There have been rare reports of inflammatory ocular disease such as scleritis and uvenitis in adults secondary to BPs therapy, most of which were associated with an acute phase reaction, occurred within 6 hr to 2 days of treatment, and subsided after discontinuation of the BPs therapy [21]. In the present study, one child with an orbital LCH lesion developed blurred vision secondary to uvenitis after 11 courses of PAM therapy without an acute phase reaction. This presentation differs from those described in previous reports of BP-induced uvenitis. It is possible that an orbital inflammatory LCH lesion might affect the development of uvenitis. Another clinically significant adverse reaction to intravenous BPs is nephrotoxicity, which is dependent upon both the dose and the infusion time, and which can be avoided by dose reduction and a prolongation of infusion time to allow the monitoring of serum creatinine levels [22]. Although there has been one report of BPs induced renal failure in a patient with LCH [15], most cases of BPs-induced nephrotoxicity have been reported in patients with multiple myeloma receiving high dose PAM, and there have been no such reports in children [22]. Osteonecrosis of the jaw (ONJ) has been described as a serious complication of BPs therapy in adults with cancer [23], but not in children [24]. With respect to the long-term safety of BPs in children, a major concern is the suppression of longitudinal bone growth. This has been shown to be mildly suppressed by ZOL in growing rabbits [25], but intravenous PAM therapy does not appear to have a detrimental effect on the growth of children with OI [26]. Thus, while continued careful monitoring is required, particularly for the development of inflammatory ocular disease in children with an orbital LCH lesion, intravenous administration of

1 mg/kg PAM once every month for a total of 6 months for children with LCH may be a safe treatment.

In addition to having resolved the bone lesions, PAM therapy may have contributed to resolution of the skin and soft tissue lesion(s) of the children in the present study. No previous study has suggested that PAM is efficacious for LCH lesions in sites other than bone. PAM suppresses the activation and longevity of osteoclast-like MGCs, which are known to be present in LCH lesions of the skin and other sites as well as in LCH bone lesions [7]. However, it should be noted that none of the children in the present study had RO involvement at the commencement of PAM therapy. In addition, 2 children from our total cohort of 21 children with LCH who are not described in this report had RO involvement at the commencement of BPs therapy and experienced disease progression despite having received BPs and chemotherapy at disease onset. Children with a first reactivation had a statistically significantly better PFS, which may indicate that patients who experience more than one reactivation might have differing biological characteristics to those who experience no or only one reactivation. These findings suggest that BP therapy may be more effective for LCH children with a first reactivation involving a non-RO site.

In the present study, 9 of the 16 children received MC in addition to PAM. MC is a non-steroidal anti-inflammatory (NSAID) agent and a selective inhibitor of cyclooxygenase-2 (COX-2). NSAIDs block the arachidonic acid-prostaglandin pathway by inhibiting COX. It has been shown that COX and prostaglandins are over-expressed by LCH lesions and that prostaglandin E2 can induce bone resorption [27,28]. Several reports have described the effectiveness of NSAIDs in the treatment of LCH bone lesions [29–32]. The combined administration of MC and PAM did not affect PFS, however, and two children in the present study experienced disease reactivation shortly after the cessation of MC. MC might therefore have acted in an additive or synergistic manner with PAM in the children in the present study.

In conclusion, the intravenous administration of 1 mg/kg PAM once every month for 6 months may be an effective and safe treatment for children with LCH bone lesions. Although the long-term effects of BPs on LCH are unknown, it is possible that by preventing further disease reactivation in bone, PAM therapy may decrease or prevent late effects such as DI without the use of cytoreductive agents. However, a weakness of this study is its retrospective approach. Another weakness was that radiographs were not evaluated by independent radiologists but instead were evaluated by radiologists at each institution. A prospective study of children with reactivated LCH and no RO involvement is warranted to confirm these results, and this should be conducted with careful attention to the development of adverse effects.

