A novel approach to treatment in childhood acute myeloblastic leukemia and myelodysplastic syndrome with high-dose methylprednisolone as a differentiation- and apoptosis-inducing agent of myeloid leukemic cells

Çocukluk yaşı akut myeloblastik lösemi ve myelodisplastik sendromunda myeloid lösemik hücrelerde farklılaşma ve apoptosisi sağlayan yüksek doz metilprednizolon ile yeni bir tedavi yaklaşımı

Gönül Hiçsönmez
Department of Hematology, İhsan Dogramacı Children's Hospital, Hacettepe University Faculty of Medicine, Ankara, Turkey

Abstract

Differentiation-inducing therapy with all-trans retinoic acid significantly improved the outcome in children with acute promyelocytic leukemia (APL). Therefore, use of agents that induce differentiation of leukemic cells in non-APL children appears to be a highly promising therapeutic approach. Based on the experimental studies in mice, we have shown that short-course high-dose methylprednisolone (HDMP) treatment can induce terminal differentiation of leukemic cells in children with various subtypes of acute myeloblastic leukemia (AML-M1,-M2,-M3,-M4,-M7). It has also been shown to induce apoptosis of myeloid leukemic cells with or without differentiation. Administration of HDMP as a single agent resulted in a rapid clinical improvement, a marked decrease in blast cells in both peripheral blood and bone marrow and dramatic decreases in the size of extramedullary leukemic mass in children with AML and myelodysplastic syndrome (MDS). Addition of HDMP to cytotoxic chemotherapy regimens increased the remission rate and improved the outcome in these children. Future clinical trials with HDMP would contribute to further improvements in the treatment results in these children. *(Turk J Hematol 2010; 27: 1-7)*

Key words: High-dose methylprednisolone, differentiation, apoptosis, acute myeloblastic leukemia, myelodysplastic syndrome, children

Received: November 8, 2009 *Accepted: November 19, 2009*

Özet

Akut Promyelositik Lösemili (APL) çocuklarda, lösemik hücrenin normal hücreye dönüşünü (farklılaşma) sağlayan all-trans retinioik asidin (ATRA) kullanılması ile tedavi sonuçlarında önemli ilerlemeler elde edilmiştir. Bu nedenle APL dışında diğer akut myeloblastik lösemi (AML) subtiplerinde lösemik hücrelerin farklılaşmasını sağlayan ilaçların kullanılmsı, bu hastalar için de önemli ve ümit verici bir tedavi yaklaşımı olacaktır. Fare myeloid lösemik hücrelerin steroid ile farklılaşmasının sağlanabileceğini gösteren in vitro çalışmalara dayanarak, biz de, literatürde ilk defa olmak üzere, AML'li çocukların farklı subtiplerinde (AML M1,-M2,-M3,-M4,-M7), kısa süreli yüksek doz metilprednizolon (YDMP) tedavisi ile lösemik hücrelerin normal hücrelere dönebileceğini gösterdik. Ayrıca YDMP tedavisi, lösemik hücreden dönüşen veya direkt olarak myeloid lösemik hücrelerin apoptotik ölümüne neden olmaktadır.

Diğer lösemi ilaçları kullanılmaksızın YDMP, hastalara verildikten kısa süre sonra , kilinik bulgularda düzelme, periferik kan ve kemik iliği lösemi hücrelerinde önemli bir azalma ve kemik iliği dışındaki lösemi hücre kitlesinin hızla küçülmesine neden

Address for Correspondence: Gönül Hiçsönmez, MD, Department of Hematology, İhsan Dogramacı Children's Hospital Hacettepe University, Ankara 06100, Turkey
Phone: +90 312-238 11 19 Fax: +90 312-324 49 90 E-mail: gsonmez@hacettepe.edu.tr

olmaktadır. YDMP' nun sitotoksik tedavi protokollarına ilavesi ile hastaların remisyon yüzdeleri artmış ve hastalıksız yaşam süreleri uzamıştır. Bu nedenlerle, YDMP ile yapılacak kilinik çalışmaların, AML ve myelodisplastik sendromlu çocukların tedavilerinden alınacak sonuçlara önemli katkısı olacağı kanısındayız. *(Turk J Hematol 2010; 27: 1-7)*
Anahtar kelimeler: Yüksek doz metilprednisolon, faklılaşma, apoptosis, akut myeloblastik lösemi, myelodisplastik sendrom, çocuklar

Geliş tarihi: 8 Kasım 2009 *Kabul tarihi: 19 Kasım 2009*

Introduction

Glucocorticoids (GCs) have been used successfully in the treatment of childhood acute lymphoblastic leukemia (ALL) since the late 1940's. To enhance their effectiveness, higher doses of GCs (250 to 1000 mg daily) were used, mostly in adults, up to the early 1960's. Massive doses of steroid induced hematologic improvement more rapidly in ALL patients, and the beneficial effect was noted in patients who became resistant to conventional-dose steroid and also in a few patients with acute myeloblastic leukemia (AML) [1-3). However, for a long period, the therapeutic benefits of GCs at higher doses were not evaluated in clinical trials. Recently, in *in vitro* studies, higher antileukemic activity [4), which may be associated with an increase in apoptosis [5,6), and favorable clinical results were obtained by increasing the dose of GCs in childhood ALL [7-12].

In contrast to ALL, in patients with AML, it is generally believed that GCs in conventional dose have limited or no effect. Despite the favorable results obtained in childhood ALL, progress in the treatment of children with AML with currently used regimens, which mainly depend on the eradication of blast cells by cytotoxic agents, remains unsatisfactory [13,14]. However, the possibility of treatment with agents that induce terminal differentiation of myeloid leukemic cells has long been proposed as a promising approach for improvement in the prognosis of patients with AML. Differentiation-inducing therapy with a derivative of vitamin A, all-trans retinoic acid (ATRA), resulted in significant improvement in the outcome in children with acute promyelocytic leukemia (APL, AML-M3) [15,16]. However, the results in non-APL patients obtained with ATRA are not encouraging. Based on the *in vitro* studies, which revealed that arsenic trioxide can induce differentiation and apoptosis of APL cell line NB4 and fresh APL cells with t (15;17) dose-dependently [17], it has also been incorporated in the treatment of adult APL patients. However, there are limited data regarding results in children with APL [18].

