



Mutagenicity of hydroxyurea in lymphocytes from patients with sickle cell disease

André Salim Khayat¹, Adriana Costa Guimarães¹, Plínio Cerqueira Cardoso¹,
Patrícia Danielle Lima de Lima¹, Marcelo de Oliveira Bahia², Lusânia M. Greggi Antunes³
and Rommel Rodríguez Burbano¹

¹Universidade Federal do Pará, Centro de Ciências Biológicas, Departamento de Biologia,
Belém, PA, Brazil.

²Universidade Federal do Pará, Centro de Ciências Biológicas, Departamento de Patologia,
Belém, PA, Brazil.

³Faculdade de Medicina do Triângulo Mineiro, Departamento de Ciências Biológicas,
Uberaba, MG, Brazil.

Abstract

Hydroxyurea is commonly used in the treatment of myeloproliferative diseases and in patients with sickle cell disease (SCD). The use of this antineoplastic agent in patients with SCD is justified because of the drug's ability to increase fetal hemoglobin levels, thereby decreasing the severity of SCD. However, high doses or prolonged treatment with hydroxyurea can be cytotoxic or genotoxic for these patients, with an increased risk of developing acute leukemia. This danger can be avoided by monitoring the lymphocytes of patients treated with hydroxyurea. Cytogenetic tests are important endpoints for monitoring the physiological effects of physical and chemical agents, including drugs. In this work, we assessed the genotoxicity of hydroxyurea in short-term cultures of lymphocytes from SCD patients. Hydroxyurea was not cytotoxic or genotoxic at the concentrations tested in the G2 phase of the cell cycle. These results support the use of hydroxyurea in the treatment of SCD, although further work is necessary to understand the effects of this drug *in vivo*.

Key words: hydroxyurea, sickle cell disease, mutagenesis.

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Introduction

Hydroxyurea (HU) is an antineoplastic agent that acts on the enzyme ribonucleotide reductase enzyme to inhibit the conversion of ribonucleotides into deoxyribonucleotides, thereby limiting DNA biosynthesis. By preventing impeding deoxyribonucleotide synthesis, HU acts as a cytotoxic and antineoplastic agent that specifically affects the S phase and interrupts the cell cycle in the G2 and S phases (Yarbro, 1992; Donehower, 1992).

This mechanism has led to clinical applications in a variety of infect-contagious diseases, neoplasias and hematological disorders (De Vita *et al.*, 1997; Mayhew *et al.*, 1999; De Montalembert, 1999; Melo *et al.*, 2000). Hydroxyurea is useful for the treatment of patients with sickle cell anemia, a hemolytic anemia caused by erythro-

cyte deformation (sickling) (Reid *et al.*, 1995), since it increases fetal hemoglobin production (HbF), thereby boosting the absolute number and proportion of HbF-carrying reticulocytes and elevating the HbF concentration in individual cells. The increase in HbF reduces the tendency of abnormal hemoglobin (HbS) to polymerize and decreases the formation of abnormal cells characteristic of sickle cell anemia (Donehower, 1992).

Despite its widespread clinical use, HU can damage DNA (genotoxic action) and produce gene and chromosomal mutations (Donehower, 1992). In this work, we assessed the cytotoxicity (mitotic index) and the genotoxicity (chromosomal aberrations and polyploidy) of HU in lymphocytes from sickle cell anemia patients treated with this compound.

Materials and Methods

Peripheral blood samples were collected from eight patients (five men and three women) by venipuncture. The

subjects were carriers for sickle cell anemia who were not yet on HU therapy, but who began using HU immediately after blood collection. The study protocol was approved by the Medical Ethics Committee of the Pará State Hemotherapy and Hematology Center (HEMOPA).

Lymphocytes isolated from the blood samples were cultured at 37 °C in HAM-F10 culture medium, complemented with streptomycin (10 µg/mL), penicillin (5 µg/mL), 19.2% fetal calf serum and 4% phytohemagglutinin, the latter used as a lymphocyte mitotic stimulator.

Hydroxyurea was obtained from 500 mg gelatinous capsules of Hydrea®, the contents of which were prepared in bi-distilled sterile water.

