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Short Communication

High-dose methylprednisolone can induce remissions in patients with fludarabine-refractory chronic lymphocytic leukaemia

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ARTICLE INFO

Article history:
Received 20 April 2010
Accepted 21 April 2010
Available online 4 June 2010

Keywords:
Chronic lymphocytic leukaemia
Methylprednisolone
Salvage therapy

ABSTRACT

Purpose: To evaluate the clinical efficacy and safety of high-dose methylprednisolone (HDMP) in patients with fludarabine-refractory chronic lymphocytic leukaemia (CLL).

Methods: Twelve patients who were refractory to fludarabine-based treatment were treated with 2–6 cycles of HDMP (1 g/m^2 for 5 days).

Results: Ten patients (83.3%) responded to treatment and three (25.0%) achieved a complete remission (CR). Two (16.7%) of which had no evidence of minimal residual disease (MRD) after treatment. Patients with leukaemia cells that have high expression of ZAP-70 or CD38, unmutated immunoglobulin heavy chain variable region (IGHV), mutated p53 or adverse cytogenetic features achieved response to treatment at rates that appeared similar to those achieved by patients who did not have such disease characteristics. With a median follow-up of 13 (4–30) months, the median overall survival (OS) and the progression-free survival (PFS) have not been achieved. Treatment with HDMP was well tolerated, notably in the patients having poor myeloid reserve and pretreated cytopaenias.

Conclusions: HDMP is an effective non-myelotoxic regimen for the treatment of patients with fludarabine-refractory CLL.

1. Introduction

There are a number of salvage regimens for relapsed or resistant chronic lymphocytic leukaemia (CLL), but few of these studies aimed specifically at patients resistant to purine analogues. Novel and effective treatments are needed, particularly for patients with more aggressive molecular risk features such as deletion of 17p13.1 or 11q22.3 on fluorescence in situ hybridisation (FISH) analysis.

Glucocorticoids are of interest in the treatment of high-risk CLL, because they can kill lymphoid cells by a p53-independent mechanism and appear to be active in patients with 17p13 deletions.[1] However, few studies have specifically evaluated the clinical activity of corticosteroid-based treatment in patients with CLL.

Based on the evidence demonstrating that high-dose corticosteroids have clinical activity in patients with CLL, we have used the high-dose methylprednisolone (HDMP) as a

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doi:10.1016/j.ejca.2010.04.021

treatment strategy for selected patients with fludarabine-relapsed/refractory CLL and those who are unable to tolerate myelosuppressive treatments.

2. Patients and methods

2.1. Patients

Twelve patients with CLL were enroled between July 2007 and October 2009. All patients provided informed consent according to our institutional guidelines. The diagnosis of CLL and the indication for treatment were based on National Cancer Institute Working Group Guidelines (NCIWG-96). The staging of CLL was performed according to the Binet staging system. In addition, all patients were required to be refractory to fludarabine. We excluded patients who had a history of diabetes, steroid-induced psychosis, active peptic ulcer disease, history of recent gastrointestinal bleeding, pancreatitis and active infection.

2.2. Treatment

HDMP was given intravenously at a dose of 1 g/m^2 daily for five consecutive days repeated at four weekly intervals. Blood pressure was monitored prior to starting treatment and half-hourly during treatment. All patients were given H2 antagonists. Patients with blood glucose of 200 mg/100 ml or more on the days of treatment with HDMP received subcutaneous regular insulin following a routine sliding scale. There were no dose adjustments for HDMP.

2.3. Evaluations and follow-up

Pretreatment evaluation consisted of a medical history and physical examination; laboratory studies including serum β2-microglobulin (β2-MG) and lactate dehydrogenase (LDH). In addition, CLL cells were evaluated for immunoglobulin heavy chain variable region (IGHV) gene and p53 gene mutational status by PCR and sequencing, ZAP-70 and CD38 expression by flow cytometry, metaphase karyotype analysis and FISH analysis using commercial CLL panel probes.

