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Original Article

Pirarubicin-based chemotherapy displayed better clinical outcomes and lower toxicity than did doxorubicin-based chemotherapy in the treatment of non-metastatic extremity osteosarcoma

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Abstract: Pirarubicin (THP) is a newer generation anthracycline anticancer drug with antineoplastic efficacy against numerous tumors. Few studies have reported its application and efficiency in anti-osteosarcoma chemotherapeutic strategies. Ninety-six non-metastatic extremity osteosarcoma patients treated with THP or doxorubicin (DOX) in combination with high-dose methotrexate (HDMTX), cisplatin (DDP) and ifosfamide (IFO) within the past 9 years at our hospital were evaluated retrospectively to compare efficacy and side effects. Among the patients, 55.2% were male, 36.5% were ≤14 years old and 59.4% presented with a large tumor (≥1/3 of bone) to our department. The 5-year disease-free survival (DFS) rate of the patients treated with the THP-based chemotherapeutic regimen was 70.2%, significantly higher than that of the DOX-based regimen-treated group (53.1%). The THP-based chemotherapeutic regimen decreased the lung metastatic rate significantly compared with the DOX-based regimen (19.1% vs. 36.7%, *P*=0.045), as well as the relapse rate (31.9% vs. 49.0%, *P*=0.067). Both regimens were generally well tolerated. However, while the THP-based chemotherapeutic regimen did not alter toxicity in the hematologic system, liver or kidneys compared with the DOX-based regimen, it showed lower rates of alopecia (63.8% vs. 85.7%, *P*=0.012), nausea and vomiting (51.1% vs. 79.6%, *P*=0.003), and mucositis (48.9% vs. 75.6%, *P*=0.003). THP also resulted in lower cardiac toxicity. Our data demonstrate that the THP-based regimen is better than the DOX-based regimen in terms of the 5-year DFS rate, pulmonary metastasis rate, relapse rate and side effects.

Keywords: Osteosarcoma, chemotherapy, pirarubicin, doxorubicin, relapse, side effects, disease-free survival, overall survival.

Introduction

Osteosarcoma defines neoplasms that share the histological properties of osteoid production in association with malignant mesenchymal cells [1]. It most often occurs in the long bones of the extremities near the metaphyseal growth plates and has been reported as the third most common cancer in adolescence [1, 2]. Because of its early onset, propensity to metastasize and common relapse, patients with osteosarcoma had a survival rate of less than 20% in the past [2]. Thankfully, chemotherapy regimens pioneered in the early 1980s markedly improved survival rates to approximately 50-70% [2-6]. After exploring and improving new anti-osteosarcoma strategies for decades, the current management of osteosarcoma involves a consensus protocol comprising neoadjuvant chemotherapy, followed by surgical removal of all detectable disease and adjuvant chemotherapy [1, 2]. In the past two decades, high-dose methotrexate (HDMTX), doxorubicin (DOX), cisplatin (DDP) and ifosfamide (IFO) have been the most effective agents in the treatment of osteosarcoma and, thus, form the pharmaceutic backbone of the neoadjuvant-adjuvant combination chemotherapy regimens for osteosarcoma [1-3, 7]. Although great progress has been made, relapse still occurs in

30-50% of patients with localized tumors, most of whom will die despite further surgical and chemotherapeutic treatments [8-10]. Studies have explored new strategies to improve the antitumor efficacy of combination regimens [11-13]. These strategies include the addition of new agents such as muramyl tripeptide [11] and increases in dose intensities [12, 13]. However, there have been no major improvements.

DOX ($C_{27}H_{29}NO_{11}$) is one of the key drugs in HDMTX-DDP-DOX-IFO combination regimens [1]. By itself, DOX has elicited response rates of up to 40% in osteosarcoma patients [8]. Nevertheless, acute and chronic cardiotoxicities, such as arrhythmia and congestive heart failure (CHF), limit DOX administration [14]. Recently, a phase II study showed the combination of DDP, IFO and epirubicin (another anthracycline anticancer agent) as an active regimen with mild cardiac toxicity in patients with non-metastatic extremity osteosarcoma [15]. However, the 5-year disease-free survival (DFS) and overall survival (OS) rates were relatively low (41.9% and 48.2%, respectively) [15]. The most prominent grade 3-4 toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) was neutropenia, which occurred in 71% of patients, followed by nausea and vomiting (50%) and mucositis (11%) [15]. Those toxicities, together with multidrug resistance (MDR), are the major barriers to long-term remission [15]. Thus, finding new agents that overcome MDR and decrease toxicities has been pursued actively.