GC-induced differentiation and/or apoptosis of human myeloid leukemic cells *in vitro*

Since the early 1970's, a number of experimental studies have shown that prednisolone (PRD) and dexamethasone (Dex) are the most potent agents for inducing differentiation of mouse myeloid leukemic cells into macrophages and granulocytes [19-22]. Moreover, a high concentration of Dex caused complete arrest of mouse myeloid leukemic cell proliferation and prolonged the survival in mice inoculated with sensitive M1 cells [23]. In further studies, differentiation- and/or apoptosis-inducing effects of Dex or methylprednisolone (MP) on human primary AML cells [24-26] and myeloid leukemia cell lines (HL-60, NB4, U937, HIMeg and t(8;21)-positive myeloid leukemia cells), in a dose-dependent manner, have been demonstrated *in vitro* [27-32]. Various effects of steroid on human and mouse myeloid leukemic cells were reviewed previously [33,34].

Other steroid derivative-induced differentiation and/or apoptosis of human myeloid leukemic cells *in vitro*

Very recently, Chow et al. [35] reported that 2-methoxyestradiol (2ME2), a natural metabolite of 17β-estradiol, can induce apoptosis in different types of AML cell lines (HL-60, NB4 and U937) and in primary human AML cells (M0, M1, M2) *in vitro* in a dose-dependent manner. Trafalis et al. [36], who evaluated the effects of two homo-aza-steroids (1 androgen and 1 estrogen derivative) on leukemic blasts obtained from patients with various subtypes of AML, indicated the capacity of differentiation. Several aminosteroid compounds have also been shown to inhibit proliferation and/or induce differentiation in HL-60 cells dose-dependently [37,38]. In addition, a cardiotonic steroid, bufalin, has been shown to induce differentiation of human myeloid leukemia-derived cell lines, HL-60, U937 and ML1, and leukemic cells from 4 of 20 AML patients in primary culture [39,40]. In all these studies, therapeutic potential of these different steroid derivatives as possible novel agents in the blood malignancies has been indicated [35-40]. Furthermore, in addition to another steroid derivative, 1α, 25-dihydroxyvitamin D_3 and its analogs, which is well known to induce differentiation [41], isomeric pregnadienedione steroids (guggulsterones, naturally occurring plant sterols) and 16-dehydroxyprogesterone with increasing concentration have been shown recently to exert antileukemic effect by inducing differentiation and apoptosis in human AML cell lines (HL-60, NB4 and U937) by Samudio et al. [42]. These compounds also induced apoptosis in primary CD34-positive leukemia cells obtained from relapsed patients with AML-M0 and M2 [42]. Interestingly, it has been indicated that the pregnadienedione structure of these steroids could offer the potential for development of novel chemotherapeutics.

Here, the results of our clinical studies with another pregnadienedione steroid derivative, MP, which induces differentiation and apoptosis of myeloid leukemic cells *in vivo*, will be reviewed.

Short-course HDMP treatment-induced differentiation of myeloid leukemic cells *in vivo*

Since 1988, we have shown the remarkable antileukemic effects of MP at high doses, in various subtypes of newly diagnosed and relapsed children with AML [43,44]. Children who had no infection were given MP sodium succinate (Prednol-L, MN Pharmaceutical, Turkey) orally, at a single daily dose of 20-30 mg/kg (not exceeding 1 g daily) together with an antacid. Based on the experimental studies on induction of differentiation of mice myeloid leukemic cells, we first demonstrated morphologic evidence of *in vivo* differentiation of myeloid leukemic cells to mature granulocytes in a boy with AML-M4 treated with HDMP alone in 1991 [45]. In our further studies, short-course (3 to 7 days) high-dose (HD)MP treatment was also shown to induce terminal differentiation of myeloid leukemic cells in children with APL (AML-M3) and in other sub-

types of AML (AML-M1,-M2,-M4,-M7) [46-49]. In addition to rapid reduction of blast cells, morphologic changes including presence of Auer rods in mature cells (Figure 1) and surface marker analysis of peripheral blood and/or bone marrow cells by flow cytometric studies confirmed the evidence of *in vivo* differentiation of myeloid leukemic cells. More recently, MP has been shown by Corsello et al. [32] *in vitro* to induce differentiation of AML cell lines with a t(8;21) in a dose-responsive manner. In this study, treatment of t(8;21)-positive Kasumi-1 cells with MP revealed loss of AML1-ETO protein expression in a proteasome- and GC receptor-dependent manner. Moreover, in three primary patient AML samples, AML1-ETO protein was reduced with *in vitro* MP treatment. MP could also be the effective agent for the targeted inhibition of dysregulated transcription factors in some other subtypes of AML. It will be interesting to explore whether or not HDMP treatment induces cellular differentiation in some other malignant diseases.

Short-course HDMP treatment-induced apoptosis of myeloid leukemic cells *in vivo*

