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P1474 MOLECURAL MECHANISM OF CHEMOTHERAPEUTIC HYDROXYUREA IS MEDIATED BY NOS2

Topic: 26. Sickle cell disease

Teodora Dragojević[1], Olivera Mitrović Ajtić[1], Miloš Diklić[1], Tijana Subotički[1], Dragoslava Đikić[1], Emilija Živković[1], Vladan Čokić[1], Milica Vukotić[1]

[1] *Department of Molecular Oncology, Institute for Medical Research, National Institute of Republic of Serbia, Belgrade, Serbia*

Background:

: Hydroxyurea (HU) is a chemotherapeutic agent that reduces ribonucleotide reductase, stops DNA synthesis and repar, and therefore causes cell proliferation inhibition and apoptosis. Due to its cytostatic properties, HU is frequently used for treatment of myeloproliferative neoplasms, ovarian cancer, and sickle cell anemia. Nitric oxide (NO), produced by nitric oxide synthase (NOS) enzymes is a potent signaling molecule involved in blood flow regulation, neutrotransmission, and immunity. Although HU treatment increases NO levels, up to date it is not clear whether it originates from activation of NOS enzymes or HU degradation.

Aims:

The aim of this study was to determine the involvement of NOS2 enzyme in the cytostatic effect of HU.

Methods:

To examine the involvement of the NOS2 enzyme in the molecular mechanism of HU, we treated erythroleukemic HEL92.7.1 cells with pan-selective NOS inhibitor L-NAME (200μM, 1mM, and 5mM), NOS2 specific inhibitor 1400W (1, 10, and 100μM), or NOS2/NOS3 inhibitor DPI (1, 5, and 10μM), in combination with hydroxyurea (200μM), and monitored their effect on proliferation and cell cycle. Immunocytochemistry for the proliferation marker Ki67 was performed to assess proliferation, while cell distribution in cell cycle phases was determined by flow cytometry after propidium iodide staining. Colony forming assay have been performed with the bone marrow cells of Nos2 null mice after oral HU treatment to corroborate the data obtained by enzymatic inhibition.

Results:

In this study, we demonstrated that treatment of HEL92.7.1 cells with HU induces a dose-dependent increase in NOS2 protein levels and two products of the enzyme NOS - NO and citrulline. HU-induced citrulline levels can be reduced by treatment with the NOS inhibitor L-NAME, indicating that NO is produced *de novo* by the NOS enzyme rather than HU degradation. Inhibition of the NOS2 enzyme by L-NAME, 1400W, or DPI was sufficient to abolish HU-mediated inhibition of proliferation. While HU increased the number of cells in S-phase of the cycle at the expanse of the G0/G1 due to blocked DNA synthesis, combined treatment with HU and L-NAME or DPI inhibitor resulted in decreased G0/G1 phase and increased S and G2/M phases pointing to increased proliferation. These data indicate that the cytostatic properties of HU are mediated by the NOS2 enzyme. A colony formation assay showed that Nos2 deficient bone marrow cells isolated from mice treated orally with HU (200mg/kg) formed significantly more erythroid colonies (BFU-E and CFU-E) and granulocyte/macrophage progenitors (CFU-GM) compared to HU treated wild-type and untreated Nos2 null mice showing the involvement of Nos2 in the molecular mechanism of HU *in vivo*.

Summary/Conclusion:

Our results show that HU induces the enzymatic activity of the NOS2 protein which in turn is involved in the HU regulation of proliferation and cell cycle. Comprehensive knowledge of the molecular mechanism of HU might help to improve its beneficial properties and decrease adverse effects.