Pirarubicin (THP, $C_{32}H_{37}NO_{12}$), a semisynthetic derivative of DOX, is a newer generation anthracycline anticancer drug [16]. The change in chemical structure allows THP to be absorbed by tumor cells approximately 170-fold faster than DOX and expedites its distribution to the cell nucleus and incorporation into DNA [17-19]. *In vitro* studies showed that THP causes G0/G1 cell cycle arrest and induces apoptosis in human osteosarcoma MG63 cells [20]. THP has also shown favorable activity in various MDR cancer cells, including P-glycoprotein (P-gp)-overexpressing breast cancer cells [21], DOX-resistant lymphoblastoma cells [22] and K562/A02 cells [23]. Our previous *in vitro* study showed that THP partially overcame DOX resistance caused by P-gp overexpression and inhibited MDR osteosarcoma cell line proliferation through induction of G2/M cell cycle arrest [24]. Clinically, THP and THP-based combination chemotherapies have been shown to be effective against various tumors without severe side effects [25-28].

There is little evidence for the superior effects of THP or THP-based chemotherapies on osteosarcoma. So far, only one retrospective study using a small sample size (30 patients) reported the superiority of a THP-based regimen over a DOX-based regimen in terms of 2-year OS rates in patients with non-metastatic osteosarcoma [25]. Our clinical studies showed that THP-based chemotherapy regimens were effective and safe as salvage chemotherapy options for refractory or recurrent osteosarcoma patients who had previously received adjuvant/neoadjuvant chemotherapy with HDMTX-DDP-DOX-IFO [29, 30]. This encouraging evidence and our over 10 years of clinical experience with THP application in osteosarcoma patients prompted us to compare the efficacy and safety between the HDMTX-DDP-THP-IFO and conventional HDMTX-DDP-DOX-IFO regimens in patients with high-grade, non-metastatic extremity osteosarcoma. In this report, 9 years of data on the clinical outcomes and side effects of THP- and DOX-based chemotherapies were evaluated retrospectively.

Materials and methods

Ethical issues

This study was conducted according to the principles of the World Medical Association Declaration of Helsinki. The study was approved specifically by the Internal Review Board of the Affiliated Sixth People's Hospital, Shanghai Jiaotong University (permission number SH6H-005). All participants provided written informed consent for this retrospective study We also obtained written informed consent from parents on behalf of the minors enrolled in our study. The ethics committee approved the consent procedure specifically for participants less than 18 years of age (permission number SH6H-005m).

Patient selection and evaluation

All of the osteosarcoma patients included in the study met the following criteria: age ≤40

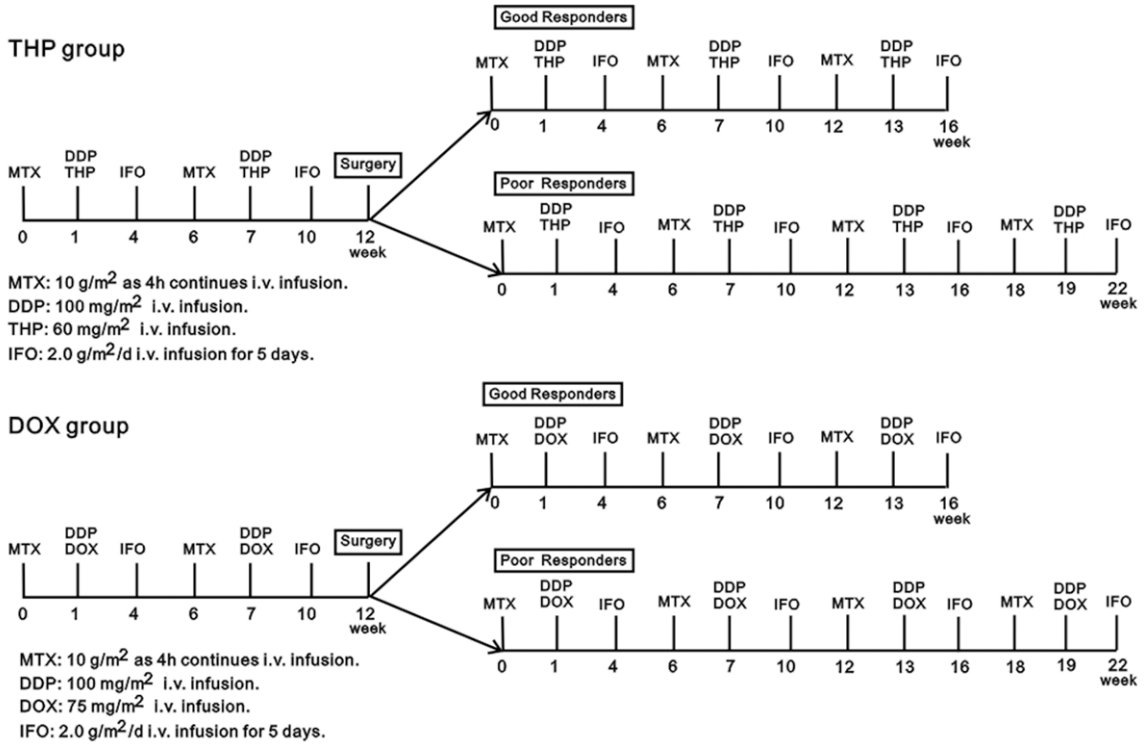

Figure 1. Schematic illustration of the THP- and DOX-based protocols. The cumulative dosages of the chemotherapeutic drugs, each chemotherapy cycle of the main regimens, the week the cycle started and the time of surgery are indicated.

