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REVIEW

AZELAIC ACID: PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES AND ITS THERAPEUTIC ROLE IN HYPERPIGMENTARY DISORDERS AND ACNE

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HISTORY

Azelaic acid (AZA) is a naturally occurring saturated nine-carbon dicarboxylic acid (COOH $(CH_2)_7$-COOH). It possesses a variety of biological actions both *in vitro* and *in vivo*. Interest in the biological activity of AZA arose originally out of studies of skin surface lipids and the pathogenesis of hypochromia in pityriasis versicolor infection.[1] Later, it was shown that *Pityrosporum* can oxidize unsaturated fatty acids to C_8-C_{12} dicarboxylic acids that are competitive inhibitors of tyrosinase *in vitro*.[2] Azelaic acid was chosen for further investigation and development of a new topical drug for treating hyperpigmentary disorders for the following reasons: it possesses a middle-range of antityrosinase activity, is inexpensive, and more soluble to be incorporated into a base cream than other dicarboxylic acids.

PHARMACOKINETIC PROPERTIES

Mechanism of Absorption

After topical application of 1 g of 20% AZA cream, a percutaneous absorption of about 3% and a correlated plasma concentration of 0.038 μg/mL (2.1×10^{-7} M) were estimated.[3] The formulation of the topical vehicle significantly affects the % amount being absorbed in a time-dependent manner. Absorption from 15% azelaic acid gel after 12 hours was higher (8%) than that from a water-soluble polyethylene glycol ointment base (3%).[4] In normal cells, dicarboxylic acids penetrating the cell membrane undergo complete metabolism by β-oxidation. Penetration of dicarboxylic acids through neoplastic cell membranes is about 3× higher with resulting higher intracellular concentrations.[5,6] Whether AZA is transported across the cell membrane via a transport carrier system or by simple diffusion remains unknown. Other dicarboxylic acids (i.e., malate, succinate, oxaloacetate) are transported by specific protein carriers.[7]

Distribution

Twelve hours after oral administration, the highest concentrations of AZA were estimated to occur in the liver, lungs, and kidneys of rats. [^{14}C]-Azelaic acid continues to accumulate in adipose tissue for about 96 hours after a dose.[8] Of the total organ radioactivity, 90% was detected in fatty tissues and in fatty acid fractions of triglycerides and phospholipids.[8,9] Azelaic acid can cross the blood-brain-barrier of dogs, after oral and intravenous administration with the cerebrospinal fluid (CSF) concentrations estimated at 2–5% of those of plasma.[10] The ocular distribution of AZA after topical (retrobulbar) and intravenous administration in rabbits was also reported. Higher concentrations were found in the aqueous humor than vitreous humor, peaking at 2 hours after a dose.[11]

Pharmacology and Metabolism

Tests on rats and rabbits indicated that AZA is nontoxic, nonmutagenic, and nonteratogenic.[12–14] In humans, AZA was considered a substrate for total parenteral nutrition.[12,13] The 15% sodium salt of AZA was given intravenously, intraarterially, and intralymphatically by continuous infusion for up to 1 week without adverse local or systemic effects.[5,12] Azelaic acid was also found in urine of patients with ketosis and disorders of mitochondrial and peroxisome β-oxidation. After administration by various routes, AZA is predominantly excreted in the urine, but also partly metabolized via mitochondrial β-oxidation to pimelic acid and partly decarboxylated.[8] Further metabolism yields malonyl-CoA and acetyl-CoA. While acetyl-CoA enters the Krebs-cycle to be completely oxidized to CO_2 and H_2O, malonyl-CoA cannot be further oxidized. Malonyl-CoA is utilized in the synthesis of other fatty acids. When given orally in man, up to 20 g a day is tolerated and about 60% of the unmetabolized form is excreted in the urine within 12 hours.[8] The serum level

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peaks at 6–75 mg/L ($3.3 \times 10^{-5} - 4.2 \times 10^{-4}$ M) 2–3 hours after oral administration of 0.5 – 5 g AZA and then falls off within 8 hours.[8] When administered intravenously at a constant rate (20 g in 4 h), serum levels can be maintained as high as 5×10^{-3} M.[10] The urinary excretion was estimated at 77% of the infused dose and the mean urine clearance rate at 8.4 L/h.[12] After topical application of 1 g of 20% AZA cream, a low serum level of about 0.04 μg/mL (2.1×10^{-7} M) was estimated. Urine excretion rates at the same time were measured at 4.5 mg over a 48-hour period.[3]

PHARMACODYNAMIC PROPERTIES

Effects on Cellular Enzymes

During investigations of hypochromia in pityriasis versicolor, C_6–C_{12} dicarboxylic acids were formed from unsaturated fatty acids (with double bonds in the 6–12 positions) added to the culture media growing *Pityrosporum*.[2] Subsequently, AZA was found among these dicarboxylic acids to have antityrosinase activity. Passi et al.[10] who chemically manipulated the electron donor or acceptor groups of C_8–C_{13} dicarboxylic acid, clearly demonstrated that AZA competitively inhibits tyrosinase, the key enzyme for melanogenesis.

