WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 8, 1504-1517. Review Article ISSN 2277– 7105

# HYDROXYUREA: ITS THERAPEUTIC POTENTIAL AND TOXICITY

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Article Received on
21 June 2016,

Revised on 11 July 2016,
Accepted on 31 July 2016

DOI: 10.20959/wjpr20168-6842

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## ABSTRACT

Hydroxyurea (HU) is an antineoplastic drug. It has widespread usage in the treatment of both malignant and nonmalignant diseases like Sickle-cell anaemia and HIV infections. It acts on the enzyme ribonucleotide reductase to inhibit the conversion of ribonucleotides into deoxyribonucleotides, thereby limiting DNA biosynthesis. The principal use of HU has been as a myelosuppressive agent in the treatment of myeloproliferative syndromes, particularly chronic myelogenous leukemia and polycythemia vera. Although treatment of chronic myelogenous leukemia with HU for many years has been reserved for patients whose disease was no long irresponsive to busulfan. 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. Studies showed that HU causes rapid cell death through the initiation of uncontrolled free radical chain reaction. Free radical reactions are able to devastate cellular metabolism quickly by inactivating enzymes, cross-linking DNA and altering membrane function through lipid auto-oxidation. Despite being very frequently used in the treatment of several malignant and non-malignant diseases, prolonged use of HU has been reported to cause mutation and clastogenic effects in several *in vitro* systems, as well as cytogenetic damage in exposed mice. It has also shown a wide variety of toxic effects, like induction of chromosomal aberrations and cytotoxicity, as well as a well established genotoxic activity in cell culture and rodent models.

**KEYWORDS:** Hydroxyurea, antineoplastic drug, ribonucleotide reductase, cytotoxic, genotoxic.

## INTRODUCTION

Hydroxyurea (HU) is a well known drug used in the treatment of several malignant and non malignant diseases. It is also known as *N*-(Aminocarbonyl) hydroxylamine; carbamohydroxamic acid; carbamohydroximic acid; carbamoyl oxime; HU; hydroxycarbamide; hydroxycarbamine and hydroxylurea. HU has been prepared by the reaction of calcium cyanate with hydroxylamine nitrate in absolute ethanol and by the reaction of potassium cyanate and hydroxylamine hydrochloride in aqueous solution. HU has also been prepared by converting a quaternary ammonium anion exchange resin from the chloride form to the cyanate form with sodium cyanate and reacting the resin in the cyanate form with hydroxylamine hydrochloride.[1] HU is primarily used in patients with chronic granulocytic leukemia although occasional responses are seen in melanoma and other solid tumors.[2] The principal use of HU has been as a myelosuppressive agent in the treatment of myeloproliferative syndromes, particularly chronic myelogenous leukemia and polycythemia vera.[3] HU is a cytotoxic, antimetabolic and antineoplastic agent which is being used for several decades to treat a variety of medical disorders, most notably myeloproliferative neoplasms[4], chronic myelogenous leukemia[5] and HIV.[6] The efficacy of HU for these varied medical conditions is due to its mechanism of action as a potent inhibitor of ribonucleotide reductase (RNR), a ubiquitous intracellular enzyme that converts ribonucleotides to deoxyribonucleotides, which are required for DNA synthesis and repair.[7] This enzyme is synthesised in low amounts and is the only highly regulated enzyme involved in the conversion of ribonucleotide precursors to DNA.[8] Thus, it forms the rate-limiting step in the *de novo* synthesis of DNA. HU is a chemically simple antimetabolite, which is cytostatic by inhibiting ribonucleotide reductase. HU was initially synthesized over 120 years ago in 1869 by Dresler and Stein (Germany) from hydroxylamine and hydrogen cyanate, but its potential biological significance was not recognized until 1928. In the late 1950s, the drug was evaluated in a large number of experimental murine tumour systems and shown to be active against a broad spectrum of tumours. It has also been utilized for the treatment of sickle cell disease (SCD). It has many characteristics of an ideal drug for Sickle cell anemia (SCA) and provides therapeutic benefit through multiple mechanism of action. HU may be given to children and adults with SCD for an extended period of time or for repeated cycles of therapy and its beneficial effect in SCD has been associated with its capacity to induce fetal hemoglobin synthesis.[9] HU is also used in the treatment of HIV infection in combination with nucleoside analogues, 2’3’-didehydro-3’ deoxythymidine (D4T), 2’3’-dideoxyinosine or abacavir.[10] HU has been used, or investigated for use, in the treatment of

a number of diseases like- Myeloproliferative syndromes, Sickle-cell haemoglobinopathy, with didanosine in the treatment of HIV/AIDS, Psoriasis, Solid tumours etc.