years; histologically proven, high-grade, localized extremity, central osteosarcoma; no previous history of cancer or prior treatments; no coexisting disease contraindicating chemotherapy; white blood cell count >3.5×10^9/L and platelets >100×10^9/L; normal liver and kidney functions; normal cardiac function with a resting left ventricular ejection fraction (LVEF) >50%; and Eastern Cooperative Oncology Group (ECOG) performance score of 0-1. The exclusion criteria included unwillingness to participate in the study and cessation of therapy.

The diagnosis of osteosarcoma was established by clinical and X-ray findings and confirmed on histologic slides of the tumor tissue biopsy. After the diagnosis of high-grade central osteosarcoma, tumors were classified retrospectively into different subtypes according to the World Health Organization criteria [31]. Data on the patients' clinical characteristics, such as age, gender, pathologic subtypes, location of primary tumor, tumor size, pathologic fracture, serum value of alkaline phosphatase and performance status, were collected.

Before primary chemotherapy, all patients underwent the following evaluation processes: a complete medical history questionnaire; thorough physical examination; and baseline imaging studies, including plain X-rays, a computed tomography (CT) scan and magnetic resonance imaging (MRI) of the primary tumor. Whole-body bone scintigraphy and chest CT scans were used to exclude bone and lung metastases. These examinations were repeated before surgery.

During chemotherapy, patients were monitored twice weekly for complete blood cell counts, hepatic and renal function tests and electrolytes (when HDMTX was administered) until recovery from toxicity. Physical examinations, complete blood cell counts, renal and hepatic function tests and electrocardiography (ECG) were repeated before each cycle of chemotherapy. X-rays of the primary site, chest CT scans, abdominal ultrasonography and echocardiography were repeated every 6 weeks. Holter monitoring was performed in cases of suspected cardiac problems after ECG or clinical manifes-

Table 1. Patient characteristics at the start of systemic therapy

Characteristics	Total (n=96)	THP group (n=47)	DOX group (n=49)	*P* value
Sex				
Male	53 (55.2%)	25 (53.2%)	28 (57.1%)	*0.427*
Age at study entry (years)				
≤14	35 (36.5%)	16 (34.0%)	19 (38.8%)	*0.394*
Primary tumor site				
Distal femur	44 (45.8%)	21 (44.7%)	23 (46.9%)	*0.493*
Proximal tibia	23 (24.0%)	11 (23.4%)	12 (24.5%)	*0.546*
Proximal humerus	10 (10.4%)	6 (12.8%)	4 (8.2%)	*0.344*
Proximal fibula	8 (8.3%)	4 (8.5%)	4 (8.2%)	*0.619*
Other bones	11 (11.5%)	5 (10.6%)	6 (12.2%)	*0.530*
Histology				
Osteoblastic	50 (52.1%)	24 (51.1%)	26 (53.1%)	*0.503*
Chondroblastic	14 (14.6%)	7 (14.9%)	7 (14.3%)	*0.580*
Fibroblastic	9 (9.4%)	4 (8.5%)	5 (10.2%)	*0.527*
Telangiectatic	6 (6.3%)	3 (6.4%)	3 (6.1%)	*0.641*
Impossible to specify	11 (11.5%)	5 (10.6%)	6 (12.2%)	*0.530*
Small cell	6 (6.3%)	4 (8.5%)	2 (4.1%)	*0.319*
Pathologic fracture				
Yes	9 (9.4%)	4 (8.5%)	5 (10.2%)	*0.527*
Alkaline phosphatase				
Elevated	55 (57.3%)	26 (55.3%)	29 (59.2%)	*0.430*
Tumor size				
Large (≥1/3 of bone)	57 (59.4%)	27 (57.4%)	30 (61.2%)	
ECOG score				
0	60 (62.5%)	32 (68.1%)	28 (57.1%)	*0.185*

Categorical data are presented as numbers (percentages). Differences in baseline characteristics were analyzed by the χ^2 test. ECOG, Eastern Cooperative Oncology Group; THP group, patients treated with the THP-based therapeutic regimen; DOX group, patients treated with the DOX-based therapeutic regimen.

tation. Bone scintigraphy was repeated every 6 months. Patients with suspected local relapse were first evaluated using X-rays and a CT scan or MRI to determine the relapse location and then underwent biopsy and/or surgery.

