THE CHEMOTHERAPY OF PSORIASIS WITH AZARIBINE: MIRAGE OR MIRACLE

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Dedicated to the memory of Professor František Šorm.

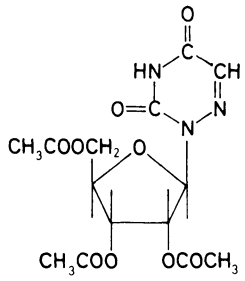
An antimetabolite, administered orally, which alleviates the symptoms and signs of severe psoriasis, azaribine (triacetyl-6-azauridine), was approved by the Food and Drug Administration (FDA of the USA) for clinical use in 1975, but 18 months later approval was withdrawn. The mechanism of action of azaribine (as a precursor of 6-azauridine 5'-monophosphate) is described and the probable cause of the infrequent vascular thromboses and a means of preventing them (by the co-administration of pyridoxine) are discussed.

1. INTRODUCTION

A review by Škoda[1] of "6-Azapyrimidine Nucleosides" serves as an introduction to the chemistry and biological activities of 6-azauracil, 6-azauridine, and azaribine [2',3',5'-triacetyl-6-azauridine or 2-(2,3,5-tri-O-acetyl-D-ribofuranosyl)-1,2,4-triazine--3,5-(2*H*,4*H*)-dione; see formula]. Even earlier reviews include those of Welch et al.[2] and Handschumacher et al.[3], while a recent article by Drell and Welch[4] has presented the reasons for the previously unexplained, serious but very infrequent toxicity of azaribine, and the means of preventing this toxic effect that had led to the withdrawal of azaribine from clinical use (because "the drug may cause life threatening or fatal blood clots in the veins or arteries"[5]).*

* Psoriasis itself is associated with an increased incidence of occlusive vascular disease[6]. Psoriatic patients have an increased incidence of hyperlipidemia[7] and moderately increased homocysteinemia[8], each of which is considered to be an independent risk factor for vascular disease.

Although 6-azathymine and 6-azathymidine were studied first[9], this reflected the ease of synthesis and other interests of those authors. In fact, the synthesis of 6-azauracil had been reported much earlier[10], but only in connection with an unrelated study of reaction mechanisms (and only in abstract form). Biologically, the study of 6-azauracil lay dormant until its resynthesis (via oxomalonic acid or glyoxylic acid) by Barlow and Welch[11], by Šorm and Škoda[12], and by Falco, Pappas and Hitchings[13], each in 1956.



Azaribine

When 6-azauracil inhibits the growth of *Streptococcus faecalis*, its ribonucleoside 6-azauridine, accumulates intracellularly[14], but a great step forward occurred when *Escherichia coli* was used[15,16] and 6-azauridine not only was formed but also excreted into the medium with such efficiency that a practicable means of its large-scale preparation was afforded. 6-Azauridine was also prepared synthetically[17–19], but these routes served primarily for proof of structure. Many derivatives of 6-azauridine have been prepared; however, of these only two have real or potential importance: 6-azacytidine, which is deaminated enzymatically to form 6-azauridine, and azaribine, the orally active form that through enzymic deacetylation serves as a source of 6-azauridine. It was developed to obviate the need for frequent or continuous iv-infusion of 6-azauridine to maintain adequate tissue levels. Azaribine, because of its remarkable activity in the oral therapy of psoriasis, a proliferative disease that mainly affects the skin and afflicts an estimated 2 to 5 percent of European and American populations[20,21], is an important addition to the list of now-orphaned drugs. It is because of the great value of azaribine in attaining remissions in this very unpleasant and incurable disease, and the long (and now successful) search for an explanation of the infrequent occurrence (about 1·6% incidence) of a serious side-effect (intravascular thrombosis) and to a means of preventing it, that this article has been written. May it serve as a memorial to Academician František Šorm, one of the pioneers in the investigations that led to the usefulness of 6-azauridine and azaribine and to the many years of fruitful collaboration and friendship between these authors and many other workers in both countries. May it also lead, in the light of

the findings described herein, to a favorable reevaluation by the U.S. Food and Drug Administration, of the safety of azaribine, when used in combination with pyridoxine hydrochloride, in the oral therapy of severe recalcitrant psoriasis.

