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Pharmacology and Toxicology of Azelaic Acid

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The results of general pharmacological studies on metabolism, smooth muscles, renal function, cardiovascular and neurotropic effects do not contra-indicate the specific surface use of azelaic acid. From specific pharmacologic studies it is assumed that azelaic acid exerts its therapeutic effect in acne by an antimicrobial, probably bacteriostatic, effect on acne-relevant microorganisms such as *Propioni bacerium* acnes and, in addition, by a strong comedolytic effect. In numerous studies it has been demonstrated that azelaic acid is not toxic.

INTRODUCTION

Pharmacological investigations on azelaic acid were carried out in order to provide a basis for clinical studies already performed (1), for safety reasons and out of interest in its mechanism of action.

General pharmacology

The investigation on general pharmacology is summarized in Table I.

Taking into consideration the fact that use of azelaic acid for acne involves use on damaged skin and thus absorption of a certain quantity, the safety of azelaic acid was evaluated in studies which disclosed its influence on the metabolism of carbohydrates and fats, on kidney function, and on cardiovascular and neurotropic effects.

To evaluate the influence of azelaic acid on serum-free fatty acid, blood glucose, lactate and pyruvate concentration, the disodium salt of azelaic acid was injected intravenously in doses of 1 000 mg/kg over 45 s into Wistar SPF rats. Fifteen minutes post injection, the lactate concentration was significantly elevated, the free fatty acid concentration was significantly reduced, and the glucose concentration was inconspicuous. Thirty minutes after injection, all the parameters measured had returned to within the normal range. These effects, of fairly short duration, should not have any clinical relevance for topical therapy, since the concentrations of azelaic acid used in the test will never be reached after local surface application for acne treatment.

In a similar study, 100 mg disodium salt of azelaic acid per kg body weight was administered intravenously to rabbits once daily on 6 consecutive days. No effect was observed on liver function, lactate, pyruvate, glucose, urea or creatinine serum concentrations. Glucose tolerance was slightly decreased under the influence of azelaic acid treatment.

In order to evaluate the influence of azelaic acid on smooth muscles, appropriate in vitro set-upts for isolated guinea pig organs were used. Azelaic acid in excess of a certain, calculated concentration, assuming immediate and 100% resorption of the topically applied drug, had no effect on smooth muscle of organs such as trachea, ileum and uterus.

Since azelaic acid is excreted in Man through the kidneys, its effect was studied on urinary excretion of the ions Na^+, K^+ and Ca^{++} and on the urinary flow of the disodium salt of azelaic acid administered intravenously up to 1 000 mg/kg. From the results it can be concluded that azelaic acid has no effect on kidney excretion after doses used clinically.

By comparison with the control group, azelaic acid did not show any effect on arterial pressure or heart rate in conscious rats after ascending doses of 10, 50, and 250 mg/kg, intravenously, up to 60 min after injection. For concentrations up to 10^{-3} mol/l it did not affect the frequency or contractile strength of the isolated guinea pig atrium with spontaneous or stimulated beat.

The Irwin test on Wistar rats after a single i.v. injection of azelaic acid disodium salt in doses up to 800 mg/kg was used to check the neurotropic effect of azelaic acid. At 30 min, 4 h and 24 h after the injection, the incidence of effects was defined on the basis of a scale of control with 58 items which included various neurological and behavioural symptoms and functions related to the autonomic nervous system. Mydriasis (400 mg/kg) and reduced locomotor activity (800 mg/kg) were observed sporadically.

All the results of these pharmacodynamic studies therefore exclude any effect which contra-indicates the specified surface use of azelaic acid.