After chemotherapy, routine clinical follow-up was conducted. It included physical examinations, complete blood cell counts, renal and hepatic function tests, X-rays of the primary site, chest CT scans, abdominal ultrasonography and ECG every 2 months for the first 2 years, every 3 months in the third year, every 4 months in the fourth year, every 6 months in the fifth year and yearly thereafter.

Treatment schedules and regimens

Pre- and postoperative chemotherapy was performed mainly based on the Italian treatment protocol IOR-OS/N-5 with dose modifications [32].

As shown in **Figure 1**, prior to surgery, patients received two cycles of high-dose HDMTX, DDP/DOX (DOX-based group) or DDP/THP (THP-based group) and IFO. HDMTX was administered via a 4-h infusion at a dose of 10 g/m^2. Calcium folinate rescue (15 mg, intravenously every 6 h, 11 times) was started 24 h after the beginning of HDMTX (the exact time of rescue was based on the concentration of HDMTX in the blood). DOX was administered at a dose of 75 mg/m^2 (DOX-based group) and THP at a dose of 60 mg/m^2 (THP-based group) as a 30-min infusion; DDP was administered at a dose of 100 mg/m^2 (2-h infusion), with pre- and postoperative hydration and mannitol diuresis. IFO was administered at a dose of 2.0 g/m^2/day (4-h infusion) with an equivalent dose of mesna for 5 consecutive days.

Postoperatively, good histological responders received three cycles of the same preoperative drugs, and poor histological responders received four cycles. Postoperative chemotherapy was started when the orthopedic surgeon determined that wound healing was adequate for initiation of chemotherapy. Generally, chemotherapy was restarted 5-7 days after surgery in amputees and 10-20 days in patients treated with limb salvage. 5-hydroxytryptamine 3 (5-HT3) receptor blockers, such as ondansetron and dexamethasone, were used as anti-emetic drugs.

Surgery was performed approximately 2-3 weeks after the end of preoperative chemotherapy. The type of surgery (amputation or limb salvage) was selected depending on the location and extent of the tumor and the patient's age, desired lifestyle and preferences. However, it is necessary to perform conserva-

Table 2. Integrity of first-line chemotherapeutic regimens and cumulative doses

Integrity and cumulative doses	THP group (n=47)	DOX group (n=49)	*P* value
Integrity of chemotherapeutic regimens			
Patients receiving a reduced dose	4 (8.5%)	4 (8.2%)	*0.62*
Patients transferred to second-line chemotherapy	0 (0%)	2 (4.1%)	*0.258*
Cumulative doses			
HDMTX (g/m^2)	52.3 ± 4.5	52.2 ± 5.0	*0.957*
DDP (mg/m^2)	527.7 ± 49.8	524.5 ± 52.2	*0.762*
IFO (g/m^2)	52.8 ± 5.0	52.2 ± 5.9	*0.641*
THP or DOX (mg/m^2)	313.2 ± 27.9	390.6 ± 37.2	*<0.001*

Continuous variables are presented as means ± standard deviations (SD), and categorical data are presented as numbers (percentages). Differences between the two groups were analyzed by χ^2 test or independent-samples t-test according to the data distribution. SD, standard deviation, HDMTX, high-dose methotrexate; DDP, cisplatin; IFO, ifosfamide; THP, pirarubicin; DOX, doxorubicin.

tive surgery when preoperative staging indicates the possibility of achieving wide surgical margins. After surgery, the surgeon and pathologists reviewed the gross specimen to evaluate the surgical margins. Radical and wide margins were considered adequate. Marginal, intralesional or contaminated margins were considered inadequate. Post-relapse treatments included surgery and salvage chemotherapy with gemcitabine and docetaxel.

Assessment of efficacy and safety

Histological response to neoadjuvant chemotherapy was assessed by estimating the percentage of necrosis in the resected specimen [33]. A good histological response was defined as ≥90% necrosis in the resected specimen and a poor histological response as <90% necrosis. DFS was defined as the period from the start of chemotherapy to the time of recurrence at any site. OS was defined as the period from the start of chemotherapy to the date of the last follow-up or death of any cause.

Maximum toxicity was evaluated for each cycle of chemotherapy according to NCI-CTC version 3.0 [34]. The following toxicities were recorded: hematological toxicities (leucopenia, anemia and thrombocytopenia), gastrointestinal toxicities (nausea and vomiting), alopecia, mucositis, liver toxicity, renal toxicity, cardiac toxicity (arrhythmia, myocardial ischemia and heart failure) and infection.