In various biological systems, 6-azauridine is readily phosphorylated by uridine kinase to 6-azauridine 5'-monophosphate; however, further phosphorylation does not occur regularly (except in certain bacteria[17], protozoa[22] and, as recently reported, in L-1210 murine leukemia cells[23]). Even if a biological mechanism were available in man for the incorporation of 6-azauridine into RNA, "dead codons" would be created that would be unable to bind aminoacyl-tRNA to ribosomes, and thus potentially incorporated 6-azauridine could not cause errors in the translation process[24].

6-Azauracil exerts modest biological activity in vivo against certain experimental neoplasms, which is attributable either to its conversion to 6-azauridine or directly to the 5'-monophosphate of the latter[25]. *6-Azauridine 5'-monophosphate functions as an inhibitor of a specific decarboxylase that converts orotidine 5'-monophosphate into uridine 5'-monophosphate* (UMP), and thus leads to the accumulation of orotidine and orotic acid, essential precursors in the biosynthesis de novo of pyrimidines. Both orotic acid and orotidine are excreted in the urine in small amounts [$\simeq 9$ μmol (1·4 mg of orotic acid) per 24 h] by normal human subjects[26]. Administration of 6-azauridine markedly increases the urinary concentration of these compounds in normal subjects[27–29], as it does in mice[30], while their concentrations are also increased in bacterial cultures[31], and in experimental tumors[32].

The antineoplastic effects of 6-azauridine were demonstrated with several experimental neoplasms[2,33–36]. Various studies of the effects of 6-azauridine on other animal neoplasms have been conducted, in some cases in combination with other drugs or with radiation[37–39]. As with other antimetabolites, resistance to 6-azauridine could be observed; this is attributable to the selection of cells deficient in uridine kinase activity[40,41].

Of particular interest is a remakable species difference in the responses to 6-azauridine; this appears to reflect the dependence of the susceptible species on the biosynthesis of pyrimidines de novo, as contrasted to the pathways involved in salvage for the acquisition of the precursors of the pyrimidine-components of nucleic acids[42]. In the mouse, rat, pig, or monkey, as in man, 6-azauridine has but little toxicity, while in the dog, the compound is very toxic: thus, 6 mg per kg given at 8-hour intervals either orally or intramuscularly, led progressively to signs of severe toxicity in the dog, with profound leukopenia prior to death[43]. Note also that 6-azauridine in the dog, in contrast to man, is effective when given orally.

6-Azauracil in relatively huge doses (3 g per kg) causes narcosis in mice[36], an effect shared to a lesser degree with 6-azathymine and other 5-alkylated derivatives of 6-azauracil[43]; however, this is seen but minimally in other species. In man, 6-aza-

uracil causes a bizarre form of neurotoxicity, which effect has not been observed in other species, including the monkey, while 6-azauridine and azaribine (when free of 6-azauracil), do not appear to exert this effect[44].

2. Clinical Studies at Yale University (U.S.A.)

Early in the history of the studies of 6-azauridine at Yale University, using 6-azauridine supplied through the National Cancer Institute and prepared by Calbiochem (La Jolla, CA), under the supervision of one of us (W.D.), clinical studies had been initiated by Paul Calabresi. The initial studies involved the use of the drug orally, in view of the findings in animal species indicating that 6-azauridine was readily absorbed. When it became evident that this was not the case in man, 6-azauridine was applied by iv-infusion. Investigations of the mechanisms of renal clearance had demonstrated that the drug was predominantly filtered at the glomerulus, although evidence in animal species was obtained for a tubular secretory component as well; the latter could be inhibited by probenecid, but with the large doses of 6--azauridine required in man, the use of probenecid was not significantly contributory[45]. After many disappointing responses in adult acute leukemic patients, and a few solid tumors[2], a truly dramatic series of responses in a child with near-terminal acute lymphoblastic leukemia was obtained, only reported elsewhere in a graphic form[2], and hence it is deemed appropriate to describe them here.