Azelaic acid was also reported to inhibit reversibly thioredoxin reductase,[15] that is involved in the biosynthesis of deoxyribonucleotides. In addition, AZA reversibly inhibits nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P-450 reductase and 5α-reductase in microsomal preparations supplemented with reduced NADPH.[16,17] It also reversibly inhibits the activity of mitochondrial respiratory chain enzymes in the rat liver: such as NADH-dehydrogenase, succinic acid dehydrogenase, and H_2CoQ cytochrome C oxidoreductase, resulting in a decreased rate of O_2-consumption.[18] It is possible that flavin nucleotide directly is involved with the mechanism of inhibition by AZA because NADH dehydrogenase, succinyl dehydrogenase, and H_2CoQ cytochrome C oxidoreductase are flavin-linked dehydrogenases. In chicken embryos, AZA was found to inhibit anaerobic glycolysis.[19] Concentrations at which AZA exerts its antienzymatic activities were found to be 10^{-3} M and beyond,[15,18] when given intravenously.

The effect of AZA on testosterone metabolism is controversial. Stamatiadis et al.[20] reported that AZA can competitively inhibit 5α-reductase, which converts testosterone to dihydrotestosterone in both human skin and hair follicles.[21,22] The latter hormone is generally considered responsible for stimulating sebaceous glands[23] and to be a possible contributing factor in the pathogenesis of human acne.[21,24] Nguyen et al. found no significant effect of AZA on 5α-reductase activity in human follicle hair cells (unpublished data). It has been suggested that zinc functions as a cofactor of 5α-reductase.[25] It is possible that AZA may form complexes with heavy metal cations,[26] and particularly with zinc ion; AZA may indirectly inhibit 5α-reductase by preventing the zinc ion from interacting with the enzyme. *In vivo* animal studies of lipogenesis in sebaceous glands of the hamster ear, Limburg et al.[27] reported inhibition by AZA; while Rach, and Topert[28] found no effect after 4 months of topical AZA administration.

Apart from mitochondria, [^{3}H]-AZA was found incorporated predominantly into the nucleus of both human and murine melanoma cells and keratinocytes *in vitro*.[29] At 10–40 mM concentrations AZA selectively inhibits DNA polymerase, assessed by inhibition of cellular incorporation of [^{3}H]thymidine and cell replication *in vitro*.[29–31] At the same concentrations, AZA has minimal inhibitory effects on RNA synthesis ([^{3}H]uridine incorporation) in murine keratinocytes or protein synthesis ([^{35}S]methionine incorporation) in cultures of human melanoma cells.[32]

Effects on Cutaneous Microflora

In vitro tests, using various strains of cutaneous microorganisms (*Staphylococcus epidermidis*, *S. aureus*, *S. capitis*, *S. hominis*, *Propionibacterium acnes*, *P. granulosum*, *P. avidum*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*), demonstrated that AZA possesses bacteriostatic activity[14,28,33–35] with minimum inhibitory concentrations (MICs) varying from 0.03 M to 0.25 M and minimum bactericidal concentrations (MBCs) of 0.25 M or greater.[35] In *S. epidermidis* and *P. acnes*, both nutrient depletion and acidic pH (5.6) enhance the bactericidal activity of AZA. The latter factor (pH) appears to increase the uptake of AZA by bacteria.[33,36] Inhibition of bacterial protein synthesis ([^{3}H]phenylalanine incorporation) mainly accounts for the bactericidal and bacteriostatic effects.[33,36] Antiviral activity *in vitro* induced by AZA has also been reported.[5,37] Brasch and Christophers[38] recently demonstrated the antimycotic activity of AZA *in vitro* against common dermatophytes, *Scopulariopsis*, *Candida*, and *Pityrosporum*.

Effects on Cell Morphology

As was pointed out earlier, the involvement of AZA with mitochondrial functions is not surprising since the compound is partly metabolized by the mitochondrial β-oxidative enzymes. Electronmicroscopic studies revealed that mitochondria are the first targets of action of AZA. In the presence of 1–100 mM AZA, mitochondrial swelling and destruction of cristae without damage to cytoplasmic organelles and cell membranes were observed in murine melanoma cells,[39–41] human melanoma cells,[42] and human choroidal melanoma cells in culture,[43] but much lesser degrees of mitochondrial swelling induced by 100 mM AZA were observed in normal human melanocytes.[40,42] In cultures of ker-

atinocytes of newborn mice 20–50 mM AZA induced, apart from mitochondrial changes, enlargement of the rough endoplasmic reticulum.[30]