Patients underwent physical and laboratory studies before each cycle, 2 months after completion of treatment and every 3–6 months until additional therapy was administered or death. All patients underwent a marrow aspiration 2 months after completing treatment to assess for minimal residual disease (MRD) by 4-colour flow cytometry evaluating for CD5 and CD19.

2.4. Response and toxicity assessments

Patients were evaluated for response at least 2 months following completion of therapy using the NCIWG-96 criteria. Those without evidence of MRD in the marrow who also satisfied criteria for a complete remission (CR) were designated as having had an MRD-negative CR. Non-haematologic toxicity was graded in accordance with the National Cancer Institutes Common Toxicity Criteria (http://ctep.cancer.gov/reporting/ctc.html). Haematological toxicity was graded according to NCIWG-96.

2.5. Statistical analysis

Demographics and baseline characteristics, response to therapy, progression-free survival (PFS) and overall survival (OS) were recorded and evaluated. Differences in CR rates of various baseline characteristics were analysed using a chi-square test. Descriptive statistics (mean ± sd) were used to analyse changes in absolute lymphocyte count (ALC), haemoglobin, platelet counts, serum immunoglobulin and ratio of CD4:CD8.

3. Results

3.1. Patient characteristics

The clinical characteristics of these patients are listed in Table 1. Eight patients were males and 4 were females (male:female ratio, 2.0). The median age was 55 years (range 47–66 years), and four (33.3%) were over age 60 years. All patients were diagnosed 9–140 months before they came to our attention (median 49 months) and met inclusion criteria for having CLL that was refractory to fludarabine-based therapy. The median number of earlier treatments was three (2–6). All patients were at Binet C. Three of these patients had experienced Richter's transformation prior to receiving treatment. Eight patients (66.7%) had CLL cells that expressed high level of ZAP-70 by flow cytometry, and 10 (83.3%) had high level of CD38 expression. Six patients (50%) had CLL cells that used unmutated IGHV genes. Chromosomal aberrations were found in 10 patients (83.3%), including hemizygous p53 gene deletion in 4 (33.3%) and 2 (16.7%) with ATM gene deletion. Four patients (33.3%) had p53 gene mutations by direct sequencing at exon 5, exon 5, exon 6 and exon 7, respectively. All patients had an ECOG performance status of either 0 or 1 at study entry.

3.2. Response to therapy

Patients received a median of four courses of HDMP (range 2–6) every 4 weeks without requiring delays or dosage reductions. All patients, except two, responded to treatment (OR rates 83.3%). Three patients (25.0%) achieved a CR, and two (16.7%) of which were MRD negative (Table 1). Of the patients with CLL cells harbouring deletions at 17p13 or 11q22.3, five (83.3%) achieved at least a PR to therapy (2 CR, 3 PR). All of the four patients with p53 gene mutations responded (2 CR, 2 PR). There was no significant difference in OR rates for patients with high-level expression of ZAP-70 or CD38, unmutated IGHV genes or adverse cytogenetics ($P > 0.05$).

3.3. Haematologic and immune parameters

Amelioration of impaired marrow function was observed throughout the treatment period with improvement in thrombocytopaenia or anaemia. Haemoglobin and platelet counts improved significantly from pretreatment values and lymphocyte counts decreased to normal levels in almost all cases (Fig. 1).

The 12 patients had mean pretreatment serum IgG, IgA and IgM levels of 8.57 ± 3.54 g/l, 0.96 ± 0.55 g/l and 3.56 ± 7.05 g/l, respectively. The patients' mean serum IgG,

Table 1 – The clinical characteristics and response to therapy of 12 patients with fludarabine-refractory CLL.