Apoptosis (programmed cell death) has been demonstrated *in vitro* to be the common mode of cell death of differentiated human myeloid leukemic cells (HL-60) [50]. We have also shown that induction of apoptosis of myeloid leukemic cells with or without differentiation is possible with HDMP treatment *in vivo* and *in vitro* [26,49,51]. In addition to ultrastructural findings, light microscopic studies revealed the morphologic evidence of apoptosis in bone marrow cells obtained from different subtypes of AML children (AML-M3,-M4 and -M7) who received short-course HDMP (4 days) as a single agent. Incubation of low (10^{-6}M) and high (10^{-3}M) concentration of MP with freshly obtained leukemic cells from AML children resulted in a dose-dependent increase in differentiated cells and in apoptosis [26]. Previously, Blewitt et al. [52] demonstrated that GCs at suprapharmacologic concentration, in contrast to low-dose, showed massive lethal effect possibly by inducing apoptosis in human myeloid leukemic cells *in vitro* [52]. It was also reported that cytolethal effects of GCs (hydrocortisone, Dex, MP) are dose-dependent, and among the GC preparations tested, the most severe cytolethal effect was obtained in human lymphoid and myeloid leukemic cells when exposed to a suprapharmacologic dose of MP (10^{-3}M) [53]. Dex- and/or MP-induced apoptosis has been demonstrated in AML cell lines (Kasumi-1 and Skno-1 cells) with a t(8;21) [28,32], HL-60 and in human acute megakaryocytic leukemia (AmegL) cell lines in a dose-dependent manner [27,30]. A plant steroid, diosgenin, has also been shown to induce megakaryocytic differentiation and apoptosis in a human erythroleukemia cell line dose-dependently [54]. In addition to the results of *in vitro* studies and the rapid hematological and morphological improvement detected four days after HDMP treatment via induction of differentiation and apoptosis of leukemic cells, as observed in our patients with AmegL [26,49], it is important to note that addition of HDMP to induction therapy appears to be a very promising treatment approach in these patients who have poor prognosis. Suppression of the Bcl-2 by MP or Dex might indicate the role GCs in inducing apoptosis of myeloid leukemic cells [32,55,56]. Dex has also been shown to significantly suppress expression of c-myc and c-myb genes during Dex-induced differentiation of mouse myeloid leukemic cells [56,57].

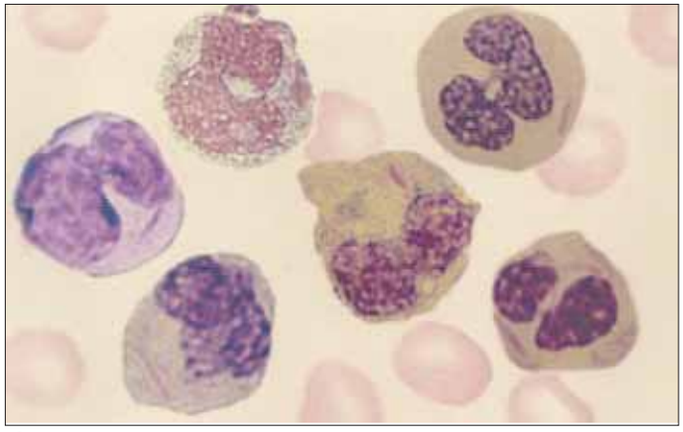

Figure 1. Four to seven days after HDMP treatment, maturing myeloid cells and granulocytes containing Auer rods are seen in the bone marrow smear of children with AML (M1,- M2,- M4) (Wright stain X 2000)

Therapeutic effects of HDMP in children with AML

Effect of HDMP on blast cells

During our long-term clinical studies, in a short-period (24 or 48 hours) after administration of HDMP treatment alone, dramatic clinical improvement (improved activities; resolution of bone pain and unexplained high fever) was observed in most of the AML children who had not received HDMP previously. In addition, rapid decrease in blast cells in both peripheral blood and bone marrow with a concomitant increase in the number of maturing and mature myeloid cells (metamyelocytes and polymorphonuclear leukocytes) was detected after short-course (4 to 7 days) HDMP without using any other antileukemic agents in different subtypes of AML children [43-49,58,59]. Changes in marrow blasts 4-7 days after HDMP treatment alone or two weeks after HDMP combined with cytosine arabinoside (Ara-c) are shown in Table 1. Marked decrease in marrow blasts was also detected in relapsed children [44]. In a patient with refractory AML, complete remission (CR) was also described by Sugawara et al. [60] using HDMP combined with granulocyte colony-stimulating factor (G-CSF). Although a similar effect was not obtained in our patients who previously received HDMP, it is important to note that 3-4 months after its discontinuation, some of the patients responded again to HDMP.

Effect of HDMP on extramedullary infiltration

More importantly, dramatic decreases in the size of extramedullary leukemic infiltration (orbita, gingiva and soft tissue) were detected in 24 hours to 7 days after initiation of HDMP treatment [43-45,58,61]. Rapid decreases in orbital and spinal mass following the administration of HDMP treatment alone were also reported by others in children with AML-M2 and t(8;21) translocation [62,63]. Furthermore, we demonstrated complete resolution of pleural effusion and marked decrease in pericardial effusion in children with chronic myelomonocytic leukemia (CMML) four days after HDMP treatment alone, which also induced differentiation and apoptosis of malignant cells in the pleural effusion [64]. Based on these results, we can suggest that addition of short-course HDMP to initial treatment would be a very effective approach for the treatment of patients presenting with extramedullary infiltration

Combined HDMP with cytotoxic chemotherapy

In our previous study reported in 1992, addition of HDMP to mild antileukemia regimens (adriamycin and Ara-c) increased

Table 1. Decrease in percent of marrow blasts after HDMP alone or HDMP combined with Ara-c in children with newly diagnosed AML and MDS

No. of Patients	Treatment	Duration of treatment (day)	Blast cells% <5%	Blast cells% 0-15%	References
37*	HDMP	4-7	12(32%)	20(54%)	58
26*	HDMP+ Ara-c	15	7(27%)	17(61%)	59
4**	HDMP+LD-Ara-c	15	4(100%)	-	66
8**	HDMP	4	7(88%)	8(100%)	79

*AML,**RAEB and RAEB-t, Ara-c (3mg/kg, 3 days a week), LD Ara-c (10 mg/m^2)
HDMP: High-dose methylprednisolone; LD Ara-c: Low-dose cytosine arabinoside; AML: Acute myeloblastic leukemia; MDS: Myelodysplastic syndrome; RAEB: Refractory anemia with excess of blasts; RAEB-t: RAEB in transformation

