Pegylated liposomal doxorubicin for myeloid neoplasms

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Pegylated liposomal doxorubicin (Peg-Dox) treatment resulted in a good outcome for patients with lymphoma and multiple myeloma, with reduced cardiotoxicity and an improved pharmacokinetic profile when compared to those of conventional doxorubicin. However, the use of Peg-Dox in myeloid neoplasms remains poorly studied. In this study, we first tested the role of Peg-Dox in the killing of myeloid cell lines and of primary myeloid leukemia cells. Then, a Peg-Dox-based protocol was used to treat patients with myeloid neoplasms. The results showed that the Peg-Dox and Peg-Dox-based protocols had a similar killing ability in myeloid cell lines and in primary myeloid leukemia cells compared to that of conventional doxorubicin. The complete remission rate was 87.5% and 100% for patients with refractory/relapsed acute myeloid leukemia and myelodysplastic syndrome with excess blasts, respectively, after treatment with Peg-Dox. All patients developed grade 3 or 4 hematological toxicity and recovered approximately 2 weeks after completing chemotherapy. No deaths or other severe complications were reported. Our results showed that Peg-Dox can be used in the treatment of myeloid neoplasms with high rates of complete remission and with mild complications.

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Introduction

Pegylated liposomal doxorubicin (Peg-Dox) is a useful drug for the treatment of various malignancies, including AIDS-related Kaposi sarcoma, ovarian cancer, lymphoma, metastatic breast cancer and multiple myeloma. In hematological malignancies, good outcomes were achieved in lymphoma and multiple myeloma by using Peg-Dox, with reduced cardiotoxicity and improved pharmacokinetic profiles when compared to those of doxorubicin [1–3]. In a recent study, Peg-Dox was used to treat elderly patients with acute lymphoblastic leukemia [4]. The outcome showed that Peg-Dox had reduced myelosuppression, reduced infections and less cardiac events with similar outcomes compared to that of continuous-infusion doxorubicin. Peg-Dox was mainly taken up by cells in the liver, spleen and bone marrow, with a higher concentration and a more prolonged period spent in these tissues compared to those of conventional doxorubicin [5, 6]. The mice with lymphocytic leukemia that were treated with Peg-Dox had a longer survival time compared to that of mice that were treated with conventional doxorubicin [6]. However, no indications and no reports have included the use of Peg-Dox in myeloid neoplasms, although non-pegylated liposomal doxorubicin has been reported for the treatment of acute myeloid leukemia (AML) [7, 8]. However, it is still unknown whether Peg-Dox can be used to treat myeloid neoplasms. In this study, we first examined the cell killing effect of Peg-Dox in myeloid cell lines and in primary myeloid leukemia cells. Then, Peg-Dox-based regimens were used to treat myeloid neoplasms.

Materials and methods

Cell killing effect of Peg-Dox in different cell lines

The K562, HL60 and MOLM13 cell lines were cultured in RPMI-1640 (Sigma-Aldrich, America) culture media containing 10% fetal calf serum (Sigma) and 1% antibiotics. Cells were harvested during logarithmic-phase growth, and 1200 cells were distributed into each well of a 384-well plate in 50 µl of growth media. One hundred nanoliters of diluted compound was added to the appropriate wells in triplicate, and the cells were cultured for 72 h at 37°C in a humidified 5% CO_2 atmosphere. Viability was determined by adding 10 µl CellTiter-Glo [Promega Corporation (Beijing) Biotechnology Co., Ltd.] and the relative light units (RLU) were measured on an Envision Plate reader. The rate of inhibition = $100\% - (RLU_{Drug} - RLU_{Background})/(RLU_{DMSO} - RLU_{Background}) \times 100\%$.