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ORIGINAL ARTICLE

Improved outcome of refractory Langerhans cell histiocytosis in children with hematopoietic stem cell transplantation in Japan

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Langerhans cell histiocytosis (LCH) that is refractory to conventional chemotherapy has a poor outcome. Hematopoietic stem cell transplanta tion (SCT) is a promising approach for refractory LCH because of its immunomodulatory effect. In this study, the outcomes of children with refractory LCH undergoing SCT in Japan were analyzed. Between November 1995 and March 2007, 15 children younger than 15 years (9 males, 6 females) with refractory LCH underwent SCT. The patients' median age at diagnosis was 8 months (range, 28 days to 28 months), and all had failed conventional chemotherapy. The median age at SCT was 23 months (range, 13–178 months). Nine had risk organ involvement at diagnosis, including liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). For SCT, a myeloablative regimen was used for 10 patients, and a reduced-intensity conditioning regimen (RIC) was used for five. The donor source varied among the patients, but allogeneic cord blood was primarily used ($n=10$). Subsequently, 11 of 15 patients have survived with no evidence of disease, with a 10-year overall survival (OS) rate (median ± standard error) of 73.3 ± 11.4%. The 10-year OS rate of nine patients with risk organ involvement at diagnosis was 55.6 ± 16.6%, whereas six without risk organ involvement have all survived with no evidence of disease ($P=0.07$). These results indicate that SCT is promising as a salvage approach for children with refractory LCH.

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Introduction

Langerhans cell histiocytosis (LCH) is a rare disease with a wide variety of clinical presentations, from localized disease to disseminated disease.[1–3] Although the risk factors for LCH have not been fully elucidated, patients younger than 2 years of age at onset, with risk organ involvement, including the hematopoietic system, liver, spleen, or lung, and disease refractory to conventional chemotherapy have a very poor outcome, with survival rates of about 20%.[4–8]

The treatment strategy for these high-risk LCH patients has not yet been established. Recently, it was reported that a combination of 2-chlorodeoxyadenosine (2-CdA) and cytarabine (Ara-C) was effective for refractory LCH.[9] Allogeneic stem cell transplantation (SCT) has also been used because of its strong immunomodulatory effects for LCH.[10,11] This report describes the improved outcomes of 15 children with refractory LCH who underwent SCT in Japan.

Patients and methods

Data collection

The HLH/LCH committee of the Japanese Society of Pediatric Hematology (JSPH) sent the first questionnaires to all hospitals in Japan where pediatric hematologists (JSPH members) worked, asking for the number of children with LCH who underwent SCT between November 1995 and March 2007. The second questionnaires were then sent to 16 hospitals where SCT was done for LCH, asking about the clinical features at onset, treatment before SCT, donor source, conditioning regimen, complications, and outcome. Thirteen hospitals responded to the second questionnaires, with a total of 15 eligible patients. The registration data of the pediatric SCT program, independently managed by the SCT committee of the JSPH, were also available to confirm the profiles of the patients who underwent SCT.

Diagnostic criteria and definition of disease state

All patients were diagnosed as having LCH by histopathological examination of the affected organs, which were positive for either CD1a or S100 staining. Each patient was divided into one of three subsets at diagnosis: single system single-site (SS-s), single system multi-site (SS-m), and multi-system (MS). In the MS subset, patients with one of the following factors were classified as the high-risk group: younger than 2 years of age at onset; risk organ involvement, including the hematopoietic system (bone marrow, BM), liver, spleen, or lung; or disease refractory to conventional chemotherapy. Patients without these factors in the MS, SS-m, and SS-s subsets were classified as the low-risk group. The characteristics of these 15 patients are shown in Table 1. The disease state was evaluated at three time-points in all patients: (1) within 6 weeks after initial diagnosis, (2) within 12 or 14 weeks after diagnosis, and (3) before SCT. A good response (GR) was defined as the disappearance of signs or symptoms of disease; a partial response (PR) was defined as regression $>50\%$ of signs or symptoms of disease with no organ dysfunction and no new lesions; a non-response (NR) was defined as regression $<50\%$ of signs or symptoms of disease with or without organ dysfunction and no new lesions; and progressive disease (PD) was defined as progressive signs or symptoms of disease and/or the appearance of new lesions.[8]

Statistical analyses

Continuous variables were compared using the Mann–Whitney *U*-test. The overall survival (OS) rate with standard error (s.e.) was estimated using the Kaplan–Meier method and compared using the log-rank test.[12] The OS was calculated for the period from the day of diagnosis until the day of death from any cause. The outcome data were updated in December 2008.

Results

Clinical course of 15 patients before SCT

Between November 1995 and March 2007, 15 children (9 males, 6 females) with LCH refractory to conventional chemotherapy underwent SCT at 13 institutions. The characteristics of all 15 patients are summarized in Table 1, and the details of each patient are shown in Table 2. The median age at diagnosis was 8 months (range, 28 days to 28 months). At initial diagnosis, 12 patients had MS type LCH, and nine had risk organ involvement, including the liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). One patient had diabetes insipidus (DI) and pituitary gland involvement at diagnosis. No CNS disorders other than DI were found in any of the patients. Two patients had SS-m type LCH at diagnosis, with multiple bone lesions. One patient had SS-s type LCH, with thymus involvement and respiratory dysfunction at initial diagnosis.