Statistics

Continuous variables are presented as the means ± standard deviations or as medians and interquartile ranges. Categorical data are presented as numbers and percentages. Differences in baseline characteristics were analyzed by student's t-test, one-way ANOVA, or χ^2 test according to the distribution of the data. Survival curves were calculated according to the Kaplan-Meier method and compared using the log-rank test. A one-tailed *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS ver. 17.0.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics at the start of systemic treatments

From December 2005 to July 2008, 47 and 49 patients with newly diagnosed non-metastatic osteosarcoma of the extremities received and completed THP-based and DOX-based regimens as the first-line treatment, respectively.

As summarized in **Table 1**, of the 96 osteosarcoma patients, 55.2% (53 patients) were male and 36.5% were younger than 14 years of age. Primary tumor sites (in the order of frequency) included distal femur, proximal tibia, proximal humerus and proximal fibula; the pathologic classification (in the order of frequency) was osteoblastic, chondroblastic, fibroblastic and telangiectatic. The proportion of patients with large primary osteosarcoma was 59.4%, and 62.5% of patients had an ECOG score of 0. No detectable metastatic focus was found in patients at the start of systemic therapy. Patients treated with the two chemotherapeutic regimens were well balanced in terms of

Table 3. Comparison of curative effects, metastasis and recurrence

Factors	THP group (n=47)	DOX group (n=49)	*P* value
Follow-up (months)	63.7 ± 16.9	55.1 ± 19.5	*0.342*
Histotype response			
Good	29 (61.7%)	29 (59.2%)	*0.482*
Poor	18 (38.3%)	20 (40.8%)	
Surgery			
Limb salvage	35 (74.4%)	33 (67.3%)	*0.294*
Amputation	12 (25.6%)	16 (32.7%)	
Surgical margins			
Adequate	44 (93.6%)	44 (89.8%)	*0.381*
Inadequate	3 (6.4%)	5 (10.2%)	
5-year DFS	33 (70.2%)	26 (53.1%)	*0.064*
5-year OS	37 (78.7%)	30 (61.2%)	*0.049*
Time to relapse (months)	26.9 ± 15.1	23.0 ± 15.4	*0.453*
Number of relapses	14 (29.8%)	23 (46.9%)	*0.064*
All lung metastases (lung, lung + bone or lung + local)	11 (23.4%)	20 (40.8%)	*0.053*
Isolated lung metastases	9 (19.1%)	18 (36.7%)	*0.045*
All bone metastases (bone, bone + lung, bone + local)	2 (4.3%)	3 (6.1%)	*0.520*
Isolated bone metastases	1 (2.1%)	1 (2.0%)	*0.742*
All local recurrence (local, local + bone, local + lung)	2 (4.3%)	3 (6.1%)	*0.520*
Isolated local recurrence	1 (2.1%)	1 (2.0%)	*0.742*
Isolated other site	1 (2.1%)	0 (0%)	*0.490*
Post-relapse outcome			
DFS after relapse	1 (7.1%)	2 (8.7%)	*0.683*
Alive with disease	3 (21.4%)	2 (8.7%)	*0.269*
Death	11 (78.6%)	20 (87.0%)	*0.407*
Time to death (months)	16.0 ± 4.0	17.0 ± 5.0	0.850

Continuous variables are presented as means ± standard deviations (SD), and categorical data are presented as numbers (percentages). Differences between the two groups were analyzed by Fisher's exact test, χ^2 test or independent-samples t-test according to the data distribution. DFS, disease-free survival; OS, overall survival.

gender, age, tumor site, histologic subtype, pathologic fracture, tumor size and serum alkaline phosphatase level at the start of systemic therapy (**Table 1**).

Therapeutic strategies and integrity

The therapeutic strategies are illustrated in **Figure 1**. Both the THP- and DOX-based chemotherapeutic regimens included two cycles of neoadjuvant chemotherapy before surgery; good responders and poor responders were treated again with three and four cycles of chemotherapy after surgery, respectively.

Four patients each in the THP and DOX groups were reduced in their drug doses due to drug-related toxicities, and the rate of reduction for each drug was no more than 20% of the dose indicated in **Figure 1** (**Table 2**). Modification of the therapeutic cycle and dosages was strictly dependent on hematologic toxicity, liver function, kidney function and pathological examination guided by relevant international organizations [34]. Two patients in the DOX group were transferred to second-line chemotherapy due to early metastasis or recurrence during first-line chemotherapy (**Table 2**).