the CR rate to 84.6% in 26 newly diagnosed AML children with or without extramedullary infiltration when compared to the CR rate (62%) of historical controls [59.65], and reduced the number of early relapses occurring within 12 months (45% vs 78%) and 24 months (76% vs 90%), respectively. However, difference in five-year disease-free survival (DFS) rate was not significant (18% vs 10%). In our subsequent studies with two different treatment protocols reported in 2004, the CR rate was 87% and 89% in 23 and 45 children, respectively, who had no extramedullary infiltration at presentation [58]. By using intensive maintenance therapy, improved outcome was achieved and the five-year DFS rate was 44% and 35% , respectively. In this study, in a small number of children (n=7) who presented with myeloblastoma, five-year DFS rate was 71% [58]. However, this improvement was not noted in children with gingival infiltration. We believe that further studies in these heterogeneous group of AML patients who have large differences in outcome should be explored with combined HDMP and more intensive chemotherapy than used in our studies, in an effort to improve the outcome. HDMP treatment was well tolerated, HDMP-related toxicity was mild, and no life-threatening events occurred [58,59]. During induction therapy, after the addition of cytotoxic agents to HDMP, mortality rate was 4% in 112 children with AML [58]. However, unlike with cytotoxic agents, in a short period after initiation of HDMP, increase in white blood cell (WBC) count, which was controlled by the administration of cytotoxic drugs, was observed in 25% of the AML children and in children with myelodysplastic syndrome (MDS) as well [58,66]. This increase was not associated with the increase in peripheral blast cells. Rapid increase in WBC count following massive-dose steroid therapy was also noted in some patients with AML in previous studies [2,3]. Therefore, patients with WBC count >25x10^9/L at diagnosis were given chemotherapy concomitantly with HDMP administration.

Since an early blast cell clearance by remission induction therapy is a major independent prognostic factor, the improved outcome in our patients could be related with the initial striking decrease in leukemic cells in the bone marrow and in the extramedullary site obtained with HDMP as a differentiating- and apoptosis-inducing agent. In addition, administration of short-course (4 days) HDMP treatment during induction therapy resulted in a rapid increase in T4+, T8+ T-cells and natural killer (NK) cells, possibly due to stimulation of marrow CD34+ cells, which may also contribute to the antileukemic effect of HDMP [67]. It has also been shown by Vitale et al. [68] that pharmacological concentration of MP can induce preferential and rapid *in vitro* differentiation of CD34+ hematopoietic precursors to NK cells.

Recently, dramatic selective blast cell reduction associated with apoptosis and improved outcome were reported by Suzuki et al. [69] in elderly patients (63-89 years) with AML secondary to MDS treated with MP alone (125 mg, body). Significant blast reduction with hematological and cytogenetic remission following HDMP (1000 mg/day) treatment alone was also demonstrated by Shimohakamada et al [70] in an adult AML patient who had pulmonary infection. However, the optimal dose of MP in inducing differentiation and apoptosis remains unknown.

The stimulation of normal myelopoiesis with HDMP treatment would be an additional benefit in the treatment of these patients. Short-course (3-5 days) HDMP treatment shortens the chemotherapy-induced neutropenic period in children with AML and ALL during induction and maintenance therapy, possibly by stimulating the CD34+ hematopoietic progenitor cells [71-73]. Pretreatment with short-course HDMP, before high-dose consolidation therapy, reduced the duration and severity of neutropenia in children with AML [74]. We suggest that the effect of short-course HDMP should be evaluated in non-leukemic malignancies in order to stimulate neutrophil recovery.

Therapeutic effects of HDMP in children with MDS

Although significant progress has been achieved in the treatment of children with MDS who could have received hematopoietic stem cell transplantation, treatment results with standard chemotherapy regimens have been generally unsatisfactory [75,76]. Since progressive impairment in differentiation of hematopoietic cell lineages is the main pathophysiological feature, there has been great interest in using differentiation-inducing agents in MDS. However, results with retinoic acid and vitamin D3 are not encouraging [77].

On the other hand, we obtained promising results in children with various subtypes of MDS (refractory anemia with excess blast (RAEB), RAEB in transformation, juvenile myelomonocytic leukemia (JMML) and CMML) with the addition of HDMP to cytotoxic chemotherapy [66,78,79], with the exception of patients with JMML and monosomy 7. As observed in AML children, a marked decrease in blast cells both in peripheral blood and bone marrow was also detected after HDMP treatment (Table 1). In addition, dramatic resolution of extramedullary infiltration (orbital, spinal and pleural), possibly due to its differentiation- and apoptosis-inducing effects, was also noted in these children [66,79]. With the use of combined HDMP and mild cytotoxic chemotherapy, higher CR rate (70%) was achieved in 17 of 25 children with different subtypes of MDS [79]. Improvement in the outcome in these children, especially in children with CMML, seems to be promising. Our preliminary data showed that short-course HDMP treatment might also be beneficial by remarkably increasing

serum levels of interleukin-2 in children with CMML [80]. As obtained with anti-thymocyte globulin and cyclosporin A, modulation of immune response by HDMP might contribute to its beneficial effect in some patients with MDS. In contrast to other immunosuppressive agents, an advantage of the use of short-course HDMP and its favorable effects have been indicated in adult patients with refractory anemia [81-83]. Decrease in serum tumor necrosis factor-alpha after short-course HDMP therapy observed in children with AML and ALL might indicate that it is also effective in MDS, leading to hematologic improvements [84]. In addition, an inhibitory effect of steroids (PRD and Dex) has been shown *in vitro* on the production of leukemia-associated inhibitor from human myeloid leukemic cells, which has been suggested by Olofsson et al. [85] as being responsible for the suppression of normal hematopoiesis. The possibility of HDMP-induced suppression of inhibitory activity of MDS abnormal clones [86] may also contribute to its effect in MDS. These possibilities might explain the favorable response to short-course HDMP treatment obtained in our patient with hypoplastic MDS as well as in a case with hypoplastic AML [87,88].

Identification of responsive MDS patients to GC therapy by *in vitro* technique was first reported by Bagby et al. [89]. In that study, it was suggested that if therapeutically achievable concentrations of cortisol enhance colony growth of marrow cells and if the colonial cells are well differentiated (granulocyte maturation was observed in 2 patients), the patients will likely respond favorably to PRD therapy. Interestingly, they described responses when higher doses of PRD were used in patients who were considered *in vitro* non-responders. Beneficial effects of high-dose steroid in patients with MDS were also reported by others [90-94]. Based on these studies, HDMP combined with chemotherapy followed by autologous stem cell transplantation can be considered as an alternative therapeutic approach in children with MDS who are not suitable for bone marrow transplantation.