Patient	Sex	age	Binet stage	Disease duration (months)	Previous treatments	Richter's transformation	ZAP-70 (%)	CD38 (%)	VH homology (%)	Cytogenetics	FISH	p53 mutation	LDH (U/l)	β2-MG (mg/l)	Response to treatments	PFS (months)	OS (months)
1	M	49	C	40	FC	No	78.1	49.2	68.30	46,XY,11q−[2]/46, XY[18]	del(11q22.3), del(13q14)	Exon 5 codon 144 CAG > TAG	126	3.1	CR	30+	30+
2	M	66	C	46	Chl, FC	No	25.0	99.0	99.58	45,XY,−2,add(19)-(q13)[1]/48, XY,dix(1;2),mar1, mar2[2]/46, XY[17]	Normal	Unmutated	187	4.2	PR	23+	23+
3	M	54	C	74	FC	No	3.3	1.0	88.01	46,XY[20]	del(13q14)	Unmutated	163	2.2	CR (MRD-neg)	21+	21+
4	F	52	C	59	CHOP, FC	Yes	97.3	59.5	99.30	47,XX,11q−, +12[8]/46, XX[12]	del(11q22.3), +12	Exon 7 codon 248 CGG > CAG	217	3.2	PR	10	14
5	F	67	C	140	FND	Yes	44.2	87.1	100	46,XX[20]	del(13q14), del(17p13)	Unmutated	278	3.3	SD		12
6	M	47	C	56	FC	No	29	42.9	89.82	45,X,−Y[12]/46, XY[8]	del(13q14), del(17p13)	Unmutated	319	5	PR	18+	18+
7	M	61	C	118	Chl, FC	Yes	12.2	0.6	93.70	45,X,−Y,−5,−7,+12, +mar[9]/46, XY[11]	+12, del(13q14)	Unmutated	165	2.8	SD		10
8	F	47	C	44	FC	No	15.5	37.8	100	45,X,Xq−,−6,+9q+, der(17) t(17;18),−18,−20, +mar[8]/46, XY[12]	del(6q23)	Unmutated	426	6.7	PR	15+	15+
9	M	48	C	52	FND	No	66.0	60.0	99.05	46,XY,t(3;11)(q29; q13)[2]/46, XY[18]	del(13q14), del(17p13)	Exon 6 codon 220 TAT > TGT	161	5.6	PR	6	9
10	M	56	C	9	F	No	6.5	76.0	94.36	NA	NA	Unmutated	595		PR	7+	7+
11	M	59	C	16	FC	No	46.7	53.8	95.60	41,XY,6q−,−9,9p+, 11q+ ,−15,−17,−19, 21q+,−22[18]/46, XY[2]	del(6q23), del(13q14), del(17p13)	Exon 5 codon 175 CGC > CAC	665	4.4	CR (MRD-neg)	5+	5+
12	F	60	C	15	FC	No	31.9	97.0	100	46,XX[20]	NA	Unmutated	381	5	PR	4+	4+

Abbreviations: β2-MG, β2-microglobulin; Chl, chlorambucil; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; CR, complete remission; F, fludarabine; FC, fludarabine, cyclophosphamide; FISH, fluorescence in situ hybridisation; FND, fludarabine, mitoxantrone, dexamethasone; LDH, lactate dehydrogenase; MRD, minimal residual disease; NA, not available; OS, overall survival; PFS, progression-free survival; PR, partial remission; SD, stable disease; VH, heavy chain variable.