The G2 phase of the cell cycle is the most suitable phase for accessing the mutagenicity of HU, because this drug is S-phase dependent and generally inhibits the cell cycle when added to phases prior to G2 (Lindenbahn and Schubert, 1983). Lymphocytes were exposed to HU (50 µg/mL and 100 µg/mL) 69 h after the onset of mitogenesis in complete HAM-F10 medium. After 1 h, the cells were incubated with colchicine (2 h) to arrest them in metaphase, and then fixed with Carnoy fixative (Acetic acid: Methanol 1:3 v/v). Peripheral blood lymphocytes in metaphase were prepared essentially as described by Moorhead *et al.* (Moorhead *et al.*, 1960), with modifications introduced by Human Cytogenetics Laboratory. at UFPA

The preparations were examined by light microscopy to assess the structural or numerical chromosomal alterations present (Rabello-Gay *et al.*, 1991).

The frequency of chromosomal aberrations (100 metaphases/culture) and the mitotic index (number of metaphases in 2,000 lymphoblasts/culture) of cells treated with HU (50 and 100 µg/mL) were determined, and compared with their respective control groups (lymphocytes cultures not exposed to HU).

The frequencies of aberrations in lymphocytes treated with HU compared to the non treated controls were compared using Student's T-test (Ayres *et al.*, 2000).

The mitotic indices of HU treated and non-treated lymphocytes were compared using the F test (ANOVA). The level of significance was set at $p < 0.05$ for all analyses (Ayres *et al.*, 2000).

Results and Discussion

In recent years, HU has become one of the most widely used drugs for the treatment of myeloproliferative disorders and some forms of solid tumours, as well as other non-malignant diseases such as sickle cell anemia (Kantarjian *et al.*, 1993; Hehlmann *et al.*, 1994; O'Brien *et al.*, 1996).

In a pilot study using lymphocytes from healthy individuals (non SCD), high concentrations of HU

(>100µg/mL) caused severe cytotoxicity in all phases of the cell cycle (unpublished results in this paper). This extensive damage made it impossible to access chromosomal alterations, while lower concentrations (<50µg/mL) caused no significant chromosomal aberrations. Based on these findings, we examined the effects of HU on peripheral blood lymphocytes from SCD patients. Two concentrations of HU (50 and 100 µg/mL) were tested in the G2 phase of the cell cycle.

The mitotic index of HU-treated lymphocytes was not significantly different from that of non tested cells (Table 1). This result may reflect the S-phase dependency of the drug and patients' hematological condition that the lymphocytes did not proliferate as much as expected. It is also possible that proliferation may have occurred only in cells resistant to the effects of HU.

Analysis of the chromosomal alterations in metaphasic lymphocytes from anemic patients (Figure 1) also showed significant differences compared to the controls (Table 2). Curiously, non-treated lymphocytes from patients IV, VI, and X contained from 2-3 chromosomal breaks, suggesting possible endogenous chromosomal instability in these individuals, which may indicate that these patients should not to be treated with this drug. Such alter-

Table 1 - Mitotic indexes in cultured peripheral blood lymphocytes from of SCD patients.

Patients	Hydroxiurea (µg/mL)		
	0 ¹	50	100
II	1.95	1.2	1.05
III	1.65	2.1	0.75
IV	1.9	1.35	1.6
VI	0.55	0.55	0.4
VII	1.6	1.35	1.75
VIII	0.55	0.3	0.6
IX	0.45	0.6	0.3
X	1.6	0.9	0.7

¹Control.



Figure 1 - Chromosomal alterations in metaphasic lymphocytes from anemic patients: (a) chromatid gap in chromosome 1 and (b) chromatid break in chromosome 16.

Table 2 - Number and type of chromosomal alterations in metaphase in cultured peripheral blood lymphocyte from SCD patients.

Patients	Hydroxyurea ($\mu\text{g/mL}$)		
	0 ¹	50	100
II	2ctg 1pol	8ctg	6ctg
III	5ctg	3ctg	2ctg
IV	3ctb 2ctg	0	0
VI	2ctb 2ctg	1ctb 2ctg	1ctb
VII	1ctg	0	1ctg
VIII	0	0	0
IX	0	0	0
X	2ctb 2ctg 1csg	1ctb 2ctg	3ctb

¹Control; ctg-chromatic gap; pol-polyploidy; ctb-chromatic break; csg-chromosomal gap.

ations were not seen in cells from healthy individuals (unpublished observations).

In conclusion, these results support use of HU in the treatment of SCD since no adverse cytotoxic or genotoxic effects were observed under the conditions used here. However studies *in vivo* are required to confirm these data.

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