Eleven patients had received conventional chemotherapy according to the study protocol JLSG-96 ($n=4$) or JLSG-02 ($n=4$) of the Japan LCH study group,[8] and DAL-HX 83 ($n=3$) of the Deutsche Arbeitsgemeinschaft fur Leukaemieforschung Histiocytosis X-83 study group.[4] Remaining four patients had received multi-drug chemotherapy following their institutional protocol. Two patients received the combination of 2-CdA and high dose Ara-C before SCT as salvage therapy and failed to achieve complete remission (patients 6 and 8). Six weeks after initial diagnosis, the disease state was PD ($n=6$), NR ($n=2$), PR ($n=5$), and GR ($n=2$). Twelve or 14 weeks after chemotherapy, the disease state was PD ($n=6$), NR ($n=0$), PR ($n=6$), and GR ($n=3$). Eleven patients with MS type, two with SS-m type, and one with SS-s type at diagnosis had risk organ involvement before SCT.

The median age at SCT of 15 patients was 23 months (range, 13–178 months). At SCT, 14 patients had risk organ involvement. The affected organs before SCT were liver ($n=10$), hematopoietic system ($n=7$), and lung ($n=4$). Five of six patients in the low-risk group had risk organ involvement at SCT.

The disease status of six patients (patients 2–6 and patient 8) in the high-risk group was PD with risk organ involvement at SCT. The disease status of patient 2 was PD despite chemotherapy according to the JLSG-02 protocol, and liver dysfunction developed gradually. BM involvement was observed in patients 3–6, patient 8, patient 9, and patient 13 despite multi-drug chemotherapy. Patient 3, patient 6, and patient 8 suffered from serious infections before SCT. All six of these patients underwent SCT during 8–11 months after diagnosis. The disease status of the other three patients (patient 1, patient 7, and patient 9) was PR or NR with risk organ involvement at SCT. These three patients underwent SCT for 12–30 months after diagnosis.

Three of the six patients in the low-risk group (patient 10, patient 12 and patient 14) had suffered from recurrent active disease for 6, 13, and 7 years, respectively, despite chemotherapy consisting of vincristine, pirarubicin, prednisone, Ara-C, vinblastine, etoposide, and cyclophosphamide with or without cyclosporine. At recurrence, lung involvement and left phrenic nerve palsy were observed in patient 10, who required oxygen for 2 months. Patient 12 had recurrence of multiple bone lesions, including the ear, and needed a hearing aid. She relapsed twice with multiple

Table 1 Summary of the clinical characteristics of the LCH patients who underwent SCT

No. of patients	15
Age, median (range)	8 months (28 days to 28 months)
Sex, male/female	9 males, 6 females
Stage at diagnosis	
MS	12
SS-m	2
SS-s	1
Age at SCT, median (range)	23 months (13–178 months)
Allo-SCT/Auto-SCT	13 allo, 2 auto
Time from diagnosis to SCT	
Median (range)	12 months (7–164 months)
Observation time	
From SCT ($n=11$), median (range)	100 months (20–158 months)
From Dx ($n=11$), median (range)	110 months (27–277 months)

Abbreviations: allo = allogeneic; auto = autologous; Dx = diagnosis; SCT = stem cell transplantation.

Table 2 Detailed characteristics of the 15 patients with refractory LCH who underwent SCT