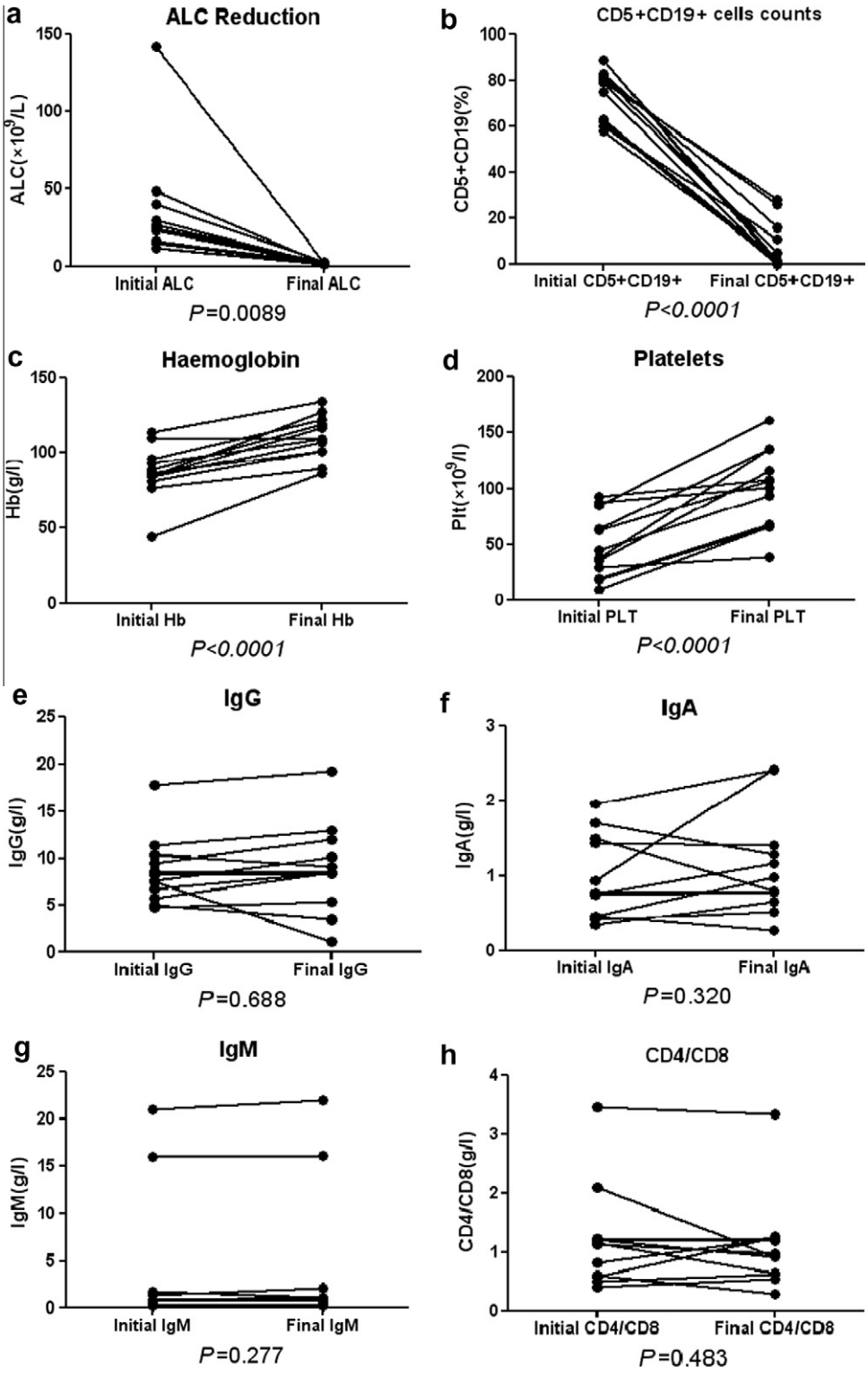

Fig. 1 – Comparison of clinical and laboratory parameters before treatment and at the end of follow-up period. (a) Absolute lymphocyte counts (ALC); (b) CD5 + CD19+ cells counts; (c) haemoglobin values; (d) platelet counts; (e) IgG levels; (f) IgA levels; (g) IgM levels and (h) ratio of CD4:CD8.

IgA and IgM levels at posttreatment were 8.88 ± 4.67 g/l, 1.12 ± 0.69 g/l and 3.70 ± 7.30 g/l, respectively. The patients' posttreatment IgG, IgA and IgM levels were not significantly different from those observed before therapy (Fig. 1). We conclude that HDMP did not worsen hypogammaglobulinaemia at posttreatment period.

On the other hand, the decreased ratio of CD4:CD8 was not observed following HDMP. The mean ratio of CD4:CD8 at

pretreatment was 1.19 ± 0.89, and a posttreatment mean ratio of 1.08 ± 0.81, which was not significantly different.