Special pharmacology

Azelaic acid is clearly indicated for the therapy of acne (1). Unfortunately, no experimental animal

Table 1. *General pharmacology of azelaic acid (di-sodium salt)*

Phamacological effect	Test-model	Dose and route of application	Result
Metabolism	Glucose, free fatty acids; lactate (rat)	1 g/kg i.v.	Slight and short-lived increase of lactate and decrease of free fatty acid
Metabolism	Glucose, lactate, pyruvate, urea, creatinine (rabbit)	6×100 mg/kg i.v. over 6 days	No effect
	Glucose tolerance (rabbit)	6×100 mg/kg i.v. over 6 days	Slight decrease
Smooth muscles (in vitro)	Isolated trachea, ileum, uterus (guinea pig)	0.13 mol/l	No effect
Renal function	Na^+, K^+, Ca^{2+} and water exretion (rat)	Up to 1 g/kg i.v.	No effect
Cardiovascular (in vivo)	Blood pressure, heart frequency (rat)	Up to 250 mg/kg i.v.	No effect
Cardiovascular (in vitro)	Isolated papillary muscle (quinea pig)	10^{-3} mol/l	No effect
Neurotropic	Irwin test (rat)	Up to 800 mg/kg i.v.	No effect

model exists for this disease. Therefore, the evaluation of pharmacodynamic activities was carried out on models which are commonly considered to be related to the basic process of acne pathology.

It is now generally accepted that bacterial propagation in the sebaceous gland and follicle is a factor causing acne. Two studies along these lines were carried out (Table II). In the first study the following was evaluated. Aerobic and anaerobic bacteria, yeast and fungi were grown in Erlenmeyer flasks under continuous shaking at 30°C for 24 to 48 h. The effect of azelaic acid at concentrations of 10^{-1} to 5×10^{-1} mol/l on the growth of the cultures was determined photometrically.

Azelaic acid at concentrations of 10^{-1} to 2×10^{-1} mol/l leads to retardation and at a concentration of 5×10^{-1}mol/l to complete inhibition of growth of the microorganism tested. From experiments where azelaic acid containing medium was replaced by fresh azelaic acid free medium, it is concluded that the effect of azelaic acid is bacteriostatic rather than bactericidal.

In a second investigation on the effect of azelaic acid on the growth of Gram-negative and Gram-positive bacteria, yeast and dermatophytes, the minimal inhibition concentration (MIC) and the minimal bactericidal concentration (MBC) were determined. MIC and MBC were evaluated visually by a serial dilution technique after appropriate incubation.

MIC of azelaic acid is in the order of 3×10^{-1} to 5×10^{-1} mol/l (*Propionibacterium acnes* 2.5×10^{-1} or 3×10^{-1} mol/l depending on the strain). The MBC is in the order of 3×10^{-1} to >1 mol/l. The results of these studies are in agreement with those of Leeming et al. (2).

Another pathogenic factor of acne is the hyperactivity of the sebaceous gland. The hamster ear lipogenesis model was used to evaluate the influence of azelaic acid on sebaceous gland activity (Fig. 1).

Lipid synthesis of the sebaceous gland of the castrated testosterone propionate-substituted hamster

Table 2. *Antimicrobial effects of azelaic acid*

MCRG = Minimum concentration reducing growth. MIC = Minimum inhibitory concentration. MBC = Minimum biocidal concentration.

	1. Study		2. Study	
Organism (strain)	MCRG (mol/l)	MIC (mol/l)	MIC (mol/l)	MBC (mol/l)
Staphylococcus				
Epidermidis	–	–	0.5	>1.0
Aureus	0.1/0.2	0.5	0.3	0.5
Propionibacterium				
Acnes	0.5	0.1	0.25	>0.5
Acnes	–	–	0.25	>0.5

