ELSEVIER

CYTOKINE

www.elsevier.com/locate/issn/10434666

Cytokine 30 (2005) 213–218

Differential effects of hydroxyurea and zileuton on interleukin-13 secretion by activated murine spleen cells: Implication on the expression of vascular cell adhesion molecule-1 and vasoocclusion in sickle cell anemia ☆

Solo Kuvibidila[a,*], B. Surendra Baliga[b], Renee Gardner[c], Lolie Yu[c], Raj Warrier[c], Maria Velez[c], David Ode[c], Johnson Haynes[b]

[a] *Department of Pediatrics, Division of Research, Louisiana State University Health Sciences Center and the Research Institute for Children 1542 Tulane Avenue, Box T8-1, New Orleans, LA 70112, USA*

[b] *University of South Alabama, College of Medicine, Department of Pediatrics and Comprehensive Sickle Cell Center, Mobile AL, USA*

[c] *Department of Pediatrics, Divisions of Hematology/Oncology, Louisiana State University Health Sciences Center 1542 Tulane Avenue, Box T8-1, New Orleans, LA 70112, USA*

Received 4 October 2004; received in revised form 22 December 2004; accepted 25 January 2005

Abstract

Background: Interleukin-13 (IL-13), a TH2 cytokine, upregulates the expression of vascular cell adhesion molecule-1 on endothelial cells, a factor involved in vasoocclusion in sickle cell disease (SCD). Hydroxyurea improves clinical status of SCD patients in part by induction of fetal hemoglobin. Its effect on IL-13 secretion has not been investigated.
Objective: To determine whether hydroxyurea and zileuton, a hydroxyurea derivative with antiinflammatory properties, affect IL-13 secretion.
Methods: We measured IL-13 in the supernatant of murine spleen cells incubated without and with hydroxyurea, zileuton (10 μg/ml), concanavalin A (2.5 μg/ml), and anti-CD3 (50 ng/ml) ($n = 8$).
Results: Hydroxyurea and zileuton do not affect baseline IL-13 secretion. Unexpectedly, hydroxyurea increases IL-13 levels above baseline (120%, 216.5%, [$p < 0.05$] after 24 h and 48 h, respectively) in lymphocytes activated by anti-CD3, while zileuton reduces them by 59%–78% ($p < 0.005$). In lymphocytes activated by concanavalin A, hydroxyurea and zileuton reduce IL-13 secretion by 24–36% and 50–87%, respectively ($p < 0.05$). Hydroxyurea, but not zileuton, significantly inhibits spleen cell proliferative responses to mitogens ($p < 0.005$).
Conclusion: Data suggest that hydroxyurea up-regulates IL-13 secretion in anti-CD3-activated lymphocytes through gene activation but not by altered cell proliferation. Increased IL-13 secretion may contribute to unresponsiveness of certain SCD patients to hydroxyurea. The potential benefit of zileuton in the management of vasoocclusion is discussed.

Keywords: Hydroxyurea; Zileuton; Sickle cell anemia; Interleukin-13; VCAM-1; Vasoocclusion

1. Introduction

Interleukin-13 (IL-13), a cytokine secreted by TH2 cells, basophils, and mast cells, induces the expression of vascular cell adhesion molecule 1 (VCAM-1) on human

☆ This work was supported by General Funds from the Department of Pediatrics, Divisions of Research and Hematology/Oncology.
* Corresponding author. Tel.: +1 504 896 2744; fax: +1 504 568 3078.
E-mail address: skuvib@lsuhsc.edu (S. Kuvibidila).

1043-4666/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2005.01.010

endothelial cells [1,2]. VCAM-1 expression contributes to the recruitment of inflammatory cells such as eosinophils, monocytes, and memory T cells during infection and inflammation. Patients with sickle cell disease (SCD) have increased expression of VCAM-1 as indicated by a higher concentration of circulating VCAM-1 during stable condition as well as during pain crisis [3–5]. Upregulation of VCAM-1 increases the adherence of sickle reticulocytes and inflammatory cells (monocytes, eosinophils, basophils, and neutrophils) that express alpha-4/beta-1 to endothelial cells [4–6]. Sickle reticulocyte adhesion to vascular endothelial cells has been implicated in sickle cell vasoocclusion [4–6].