This case involved an acutely ill child with an initial leukocyte count of about 60 000 cells per cmm of blood and with widespread leukemic nodular infiltrations of the skin. She was given 6-azauridine by iv-infusion at a dose of 100 mg per kg; after only 2 days, however, when her total white blood cell count had fallen precipitously to a normal level, the drug was withdrawn (at this time, the skin infiltrations had disappeared, and the unmistakable signs of clinical improvement had occurred). By the 18th day, however, the leukocyte count began to rise and 6-azauridine-infusion was again begun — at first, at 100 mg, then 120 mg, and finally during a 2-week period, to 160 mg per kg daily. The leukocyte count again fell from about 20 000 cells per cmm to normal levels, but in another 6 days it rose again, reaching about 40 000 cells per cmm of blood, while the nodular skin infiltrations recurred. Because of a shortage in the supply of 6-azauridine, iv-infusion was begun again with only 100 mg per kg, and after two days the infusion had to be arrested; nevertheless, the leukocyte count fell to a nearly normal level and the skin infiltrations again regressed.

[It should be noted that, at this stage, our supply of 6-azauridine was exhausted, and only thanks to the efforts of Dr. R. E. Handschumacher in recovering the drug from the child's urine were we able to continue the study and to keep the little girl alive for several weeks.*] Also, at

* Here it is appropriate to pay tribute to two colleagues without whose efforts this section of the paper could not have been written: Dr Paul Calabresi, whose patient it was and under whose expert guidance the study was done, and Dr Robert E. Handschumacher, without whose indefatigable labor in recovering 6-azauridine from the urine of this patient, the study would have been terminated long before its 80-day duration. Each of these colleagues knew well and respected greatly the eminent scientist and distinguished leader in scientific affairs, Academician František Šorm, to whose memory this article is dedicated.

this stage, medical ethics required that therapy with other drugs, e.g., prednisone and soon with 6-mercaptopurine, be begun, but these led to no improvement; indeed, while both of these secondary drugs were being given, the leukocyte count rose again to about 60 000 cells per cmm of blood and the skin nodules returned. At this time, 6-azauridine had been provided in small amount and infusion was begun at 100 mg per kg daily, but only for 2 days. The peripheral leukocyte count, nevertheless, fell to 15 000 per cmm of blood and the skin nodules disappeared, but the peripheral count then rose sharply to about 70 000 cells per cmm, to be brought down to about 30 000 cells per cmm with the last available dose of the recovered 6-azauridine. The white blood cell count then rose dramatically and the inexorable course of the disease prevailed. Death occurred after a total of 80 days from the initiation of therapy.

It was evident from simultaneous examinations of specimens of bone narrow, withdrawn during and after the treatment periods, that lymphoblasts therein were not obliterated from this tissue by 6-azauridine, as were the majority of the abnormal leukocytes in the peripheral blood and in the infiltrations of the skin, and that these 6-azauridine-insensitive marrow cells probably served as a source for the repopulation of the blood and other tissues. A reasonable explanation for this phenomenon could be that, in the marrow cells, either the concentration of uridine was high enough to be competitive for 6-azauridine in its phosphorylation by uridine kinase in that tissue or the uridine kinase activity was there too low.

Investigations with 6-azauridine in other children with acute lymphoblastic leukemia demonstrated its effect on neoplastic lymphocytes, but in no case was such a striking response seen as occurred in this initial child – a circumstance perhaps not unique in the annals of chemotherapy.

3. Clinical Studies in Czechoslovakia

In Czechoslovakia, the first use of 6-azauridine also was in acute leukemias[46], but the main emphasis was on the treatment of solid tumors, in which conditions it unfortunately exerted no appreciable activity[47], except that in carcinomas of placental origin striking effects were often seen[48]. Šmahel et al.[49] reported, after studies during several years, on the efficacy of 6-azauridine in the therapy of a variety of gestational trophoblastic neoplasms, including hydatidiform mole. In mycosis fungoides, a neoplasm primarily involving the skin and of controversial origin, Záruba et al.[50] were the first to report on the remakable activity of 6-azauridine and this was confirmed by Calabresi and Turner[51] and by McDonald and Calabresi[52], who found oral therapy with azaribine to be effective and less toxic than other chemotherapeutic agents.

Mention also should be made of the antiviral activities of 6-azauridine, although this field has not been developed greatly. First observed with vaccinia virus in a culture of chicken fibroblasts[53], a variety of other virostatic actions has been described (for a brief review of this field, see ref.[1]). It has been reported[47] that, in an experiment with only six non-vaccinated patients, the expected mortality was reduced by half with 6-azauridine.

