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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

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Abstract

Several in vitro studies have shown that dexamethasone (Dex) and prednisolone can induce differentiation of some mouse and human myeloid leukemic cells to macrophages and granulocytes. Based on in vitro experiments, we have shown that short-course (3–7 days) high-dose methylprednisolone (HDMP) (20–30 mg/kg/day) treatment can induce differentiation of myeloid leukemic cells in vivo in children with different subtypes of acute myeloblastic leukemia (AML) (AML-M1, -M2, -M3, -M4, -M7). We have also shown that induction of apoptosis of myeloid leukemic cells with or without differentiation is possible by short-course HDMP treatment. In addition, short-course HDMP treatment has been shown to be effective in accelerating leukocyte recovery, possibly stimulating normal CD34-positive hematopoietic progenitor cells. Addition of HDMP to mild cytotoxic chemotherapy (low-dose cytosine arabinoside (LD-Ara-c), weekly mitoxantrone and Ara-c or 6-thioguanine) increased the remission rate (87–89%) and improved the outcome of AML children. We believe that the results of our 17-year clinical experience will provide important benefits to AML patients.

Keywords: High-dose methylprednisolone; Differentiation; Apoptosis; $CD34^+$ cells; Childhood AML; ALL

1. Introduction

Acute myeloblastic leukemia (AML) is characterized by the accumulation of malignant myeloid cells associated with an arrest in different stages of differentiation. In the early 1960s, the establishment of a cell culture system for the clonal development of hematopoietic cells by Sachs and co-workers [1,2] facilitated the demonstration that some mouse myeloid leukemic (Ml) cells could be induced to differentiate into normal macrophages and granulocytes in vitro and in vivo [3–5]. Furthermore, it has been shown that human myeloid leukemia cells can also be induced to differentiate terminally into mature cells in vitro [6,7]. From these results, treatment with differentiation inducers has long been proposed as a promising approach for patients with AML [8–11].

The effect of various agents on the differentiation of myeloid leukemic cells has been extensively studied in vitro [9–11]. The potential effect on induction of differentiation of leukemic cells by retinoic acid (RA), a derivative of Vitamin A, was first demonstrated in cultured HL-60 cells and cells obtained from patients with acute promyelocytic leukemia (APL) [12,13]. The in vitro effect of RA was transformed into a clinical benefit for patients with APL by Huang et al. in 1988 [14]. Since 1991, when all-*trans* retinoic acid (ATRA) became available on the market for clinical use [15], numerous clinical trials have been conducted and it

Abbreviations: AML, acute myeloblastic leukemia; RA, retinoic acid; APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; A_2O_3, arsenic trioxide; Dex, dexamethasone; PB, peripheral blood; BM, bone marrow; MP, methylprednisolone; HIMeg, human megakaryoblastic leukemia cell line; HDMP, high-dose methylprednisolone; G-CSF, granulocyte-colony-stimulating factor; CR, complete remission; Ara-c, cytosine arabinoside; DFS, disease-free survival; ALL, acute lymphoblastic leukemia; WBC, white blood cell

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has been confirmed that ATRA, as a differentiation inducer, is effective in patients only with APL [15–17]. Since 1996, arsenic trioxide (As_2O_3) has been incorporated in treatment of APL patients [18]. In vitro studies have shown that As_2O_3 induces differentiation and apoptosis of APL cell line NB4 and fresh APL cells with t(15;17) dose dependently [19]. Despite the success obtained in APL, the results obtained with ATRA or As_2O_3 in non-APL patients are not encouraging. For this reason enormous efforts have been made to provide effective differentiating agents for AML patients other than APL. Despite the fact that induction of differentiation has been shown to be possible with various inducers in vitro, it is generally considered that no effective agent has yet been provided for clinical use [9–11]. Patients suffering from other subtypes of AML are therefore still looking for a new differentiation-inducing agent which will improve their outcome.

2. The effect of steroid on myeloid leukemic cells in vitro

2.1. In mice

The initial observation of the steroid effect on the murine myeloid leukemia cell line was made by Lotem and Sachs in 1974 [20,21]. Subsequently, a number of their studies have shown that there are some clones of mouse myeloid leukemic cells that can be induced by certain steroid hormones (dexamethasone (Dex), prednisolone) to differentiate normally to mature macrophages and granulocytes in vitro [22–24]. Moreover, some stages of differentiation (induction of C3 and Fc receptors on the cell surface, phagocytosis and secretion of lysozyme) of mouse myeloid leukemic cells which were not induced by the macrophage-granulocyte inducer (MGI) have been induced by Dex [22,24]. Steroid hormones are considered among the most potent differentiating agents.