**MYELOPROLIFERATIVE SYNDROMES**

Myeloproliferative disorders (MPD) are group of bone marrow diseases, including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF), with various degrees of changes in the myelopoiesis and circulating number of erythrocytes and platelets.[11] HU as a chemotherapeutic agent has had a long history in oncology. Its primary use as a chemotherapeutic agent has been for leukemia and polycythemia diseases. HU has been widely used for the treatment of human malignancies, particularly chronic myelogenous leukaemia (CML) and other myeloproliferative neoplasms (MPN).[12] CML is the only MPN that is characterized by the chromosomal translocation t (9;22), BCR–ABL fusion gene. The most commonly recognized mutation in the remainder of the MPN is Janus kinase 2 (JAK2) V617F. It is present in greater than 90% of patients with PV and approximately half of those with primary myelofibrosis PMF or ET.[13] This mutation substitutes phenylalanine for valine at position 617 in the JH2 domain (Val617Phe, V617F) of exon 14, leading to constitutive action of the JAK-STAT and other pathways resulting in uncontrolled cell division.[14] The first drug found to be effective in the treatment of CML was busulfan. In a multicentric randomized study conducted by Helhmann et. al., a comparative study between these two drugs i.e. busulfan and HU was conducted among patient and found significant result in the favour of HU. It has proved to be an effective radiation sensitiser and has been employed in this role in the treatment of head and neck cancer.[15] HU has also been used with some success in advanced cervical carcinoma, producing an increase in response and survival with concurrent therapy.[16]

Increased red blood cell production in PV leads to an increased red cell mass and increased blood viscosity. This inturn can lead to arterial or venous thrombosis, bleeding or both.[17] PV typically presents at a median age of 60 years and is characterized by the occurrence of vascular complications.[18] Over time, PV may undergo hematologic evolution to myelofibrosis (MF), acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Although the mechanisms behind these evolutions remain unclear, there are some evidences suggesting that PV therapy, such as radioactive phosphorus or alkylating agents, may increase the frequency of progression.[19] HU and interferon alfa (IFN-α) are recommended as first-line treatment in high-risk patients with PV.[20] HU displays a good efficacy and

tolerability profile and is associated with significant reductions in thrombotic complications.[21]

ET, previously called hemorrhagic thrombocythemia, was first described by Epstein and Goedel in 1934. ET is characterized by a sustained clonal proliferation of megakaryocytes in the bone marrow[22], with a peripheral blood platelet count greater than 600 x $10^9$/L. This platelet count threshold has been decreased to greater than 450 x $10^9$/L in the most recent WHO classification.[13] A randomized study has demonstrated that HU decreases the risk of thrombosis in high-risk patients who have ET from 24% to less than 4% (P = 0.003), compared with no treatment, when the platelet count is decreased to less than 600 x $10^9$/L.[23]

**SICKLE-CELL HAEMOGLOBINOPATHY**

Sickle cell disease is a genetic disorder that decreases life expectancy by 25 to 30 years and affects approximately 100,000 Americans.[24] Sickle cell disease refers to a group of disorders in which the red blood cell undergoes sickling when deoxygenated. The abnormality was subsequently identified as the result of an exchange of the amino acid valine for glutamine in the β-globin chain of the hemoglobin molecule at sixth position. This abnormal hemoglobin becomes polymerized, causing the red blood cell to assume a sickle shape and making the cell both rigid and fragile. These distorted cells obstruct the blood vessels and may disrupt endothelial cell function, leading to tissue hypoxia and clinical complications. Patients with sickle cell disease experience both chronic and episodic pain and have a reduced quality of life.[25] Painful crisis is the most common reason for emergency department use by patients with sickle cell disease.[26] HU is the only drug approved by the Food and Drug Administration (FDA) in February 1998 for the treatment of sickle cell disease. In 2002, The National Heart Lung and Blood Institute issued a recommendation that practitioners should consider using HU daily in selected patients with sickle cell disease. Since HU increases fetal hemoglobin (HbF) production, 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.[12] The initial pilot and Phase I/II studies, the clinical and laboratory benefits of HU for patients with SCA have been repeatedly and consistently demonstrated. The hematological effects of HU therapy are important for obtaining clinical benefits, but also are the parameters used for dose escalation to maximum tolerated dose (MTD). Laboratory effects include a predictable rise in the

hemoglobin concentration, %HbF and mean corpuscular volume along with a concurrent decrease in absolute reticulocyte count, total leukocyte count and absolute neutrophil count. These laboratory values, in addition to examination of the peripheral blood smear for erythrocyte morphology, are used to escalate patients to MTD and also can be used to monitor medication adherence.[27] The clinical efficacy of HU therapy for patients with SCA was most notably demonstrated in the Multicenter Study of HU in Sickle Cell Anemia, an NHLBI-funded phase III double-blinded, placebo-controlled randomized trial of 299 adults with severe SCA, which was halted early due to highly significant reductions in the time to first painful vaso occlusive event. Subjects randomized to the HU treatment arm also showed a significant reduction in incidence of painful events, acute chest syndrome, hospitalizations and number of blood transfusions.[28]