4. Side-Effects of Azaribine and Their Origin

In the studies of azaribine in the treatment of psoriasis, it was observed that a prevalent "side-effect" was a reduction in the red cell count. Actually, this effect on the production of erythrocytes was mediated by what has been termed "pyrimidine starvation", with resultant depression of RNA-biosynthesis; it could be nullified by the administration of uridine. Extensive studies of azaribine, as a means of lowering the erythrocyte count in polycythemia vera (erythrocytosis), an often fatal disease hitherto treated by bleeding or by possibly leukemogenic injections of radioactive (^{32}P)-phosphate, led to a recommendation[54] of azaribine as a new form of therapy for polycythemia vera. This use of the drug, however, was not followed by an application for approval to the FDA, despite its unequivocal amelioration of the symptoms of erythrocytosis, i.e., decrease in splenomegaly, and the progressive reduction of hematocrit values, which on withdrawal of the drug would gradually return to the pretreatment level within a month or so.

The first clue to the elucidation of the mystery concerning the infrequent occurrence of the vascular thromboses (which later was found to average 1·6% of over 500 patients with psoriasis given azaribine), came from studies by Slavik et al.[55–57], which demonstrated that azaribine can cause the abnormal appearance of certain amino acids in the serum and urine of psoriatic patients, as well as in rabbits. These effects resemble those seen in inborn hyper-β-alaninemia, hyperhistidinemia, and homocystinemia*; indeed, serum levels of methionine also were raised in man, while decreased blood levels of Cu and Zn were observed[60].

It was later proposed by Slavik et al.[61] that potential catabolites of the 6-azauracil-moiety of azaribine, e.g., semicarbazide ($NH_2NHCONH_2$), carboxymethylhydrazine (NH_2NHCH_2COOH), or glyoxylic acid hydrazone ($NH_2N{=}CHCOOH$), might be responsible for the inactivation, through condensation with the aldehyde group of pyridoxal phosphate, the vitamin B_6-derived coenzyme for the function of enzymes involved with the metabolism of several amino acids. Indeed, it was demonstrated in 1986 by Slavik et al.[62] that semicarbazide HCl, administered daily in doses of 50 mg per kg by gavage to rabbits, reduced within 5 days their serum levels of pyridoxal phosphate to about 2% of their pretreatment values. In agreement with this concept were early studies by Handschumacher and Davis[63], which

* Homocystinuria and homocystinemia are the classical names for the genetic disease characterized by the appearance of large amounts of homocyst(e)ine in blood and urine without regard to the oxidized or reduced form in which it is found, excreted or isolated. They are manifested in part by multiple thromboembolic events, and, if untreated, more than half of the homozygotes die before the age of 20, while three-quarters are dead by the age of 30 (ref.[58]). Over 50% of the patients respond to therapeutic doses of pyridoxine HCl by reduction in the levels of homocysteine in the serum and in the rate of occurrences of initial thromboembolic events in late-detected patients[59].

showed that, in mice, 6-azauracil labeled with ^{14}C is catabolized partially to glyoxylic acid semicarbazone. These findings lend support to the proposal[64,65] that azaribine-induced homocystinemia might be the cause of the thromboembolic episodes in patients treated with azaribine. The daily administration of azaribine to rabbits by gavage led to a rapid and severe depletion of pyridoxal phosphate in the serum (to 25% of the pre-treatment values within 5 days) and the appearance of homocystinemia in 6 of 12 animals by the 4th or 5th day[61]; these results could be prevented by the simultaneous administration of pyridoxine HCl. It may be noted that increased levels of homocysteine in the plasma and urine are associated with a deficiency of vitamin B_6 in rats, pigs, rabbits, dogs and monkeys, together with the development in rabbits, dogs and monkeys of arterial fibrous plaques (for references, see Drell and Welch[4]). Slavik[66] has recently demonstrated that, as in rabbits, azaribine causes in man a reduction in the level of pyridoxal phosphate and an increase in the concentration of homocysteine in the plasma.

The stage is now set for the final act, in which it may be demonstrated that the administration in humans of pyridoxine together with azaribine will convert the latter into a safe, as well as effective, means of oral treatment of severe psoriasis. The putative mirage now promises to become not only something of a miracle but a scientific fact supported by hard and convincing evidence.

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