Subsequent experiments with steroids were carried out in mice by a Japanese group from the Saitama Cancer Center. They have also shown that Dex can induce differentiation of mouse myeloid leukemic cells in vitro and in vivo [25–30]. Abe et al. have also observed that the degree of cell differentiation in various markers (Fc, C3 receptors, lysozyme activity, formation of macrophage) induced by 12 nM 1α,25 dihydroxyvitamin D3 was nearly equivalent to that induced by 1 μM Dex [31]. In addition, changes in phospholipid composition and prostaglandin synthesis during differentiation of cultured mouse myeloid leukemic cells with Dex have been demonstrated [32–33]. Furthermore, with an increase in the concentration of Dex, a complete arrest of mouse myeloid leukemic cells has been observed [34]. Treatment with Dex at a certain dose was also effective in prolongation of the survival time of mice bearing sensitive myeloid leukemic cells [27,34]. Some other effects of steroid on mouse myeloid leukemic cells were reviewed in [35].

Recently, a novel 2-aminosteroid, 2-(4′-methyl-l′piperazinyl)-3α-hydroxyl-5α-androstane-17-one), has also been shown to suppress proliferation, and to induce apoptosis and differentiation of the murine myelomonocytic leukemia cell line (WEHI-3B) toward mature macrophage-like cells in vitro in a dose-dependent manner; it has also been shown to down-regulate expression of c-myc mRNA in WEHI-3B leukemia cells [36]. In addition, a suppression of expression of c-myc protooncogene by Dex has been demonstrated during Dex-induced differentiation of mouse myeloid leukemic cells in vitro [37,38]. Administration of 2-aminosteroid, especially at a high-dose (20 mg/kg), decreased the blast cells in both the peripheral blood (PB) and the bone marrow (BM) of BALB/c mice burdened with WEHI-3B cells in vivo [36].

2.2. In human

The effects of steroid on human myeloid leukemic cells have been demonstrated in vitro by Brandt et al. and Skubitz et al. in 1981 and 1982, respectively [39,40]. In these studies Dex was seen to increase the number of chemotactic receptors in cultures of differentiating human myeloid HL-60 cells in a dose-dependent manner and to potentiate the morphological differentiation of HL-60 cells induced by *N*, *N*-dimethylformamide or *N*-dimethylsulfoxide. In addition, Dex has been shown to promote the chemotactic activity in differentiating HL-60 cells induced by RA and a human T-cell-derived lymphokine [41]. Moreover, Dex markedly enhanced the RA-induced differentiation of HL-60 cells to neutrophils [42]. Recently, it has been demonstrated that different signal transduction pathways are used during differentiation of HL-60 cells by methylprednisolone (MP) or As_2O_3 [43]. In contrast to As_2O_3, MP-induced granulocytic differentiation is related with serine/threonine protein phosphatases type 2A subunit upregulation. As with the RA, the combination of As_2O_3 and MP had also a synergistic effect on differentiation of HL-60 cells [41–43]. Recently, Dex has been shown to induce differentiation and inhibit proliferation of the human megakaryoblastic leukemia cell line (HIMeg) in a dose-dependent manner by Song and Cheng [44]. Interestingly, the synergistic effect of Dex and RA on the differentiation of HIMeg cells was also observed in this study. Moreover, in vitro experiments showed that Dex did not inhibit the RA-induced differentiation and proliferation of t(15;17) NB4 cells [45]. Rather, it showed antiproliferative activity. Interestingly, Nakamaki et al. in 1989 found that Dex at 10^{-6} or 10^{-7} M concentration induced differentiation of leukemic cells in vitro in 10 out of 17 patients with different subtypes of AML (AML-M2, -M4, -M5); they suggested that steroid can be used as the drug for differentiation induction therapy in AML [46]. A novel aminosteroid, 2β-(4′-methyl-l′piperazinyl)-3α,17 β-dihidroxyl-5α-androstane, has been shown to induce differentiation of HL-60 cells dose dependently by He and Jiang in 1999 [47].