Effects on Nontumor Cell Proliferation and Viability

At a low concentration (1mM), AZA has no significant antiproliferative or cytotoxic effect on normal human dermal fibroblasts[31] or normal epidermal melanocytes.[44] In the 10–40 mM range, no cytostatic and cytotoxic effects were observed in normal murine fibroblasts[6] or keratinocytes.[32] At the same concentrations, AZA exerts a reversible concentration- and time-dependent antiproliferative activity in cultured murine keratinocytes,[30] and in human keratinocytes,[29,45] mainly by inhibiting DNA synthesis. At concentrations in excess of 40 mM, cytotoxic effects were evident in both murine and human keratinocytes[29] (Table 1).

As was mentioned earlier, neoplastic cells may possess defective cellular membranes, thus allowing AZA to diffuse readily into the cytoplasm and mitochondria, and become more vulnerable to the activity of AZA.[10] Since dicarboxylic acids are more polar than monocarboxylic acids or esters of dicarboxylic acids, they would diffuse less readily through normal cell membranes. This may account for the insignificant effects of AZA on normal cells. Once inside the cell, cytoplasmic esterases would cleave the monocarboxylic or dicarboxylic acid esters into dicarboxylic acid forms that are likely the active drugs. It would be interesting to find out whether a prodrug, monocarboxylic or dicarboxylic acid ester, could produce similar antiproliferative and cytotoxic effects as AZA in normal cells. This would allow one to test the hypothesis that the lack of AZA transport in normal cells accounts for its ineffectiveness in these cells.

Azelaic acid acts as an antikeratinizing agent and influences the differentiation of human keratinocytes *in vivo*. It retards the synthesis of filaggrin, a keratin filament aggregating protein.[46] Examination of the epidermis by light and electron-microscopy revealed intercellular edema, thickness of the horny layer in acroinfundibular areas, and reduction of keratohyaline granules and tonofilament bundles in the stratum corneum.[47,48] Immunocytochemistry studies suggested that AZA may influence the terminal phase of epidermal keratinization and cause restoration of the normal pattern of filaggrin distribution within the epidermal granular and horny layers of the affected skin.[46–48]

Effects on Tumor Cells

At 10–100 mM concentrations AZA was shown to exhibit time- and concentration-dependent antiproliferative effects *in vitro* in human and murine malignant melanoma,[32,40–43] mainly by inhibiting DNA synthesis. Cytotoxicity was observed at AZA concentrations greater than 40 mM, probably due to inhibition of mitochondrial respiration and inhibition of DNA synthesis. Other nontyrosinase metabolizing tumor cells, such as human lymphoma and leukemia-derived cell lines, lymphoblastoid cells,[6,32] and squamous carcinoma cells,[49] manifested similar effects when exposed to 10–50 mM AZA (Table 2).

It must be noted that AZA is not active against any cell lines until its concentrations approach 1–10 mM.[10] At 100 mM it also begins to affect nonmalignant cell lines. At these extreme pharmacologic levels that are much higher than the generally accepted 10^{-5}–10^{-9} M concentrations commonly employed for pharmacologically active drugs in cell culture, one must account for a variety of nonreceptor-mediated, mass action mecha-

Table 1. Effects of Azelaic Acid on Normal Cell Lines in Culture

References	*Cell Lines*	*Concentration (mol/L)*	*Effects Observed*
Breathnach et al., 1979[44]	Human melanocyte	10^{-3}	No effect on melanogenesis
Leibl et al., 1985[32]	Murine epidermal keratinocyte	4×10^{-2}	No effect on DNA synthesis
Picardo et al., 1985[6]	Normal lymphocyte, murine fibroblast stimulated lymphocyte	10^{-3}	No effect on growth or DNA synthesis
Breathnach et al., 1984[5]	Human melanocyte	$> 10^{-2}$	Mitochondrial swelling
Hu et al., 1986[39]	Monkey choroidal melanocyte	10^{-3}	No effect on mitochondria
Geier et al., 1986[31]	Human fibroblast	10^{-3}	No effect on growth
Detmar et al., 1989[30]	Neonatal murine keratinocyte	$2–5 \times 10^{-2}$	Growth inhibition, reduced DNA* and RNA† synthesis, mitochrondrial and RER‡ swelling and vacuolation
Detmar et al., 1988[45]	Human keratinocyte	$1–4 \times 10^{-2}$	Growth inhibition, reduced DNA synthesis
Galhaup, 1989[29]	Human and murine keratinocyte	$1–4 \times 10^{-2}$	Growth inhibition, reduced DNA synthesis
		$> 4 \times 10^{-2}$	Cytotoxicity

* DNA synthesis was assessed by ^{3}H-thymidine incorporation.
† RNA synthesis was assessed by ^{3}H-uridine incorporation.
‡ RER: rough endoplasmic reticulum.

nisms, by which AZA may be exerting its activity.[50] Wilkerson[50] pointed out that AZA may form complexes with essential divalent ions and interfere with cellular functions.