**HU IN THE TREATMENT OF HIV/AIDS**

A principal component of the treatment of patients infected with HIV is the administration of nucleoside analogues such as zidovudine and didanosine. However, the therapeutic benefits of these compounds are temporary and improved therapy is urgently needed. *In vitro* studies have shown that HU inhibits HIV replication.[29] This effect is greatly enhanced when HU is used in combination with nucleoside analogues, particularly didanosine. The antiretroviral effects of HU are because of the depletion of intracellular deoxyribonucleotides. This depletion further permits increased cellular uptake of nucleoside analogues. HU is an inhibitor of the enzyme ribonucleoside diphosphate reductase[30] that catalyzes the conversion of ribonucleotides to deoxyribonucleotides, an essential step in DNA synthesis. This ability of HU to deplete cellular pools of deoxyribonucleotides suggested that it might enhance the activity of nucleoside analogues by increasing their ability to compete with endogenous nucleotides for incorporation into the human immunodeficiency virus-type 1 (HIV-1) chain.[31,32] The ability of this drug to reach the central nervous system (CNS) is of special interest, as the brain is a site of infection, viral replication and sanctuary for HIV-1. Certain drugs may enter the brain either directly across the blood–brain barrier (BBB), located at the level of the cerebral capillary endothelial cells and/or indirectly across the blood–cerebrospinal fluid (CSF) barrier (choroid plexuses and arachnoid membrane) into the CSF and then enter the subependymal brain tissue. It is known that hydroxyurea is distributed into human CSF and is transported from the CSF to subependymal brain sites.[33,34] HU is a member of the family of cellular dNTP modulators. This drug is a well-studied compound that has been widely used in clinical therapy. It has been shown to be readily absorbed after

oral ingestion, to be rapidly distributed in the body fluids including the cerebrospinal fluid, and to enter cells by passive diffusion very efficiently.[12] Indirect inhibition of reverse transcriptase and impairment of HIV-1 DNA synthesis by HU were observed in experiments in the absence of apparent toxic effects and resulted in the formation of incomplete HIV-1 DNA. DNA of this nature is expected to be labile and rapidly degraded in peripheral blood lymphocytes (PBLs). Therefore, data suggest that there is a class of anti-HIV-1 drugs able to modulate cellular dNTP pool at pharmacological ranges. Blocking the activity of a cellular enzyme instead of a viral enzyme would have the advantage to reduce the possibility of viral escape since cellular proteins do not mutate in physiological conditions, in contrast to the high variability of HIV-1 proteins. Furthermore, by depleting the cellular dNTP pool, HU is expected to increase the therapeutic effect of nucleoside analogs 3'-azido-3'- deoxythymidine, dideoxyinosine, or dideoxycytosine, which act as competitors of cellular dNTP.[31] Several clinical trials have been performed to evaluate the effects of HU alone.[35,36] and combined administration of HU and nucleoside analogues in patients with HIV.[37,38] Overall, the combination therapy is well tolerated and accompanied by a significant reduction in plasma viral load that is related to HU dosage. Monotherapy with HU has not been shown to be beneficial in patients with HIV infection.

## FREE RADICALS AND ROS GENERATION

The term ‘reactive oxygen species’ (ROS) applies to any mixture of molecules, ions and free radicals containing derivatives of molecular oxygen that are more reactive than oxygen itself. The ROS formed in living cells commonly include hydrogen peroxide, hydroxyl radical and superoxide anion. The normal process of respiration in mitochondria is a major source of ROS, and production of ROS is enhanced when mitochondrial function is disturbed during apoptosis. During apoptosis, and also in some types of necrotic cell death, unusually large amounts of ROS can be released and can contribute, by extensive oxidation of macromolecules, to the killing of cells. In some types of apoptosis, ROS also serve essential signaling functions.[39] HU has been of scientific and clinical interest for more than 100 years.[40] HU is structurally related to hydroxamic acids, known as metal chelators and microbial siderophores characterised by diverse biological activities such as antibacterial, antifungal, antitumor and anti-inflammatory activities.[41, 42, 43] Structural and electronic properties and chemical fate of free radicals generated from HU and its methylated analogues *N*-methylhydroxyurea (NMHU) and *O*-methylhydroxyurea (OMHU) are of utmost importance for their biological and pharmacological effects.[44] The pharmacological effects