The cumulative doses of each drug in the THP- and DOX-based chemotherapy regimens are also summarized in **Table 2**. There was no significant difference in the cumulative doses of HDMTX, DDP or IFO between the two groups. The cumulative doses of THP and DOX were 313.2 ± 27.9 and 390.6 ± 37.2 mg/m^2, respectively. The dose of THP was significantly lower than that of DOX (**Table 2**).

All patients who completed the treatment indicated in **Figure 1** were given second-line chemotherapy if they met the clinical criteria. The second-line chemotherapy consisted of mainly docetaxel and gemcitabine.

Increased 5-year DFS and OS rates with the THP-based chemotherapeutic regimen

To compare the efficacies of THP-based and DOX-based chemotherapeutic regimens, the outcomes of all patients were followed up. The mean follow-up durations were 63.7 ± 16.9 and 55.1 ± 19.5 months for the THP and DOX groups, respectively (**Table 3**). The rates of

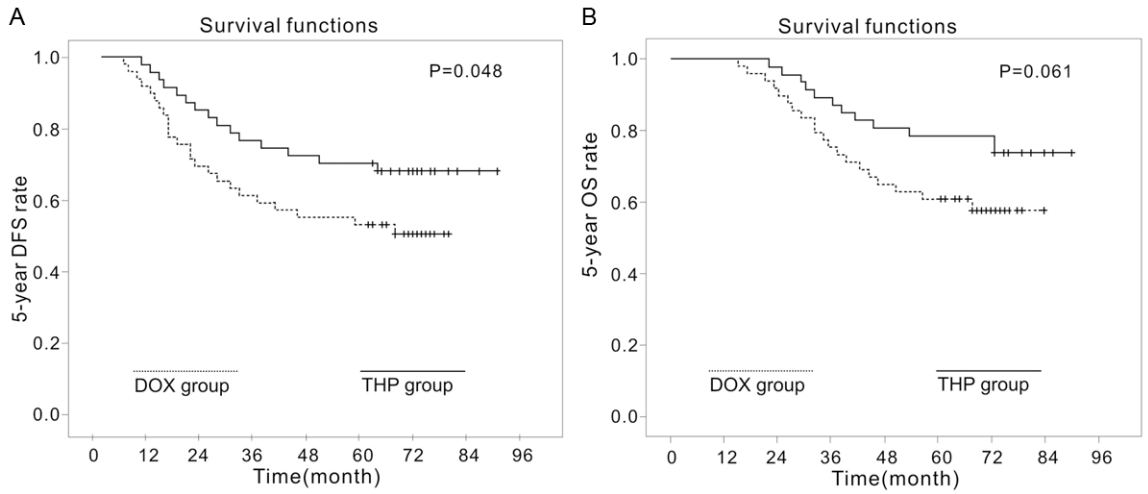

Figure 2. Survival curves for the THP- and DOX-based chemotherapeutic regimens. The survival curves were calculated according to the Kaplan-Meier method and were compared using the log-rank test. Crosses indicate the regimen endpoints for each patient. A. Comparisons of disease-free survival (DFS) between the groups. B. Comparisons of overall survival (OS) between the groups.

good histotype response in the THP and DOX groups were 61.7% and 59.2%, respectively (**Table 3**). The rates of limb salvage in the THP and DOX groups were 74.4% and 67.3%, respectively (**Table 3**). There were no significant differences in time to relapse and post-relapse outcomes between the two groups (**Table 3**). Interestingly, the rate of relapse tended to be lower in the THP group (31.9%) than the DOX group (49.0%). The 5-year DFS rate tended to be higher in the THP group (70.2%) than the DOX group (53.1%) (**Table 3**). The 5-year OS rate was significantly higher in the THP group (78.7%) than in the DOX group (61.2%) (**Table 3**). When survival curves were created according to the Kaplan-Meier method and compared using the log-rank test, the THP group displayed a significantly more favorable DFS rate during the follow-up period compared with the DOX group (**Figure 2A**). The OS rate was also higher in the THP group than in the DOX group, although this difference was not statistically significant (**Figure 2B**).

Decreased rates of relapse and pulmonary metastasis with the THP-based chemotherapeutic regimen

Relapse occurred in 14 patients (29.8%) in the THP group and in 23 patients (46.9%) in the DOX group (**Tables 3** and **4**). The relapse rate tended to be lower in the THP group (P=0.064). The mean time to relapse was 26.9 ± 15.1 months in the THP group and 23.0 ± 15.4 months in the DOX group; there was no significant difference between the two groups (**Table 3**). The main sites of metastasis were the lungs and bone. There were no significant differences in the rates of all bone metastases, isolated bone metastases, all local relapse or isolated local relapse between the THP and DOX groups (**Table 3**). It is noteworthy that the overall lung metastatic rate tended to be lower in the THP group (23.4%) than in the DOX group (40.8%) (**Table 3**). The rate of isolated lung metastases was significantly lower in the THP group (19.1%) than in the DOX group (36.7%) (**Table 3**).