Effects on Neutrophil Functions and Reactive Oxygen Species

Neutrophil chemotaxis and phagocytosis as well as reactive oxygen species (ROS) generated in a xanthine-xanthine oxidase system are not significantly affected in the presence of AZA; however, it markedly reduces superoxide and hydroxyl radicals generated by neutrophils.[51] *In vitro*, AZA (0.05–1.0 mM) acts as a scavenger of hydroxyl radicals and can inhibit the hydroxylation of l-tyrosine to l-DOPA that requires hydroxyl radicals produced by the Fenton reaction. It also inhibits peroxidation of arachidonic acid induced by ROS.[52] But AZA is not a scavenger of superoxide radicals generated by the xanthine-xanthine oxidase system.[52] At nontoxic concentrations (< 20 mM), it reduces the cytotoxic effects of hydroxyl radicals generated by UV irradiation or diphenol autooxidation on melanoma and lymphoma-derived cell lines.[53,54]

THERAPEUTIC APPLICATION

Disorders of Pigmentation

Topical AZA (15–20%) has no depigmentation effect on normal skin, solar freckles, senile freckles, lentigo simplex, pigmented seborrheic warts, and nevi; but has been reported to be effective against hypermelanosis caused by physical or photochemical agents, postinflammatory melanoderma, melasma, chloasma, lentigo maligna (LM), and primary lesions of lentigo maligna melanoma and malignant melanoma (MM). These conditions are characterized by either hyperactivity or abnormal proliferation of melanocytes.[3,32,55–61] In these cases, AZA induces direct cytotoxic effects toward hyperactive and malignant melanocytes by inhibiting mitochondrial enzymes and DNA synthesis (see Table 2).

Postinflammatory, Physical, and Photochemical Hyperpigmentation. Breathnach et al.[3] claimed that 3–4 months of topical 20% AZA treatment provided satisfactory results in hyperpigmentation after burns, physical trauma, herpes zoster, acne vulgaris, and inflammation. Chemically (i.e., fertilizer, disinfectants) induced hyperpigmentation also responded to AZA therapy;[3] however, 20% AZA cream has no depigmenting or preventative effects on the normal skin pigmentation occurring after exposure to UVA, UVB, and visible light. Interrupting or continuing AZA treatment after skin irradiation has no influence on the resulting pigmentation.[62] No other clinical studies validate the above reports.

Melasma. Melasma is an acquired macular hypermelanosis of sun-exposed areas, commonly encountered among darker-skinned individuals. Factors implicated in the etiology of melasma are UVA and UVB light, pregnancy, racial predisposition, and certain cosmetics and medications. The drug most frequently used in the treatment of melasma is hydroquinone (HQ), alone or combined with tretinoin and corticosteroids.[63] A few clinical studies suggested that topical 20% AZA, when applied twice daily with a broad spectrum sunscreen, is effective in reducing the pigmentary intensity and size of the lesions of melasma (Table 3).

Breathnach et al.[3] reported their experience with over 300 cases, that topical AZA cream (dose and duration of treatment not specified) provided satisfactory treatment. In a noncomparison study by Rigoni et al.,[60] 39 patients (predominantly women) with melasma (mean duration = 5 years) were treated with 20% AZA cream twice a day for 6 months. A mean reduction in pigmentation of 51.3% (compared to baseline) was reported. A randomized double-blind study with 155 patients of Indo-Malay-Hispanic origin compared the efficacy of twice daily 20% AZA versus 2% hydroquinone cream. After 24 weeks, 73% of the AZA treated patients, compared with 19% of the hydroquinone group, showed a significant reduction of the pigmentary intensity and size of their melasma.[61] Another multicenter, controlled, randomized double-blind comparison (n = 329 women) of 20% AZA cream and 4% HQ over 24 weeks indicated an equal efficacy, both in terms of lesion size and reduction in pigmentation intensity.[55] Similar results were obtained by Piquero-Martin et al.[64] in a double-blind study of 60 women on oral contraceptives. After twice daily application of 20% AZA compared to 4% HQ for 24 weeks, AZA was not better than HQ in the treatment of melasma (see Table 3).