The following four groups were included: (1) Peg-Dox (40 mg/m^2) (CSPC Ouyi Pharmaceutical Co., Ltd., Hebei, China) and doxorubicin (40 mg/m^2) (Shenzhen

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Wan Le Pharmaceutical Co., Ltd., China); (2) PLDAC1 [cladribine (5 mg/m^2) (Hanhui Pharmaceutical Co., Ltd., Hangzhou, China) combined with cytarabine (1 g/m^2) (Pfizer Pharmaceuticals Ltd; America) and Peg-Dox (40 mg/m^2)] and DAC1 [cladribine (5 mg/m^2) combined with cytarabine (1 g/m^2) and doxorubicin (40 mg/m^2)]; (3) PLDAC2 [cladribine (5 mg/m^2) combined with cytarabine (100 mg/m^2) and Peg-Dox (40 mg/m^2)] and DAC2 [cladribine (5 mg/m^2) combined with cytarabine (100 mg/m^2) and doxorubicin (40 mg/m^2)] and (4) HAPLD [homoharringtonine (2 mg/m^2) (Hangzhou people's livelihood Pharmaceutical Group Co., Ltd., China) combined with cytarabine (15 mg/m^2) and Peg-Dox (40 mg/m^2)] and HAD [homoharringtonine (2 mg/m^2) combined with cytarabine (15 mg/m^2) and doxorubicin (40 mg/m^2)].

Cell killing effect of Peg-Dox in primary myeloid leukemia cells

Bone marrow was collected from three patients with newly diagnosed AML. The red cells were removed with red blood cell lysis buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The primary leukemia cells were maintained in RPMI-1640 culture media containing 10% fetal calf serum and 1% antibiotics. Cells were harvested during logarithmic-phase growth, and 5000 cells were distributed into each well of a 384-well plate with 50 µl of growth media. One hundred nanoliters of diluted compound was added to appropriate wells in triplicate, and the cells were cultured for 72 h at 37°C in a humidified 5% CO_2 atmosphere. Viability was determined by adding 10 µl CellTiter-Glo (Promega, Wisconsin, American), and the RLU were measured on an Envision Plate reader. The groups were divided as described above for the cell lines. The rate of inhibition = $100\% - (RLU_{Drug} - RLU_{Background})/(RLU_{DMSO} - RLU_{Background}) \times 100\%$.

Study population

Patients were eligible for study enrollment if they had relapsed/refractory AML and newly diagnosed myelodysplastic syndrome-refractory anemia with excess blasts (MDS-RAEB), according to the 2008 WHO classification [9, 10]. Patients with relapsed AML had received at least one cycle of chemotherapy, and the blast cells had decreased by less than 50%. This study was approved by the ethics committee of Xinqiao Hospital, and written informed consent was obtained from the patients in accordance with the Declaration of Helsinki. The patients provided informed consent for the publication of the cases and gave permission to be included in the article.

Treatment plan

The patients with relapsed/refractory AML received the PLDAC protocol: Peg-Dox (15 mg/m^2/day for 3 days) combined with cytarabine (1 g/m^2/day for 5 days) and cladribine (5 mg/m^2/day for 5 days). The patients with newly diagnosed MDS-RAEB received the HAPLDG protocol: homoharringtonine (2 mg/m^2/day for 7 days) combined with cytarabine (15 mg/m^2 q12 h for 7 days) and Peg-Dox (40 mg/m^2 divided into 3 days), and recombinant human granulocyte colony-stimulating factor (rhG-CSF) was used before chemotherapy (300 µg/day for 8 days).

The toxic effects were continuously monitored. The response to treatment was assessed according to the International Working Group's response criteria [10]. The primary endpoint was the complete remission (CR) rate after chemotherapy. The secondary objective was toxicity. A CR was defined as the presence of <5% blasts in the bone marrow aspirate with $>1 \times 10^9$/L neutrophils and $\geq 100 \times 10^9$/L platelets in the peripheral blood, with no evidence of extramedullary disease. A CR with negative minimal residual disease (MRD) was defined as a response meeting the criteria for CR and MRD-negativity, as detected by multiparameter flow cytometry performed on remission bone marrow specimens at the time of achievement of CR.

Statistical analysis

The median and range are used to report noncategorical data. The SPSS software (version 19.0, SPSS, Inc. Chicago, IL) was used to perform statistical evaluation using *t*-tests. A value of $P < 0.05$ was considered significant.