3.4. *Disease progression and survival*

With a median follow-up of 13 (4–30) months, the median OS and PFS for the patients have not been reached. The estimated mean OS and PFS time of patients were 21.7 months (95% confidence interval (CI), 15.5–27.8 months) and 24.08 months (95% CI, 17.04–31.1 months), respectively. No patient who achieved an MRD-negative CR has required additional therapy. One stable disease (SD) patient developed myelodysplasia, then to acute myelogenous leukaemia and died at 10 months. The other SD patients died at 12 months for progression of CLL. Two PR patients relapsed following HDMP treatment and died at 9 and 14 months, respectively.

3.5. *Toxicity*

The most common adverse events were insomnia and hyperactivity. Fluid retention occurred in five patients (41.7%), particularly in the lower extremities. Four patients (33.3%) experienced grades II–III hyperglycaemia and required temporary insulin therapy only during the administration of methylprednisolone. No patient had sustained hyperglycaemia, or developed diabetes. Neutropaenia, worsening anaemia and thrombocytopaenia were not observed. For a total of 45 administered cycles there was only one red blood cell transfusion and no platelet transfusions. There were no deaths during treatment or immediate follow-up period.

4. Discussion

Among the biological prognostic factors so far identified in CLL, mutation or deletion of the p53 gene encoded at 17p13 is the most powerful predictor of short survival. In keeping with the fact that p53-mediated apoptosis underpins the cytotoxicity of many anticancer drugs,[2] p53 defects in CLL have been strongly linked to resistance to alkylating agents and purine analogues in *ex vivo* experiments, retrospective clinical studies and most importantly in three large prospective clinical trials of first-line chemotherapy.[3–5] In the light of these observations, it is clear that one of the most pressing therapeutic challenges in CLL is to devise novel and effective ways of treating patients with p53 defects.

Corticosteroids have long been recognised to have lympholytic effects in CLL and other lymphoproliferative diseases. Bosanquet and colleagues first reported on the efficacy of HDMP (1 g/m^2 for 5 days) in seven patients with CLL and observed an OR rate of 57%.[6] Thornton et al.[1] reported the efficacy of HDMP in 25 patients who were refractory to fludarabine and 10 who had del(17p13) or p53 dysfunction. HDMP was quite effective, with 77% of patients having a clinical response and a PFS of 12 months (range, 7–23 months). Responses were noted in five of 10 of the del(17p13) patients.

Myelosuppression is one of the most frequent toxicities of salvage regimens for patients with relapsed and refractory CLL. Contrary to this, HDMP regimen had a much lower incidence of neutropaenia or thrombocytopaenia.

In this study, patients treated with HDMP achieved a high OR rate (83.3%). Patients with leukaemia cells that have high expression of ZAP-70 or CD38, unmutated IGHV, mutated p53 or adverse cytogenetic features achieved response to treatment at rates that appeared similar to those achieved by patients who did not have such disease characteristics. The similar response rates might be attributable to HDMP killing lymphoid cells by p53-independent apoptosis, being effective in patients with p53 abnormalities.

Of note, fludarabine-containing regimens used for the treatment of CLL have been associated with CD4 T-lymphopaenia, a characteristic associated with increased infectious complications. However, in this study, decreased ratio of CD4:CD8 and worsening hypogammaglobulinaemia were not observed at posttreatment period.

Overall, HDMP may be an effective non-myelotoxic regimen for the treatment of patients with fludarabine-refractory disease. Further evaluation of this regimen in controlled trials appears warranted.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by National Natural Science Foundation of China (30871104, 30971295, 30971296), Jiangsu Province's Outstanding Medical Academic Leader Program (LJ200623), Jiangsu Province's Medical Elite Program (RC2007042), 'Qing Lan' project of Jiangsu Province and 'Liu Da Ren Cai Gao Feng' of Jiangsu Province.

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