Lentigo Maligna. Lentigo maligna is a hyperpigmentary disorder characterized by abnormal proliferation of melanocytes and insidious progression to malignant melanoma. These lesions typically occur on sun-exposed areas of elderly individuals. The clinical experience in using topical AZA for the treatment of lentigo maligna is still limited to date and available evidence is inadequate to support the use of AZA as a primary agent. The melanocytotoxic effect observed in experimental animals led Nazzaro-Porro and his colleagues to investigate further AZA as a potential therapeutic agent in the treatment of lentigo maligna.[59] Subsequently, three cases of LM treated with 15% topical AZA cream for 3 months with remarkable improvement were described.[56] Additional noncontrolled clinical studies with 5–10-years follow-up were carried out by the same group of investigators, who reported that twice daily application of 15–20% AZA for 3–12 months in 50 patients resulted in complete clinical and histological resolution. Twenty-seven out of 50 patients were still disease-free from 5–10 years after treatment.

Table 2. Effects of Azelaic Acid on Various Cell Lines in Culture

References	*Cell Lines*	*Concentration (mol/L)*	*Effects on Cellular Proliferation, Organelles, DNA* and RNA† Synthesis*
Schachtschabel, 1984[80]	Harding–Passey‡	10^{-3}	Growth inhibition, reduced DNA and melanin synthesis
Leibl et al., 1985[32]	Cloudman S-91,‡ Human‡ and Rajii	$10^{-3} \times 10^{-2}$	Growth inhibition, reduced viability and DNA synthesis
Mensing et al., 1985[81]	Line ?	10^{-3}, 10^{-1}	Decreased PAA‡ and CTX
Pathak et al., 1985[82]	Human‡	10^{-5}, 10^{-2}	Growth inhibition
Picardo et al., 1985[6]	Human lymphoma and leukemia	$1–5 \times 10^{-2}$	Reduced DNA synthesis
Reith et al., 1985[37]	Viral§ infected Hela and Vero cells	$10^{-4} – 10^{-2}$	45% Reduction in viral DNA synthesis
Robins et al., 1985[40]	Human‡	$10^{-2} – 10^{-1}$	Growth inhibition, reduced DNA synthesis, mitochondrial swelling
Robins et al., 1985[41]	Harding-Passey‡ and Cloudman S = 91‡	$10^{-3} – 10^{-1}$	Growth inhibition, reduced viability, mitochondrial swelling
Breathnach et al., 1986[42]	Human‡	5×10^{-2}	Growth inhibition, reduced viability, mitochondrial swelling
Breathnach et al., 1989[43]	Human choroidal‡	$10^{-2} – 10^{-1}$	Growth inhibition, reduced viability and DNA synthesis, mitochondrial swelling
Hu et al., 1986[39]	Mouse-B16‡	10^{-3}	Mitochondrial swelling
Geier et al., 1986[31]	Human‡	$10^{-4} – 10^{-3}$	Growth inhibition
Patzold et al., 1989[49]	Human squamous carcinoma	$1–5 \times 10^{-2}$	Growth inhibition, reduced viability and DNA synthesis
Zaffaroni et al., 1990[83]	Human‡	$0.5–5 \times 10^{-2}$ $2.5–5 \times 10^{-2}$	Growth inhibition Reduced DNA and RNA synthesis

* DNA synthesis was assessed by ^{3}H-thymidine incorporation; † RNA synthesis was assessed by ^{3}H-uridine incorporation; ‡ Melanoma cell line; § Vaccinia virus (Lister strain).
PAA = Plasminogen activator activity; CTX = Chemotaxis.

Eleven cases of recurrent LM were found, but all resolved on renewed AZA treatment. Clinical improvements were marked by progressive reduction of the intensity of pigmentation, flattening of the elevated and indurated surface, and shrinkage in size without apparent hypochromia or scarring.[58,59] Biopsy at the end of treatment revealed that the general organization of the epidermis, appendages, and dermis appeared essentially normal. The melanocytes were present in normal numbers and relatively inactive with normal morphologic appearance.[3] Leibl et al.[32] also reported good results in treating nine patients with AZA and further correlated their clinical findings with those of cultured melanoma cells.

McLean and Peter,[65] in a small study of nine patients treated with 15–35% AZA cream twice daily for 12–64 weeks, reported complete clearing in one and clinical improvement (confirmed by biopsy) in four patients. Two patients developed invasive lentigo malignant melanoma while on treatment. It is possible that some LM cells do not respond to AZA. Variable responses were noted when one part of the lesion faded, while another area apparently progressed.[65] The authors recommended that the first line therapy for lentigo maligna remains surgical excision and AZA should be used only when alternative proven forms are not possible. Lentigo maligna, even when left untreated, in aged individuals rarely results in death from metastasis. The aggressiveness of this tumor is much less than that of malignant melanoma and one has to question, how malignant each lesion actually is.[50] In considering AZA treatment, patients must be carefully selected and informed of alternative forms of therapy. It should be considered for early or recurrent cases, according to site and extent of the lesion, and for patients who are not suitable for surgery because of age, concurrent morbidity, or who refuse surgery and other forms of treatment.[3]

