Benzylacyclouridine Reverses Azidothymidine-Induced Marrow Suppression Without Impairment of Anti-Human Immunodeficiency Virus Activity

By Paul Calabresi, Alfredo Falcone, Marty H. St. Clair, Michael C. Wiemann, Shih H. Chu, and James W. Darnowski

Increased extracellular concentrations of uridine (Urd) have been reported to reduce, in vitro, azidothymidine (AZT)-induced inhibition of human granulocyte-macrophage progenitor cells without impairment of its anti-human immunodeficiency virus (HIV) activity. Because of the clinical toxicities associated with chronic Urd administration, the ability of benzylacyclouridine (BAU) to effect, in vivo, AZT-induced anemia and leukopenia was assessed. This agent inhibits Urd catabolism and, in vivo, increases the plasma concentration of Urd in a dose-dependent manner, without Urd-related toxicity. In mice rendered anemic and leukopenic by the administration of AZT for 28 days in drinking water (1.5 mg/mL), the continued administration of AZT plus daily BAU (300 mg/kg, orally) partially reversed AZT-induced anemia and leukopenia (P < .05), increased peripheral reticulocytes (to 4.59%, P < .01), increased cellularity in the marrow, and improved megaloblastosis. When coadministered with AZT from the onset of drug administration, BAU reduced AZT-induced marrow toxicity. In vitro, at a concentration of 100 μM/L, BAU possesses minimal anti-HIV activity and has no effect on the ability of AZT to reverse the HIV-induced cytopathic effect in MT4 cells. The clinical and biochemical implications of these findings are discussed.

AZIDOThYMIDINE (AZT), a thymidine (dThd) analogue that is a potent inhibitor of retroviral reverse transcriptase,1 is the only established effective treatment for patients with acquired immunodeficiency syndrome (AIDS).2 Marrow suppression, which results in anemia and neutropenia, is the most frequent dose-limiting toxicity and occurs in almost 50% of treated patients. This hematotoxicity most often occurs within 8 weeks from the initiation of therapy and may necessitate transfusions, reduction of dose, or discontinuation of treatment.3,5

Cytopenias associated with AZT therapy appear to result from a direct suppression of committed stem-cell growth and differentiation. In vitro, AZT inhibits the growth of granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) colonies from normal human marrow.6,7 The addition of dThd to media can prevent the inhibitory effects of AZT on the growth of human marrow colonies, but at concentrations that decrease its antiviral activity.6,8 In contrast, Sommadossi et al9 have reported that, in vitro, uridine (Urd) can prevent and reverse the toxic effects of AZT on granulocyte-macrophage progenitor cells without loss of antiretroviral activity, even at a Urd/AZT molar ratio of 10,000/1. In these studies, maximal effects were achieved at the relatively low Urd/AZT molar ratio of 10/1 (Urd concentration of 50 μM/L). Unfortunately it has been shown that the clinical utility of long-term Urd therapy is impaired by serious Urd-related toxicities, particularly hyperthermia and phlebitis.9

Benzylacyclouridine (BAU) is a potent inhibitor of rodent and human Urd-phosphorylase.10,11 This enzyme is responsible for the catabolism of Urd to uracil and thus appears to have a central role in the control of Urd concentrations in plasma.12 The efficiency of this catabolic enzyme is reflected in the observation that, to maintain elevated levels of Urd in plasma (50 to 100 μM/L), large, frequent, and, subsequently, toxic doses of exogenous Urd are required. While the exact mechanism by which Urd induces its toxic effects in vivo is not known, Peters et al13 have presented evidence that suggests that Urd toxicity in mice and rats might be related to the elevated plasma uracil concentrations induced by the catabolism of Urd. By inhibiting Urd catabolism, the administration of BAU produces a dose-dependent and sustained increase in the concentration of Urd in plasma and tissues without evidence of Urd-related toxicity.14,15 We have demonstrated the potential clinical utility of this compound by using BAU to increase the antineoplastic activity of fluorodeoxyuridine in vitro.16 In addition, by elevating plasma and tissue Urd pools, BAU can reduce FUra-related toxicity in vivo.17,18

The present study was undertaken to determine if: (1) BAU, by virtue of its ability to increase the plasma concentration of Urd, could reduce AZT-induced anemia and leukopenia, in vivo, and (2) BAU effects the anti-human immunodeficiency virus (HIV) activity of AZT. Preliminary reports have been presented.19,20

MATERIALS AND METHODS

Mice. All experiments used 6- to 12-week-old female heterozygous Balb/c mice [(Balb/c AnNCr) × nu], obtained from the Roger Williams Cancer Center (Providence, RI).