Patient No.	*Sex*	*Onset*				*At SCT*					*Outcome*[b]
		Age	*Involved organ*	*Type of LCH*	*Initial response*	*Age*	*Involved organ*	*Disease status*	*Donor source*	*Conditioning regimen*[a]	
1	M	3 months	LIV, SPL, LU, skin, bone, LN	MS	PR	23 months	LIV, skin, bone, LN	PR	CB (sibling)	RIC (Flu, PAM)	Alive in CR (+73 months)
2	M	5 months	LU, skin	MS	PD	13 months	LIV	PD	PB (mother)	RIC (Flu, PAM)	Died (18 days)
3	F	5 months	LIV, SPL, LN	MS	NR	14 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, ATG)	Alive in CR (+92 months)
4 (1st)	F	8 months	BM, LIV, skin, bone, LN, thymus	MS	PD	17 months	BM, LIV	PD	PB (father)	RIC (Flu, CY, TBI)	
4 (2nd)						19 months	BM, LIV, LU	PD	PB (father)	RIC (TBI)	Relapse and died (271 days)
5	M	8 months	BM, LIV, SPL, LU, skin, bone	MS	PD	17 months	BM, LIV	PD	UCB	Myeloab (TBI, VP16, PAM)	Alive in CR (+107 months)
6	M	8 months	LIV, SPL, skin, middle ear, LN	MS	NR	16 months	BM	PD	UCB	RIC (Flu, PAM, TBI)	Alive in CR (+20 months)
7	F	9 months	BM, LIV, SPL, LU, skin, bone, thymus, pancreas	MS	PD	21 months	LIV, SPL, skin, bone, thymus	NR	UCB	Myeloab (TBI, CY)	Relapse and died (47 days)
8	F	15 months	LU, skin, bone	MS	PR	27 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, VP16)	Died (188 days)
9	M	21 months	BM, pituitary, DI	MS	PR	51 months	BM, pituitary, DI, bone	NR	BM (sibling)	Myeloab (CY, VP16)	Alive in CR (+158 months)
10	M	28 days	Skin, LN, bone, mediastinal mass	MS	PD	83 months	LU	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+109 months)
11	F	6 months	Skin, intestine	MS	PD	16 months	LIV, LU	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+39 months)
12	F	13 months	LN, middle ear	MS	GR	178 months	Bone, pituitary, DI	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+113 months)
13	M	4 months	Bone	SS-m	PR	35 months	BM, LIV	PD	UCB	RIC (Flu, PAM, ALG, TLI)	Alive in CR (+38 months)
14	M	28 months	Bone	SS-m	GR	122 months	LIV	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+144 months)
15	M	9 months	Thymus	SS-s	PR	22 months	LU, skin, thymus, LN, gingiva	PR	UCB	Myeloab (TBI, CY, VP16)	Alive in CR (+110 months)

Abbreviations: ATG = antithymocyte globulin; auto = autologous; BM = bone marrow; CR = complete remission; CY = cyclophosphamide; DI = diabetes insipidus; F = female; LIV = liver; LN = lymph node; PAM = melphalan; LU = lung; M = male; MS = multisystem; myeloab = myeloablative; NR = non-response; PB = peripheral blood; PD = progressive disease; PR = partial response; RIC = reduced-intensity conditioning; SCT = stem cell transplantation; SPL = spleen; SS-m = single system multisite; SS-s = single system single site; TBI = total body irradiation; TLI = total lymphoid irradiation; UCB = unrelated cord blood; VP16 = etoposide.

Nine patients (patients 1–9) are classified in the low-risk group, and six (patients 10–15) are in the low-risk group.

[a]Dose of TBI/TLI was 10–12 Gy in the myeloablative regimen and 2 Gy in the reduced conditioning regimen.

[b]Values in parentheses indicate the duration from SCT to the final observation.

skull lesions and occurrence of DI, despite chemotherapy. Patient 14 developed systemic xanthogranuloma 3 years after diagnosis. He suffered from liver dysfunction, ascites, pleural effusion, fever, and pancytopenia before SCT. Among the remaining three patients, patient 13, who relapsed during maintenance therapy, became refractory to more intensive chemotherapy, and BM and CNS involvement occurred at SCT. Patient 11 suffered from diarrhea, bloody stool, and protein-losing gastroenteropathy at initial diagnosis. After 2 months, skin rash, hepatosplenomegaly, and disseminated intravascular coagulation were seen and she was diagnosed based on a rectal biopsy. She failed to achieve remission after JLSG Induction regimen A and B and received cisplatin according to the neuroblastoma regimen after disease activation. Patient 15 failed to achieve remission after the JLSG Induction regimen and received chemotherapy according to the non-Hodgkin lymphoma regimen. Patient 15, who had thymus involvement and respiratory dysfunction at diagnosis, obtained only a PR clinically and radiographically, and the skin, lung, gingiva, and palpebral conjunctiva became involved 6 months after diagnosis.