In conclusion, our clinical studies indicate that addition of short-course HDMP as a differentiation- and apoptosis-inducing agent to cytotoxic chemotherapy regimens represents a promising approach in the treatment of children with AML and MDS. In further studies, its effective dosage, duration of administration and role in maintenance therapy should be evaluated. Although the factors involved in the mechanisms of HDMP effect in inducing differentiation, apoptosis and stimulation of myelopoiesis are not well known, it may be effective through complex mechanisms to target several antileukemic pathways. Further studies, enhancing the understanding of the metabolic and molecular basis of these effects, could provide important therapeutic benefit in children with AML and MDS. Based on the results of our clinical studies, short-course HDMP also deserves evaluation regarding its effect in the treatment of some other malignancies.

Acknowledgements

I would like to particularly thank Prof. Dr. M. Çetin and all my colleagues who made valuable contributions during these studies, as indicated in the list of cited references of the published papers. I also warmly thank all doctors, nurses and the technical personnel who also made valuable contributions during the long clinical follow-up period of these children. I would like to acknowledge Prof.Dr. E. Erdemli and Prof.Dr. M. Tekelioğlu from the Department of Histology at Ankara University for providing electron microscopic studies.

Conflict of interest

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

References

1. Ranney HM, Gelhorn A. The effect of massive prednisone and prednisolone therapy in leukemia and lymphoma. Am J Med 1957;22:405-13.
2. Shanbrom E, Miller S. Critical evaluation of massive steroid therapy of acute leukemia. N Engl J Med 1962;266:1354-8.
3. Granville NB, Rubio F, Unugur A, Schulman E, Dameshek W. Treatment of acute leukemia in adults with massive doses of prednisone and prednisolone. N Engl J Med 1958;259:207-13.
4. Haarman EG, Kaspers GJL, Pieters R, Rottier MMA, Veerman AJP. Circumvention of glucocorticoid resistance in childhood leukemia. Leuk Res 2008;32:1417-23.
5. Uckan D, Yetgin S, Cetin M, Özyürek E, Okur H, Aslan D, Tuncer M. The effects of high-dose methylprednisolone on apoptosis in children with acute lymphoblastic leukemia. Clin Lab Haematol 2003;25:35-40.
6. Erduran E, Tekelioglu Y, Karakas T, Gedik Y, Mert FM. Comparison of the apoptotic effects on lymphoblasts and on increase of myeloid lineage cells of a short-time high-dose methylprednisolone and the conventional-dose prednisolone treatments in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol 2006;23:587-98.
7. Schwartz E, Thompson EB, Gelber RD, Young RD, Chilton D, Cohen HJ, Sallan SE. Improved response with higher corticosteroid dose in children with acute lymphoblastic leukemia. J Clin Oncol 2001;19:1040-6.
8. Ryalls MR, Pinkerton CR, Meller ST, Talbot D, McElwain TJ. High-dose methylprednisolone sodium succinate as a single agent in relapsed childhood acute lymphoblastic leukemia. Med Pediatr Oncol 1992;20:119-23.
9. Hicsonmez G, Ozsoylu S, Onat N, Prozorva VZ, Gümrük F, Tuncer M. High-dose methylprednisolone in resistant and relapsed children with acute lymphoblastic leukemia. Med Pediatr Oncol 1994;22:68-9.
10. Hicsonmez G, Gümrük F, Zamani VP, Tuncer MA, Yetgin S, Gürgey A, Atahan L, Özsoylu S. High-dose methylprednisolone for children with acute lymphoblastic leukemia and unfavorable presenting features. Eur J Haematol 1997;58:26-31.
11. Yetgin S, Cetin M. The dose related effect of steroids on blast reduction rate and event free survival in children with acute lymphoblastic leukemia. Leuk Lymphoma 2003;44:489-95.
12. Yetgin S, Tuncer MA, Cetin M, Gümrük F, Yenicesu, I, Tunc B, Öner AF, Toksoy H, Koç A, Aslan D, Özyürek E, Olcay L, Atahan L, Tunçbilek E, Gürgey Ae. Benefit of high-dose methyl-prednisolone in comparison with conventional-dose prednisolone during remission induction therapy in childhood acute lymphoblastic leukemia for long-term follow-up. Leukemia 2003;17:328-33.
13. Rubnitz JE, Gibson B, Smith FO. Acute myeloid leukemia. Pediatr Clin N Am 2008;55:21-51.
14. Woods WG. Curing childhood acute myeloid leukemia (AML) at the half-way point: promises to keep and miles to go before we sleep. Pediatr Blood Cancer 2006;46:565-9.
15. Testi AM, Biondi A, Lo Coco F, Moleti ML, Giona F, Vignetti M, Menna G, Locatelli F, Pession A, Barisone E, De Rossi G, Diverio D, Micalizzi C, Aricò M, Basso G, Foa R, Mandelli F. GIMEMA-AIEOP AIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. Blood 2005;106:447-53.
16. Mantadakis E, Samonis G, Kalmanti M. A comprehensive review of acute promyelocytic leukemia in children. Acta Haematol 2008;119:73-82.
17. Chen Z, Chen GQ, Shen ZX, Chen SJ, Wang ZY. Treatment of acute promyelocytic leukemia with arsenic compounds: in vitro and in vivo studies. Semin Hematol 2001;38:26-36.