Malignant Melanoma. At present, insufficient information from clinical studies are available to define the role of AZA in the treatment of malignant melanoma. A preliminary noncontrolled study of 23 patients with cutaneous MM (superficial spreading and nodular type with and without local or disseminated metastasis), treated with a combination of 10–15 g/day orally and 15% topical cream twice daily for 2–12 weeks before a wide surgical excision, has suggested beneficial effects on the primary lesions. Progressive reduction in the intensity of pigmentation, arrest, and regression of the advancing margin of the lesions, and flattening of the nodular areas were observed.[57] In seven patients each with a single local lesion without evidence of local lymphatic involvement, a complete clinical resolution of the lesion was observed and confirmed by biopsy. Light and electron microscopy displayed a normal epi-

Table 3. Summary of Clinical Studies Evaluating the Therapeutic Efficacy of Topical Azelaic Acid (AZA) and Hydroquinone Cream (HQ) in the Treatment of Melasma*

References	*Study Design*	*N*	*Dosage Regimen*	*Treatment Duration (wks)*	*Clinical Response†*	*Conclusion‡*
Rigoni et al., 1989[60]	nb	39	20% AZA b.i.d.	24	51.3%	Effective
Verallo-Rowell et al., 1989[61]	r,db	77	20% AZA b.i.d.	24	73%	AZA > HQ
		78	versus 2% HQ b.i.d.	24	19%	
Balina and Graupe, 1991[55]	r,db	164	20% AZA b.i.d.	24	64.8%	AZA = HQ
		165	versus 4% HQ b.i.d.	24	72.5%	
Piquero-Martin et al., 1988[64]	db	30#	20% AZA b.i.d.	24		AZA = HQ
		30#	versus 4% HQ b.i.d.	24		

N = Number of patients assigned to each treatment group. * A broad spectrum sunscreen was applied concomitantly. † Percentage of patients who achieved good-to-excellent clinical response on completion of treatment. ‡ Overall efficacy: = denotes equivalent efficacy; > denotes superior efficacy. # Patients were on oral contraceptives during the study. nb = nonblind; db = double-blind; r = randomized.

dermal and dermal organization and absence of malignant melanocytes. At 10-year-follow-up, six of these seven patients remained in clinical remission, and one developed cutaneous metastasis.[3] Sowden et al.[66] reported an isolated case of a 69-year-old man with lesions of malignant melanoma arising from a scar of lupus vulgaris that responded to 20% AZA cream twice daily. Three months after this treatment the biopsy showed a striking reduction in the number of atypical melanocytes, but there remained an abundance of melanin within basal keratinocytes and dermal macrophages that contributed to the persistent pigmentation of the lesions.

While Mingrone et al.[11] reported the pharmacokinetic distribution of radiolabeled AZA into ocular membranes and fluids of rabbits resulting from continuous intravenous infusion of the compound, oral administration 12 g/day AZA (5 × 600 mg capsule q.i.d.) for 3 months appeared ineffective in the management of four patients with ocular and adnexal melanoma.[67] The problem in this case was most likely the 12 g/day oral dose, which was insufficient to achieve the therapeutic levels in serum ($\geq 10^{-2}$ M) required to affect the malignant cells (see section Pharmacology and Metabolism). If given by continuous intravenous infusion, the results might have turned out differently.

While the above reports suggested a direct cytotoxic effect of AZA on melanocytes of cutaneous malignant melanoma, it must be stressed that AZA should not be used as a primary treatment or replace the standard surgical excision for this condition.[3,66]

Reticulate Acropigmentation of Kitamura. Reticulate acropigmentation of Kitamura (RAPK) was first described in Japan in 1943. Cases of RAPK have been reported from other countries. Patients were found to manifest reticulate, brown macules on the trunk, dorsum of hands and feet, and "pits" on the palms. Kameyama[68] reported an isolated case of a 50-year-old Japanese woman with RAPK, who was successfully treated with 20% AZA cream twice a day for 2 months. Histologic findings revealed that melanin production and its transfer to keratinocytes were greatly increased in patients with RAPK. In the affected areas, AZA suppressed the proliferation of melanocytes. Nevertheless, the efficacy of AZA in treating RAPK still needs to be confirmed by additional clinical studies.

Other Hyperpigmentary Skin Disorders. Topical application of azelaic acid over a period of 3–4 months has been reported effective in the treatment of isolated cases of rosacea and solar keratosis.[79] To date, no other clinical studies confirmed such findings.