of HU and its derivatives has been attributed not only to enzyme inhibition or metal chelation, but also to its ability to serve as nitroxyl (HNO/NO-) and nitric oxide donors.[28] It has been reported that HNO and NO are formed upon oxidation of HU chemically,[45] enzymatically,[46, 47] or by the combination of heme proteins and $H_2O_2$.[48] Nitric oxide formation from HU requires a Three electron oxidation, which may proceed either through the nitroxide radical or a C nitroso intermediate.[49, 46] The remainder of the HU molecule may decompose into formamide or carbon dioxide and ammonia, depending on the conditions and type of oxidant employed.[50] However, the mechanism for the formation of reactive nitrogen species from HU is not clear and requires further elucidation. The most common ROS released from the respiratory chain is superoxide.[51] Studies demonstrate a connection between toxic activity and membrane stress, together with earlier results showing that δcydB is substantially less sensitive to HU treatment, suggest that HU-induced changes in membrane composition and function may lead to superoxide formation. Superoxide is converted to hydrogen peroxide ($H_2O_2$) that can react with ferrous iron to produce $OH^{\cdot}$.[52] Bryan and colleagues[53], propose the mechanism whereby HU treatment results in cell death. HU inhibits RNR, leading to dNTP depletion and an arrest of normal DNA replication. dNTP depletion results in the activation of toxins MazF and RelE. The activity of these toxins produces improperly translated proteins, leading to membrane stress and activation of membrane stress responses. These effects alter the properties of one of the cell's two terminal cytochrome oxidases in the electron transport chain, causing an increase in the production of superoxide, which is then converted to hydrogen peroxide. The hydrogen peroxide reacts with free ferrous iron, resulting in the formation of $OH^{\cdot}$, which contribute to cell death.

## TOXICITY

Despite its beneficial effect, it is known that the treatment with HU is associated with side effects such as cytotoxicity and myelosuppression. In the literature, the HU ability to cause cancer is controversial and the long-term efficacy and safety of HU in treating patients with SCA remain incompletely defined.[54] Some studies have shown that hydroxyurea is genotoxic while other studies suggest that HU has low mutagenicity *in vivo*.[55,56] Some reports related that HU acts as a competitive inhibitor of catalase mediated hydrogen peroxide decomposition and this effect could be related to *in vivo* toxicity.[57] In eukaryotic cell, a study of HU, in mammalian (V79) cells, reported microsomal activation-dependent mutagenicity and found that the addition of catalase inhibited microsome mediated mutagenicity, indicating that hydrogen peroxide was involved in the formation of mutagenic

DNA lesion.[58] Side effects of HU therapy in young patients with SCA are usually mild and most children tolerate HU without difficulty. Ocassionally patients will describe headach or mild gastrointestinal symptoms, including abdominal discomfort or nausea.[59] Some children develop dermatologic changes, including skin hyperpigmentation or darkening of the nails (melanonychia), which are sporadic and not dose dependent.[60] The greatest fear regarding HU for patients with SCA, particularly children who might receive decades of treatment, is the possibility of mutagenicity and carcinogenicity. Because HU reduces intracellular deoxynucleoside triphosphate pools,[61, 62] it affects not only DNA Synthesis required for cell division but also DNA repair. *In vitro*, DNA damage that develops spontaneously or from environmental mutagens cannot be fully repaired in the presence of HU, possibly leading to *in vivo* accumulation of somatic mutations and chromosomal damage.[63] HU toxicity is dose-dependent: a high frequency of toxic events is observed at the doses usually employed in anticancer therapy (40–80 mg/kg as single or repeated administration, depending on the protocol adopted). However, at the lower doses used in non-neoplastic diseases, usually 15 mg/kg per day, HU is surprisingly well tolerated; there are reports of long-term (up to 1 year) treatments in AIDS patients without significant toxic effects.[64, 37, 35, 38]

## CONCLUSION

HU being used in the treatment of both malignant and non-malignant diseases, there are various good outcomes in the favour of this drug. It is the only known medicine used in the treatment of SCA. Preliminary results are promising that in addition to the well-documented clinical effects of HU on reducing painful events and hospitalizations, HU also reduces mortality and may have benefits for preventing chronic organ damage associated with SCA. Different studies showed the prolong use of HU causes free radical generation and ROS production which ultimately leads to cell death or apoptosis and other various forms of chromosome damage, measured as chromosome breaks and chromatid breaks on standard karyotype analysis, as well as double-stranded DNA breaks observed as micronucleated reticulocytes and specific DNA damage.

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