18. Zhang L, Zhao H, Zhu X, Chen Y, Zou Y, Chen X. Retrospective analysis of 65 Chinese children with acute promyelocytic leukemia: a single center experience. Pediatr Blood Cancer 2008;51:210-5.
19. Lotem J, Sachs L. Induction of specific changes in the surface membrane of myeloid leukemic cells by steroid hormones. Int J Cancer 1975;15:731-40.
20. Krystosek A, Sachs L. Steroid hormone receptors and the differentiation of myeloid leukemic cells. J Cell Physiol 1977;92:345-52.
21. Sachs L. Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia. Nature 1978;274:535-9.
22. Honma Y, Kasukabe T, Okabe J, Hozumi M. Glucocorticoid induced differentiation of cultured mouse myeloid leukemic cells. Gunn 1977;68:241-6.
23. Kasukabe T, Honma Y, Hozumi M, Suda T, Nishii Y. Control of proliferating potential of myeloid leukemia cells during long-term treatment with vitamin D3 analogues and other differentiation inducers in combination with antileukemic drugs. In vitro and in vivo studies. Cancer Res 1987;47:567-72.
24. Nakamaki T, Sakashita A, Sano M, Hino K, Suziki K, Tomayasu S, Tsuruoka N. Differentiation induction of myeloid leukemia cells by glucocorticoid - the differentiation induction effect in vitro and in vivo. Rinsho Ketsueki 1989;30:149-57.
25. Ovali E, Özdemir F, Aydın F, Kavgacı H, Tekelioglu Y, Büyükcelik A, Yılmaz M. The effects of dexamethasone on leukemic cells derived from patients with AML. J Exp Clin Cancer Res 2003;22:85-9.
26. Özbek N, Erdemli E, Hicsonmez G, Okur H, Tekelioglu M. Effect of methylprednisolone on human myeloid leukemic cells in vitro. Am J Hematol 1999;60:255-9.
27. Song LN, Cheng T. Glucocorticoid-induced growth inhibition and differentiation of a human megakaryoblastic leukemia cell line. Involvement of glucocorticoid receptor. Stem Cells 1993;11:312-8.
28. Miyoshi H, Ohki M, Nakagawa T, Honma Y. Glucocorticoids induce apoptosis in acute myeloid leukemia cell lines with a t (8;21) chromosome translocation. Leuk Res 1997;21:45-50.
29. Uzunoglu S, Uslu R, Töbü M, Saydam G Terzioglu E Büyükkeçeci F. Augmentation of methylprednisolone-induced differentiation of myeloid leukemia cells by serine/threonine phosphatase inhibitors. Leuk Res 1999;23:507-12.
30. Omay SB, Saydam G, Aydın HH, Selvi N, Öktem G, Sanlı UA, Tobu M, Büyükkeçeci F. Potential involvement of calcineurin regulating the state of differentiation and apoptosis of HL-60 cells during methylprednisolone treatment. Turk J Hematol 2003;20:143-51.
31. Özcimen A, Cetin M. Methylprednisolone induces terminal differentiation in the U-937 human myelomonocytic leukemia cells. Turk J Immunol 2008;13:10-4.
32. Corsello SM, Roti G, Ross KN, Chow KT, Galinsky D, DeAngelo DJ, Stone RM, Kung AL, Golup TR, Stegmaier K. Identification of AML1-ETO modulators by chemical genomics. Blood 2009;113:6193-205.
33. Hicsonmez G, Ozsoylu S, Tuncer AM. Differentiation of myeloid leukemic cells induced by high-dose methylprednisolone in patients with acute myeloblastic leukemia and its therapeutic potential. Leuk Res 1991;15:537-41.
34. Hicsonmez G. The effect of steroid on myeloid leukemic cells: the potential of short-course high-dose methylprednisolone treatment in inducing differentiation, apoptosis and in stimulating myelopoiesis. Leuk Res 2006;30:60-8.
35. Chow JM, Liu CR, Lin CP, Lee CN, Cheng YC, Lin S, Liu HE. Downregulation of c-Myc determines sensitivity to 2-methoxyestradiol-induced apoptosis in human acute myeloid leukemia. Exp Hematol 2008;36:140-8.
36. Trafalis DTP, Sambani C, Kapsimali V, Economidou J, Politis G, Catsoulacos P. Effects of homo-aza-steroids on acute nonlymphoblastic leukemia cell proliferation in vitro. Br J Haematol 1995;91:907-14.
37. He Q, Jiang D. A novel aminosteroid is active for proliferation inhibition and differentiation induction of human acute myeloid leukemia HL-60 cells. Leuk Res 1999;23:369-72.
38. Thibeault D, Roy J, DeRoy P, Poirier D. Chemical synthesis of 2β-amino-5α-androstane-3α, 17β -diol N-derivatives and their antiproliferative effect on HL- 60 human leukemia cells. Bioorg Med Chem 2008;16:5062-77.