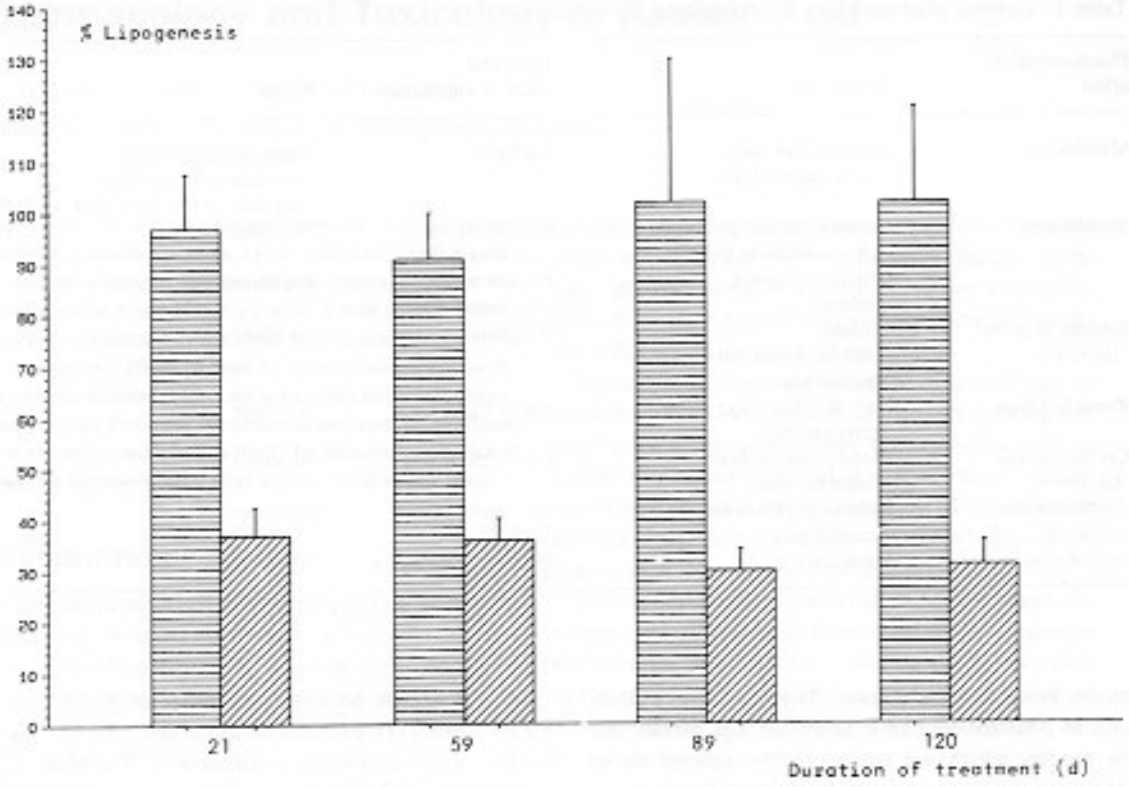
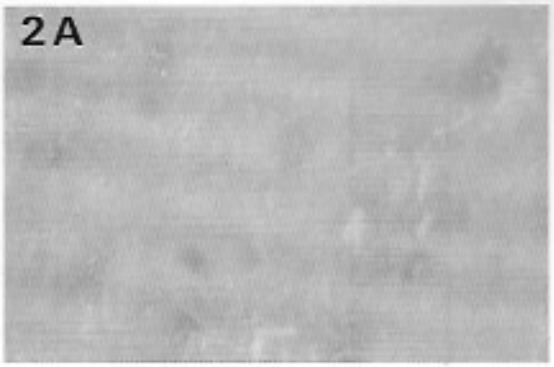
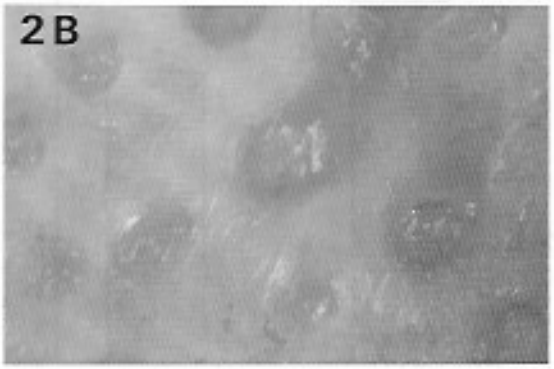


Fig. 1. Sebaceous gland lipogenesis in the ear of the castrated testosterone propionate-substituted (TP-substituted) hamster following a treatment with azelaic acid over a period up to 4 months. Results expressed as a percentage of castrated TP-substituted controls (100%). Vertical range bars indicate standard deviation. ▨, 10% azelaic acid (ethanolic solution); ▧, castrated control.