Acne Vulgaris

While treating patients suffering from benign hyperpigmentary disorders with AZA cream, Nazzaro-Porro et al.[69] observed significant simultaneous improvement of acne lesions within the treated areas. Subsequently, the therapeutic efficacy of topical 20% AZA cream was evaluated in several controlled, clinical trials and compared with vehicle and other established antiacne products such as tretinoin, benzoyl peroxide, erythromycin, and tetracycline (Table 4). Under controlled conditions, twice daily topical application of 20% AZA cream (over a 3-month period) appeared more effective (64%) than placebo (36%) in reducing comedonal, papular and pustular lesions in mild-to-moderate acne.[70,71] After treatment for 6 months, topical 20% AZA cream applied twice daily was of comparable efficacy to topical 0.05% tretinoin cream,[71] topical 5% benzoyl peroxide gel,[72] topical 2% erythromycin cream,[73] and oral tetracycline 0.5–1.0 g/day in comedonal and mild-to-moderate (80%) and moderate-to-severe (60%) inflammatory types of acne.[74,75] The same treatment appears less effective for conglobate acne when compared to 0.5–1.0 mg/kg/day oral isotretinoin.[76]

Acne is a chronic inflammatory disorder of the pilosebaceous unit. The physiopathologic mechanism of acne seems to depend on several factors; (1) a hyperkeratinization process of the follicular channels; (2) microbial colonization of the pilosebaceous units; (3) perifollicular inflammation; (4) sebum production and

excretion; and (5) differential rates of conversion of testosterone to dihydrotestosterone. When compared to normal skin, acne-bearing skin was found to produce from 2 to 20 times more dihydrotestosterone,[21] generally considered to stimulate the pilosebaceous unit and a possible contributing factor in the pathogenesis of acne.[24,77] Azelaic acid appears to retard the conversion of testosterone to dihydrotestosterone through competitive inhibition of 5α-reductase.[20] This may be one mechanism that AZA is effective in treating acne, but Nguyen et al. found that AZA has no effect on the 5α-reductase activity in cells of human hair follicle. *In vivo* animal studies also reported conflicting results: lipogenesis in sebaceous glands of the hamster ear was not significantly affected by topical application of AZA up to a 4-month period.[27,28] In acne patients, application of 20% AZA cream over a 3- to 6-month period did not affect the excretion rate,[47,70,72] or composition of sebum,[47,70] or the morphology of sebaceous glands.[47] Nevertheless, patients with acne reported subjectively gradual and progressive reduction in skin greasiness after 1–2 months of treatment.[69,78] Histologic findings showed normal skin possesses smaller sebaceous glands than seborrheic or acne skin, the latter having larger sebaceous glands.[47]

Mayer-da-Silva et al.[46,47] demonstrated that AZA is an antikeratinizing agent, displaying an antiproliferative cytostatic effect on keratinocytes (via inhibition of DNA synthesis) and modulating the early and terminal phases of epidermal differentiation (via inhibition of cytoplasmic protein synthesis). The infundibular epidermis of individuals with acne showed marked reduction of thickness of the horny cell layer, widening of the horny cell cytoplasm, and normalization of filaggrin distribution.

So far, data accumulated from physiobiochemical and ultrastructural studies have suggested that AZA may achieve its antiacne activity through its antikeratinizing effects on the follicular epidermis and its antimicrobial action rather than by direct inhibition of sebaceous gland function. Cunliffe and Holland[70] proposed that direct modification of comedogenesis, by normalization of the disorganized keratinization of the follicular infundibulum, may cause rapid reversal of noninflamed acne lesions in response to AZA therapy. On the other hand, the antimicrobial action of the

Table 4. Summary of Clinical Studies Comparing the Therapeutic Efficacy of Azelaic Acid (AZA) against Established Antiacne Products

References	*Study Design*	*N*	*Dosage Regimen*	*Treatment Duration (months)*	*Clinical Response**				
					Inflammatory	*Noninflammatory*	*Nodulo-cystic*	*Overall Response†*	*Conclusion‡*
Vehicle (V)									
Cunliffe and Holland, 1989[70]	r,db	20	20% AZA cream b.i.d.	3	49	50		50–55	AZA ≥ V
		20	versus Vehicle	3	12	27		5–20	
Katsambas et al., 1989[71]	r,db,mc	43	20% AZA cream b.i.d.	3	72	56		64	AZA > V
		49	versus Vehicle	3	47	0		36	
Benzoyl peroxide (BP)									
Cavicchini and Caputo, 1989[72]	r,sb,mc	309	20% AZA cream b.i.d.	6	84			66	AZA = BP
			versus 5% BP gel b.i.d.	6	83			70	
Tretinoin (TN)									
Katsambas et al., 1989[71]	r,sb,mc	143	20% AZA cream b.i.d.	6		79		65	AZA = TN
		146	versus 0.05% TN cream b.i.d.	6		82		69	
Erythromycin (ER)									
Graupe and Zaumseil, 1991[73]	r,db	154	20% AZA cream b.i.d.	5	79	68		71	AZA = ER
		152	versus 2% ER cream b.i.d.	5	76	69		67	
Isotretinoin (ITN)									
Gollnick and Graupe, 1989[76]	nb,mc	84	20% AZA cream b.i.d.	6	69			33	ITN > AZA
			versus ITN 0.5--1.0 mg/kg/day p.o.	6	100			91	
Tetracycline (TC)									
Bladon et al., 1986[74]	db	23	20% AZA cream b.i.d.	6			42		AZA = TC
		22	versus TC 0.5–1.0g/d p.o.	6			54		
Hjorth and Graupe, 1989[75]	r,db,mc	164	20% AZA cream b.i.d.	5	83			82	AZA = TC
		169	versus TC 0.5–1.0g/d p.o.	5	86			86	
	r,db,mc	126	20% AZA cream b.i.d.	6	79		80	62	AZA = TC
		135	versus TC 0.5–1.0g/d p.o.	6	79		83	61	