39. Zhang L, Nakaya K, Yoshida T. Induction by bufalin of differentiation of human leukemia cells HL60, U937, and ML1 toward macrophage/monocyte-like cells and its potential synergistic effect on the differentiation of human leukemia cells in combination with other inducers. Cancer Res 1992;52:4634-41
40. Yamada K, Hino K-I, Tomoyasu S, Honma Y, Tsuruoka N. Enhancement by bufalin of retinoic acid-induced differentiation of acute promyelocytic leukemia cells in primary culture. Leuk Res 1998;22:589-95.
41. Zhou JY, Norman AW, Akashi M, Akashi M, Chen DL, Uskokovic MR, Aurrecoechea JM, Koeffler HP. Development of a novel 1,25 (OH)2-vitamin D3 analog with potent ability to induce HL-60 cell differentiation without modulating calcium metabolism. Blood 1991;78:75-82.
42. Samudio I, Konopleva M, Safe S, McQueen T, Andreeff M. Guggulsterones induce apoptosis and differentiation in acute myeloid leukemia: identification of isomer-specific antileukemic activities of the pregnadienedione structure. Mol Cancer Ther 2005;4:1982-92.
43. Hicsonmez G, Ozsoylu S, Tuncer M, Erer B. High-dose intravenous methylprednisolone in the treatment of acute nonlymphoblastic leukemia with ocular involvement. Turk J Pediatr 1988;30:181-3.
44. Hicsonmez G, Ozsoylu S, Gurgey A, Zamani VP, Irken G. High-dose methylprednisolone for remission induction in children with acute nonlymphoblastic leukemia. Eur J Haematol 1989;42:498-500.
45. Hicsonmez G, Ozsoylu S, Tuncer A, Ertürk G, Ozbek N, Karadeniz N. Direct morphological evidence of high-dose methylprednisolone-induced maturation of leukemic cells in children with acute nonlymphoblastic leukemia. Exp Hematol 1991;19:232-3.
46. Hicsonmez G, Tuncer AM, Guler E, Tan E, Tekelioglu M. The potential role of high-dose methylprednisolone on the maturation of leukemic cells in children with acute promyelocytic leukemia (APL). Exp Hematol 1993;21:599-601.
47. Hicsonmez G, Tuncer AM, Toksoy HB, Yenicesu I, Cetin M. Differentiation of myeloid leukemic cells induced by short-course high-dose methylprednisolone in patients with different subtypes of acute myeloblastic leukemia. Leuk Lymphoma 1999;33:573-80.
48. Hicsonmez G, Cetin M, Aslan D, Ozyurek E. The role of short-course of high-dose methylprednisolone in children with acute myeloblastic leukemia (FAB M2) presented with myeloid tumor. Pediatr Hematol Oncol 2003;20:373-9.
49. Hicsonmez G, Cetin M, Okur H, Erdemli E, Gürgey A. The potential effect of short-course high-dose steroid on the maturation and apoptosis of leukemic cells in a child with acute megakaryoblastic leukemia. Leuk Lymphoma 2003; 44:1037-42.
50. Martin SJ, Bradley JG, Cotter TG. HL-60 cells induced to differentiate towards neutrophils subsequently die via apoptosis. Clin Exp Immunol 1990;79:448-53.
51. Hicsonmez G, Erdemli E, Tekelioglu M, Tuncer AM, Ozbek N, Cetin M, Cotter TG. Morphologic evidence of apoptosis in childhood acute myeloblastic leukemia treated by high-dose methylprednisolone. Leuk Lymphoma 1996;22:91-6.
52. Blewitt RW, Abbott AC, Bird CC. Mode of cell death induced in human lymphoid cells by high and low doses of glucocorticoid. Br J Cancer 1983;47:477-86.
53. Bird CC, Robertson AMG, Read J, Currie AR. Cytolethal effects of glucocorticoids in human lymphoblastoid cell lines. J Pathol 1977;123:145-56.
54. Cailleteau C, Liagre B, Battu S, Jayat Vignoles C, Beneytout JL. Increased cyclooxygenase-2 and thromboxane synthase expression is implicated in diosgenin-induced megakaryocytic differentiation in human erythroleukemia cells. Anal Biochem 2008;380:26-34.
55. Lotem J, Sachs L. Regulation of bcl-2, bcl-XL and bax in the control of apoptosis by hematopoietic cytokines and dexamethasone. Cell Growth Differ 1995;6:647-53.
56. Lotem J, Sachs L. Control of sensitivity to induction of apoptosis in myeloid leukemic cells by differentiation and bcl-2 dependent and independent pathways. Cell Growth Differ 1994;5:321-7.