Results

Cell killing effect of Peg-Dox in myeloid cells

In cell lines, the cell killing effect of Peg-Dox was similar in the K562, HL60 and MOLM13 cell lines compared to that of doxorubicin in these cell lines ($P > 0.05$) (Fig. 1a–c). A similar cell killing effect was found for the PLDAC1 protocol compared to that of the DAC1 protocol, for the PLDAC2 protocol compared to that of the DAC2 protocol, and for the HAPLD protocol compared to that of the HAD protocol ($P > 0.05$) (Fig. 1a–c).

In primary AML cells, the cell killing effect of Peg-Dox was similar in the cells from three patients compared to that of doxorubicin in those cells ($P > 0.05$) (Fig. 1d–f). A similar killing effect was found for the PLDAC1 protocol compared to that of the DAC1 protocol, for the PLDAC2 protocol compared to that of the DAC2 protocol, and for the HAPLD protocol compared to that of the HAD protocol ($P > 0.05$) (Fig. 1d–f).

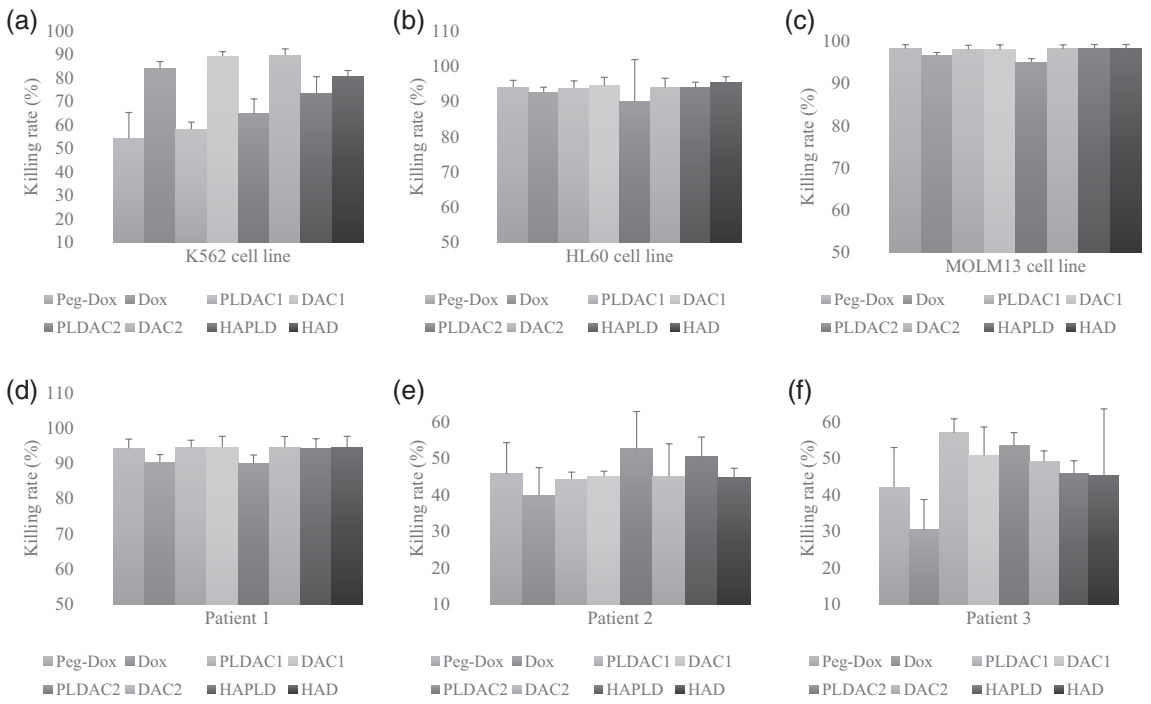
Patient characteristics

Eight patients with relapsed/refractory AML and three patients with newly diagnosed MDS-RAEB were enrolled in this study. Most of these patients had gene mutations and abnormal karyotypes (Table 1).

Outcome of treatment

Seven patients (87.5%) achieved CR with a 75.0% negative-MRD rate (6/8) for patients with refractory/relapsed AML after the first cycle. The CR rate with negative MRD was 87.5% after two cycles of chemotherapy.