Reduced side effects with the THP-based chemotherapeutic regimen

The chemotherapeutic treatment of osteosarcoma is associated with important short- and long-term toxic effects [35]. To compare differences in side effects between the THP-based and DOX-based chemotherapeutic regimens, hematological toxicities, hepatic and renal function, cardiac toxicities, mucositis, infection, alopecia, and nausea and vomiting were monitored during treatment. Both chemotherapy regimens displayed toxicities to the hematologic system, liver, kidneys, heart and mucosa (**Table 4**). However, these side effects were well tolerated in general. No one died of chemotherapy-related toxicity or unrelated causes or developed a second tumor during the follow-up period.

Table 4. Toxicities caused by the two regimens

Toxicity and grade	THP group (n=47)	DOX group (n=49)	*P* value
Hematological toxicities			
Leucopenia			
All	42 (89.4%)	46 (93.9%)	*0.334*
3	12 (25.5%)	16 (32.7%)	*0.294*
4	5 (10.6%)	7 (14.3%)	*0.410*
Anemia			
All	25 (53.2%)	29 (59.2%)	*0.350*
3	3 (6.4%)	5 (10.2%)	*0.381*
Thrombocytopenia			
All	22 (46.8%)	26 (53.1%)	*0.341*
3	2 (4.2%)	3 (6.1%)	*0.520*
Nausea and vomiting			
All	24 (51.1%)	39 (79.6%)	*0.003*
3-4	9 (19.1%)	20 (40.8%)	*0.018*
Hepatic dysfunction			
All	24 (51.1%)	27 (55.1%)	*0.424*
3-4	9 (19.1%)	10 (20.4%)	*0.541*
Renal dysfunction			
1-2	3 (6.4%)	4 (8.2%)	*0.524*
Alopecia			
1-2	30 (63.8%)	42 (85.7%)	*0.012*
Mucositis			
All	23 (48.9%)	38 (75.6%)	*0.003*
3-4	1 (2.1%)	5 (10.2%)	*0.112*
Cardiac toxicities			
Arrhythmia			
1-2	3 (6.4%)	9 (18.4%)	*0.070*
Heart failure			
1-2	0 (0%)	2 (4.1%)	*0.258*
Myocardial ischemia			
1-2	0 (0%)	3 (6.1%)	*0.129*
Infection			
1-3	2 (4.2%)	4 (8.2%)	*0.359*

Categorical data are presented as numbers (percentages). Differences were analyzed by Fisher's exact test or χ^2 test according to the data distribution.

The risk of cardiac toxicity with DOX is related to both the dose intensity and total cumulative dose [1]. In our study, no grade 3 or 4 toxicities were observed. The rate of chemotherapy-related arrhythmia tended to be lower in the THP group (6.4%) than in the DOX group (18.4%) (**Table 4**). Other toxicities are summarized in **Table 4**, no significant differences between the two groups were observed for hematological toxicities (including the rates of leucopenia, anemia and thrombocytopenia), hepatic and renal dysfunctions, cardiac toxicities and infection, while the rates of nausea and vomiting, alopecia and mucositis were 51.1%, 63.8% and 48.9%, respectively, in the THP group, they were significantly lower than the corresponding rates (79.6%, 85.7% and 75.6%, respectively) in the DOX-treated group.

Discussion

In the present study, we evaluated the efficacy and toxicity of a THP-based combination regimen and compared it with a conventional DOX-based regimen in patients with non-metastatic osteosarcoma. Our data indicate that the THP-based regimen had better efficacy compared with the DOX-based regimen in terms of 5-year DFS and OS, which were evaluated by two statistical approaches. When 5-year DFS and OS rates were analyzed only by the total numbers of individuals who met the 5-year DFS or OS criterion, ignoring the time factor, the 5-year OS rate was significantly higher and the 5-year DFS rate tended to be higher in the THP group compared with the DOX group (**Table 3**). When survival curves were created according to the Kaplan-Meier method and compared using the log-rank test, the THP group displayed a significantly more favorable DFS rate (70.2%) compared with the DOX group (53.1%) (**Figure 2**). Our main focus in this study was the efficiency of THP in combination with other first-line drugs in complete first-round therapeutic strategies. The 5-year DFS rate derived from our clinical experience is encouraging and noteworthy. In fact, clinical experiences revealed that first-round 5-year DFS is critical for osteosarcoma patients [36]. Our data support this view. The number of patients reaching 5-year OS increased only by four in both the THP and DOX groups, which suggests that second-line chemotherapeutic regimens and other treatments could not improve the survival rate significantly (**Table 3**). Although the 70.2% 5-year DFS rate did not exceed the best-reported rates, 59.4% of the patients evaluated who visited our hospital had large tumor sizes (≥1/3 of bone), suggesting that a delay in proper treatment in China might impact the 5-year DFS rate.