Drugs. AZT was provided by the Burroughs Wellcome Co (Research Triangle Park, NC) and dissolved in water at 1.5 mg/mL. This solution replaced drinking water and was administered to mice ad libitum.21 BAU was synthesized as previously described22 and administered orally at 300 mg/kg, once daily, as a suspension in 1% methylcellulose. Mice received 0.1 mL/10 g of body weight.

Hematology. Blood samples (50 to 60 μL) were obtained from the orbital sinus in heparinized Natelson pipettes. Hemoglobin concentration (Hb) was measured spectrophotometrically (Hemoglo-
binometer; Coulter Electronics, Hialeah, FL), and peripheral white blood cell counts (WBC) were electronically determined (Coulter Electronics, Model ZM). Reticulocyte stains were prepared from whole blood and peripheral reticulocytes (RTC) expressed as a percentage of the red blood cells examined. Marrow for histologic examination was obtained from the femur.

Quantitation of plasma concentrations of BAU, Urd, and AZT. Plasma concentrations of BAU and Urd were determined by high performance liquid chromatography (HPLC) methods as previously reported in detail.14,17 To quantitate plasma AZT, blood samples were processed as previously described14 and analyzed by HPLC methods using a Rainin Dynamax C-18 reverse-phase column (4.6 mm × 25.0 cm) maintained at 20°C and eluted isocratically at 1.0 mL/min with 30% MeOH in H2O. Column effluent was monitored at 254 nm. Under these conditions the retention time for AZT was 12 minutes.

Treatment protocols. To evaluate the ability of BAU to reverse AZT-induced anemia and leukopenia, AZT alone was administered to mice in their drinking water for 28 days. At this time Hb, WBC, and RTC were again determined, and mice with a decrease in the Hb concentration of more than 3 g% (about 80% of treated animals) were culled for experiments. These mice were divided into groups balanced for Hb, WBC, and RTC. All groups continued to receive AZT, while some also began concomitant therapy with BAU. After an additional 12 and 22 days of drug administration, Hb, WBC, and RTC were again determined. At day 22, plasma AZT levels were also determined and representative animals killed to prepare marrow smears.

To evaluate if BAU could prevent AZT-induced marrow toxicity, nontreated mice were divided in two groups well balanced for Hb and WBC. One group received only AZT (1.5 mg/mL) and the other received concomitant AZT + BAU. After 30 days Hb and WBC were determined again, as well as plasma AZT levels. Representative marrow smears were also prepared.

All experiments were performed in triplicate and results pooled. Each pooled treatment group contained from 20 to 27 animals. Student's t-test was used for statistical evaluation and P values of 0.05 or less were considered significant.

In vitro evaluation of the anti-HIV activity of AZT, BAU, and AZT + BAU. MT4 cells were incubated in the presence of 100X TCID50 HIV-38 for 1 hour before exposure to medium (RPMI 1640 supplemented with 10% fetal bovine serum and 10% human T-cell growth factor) which contained AZT, BAU, or AZT + BAU at concentrations between 1 and 0.0004 μmol/L for AZT and 1.04 and 100 μmol/L for BAU. The cells were allowed to incubate at 37°C in an atmosphere of 5% CO2, for 5 days. HIV-induced cytopathic effect (CPE) was analyzed by the propidium iodide DNA intercalation assay as described by Averett,22 and IC50 were determined.

RESULTS

The rapid absorption and high bioavailability of BAU after oral administration is evidenced by our findings that, after oral administration at 300 mg/kg in 1% methylcellulose, peak concentrations of approximately 300 μmol/L are achieved 30 minutes after dosing. Thereafter, BAU clearance appeared to obey first order kinetics with a plasma t1/2 of approximately 90 minutes (Fig 1A). Peak Urd levels in plasma (47 μmol/L) were achieved 3 hours after drug administration, and the Urd concentration remained elevated for up to 7 hours (Fig 1B).

It has been reported that, in mice, daily administration of AZT in drinking water produces hematologic toxicities similar to those seen clinically.19 After 4 weeks of AZT administration to mice, a megaloblastic anemia and leukopenia was evident in 80% of treated animals. These mice also developed megaloblastic changes in the marrow (see Fig 3A). Daily oral administration of BAU (300 mg/kg) alone to normal mice did not affect their Hb and WBC (data not shown).