Fig. 1



The cell killing effect of the pegylated liposomal doxorubicin-based protocol in cell lines and in primary myeloid leukemia cells. The cell killing ability of the Peg-Dox-based protocol was similar to that of the doxorubicin-based protocol in cell lines and in primary myeloid leukemia cells ($P > 0.05$). (a) K562 cell line; (b) HL60 cell line; (c) MOLM13 cell line; (d) Patient 1; (e) Patient 2; (f) Patient 3. Peg-Dox, pegylated liposomal doxorubicin; PLDAC1, cladribine, cytarabine and Peg-Dox; DAC: cladribine, cytarabine and doxorubicin; HAPLD, homoharringtonine, cytarabine and Peg-Dox; HAD, homoharringtonine, cytarabine and doxorubicin.

Table 1 Patient characteristics and outcomes

						One cycle chemotherapy	
Pt.	Sex	Age	Diagnosis	Mutations	Karyotyping	CR	MRD (%)
Pt. 1	F	12	M2	ETO,WT1, KIT	+4, t(8;21)	Y	0.73
Pt. 2	F	14	MAL	Normal	+6, +r(7)	Y	0
Pt. 3	M	31	M4	Normal	Normal	Y	0
Pt. 4	F	33	MAL	FLT3-ITD, DNMT3A	Normal	Y	0
Pt. 5	2	F	M0	DNMT3A, TET2, WT1, RP53, ASXL1, ETO, AT1	t(1;16) (q42; q24)	Y	0
Pt. 6	F	36	M4EO	CBFβ/MYH11	der(2)t(2;8)(p21;q13)del(7)q(21)t (8;8)(p10;p10)inv(16) (p13q20)	N	N/A
Pt. 7	F	22	M2	FLT3-ITD, WT1, DEK/CAN	t(6;9)(p23; q34)	Y	0
Pt. 8	F	46	M5	FLT3-ITD	Normal	Y	0
Pt. 9	M	64	MDS-RAEB	WT1, DNMT3A	Normal	Y	0
Pt. 10	F	49	MDS-RAEB	TP5, TET2	50, XX, del(5)(q13), −6, +8, der(18)t(13;18) (q11;p11), +mar1,mar2[5]46, XX[5]	Y	0
Pt. 11	F	41	MDS-RAEB	Normal	Normal	Y	3.46

CR, complete remission; MAL, mixed acute leukemia; MDS-RAEB, myelodysplastic syndrome-refractory anemia with excess blasts; MRD, minimal residual disease; Pt., patient.

Three patients (100%) achieved CR with a 66.8% negative-MRD rate (2/3) for patients with MDS-RAEB after the first cycle. The CR rate with negative MRD was 100% after two cycles of chemotherapy (Table 1).

Adverse effects

All patients with AML developed grade 3 or 4 hematological toxicity, and four patients experienced infections. All patients recovered approximately 2 weeks after completing chemotherapy. No treatment-related deaths occurred. No other serious complications were observed.

All patients with MDS-RAEB developed grade 3 or 4 hematological toxicity, and two patients experienced infections. The patients recovered approximately 2 weeks after completing chemotherapy. No treatment-related

deaths occurred. No other serious complications were observed.

Discussion

This is the first study to explore the treatment outcome of Peg-Dox in myeloid neoplasms. Our study primarily showed that the Peg-Dox-based protocols achieved good outcomes in the treatment of refractory/relapsed and newly diagnosed myeloid neoplasms.

Although Peg-Dox has been used to treat many types of tumors, it is still unclear whether Peg-Dox can be used to treat myeloid neoplasms. In this study, we first explored the cell killing effect of Peg-Dox in myeloid cell lines, and the outcome showed that Peg-Dox had a similar cell killing ability compared to that of conventional doxorubicin, which is commonly used in the treatment of leukemia. In primary cells from patients with AML, similar results were also observed. Further experiments also showed that the Peg-Dox-based protocol had a similar cell killing ability compared to that of the conventional doxorubicin-based protocol.