Donor source and conditioning regimen

The donor source and conditioning regimen for SCT are also summarized in Table 2. A myeloablative conditioning regimen was used in 10 patients; total body irradiation was used in five, while the other five received a non-total body irradiation regimen. Five patients received a reduced-intensity conditioning regimen (RIC), which consisted of fludarabine, melphalan, or cyclophosphamide, and low-dose total body irradiation/total lymph node irradiation and/or antithymocyte globulin. Nine patients underwent unrelated cord blood transplantation. One patient received cord blood from an HLA-matched sibling. Overall, 10 of 15 patients received CBT with a median of 1.4×10^6/kg $CD34^+$ cells (range, 0.19–7.5×10^6/kg) or a median of 0.91×10^8/kg nucleated cells (range, 0.86–1.4×10^8/kg). Peripheral blood (PB) from haploidentical parental donors was used in two patients, and autologous PB was used in two patients, with a median of 10.9×10^6/kg $CD34^+$ cells (range, 7.5–13.0×10^6/kg). One patient received BM from an HLA 2 loci-mismatched sibling, with 3.0×10^8/kg nucleated cells. Prophylaxis for graft-versus-host disease and graft rejection consisted primarily of methotrexate and either cyclosporine or tacrolimus.

Clinical course of 15 patients after SCT

The clinical course of 15 patients after SCT is listed in Table 2. Engraftment with $>500/\mu l$ absolute neutrophil count was seen in all patients except for one who died of multi-organ failure on day 18. Regimen-related toxicity was seen in six of 15 patients; mucositis of grade 1 to grade 4 according to the common terminology criteria for adverse events[13] was the most common, and three patients had liver dysfunction of grade 2 or grade 3. One patient had thrombotic microangiopathy, which resolved without long-term sequelae. Four patients had various infections, such as sepsis, herpes simplex virus, and cytomegalovirus. Three patients who underwent unrelated cord blood transplantation had acute graft-versus-host disease (grades I, III, IV). One of them and another patient developed chronic graft-versus-host disease extensive type.

After SCT, two patients never entered remission and died on day 18 and day 188, respectively (patient 2 and patient 8). Patient 7 relapsed on day 20 and died on day 47 after SCT, due to sepsis, veno-occlusive disease, and gastrointestinal bleeding. Patient 4 underwent a second SCT on day 49 after the first SCT because of graft failure. She relapsed on day 194 and died on day 271 after the second SCT due to liver dysfunction, pancytopenia, and sepsis. Two patients (patient 10 and patient 12) relapsed at 8 months and 4 months after auto-PBSCT, respectively. Patient 10 had recurrence involving the cervical spine and received prednisone, vinblastine, and cyclosporine for 4 years. Patient 12 had recurrence of multiple bone lesions, including the femur, skull, and scapula in turn, and was treated with radiation therapy to control the bone lesions for 2 years. Patient 14 had macrophage activating syndrome after SCT, and TNF-α blocker and dexamethasone palmitate were administered for several months. Finally, 11 of 15 patients remain alive with no evidence of disease. The 10-year OS rate (median $\pm$ s.e.) in these patients was $73.3 \pm 11.4\%$ (Figure 1). The 10-year OS rate of nine patients who had risk organ involvement at diagnosis was $55.6 \pm 16.6\%$, whereas six patients who had no risk organ involvement at diagnosis have all survived with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$), because of the small number of patients.

Late toxicities associated with SCT included short stature with body height <-2.0 s.d. in five patients, whereas DI and hearing disturbance were seen in two patients each. One patient, who had a hip fracture and hearing disturbance, was evaluated as being intellectually retarded. Another patient, who suffered from a CNS lesion before SCT, had speech delay. Except for one infant who was too young to evaluate, the Karnofsky score[14] of the remaining eight survivors was 100%.

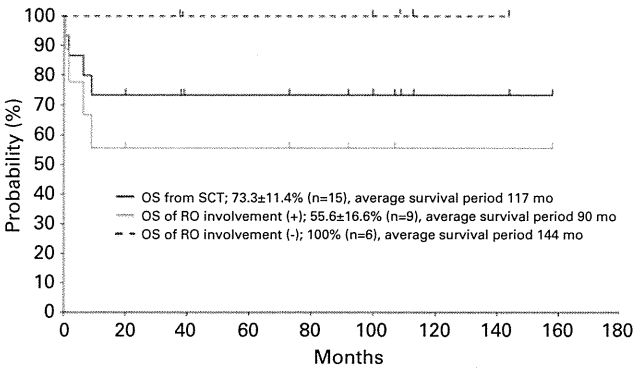

Figure 1 Overall survival (OS) of patients with LCH who underwent SCT. The 10-year OS rate of the 15 patients with refractory LCH who underwent SCT was $73.3 \pm 11.4\%$. The 10-year OS rate of the nine patients who had risk organ involvement at diagnosis was $55.6 \pm 16.6\%$, whereas all six patients who had no risk organ involvement at diagnosis remain alive with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$). RO, risk organ.