Hydroxyurea reduces major complications associated with SCD including pain crisis, blood transfusion needs, and frequency and duration of hospitalization [7–10]. Although increased concentration of fetal hemoglobin is thought to be responsible for clinical improvement, many clinical benefits are observed before a significant increase in hemoglobin levels [7–10]. Recently, Haynes et al. observed that the antiinflammatory agent zileuton, a hydroxyurea derivative used for treatment of asthma, induced fetal hemoglobin synthesis in the K562 erythroid cell line and in human erythroid progenitors as well as hydroxyurea [11]. Zileuton was also shown to attenuate the retention of sickle red blood cells in rat lungs [12]. These observations imply that zileuton may also have some beneficial effects in the management of vasoocclusion in patients with SCD.

We hypothesize that hydroxyurea and zileuton may decrease complications associated with inflammation in sickle cell anemia without significant changes in fetal hemoglobin concentration by downregulation of certain cytokines, such as IL-13, that modulate the expression of VCAM-1. The effect of either drug on IL-13 secretion has not been previously investigated. The current study was therefore designed to determine whether hydroxyurea and its derivative zileuton, alter secretion of IL-13 by murine spleen cells.

2. Results

2.1. Levels of IL-13 in supernatant

At the four time points studied, hydroxyurea and zileuton had no significant effect on baseline IL-13 levels in murine spleen cells (Fig. 1A), or in cells that were activated by concanavalin A (Fig. 1B) or anti-CD3 (Fig. 1C) cells for 6 h. At 12 h, whereas hydroxyurea had no significant effect on IL-13 levels in the supernatant of concanavalin A or anti-CD3-stimulated cultures, zileuton reduced the cytokine levels by 50% (concanavalin A, $p = 0.05$, anti-CD3, $p < 0.05$, Student's t-test). When cells were incubated with concanavalin A for 24 h and 48 h, hydroxyurea decreased IL-13 levels by 23% and 36% ($p < 0.05$), respectively, compared with 75.3% and 86.6% for zileuton (Fig. 1B, $p < 0.005$). Interestingly, hydroxyurea significantly increased IL-13 levels in anti-CD3-activated cells at 48 h (216.5% of baseline) ($p < 0.05$) and non-significantly at the 24 h time point. In contrast to hydroxyurea, zileuton decreased IL-13 levels by 58.7% at 24 h and 78% at 48 h ($p < 0.005$) in anti-CD3-activated cells. In the absence and presence of hydroxyurea and zileuton, spleen cell activation by concanavalin A and anti-CD3 antibody was associated with a significant increased secretion of IL-13 in a time dependent fashion ($p < 0.05$).

2.2. Lymphocyte proliferation

As expected due to its inhibitory effect on ribonucleotide reductase [13], hydroxyurea significantly reduced lymphocyte proliferative responses to the three mitogens (Fig. 2, $p < 0.005$). In contrast to hydroxyurea, zileuton did not significantly affect spleen cell proliferation in response to any of the mitogens studied.

3. Discussion

Our data show that hydroxyurea does not negatively affect the production of IL-13 by anti-CD3-treated spleen cells. In fact, it upregulates the secretion of this cytokine, especially at 48 h. In concanavalin A-activated cells, hydroxyurea slightly decreased IL-13 secretion, but the decrease was less than that observed with zileuton. The differential effect of hydroxyurea on IL-13 secretion in concanavalin A-treated versus anti-CD3-treated cells may be partially due to the fact that concanavalin A also induces apoptosis [14]. Additionally, while anti-CD3 antibody activates T lymphocytes through the T cell receptor (TCR)/CD3 complex, concanavalin A non-specifically binds to lymphocytes, monocytes, and non-immune cells through carbohydrates (alpha-mannosyl residues on cell membrane glycoproteins and glycolipids) [15–17]. The possible role of hydroxyurea in increasing concanavalin A binding to its receptor on lymphocytes and further promoting apoptosis requires further investigation.

Despite the structural similarities of hydroxyurea and zileuton, they appear to have different effects on T cell function. The difference in molecular weight (269 for zileuton and 76.6 for hydroxyurea) is not the reason for differential effects of hydroxyurea and zileuton on IL-13 secretion because hydroxyurea was associated with the expected inhibitory effect on DNA synthesis [13]. The mechanisms of altered IL-13 secretion by hydroxyurea and zileuton in activated spleen cells are not related to cell proliferation. They are very likely due to modulation of gene expression (up-regulation by hydroxyurea and down-regulation by zileuton). Experiments are planned to elucidate these mechanisms.