A protocol that used cladribine combined with cytarabine and daunorubicin (DAC) has better outcomes with regard to remission and long-term survival for AML patients compared to that of a protocol that used cytarabine and daunorubicin (DA) or fludarabine combined with cytarabine and daunorubicin [11]. The DAC protocol also resulted in more CR than that of the DA protocol for AML with FLT3-ITD positivity and a normal karyotype [12]. A meta-analysis thoroughly compared the effect of idarubicin versus that of other anthracyclines for induction therapy for newly diagnosed leukemia [13]. The outcome showed that idarubicin increases the CR rate compared to that of daunorubicin and doxorubicin in newly diagnosed AML. No difference was found between the CR rates of idarubicin and mitoxantrone. In patients with refractory/relapsed AML, anthracyclines have been used in the past. Therefore, new drugs should be explored. In the DAC protocol, we switched from daunorubicin to Peg-Dox to treat patients with refractory/relapsed AML. Surprisingly, these patients achieved a high CR with high MRD negativity.

MDS is a clonal hematopoietic disorder. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for MDS-RAEB. However, the blast cell level before transplantation is an independent factor that correlates with the outcome after allo-HSCT [14, 15]. In addition, a study showed that MRD negativity after allo-HSCT is related to a better outcome for patients with MDS-RAEB [16]. Therefore, it is very important to achieve CR with MRD negativity for patients with MDS-RAEB before transplantation. Hypomethylating agents, such as decitabine and 5-azacitidine, and AML-like chemotherapy, such as aclacinomycin, cytarabine and granulocyte colony-stimulating factor, are common protocols for the treatment of patients with MDS-RAEB [17–19]. However, the CR rate was very low after the first two cycles [20, 21]. After most of the patients received several cycles, the CR rate remained low, at approximately 30% [22, 23]. All of these factors affect the treatment outcomes and limit the performance of allo-HSCT, increasing the nonrelapse mortality due to the decreases in the tolerance of patients during long-term chemotherapy. Therefore, a new treatment protocol should be used to help the patients to achieve CR or CR with negative MRD after the first or second cycles. Homoharringtonine, a G1 and G2 cell cycle phase-specific drug, blocks protein synthesis by competing with the amino acid side chains of incoming aminoacyl-tRNAs for binding to the A-site cleft in the peptidyl transferase center of the ribosome. Cytarabine, an S cell cycle phase-specific drug, can incorporate into DNA and interfere with DNA synthesis. Doxorubicin, a cell cycle-nonspecific agent, can inhibit cancer cell growth by acting as a DNA intercalator that inhibits topoisomerase II. The rhG-CSF priming can cause more tumor cells to enter the cell cycle and become sensitive to these drugs. In addition, Peg-Dox has a higher concentration and a more prolonged period of residence in the bone marrow compared with those of doxorubicin, and Peg-Dox causes milder myelosuppression compared to that of doxorubicin [5, 6]. Therefore, HAPLDG was used to treat MDS-RAEB in this study. The outcome showed that all patients reached CR without severe adverse effects, although the case numbers were small.

The rhG-CSF-primed low-dose chemotherapy caused good outcomes for refractory and relapsed AML treatment [24]. The recommended dose of Peg-Dox is usually 40 mg/m^2 for one day [25, 26]. In this study, we divided the total dose of 40 mg/m^2 Peg-Dox into 3 days and combined it with rhG-CSF priming, which may have enhanced the curative effect and further decreased the toxicity.

Non-pegylated liposomal doxorubicin has been shown to improve therapeutic efficacy by significantly reducing the risk of cardiotoxicity compared with that of conventional doxorubicin [27]. Peg-Dox also had a reduced cardiotoxicity and an improved pharmacokinetic profile compared to those of doxorubicin in the treatment of various malignancies [1–3]. Peg-Dox was also associated with milder myelosuppression and lower number infections compared to those of conventional doxorubicin [4]. In this study, slight myelosuppression and mild infections were observed with Peg-Dox treatment, although there are no data comparing those of Peg-Dox to those of non-pegylated liposomal doxorubicin and of conventional doxorubicin.

This study indicated that Peg-Dox can be used in myeloid neoplasms with a good outcome and low toxicity, which extends the scope of Peg-Dox in the treatment

of tumors. This treatment is especially suitable for older patients and for patients with heart dysfunction because of the low cardiotoxicity and mild myelosuppression of Peg-Dox.

Altogether, our study showed that the Peg-Dox-based protocol can be used in myeloid neoplasms. However, additional patients and well-designed, double-blinded, randomized and controlled clinical trials that evaluate the Peg-Dox-based protocol in myeloid neoplasms are warranted.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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