57. Kasukabe T, Okabe-Kado J, Hozumi M, Honma Y. Inhibition by interleukin 4 of leukemia inhibitory factor-, interleukin 6-, and dexamethasone-induced differentiation of mouse myeloid leukemia cells: role of c-myc and junB proto-oncogenes. Cancer Res 1994;54:592-7.
58. Hicsonmez G, Cetin M, Tuncer AM, Yenicesu I, Aslan D, Ozyürek E, Unlü S. Children with acute myeloblastic leukemia presenting with extramedullary infiltration: the effects of high-dose steroid treatment. Leuk Res 2004;28:25-34.
59. Hicsonmez G, Karadeniz N, Prozorova ZV, Tuncer AM, Gümrük F, Ertürk G, Gürgey A, Ozsoylu S. High remission rate in acute myeloblastic leukemia in children treated with high-dose methylprednisolone. Eur J Haematol 1992;48:215-20.
60. Sugawara T, Sato A, Shishido T, Okuda M, Kameoka J, Meguro K, Endo K, Yoshinaga K. Complete remission in acute myeloid leukemia after treatment with recombinant human granulocyte-colony stimulating factor and high-dose intravenous methylprednisolone. Br J Haematol 1991;77:561-2.
61. Hicsonmez G, Kale G, Erdem G, Bilgic S, Hazar V. Dramatic effect of high-dose methylprednisolone on orbital granulocytic sarcoma. Pediatr Hematol Oncol 1996;13:187-90.
62. Ozyurek E, Alioglu B, Coskun M, Ozbek N. Successful use of short-course high-dose methylprednisolone in a child with acute myeloblastic leukemia (FAB M2) and myeloid tumor. Leuk Lymphoma 2006;28:97-104.
63. Takeda M, Yamaguchi S, Eguchi K, Kajıume T, Nishimura S, Kobayashi M, Kurisu K. Spinal epidural granulocytic sarcoma in a child precedent to clinical manifestation of acute myeloid lymphoma. Neurol Med Chir. 2009;49:221-4.
64. Hicsonmez G, Cetin M, Tunc B, Tuncer AM, Gümrük F, Yenicesu I. Dramatic resolution of pleural effusion in children with chronic myelomonocytic leukemia following short-course high-dose methylprednisolone. Leuk Lymphoma 1998;29:617-23.
65. Hicsonmez G, Gurgey A, Zamani VP, Yetgin S, Altay C, Ozsoylu S. Poor prognosis in children with acute nonlymphoblastic leukemia in Turkey. Am J Pediatr Hematol/Oncol 1988;10:268-73.
66. Hicsonmez G, Tuncer AM, Saylı T, Güler E, Cetin M, Mufti GJ. High-dose methylprednisolone, low-dose cytosine arabinoside and mitoxantrone in children with myelodysplastic syndromes. Hematol Pathol 1995;9:185-93.
67. Tunc B, Oner AF, Hicsonmez G. The effect of high-dose methylprednisolone on peripheral blood lymphocyte subsets in children with acute leukemia during remission induction treatment. Leuk Res 2003;27:19-21.
68. Vitale C, Cottalasso F, Montaldo E, Moretta L, Mingari MC. Methylprednisolone induces preferential and rapid differentiation of CD34+cord blood precursors toward NK cells. Int Immunol 2008;20:565-75.
69. Suzuki K, Ohishi K, Sekine T, Masoya M, Katayama N. Selective blast cell reduction in elderly patients with acute myeloid leukemia secondary to myelodysplastic syndrome treated with methylprednisolone. Int J Hematol 2007;85:344-9.
70. Shimohakamada Y, Shinohara K, Fukuda N. Remission of acute myeloblastic leukemia after severe pneumonia treated with high-dose methylprednisolone. Int J Hematol 2001;74:173-7.
71. Tuncer AM, Hicsonmez G, Gumruk F, Albayrak D, Duru F, Güzel E, Saylı T. The effect of high-dose methylprednisolone combined chemotherapy on CD34-positive cells in acute lymphoblastic leukemia. Hematol Pathol 1994;8:169-75.
72. Cetin M, Hicsonmez G, Tuncer AM, Kansu E, Canpınar H. The effect of short-course high-dose corticosteroid therapy on peripheral blood CD34+progenitor cells in children with acute leukemia. Exp Hematol 1996;24:1191-4.
73. Tunc B, Oner AF, Hicsonmez G. The effect of short-course high-dose methylprednisolone on peripheral blood CD34+ progenitor cells of children with acute leukemia during remission induction therapy. Turk J Pediatr 2002;44:1-4.
74. Elmas SA, Cetin M, Tuncer M, Hicsonmez G. Myeloprotective effect of short-course high-dose methylprednisolone treatment before consolidation therapy in children with acute myeloblastic leukemia. Am J Hematol 2005;80:1-5.
75. Niemeyer CM, Baumann I. Myelodysplastic syndrome in children and adolescents. Semin Hematol 2008;45:60-70.
76. Creutzig U, Bender- Götze C, Ritter C, Zimmermann M, Stollmann-Gibbels B, Körholz D, Niemeyer C. The role of intensive AML-specific therapy in treatment of children with RAEB and RAEB-t. Leukemia 1998;12:652-9.
77. Morosetti R, Koeffler HP. Differentiation therapy in MDS. Semin Hematol 1996;33:236-45.
78. Tuncer AM, Pagliuca A, Hicsonmez G, Yetgin G, Ozsoylu S, Mufti GJ. Primary myelodysplastic syndrome in children: the clinical experience in 33 cases. Br J Haematol 1992;82:347-53.
79. Hicsonmez G, Cetin M, Yenicesu I , Olcay L, Koc A, Aktas D, Tuncbilek E, Tuncer AM. Evaluation of children with myelodysplastic syndrome: importance of extramedullary disease as a presenting symptom. Leuk Lymphoma 2001;42:665-74.
80. Yenicesu I, Hicsonmez G, Tuncer AM. Effect of short-course high-dose methylprednisolone treatment on serum IL-2 levels in children with myelodysplastic syndromes. Leuk Res 1997;21:789-91.
81. Yamada T, Tsurumi H, Kasahara S, Hara T, Sawada M, Moriwaki H. Immunosuppressive therapy for myelodysplastic syndrome: efficacy of methylprednisolone pulse therapy with or without cyclosporine A. J Cancer Res Clin Oncol 2003;129:485-91.
82. Motoji T, Teramura M, Takahashi M, Oshimi K, Okada M, Kasukabe K, Mizoguchi M. Successful treatment of refractory anemia with high-dose methylprednisolone. Am J Hematol 1990;33:8-12.
83. Motomura S, Motoji T, Okutomi K, Nishikawa T, Kasajima T, Mizoguchi H. Successful treatment of refractory anemia by high-dose methylprednisolone associated with an increment in CD68-positive cells in bone marrow. Am J Hematol 2001;66:80-4.
84. Tuncer AM, Hiçsönmez G, Gümrük F, Saylı T, Güler E, Çetin M, Okur H. Serum TNF-alpha, gamma-INF, G-CSF and GM-CSF levels in neutropenic children with acute leukemia treated with short-course high-dose methylprednisolone. Leuk Res 1996;20:265-9.
85. Olofsson T, Sallerfors B. Modulation of the production of leukemia associated inhibitor (LAI) and its interaction with granulocyte-macrophage colony-forming cells. Exp Hematol 1987;15:1163-7.
86. Ohmori S, Ohmori M, Yamagishi M, Okuma M. MDS-macrophage derived inhibitory activity on myelopoiesis of MDS abnormal clones. Br J Haematol 1993;83:388-91.
87. Hicsonmez G, Tunc B, Olcay L, Tuncer AM. Effect of short-course high-dose steroid therapy in a child with myelodysplastic syndrome. Pediatr Hematol Oncol 2001;18:525-9.
88. Hiçsönmez G, Çetin M, Tuncer AM, Gümrük F, Özbek N, Kara A, Gürgey A. High-dose methylprednisolone for remission induction in hypoplastic acute myeloid leukemia. Eur J Haematol 1995;54:125-6.
89. Bagby GC, Gabourel JD, Linman JW. Glucocorticoid therapy in the preleukemic syndrome (hematopoietic dysplasia): identification of responsive patients using in vitro techniques. Ann Int Med 1980; 92:55-8.
90. Hino K, Nakamaki T. Differentiation therapy for myelodysplastic syndrome. Rinsho Ketsueki 1993;34:283-8.
91. Gumruk F, Tuncer AM, Hiçsönmez G. Pyoderma gangrenosum in a child with myelodysplastic syndrome. Pediatr Hematol Oncol 2000;22:362-4.
92. Pyrstowsky JH, Johnson BU, Bolwell BJ, Lazarus GS. Treatment of cutaneous granulocytic sarcoma in a patient with myelodysplasia. Am J Med 1989;86:477-80.
93. Imai Y, Fukuoka T, Nakatani A, Ohsaka A, Takahashi A. Sustained trilineage recovery and disappearance of abnormal chromosome clone in a patient with myelodysplastic syndrome following combination therapy with cytokines (granulocyte colony-stimulating factor and erythropoietin) and high-dose methylprednisolone. Br J Haematol 1996;93:146-50.
94. George SW, Newman ED. Seronegative inflammatory arthritis in the myelodysplastic syndromes. Semin Arth Rheum 1992;21:345-54.