The evaluation of metastatic sites showed that the THP-based chemotherapeutic regimen decreased the lung metastasis rate significant-

ly (**Table 3**). Approximately 90% of relapses are lung metastases, which usually occur during the first 2-3 years and are a cause of death [37]. Thus, reduced pulmonary metastasis after THP treatment is a finding of value. The rate of relapse tended to be lower in the THP group than the DOX group (**Table 3**). Although the difference was not statistically significant, we speculate that in a sufficiently large study population, THP treatment would result in a decrease in the relapse rate.

Although the THP-based chemotherapeutic regimen did not alter toxicities in the hematologic system, liver or kidneys, there were few high-grade toxicities. THP caused significantly lower rates of nausea and vomiting, alopecia and mucositis. This is extremely important for quality of life and psychological health in patients. Meanwhile, the rate of chemotherapy-related arrhythmia tended to be lower in the THP group than the DOX group.

Leukopenia is an acute toxicity caused by both THP and DOX [1, 38-40]. Leukopenia has been reported after treatment with THP at a dose of 50 mg/m^2, which is comparable to 60 mg/m^2 DOX. A clear dose-toxicity relationship exists for THP at doses ranging from 45 to 75 mg/m^2 [38]. Niitsu *et al.* showed that the THP-COPBLM regimen (THP in combination with cyclophosphamide, vincristine, prednisone, bleomycin and procarbazine) was highly effective and suggested that THP could be used at a dose of 50-100 mg/m^2 in the treatment of elderly patients with non-Hodgkin lymphoma [39, 40]. Furthermore, our earlier analysis showed that 50 mg/m^2 THP in combination with 100 mg/m^2 DDP infusion was effective and safe in patients with recurrent osteosarcoma who had received intensive adjuvant chemotherapy previously [29]. It was anticipated that untreated patients would have better bone marrow reserve function. Therefore, in this study, THP was given at a dose of 60 mg/m^2, although we did not demonstrate a clear dose-activity relationship between low and high doses of THP. In our study, hematological toxicities including leukopenia, anemia and thrombocytopenia were similar in both groups.

Raber *et al.* suggested that a lower concentration of THP in cardiac tissue might explain the lower level of cardiotoxicity [41]. In the current study, fewer patients tended to suffer from arrhythmia with the THP-based regimen, and no patients developed myocardial ischemia or heart failure. Although the cumulative doses of THP administered in the THP group were lower than those of DOX in the DOX group, studies performed by other groups using identical doses of THP and DOX suggest that THP causes less cardiotoxicity than does DOX in patients with various tumors [38, 40, 42]. DDP/DOX combination chemotherapy is highly emetogenic. Interestingly, a lower incidence of grade 3-4 nausea and vomiting (19.1 vs. 40.8%; P=0.018) was observed in the THP group. In addition, incidences of grade 1-2 alopecia (63.8 vs. 85.7%; P=0.012) and mucositis (48.9% vs. 75.6%; P=0.003) were also lower in the THP group. In agreement with our data, similar superiority of THP over DOX was observed in other cancer treatments [25, 38, 42].

In this study, we did not compare the efficacies of the two chemotherapeutic regimens against histologic subtype osteosarcomas, because our sample size failed to meet the required power in a statistical analysis. Few studies have compared the outcomes of THP-based and DOX-based regimens in the treatment of osteosarcoma. In a 2-year follow-up study, Shinozaki *et al.* enrolled 19 patients receiving THP-based regimens and 11 patients receiving DOX-based regimens and found that the survival of osteosarcoma patients was significantly better with THP than with DOX [25]. However, the patient sample was small and the follow-up period too short. In addition, the treatment regimens and criteria were not identical between the two groups.

According to our [24, 29, 30] and other authors' results [21-23] reported previously, the activity of the THP-based regimen in this study may be associated partly with overcoming MDR in osteosarcoma cells. Moreover, it was revealed previously that the THP level in the lung was 100-fold higher than that in the plasma after intravenous administration, which may partly explain the reduction in lung metastasis and the corresponding improvement in 5-year survival [43]. As the precise mechanism of the THP-based regimen has yet to be elucidated, we cannot exclude the possibility that synergistic effects among combination agents may play some role.

In conclusion, our data suggest that the THP-based combination regimen yielded better clinical outcomes, a lower lung metastatic rate and

a lower incidence of toxic events. These results should be valued in clinical practice. Further evaluation of the optimal dosage and strategies to reduce side effects are expected.

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Disclosure of conflict of interest

The authors have declared that no competing interests exist.

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