The administration of AZT (1.5 mg/mL) for 28 days (day 0 of “rescue”) resulted in a decrease in Hb from 17.2 g% to 11.3 g%. WBC also decreased from 6.3 to 4.3 × 10⁹/mm³ and RTC from 1.5% to 1.2%. Continuation of AZT administration for an additional 12 days resulted in a further drop in Hb to 9.6 g% (−15.0% v day 0 of “rescue”). This regimen also caused WBC to decrease to 4.2 × 10⁹/mm³ (−2.3%). However, RTC were slightly increased to 1.6%. In contrast, mice, who after 28 days of AZT received AZT and BAU daily, had an increase in both Hb (to 11.7 g%), and WBC (to 6.3 × 10⁹/mm³). Most significant, the RTC in these mice increased to 4.9% (P = .001 v AZT alone) (Fig 2). In mice that received AZT alone, an additional 10 days of drug administration resulted in a continued decrease in Hb and WBC to 9.1 g% (−19.5%) and 3.6 × 10⁹/mm³ (−16.3%), respectively. Administration of AZT + BAU for an additional 10 days further improved the Hb and WBC to 12.3 g%
Coadministration of AZT and BAU from the onset of drug administration and continuing for 30 days partially prevented AZT-induced toxicity. After 30 days of receiving AZT alone there was a 24.6% decrease in Hb to 12.9 g% and a 36.0% decrease in WBC to 3.9 x 10³/mm³. In mice that received AZT + BAU, Hb decreased only 12.5% to 15.0 g% (P < 0.01 vs AZT alone), and WBC 24.6% to 4.5 x 10³/mm³ (.1 > P > .05, not significant). Megaloblastic changes and hypocellularity in the marrows were less pronounced in mice that received the combination.

The plasma clearance of BAU, as well as its effect on the plasma concentration of Urd, was comparable between the mice involved in "rescue" and "prevention" experiments (data not shown). Additionally, the amount of AZT solution consumed by animals during these experiments was carefully monitored and did not significantly differ among the treatment groups. The calculated dose administered corresponded to approximately 150 mg/kg/d in all groups. AZT plasma levels in mice that received AZT alone and AZT + BAU, determined at the end of these experiments, were similar and averaged 2.8 μmol/L.

In vitro studies to determine if BAU affected the anti-HIV activity of AZT showed that BAU alone possessed minimal anti-HIV activity in the MT4 cell system against HIV 3B (100 μmol/L reversed induced CPE by only 16%). More important, however, BAU at concentrations that ranged between 1.04 and 100 μmol/L did not interfere with the anti-HIV activity of AZT. As summarized in Fig 4, BAU did not affect the IC₅₀ of AZT in this system (~4 nmol/L ± BAU). BAU was not cytotoxic to MT4 cells at the concentrations analyzed.

**DISCUSSION**

Although AZT reduces the incidence of opportunistic infections and prolongs the survival of patients with AIDS, its usefulness is limited by marrow suppression. While the mechanism(s) responsible for these toxic manifestations is unknown, studies from several laboratories suggest that AZT may produce a spectrum of biochemical effects which together produce the observed marrow toxicity. Studies by Gallicchio et al23 and Rudnick24 suggest that AZT may disrupt hematopoietic growth factor synthesis or function. Their studies indicate that AZT-induced marrow toxicity can be partially reduced by the administration of interleukin-1 or erythropoietin. In vitro and biochemical studies have also suggested that AZT toxicity may be related to perturbations in pyrimidine nucleotide biosynthesis, perhaps related to the ability of AZT-MP to inhibit thymidylate kinase and accumulate in cells.25,26 Alternatively, Sommadossi et al27 suggest that AZT cytotoxicity may be a reflection of its incorporation into cellular DNA. These investigators also suggest28 that AZT-induced marrow toxicity may reflect a more subtle disruption of de novo pyrimidine biosynthesis, because in vitro, 50 μmol/L Urd can reverse and prevent AZT-induced suppression of marrow stem-cell proliferation without impairment of its antiviral activity. Additional support for this hypothesis is found in our previous findings with triacetyl-6-azauridine, when used to treat patients with psoriasis.29 This agent is a pyrimidine antimetabolite that
Fig 3. Photomicrographs of bone marrow smears representative of mice on chronic AZT administration (1.5 mg/mL in drinking water) (A) before and (B) after concomitant rescue therapy with BAU (300 mg/kg/d). After BAU rescue both cellularity and megaloblastic changes were improved.
inhibits orotidylate decarboxylase, the enzyme that converts orotidylate to uridylate. This biochemical action also produces megaloblastic anemia and leukopenia. Similarly, megaloblastic anemia is observed in patients with congenital orotic aciduria. This condition is an inherited deficiency in the activities of orotidyl decarboxylase and phosphorylase and results in disturbances in de novo pyrimidine biosynthesis and, ultimately, in the inadequate formation of uridylate.\textsuperscript{20} These observations are consistent with the possibility that AZT, or one of its metabolites, may partially contribute to the inhibition of de novo pyrimidine biosynthesis before the formation of uridylate. Accordingly, the administration of Urd, or an agent like BAU, which markedly increases plasma Urd, or an agent like BAU, which markedly increases plasma Urd levels.

Calculated values represent the mean ± SE.

**REFERENCES**


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