N = Number of patients assigned to each treatment group. *Average percent reduction in number of primary lesions. Response rates were obtained on completion of treatment. †Percent of patients achieved good-to-excellent clinical response, defined as ≥ 50% reduction in primary or total lesion count on completion of treatment. ‡Conclusions based on the proportion of patients who achieved good-to-excellent clinical response: = denotes equal therapeutic efficacy; ≥ denotes superior efficacy; > denotes a statistically significant advantage over comparable agent. nb = nonblind; sb = single blind; db = double blind; r = randomized; mc = multicenter; b.i.d. = twice a day; p.o. = orally.

drug on cutaneous bacteria and its oxyradical scavenging properties may attribute to the reduction of inflamed acne lesions.[70]

ADVERSE AND CLINICAL SIDE EFFECTS OF AZELAIC ACID

In numerous studies, including acute, chronic, those involving reproduction toxicology, investigations of the mutagenicity and sensitizing potential, and observations of local tolerance in various animals (i.e., mouse, rat, guinea pig, rabbit, dog, and monkey), AZA was found to be nontoxic.[13,14] Continuous infusion of 10 g of AZA over 80–90 min in healthy subjects posed no adverse effects.[12] Topical application of 20% AZA is well tolerated in humans and overt systemic toxicity has not been reported. One isolated case of hypokalemia occurred following oral intake of 12 g/day AZA for 12 weeks.[67] The allergic reaction most commonly encountered is a local type of irritant, erythematous lesion that appears mild and transient.[55,65] The associated symptoms reported were burning, itching, and/or stinging,[61] but they generally subsided after 2–4 weeks of therapy.[70,72,76] Most of the local side effects were related to unsuitable cleansing, followed by excessive application and vigorous rubbing-in of the AZA cream.[61] Mild scaling[55] and absent phototoxic potential[62] have been reported.

THE ROLE OF AZELAIC ACID IN THERAPY

The therapeutic efficacy of AZA has been demonstrated in clinical trials of patients with acne and melasma (see Tables 3 and 4). Results of these studies suggested that topical 20% AZA is as effective as topical 5% benzoyl peroxide, 0.05% tretinoin, 2% erythromycin, and 0.5–1 g/d oral tetracycline in ameliorating comedonal, papulopustular, and nodulocystic acne, but much less effective than oral isotretinoin in a dose of 0.5–1 mg/kg/day in reducing conglobate acne. The few encountered side effects of AZA in topical administration and the lack of overt systemic toxicity indicate that its chronic use may be better tolerated than other agents. Topical 20% AZA has been shown as effective as 4% hydroquinone cream and superior to the 2% formulation. The lower incidence of allergic sensitization, exogenous ochronosis, or residual hypopigmentation at the application sites produces an advantage over conventional drugs. Thus, AZA may be used as an alternative therapy for melasma in addition to the use of hydroquinone monotherapy or in combination with a corticosteroid.

CONCLUSION

Inadequate evidence supports the use of AZA as primary agent in the treatment of both lentigo maligna and malignant melanoma; however, AZA may be considered as an alternative agent when surgical excision and other forms of therapy prove impractical.

Interestingly, patients with multiple sclerosis were found to develop antibody against endogenous AZA.[84] Therefore, the compound may show different therapeutic effects when administered to these patients.

In Europe, AZA is available in 20% topical cream formulation for cutaneous lesions. It can be applied once daily for the first week and twice daily thereafter for periods of 2–3 months up to 1 year. Treatment may be repeated in recurrent cases. In USA, the patent of AZA is currently licensed to Allergen Herbert, Inc. Its therapeutic applications in the treatment of skin disorders have not yet been approved by the Food and Drug Administration. Azelaic acid has not yet been marketed in USA, but once released it would be most likely used for treating acne and melasma.

DRUG NAMES

hydroquinone: Artra Skin Tone Cream, Black and White Bleaching Cream, Derma-Blanch, Eldopaque Cream
tretinoin: Retin-A

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