Table 3. *Toxicity studies (summary)*

Acute toxicity	*Toxicity with repeated administration*
Administration of the compound as microcristalline suspension (mouse, rat, dog)	Subacute toxicity trials Rat, oral, 30 days Monkey, oral, 30 days
Foetal toxicity and fertility studies	*Mutagenic potential*
Tests for teratogenicity Rat, oral Rabbit, oral Monkey, oral	In vitro studies Ames-test, HGPRT-test, Human lymphocyte test In vivo study (dominant lethal assay)
Local tolerance	*Sensitizing effects*
Rabbit, dermal 28 days Dog, dermal, 27 weeks Eye irritation studies (rabbit)	Maximization test (guinea pig)





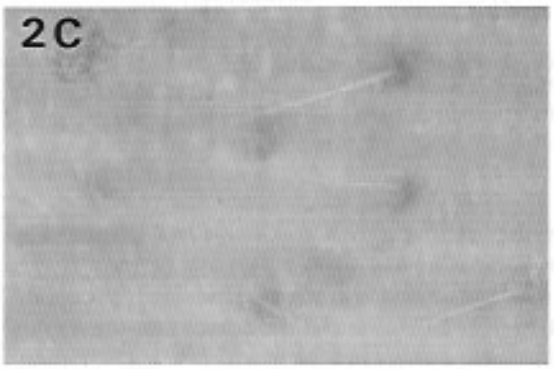
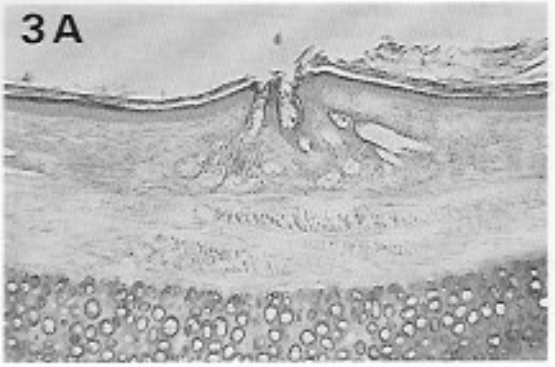


Fig. 2. Photographs of the surface (magnification ×34) of ears from non-treated rabbits (*A*), of ears from rabbits treated with tetradecane + ethanol/acetone (*B*) and of ears from rabbits treated with tetradecane + azelaic acid in ethanol/acetone (*C*).



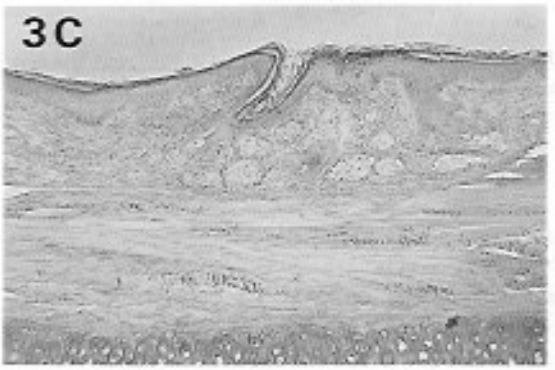


Fig. 3. Photographs of histology (stain: hematoxylin/eosin, ×135) of ears from non-treated rabbits (*A*), of ears from rabbits treated with tetradecane + ethanol/acetone (*B*) and of ears from rabbits treated with tetradecane + azelaic acid in ethanol/acetone (*C*).

was not affected following treatment with an ethanolic solution of 10% azelaic acid over a period up to 4 months (3).

Azelaic acid has been detected autoradiographically in mitochondria in high concentrations, according to Ward et al. (4). Therefore, it could be suggested that azelaic acid exerts its activity by influencing mitochondrial respiration. From studies by Passi et al. (5) on isolated rat liver mitochondria it emerged that at concentrations ranging from 5×10^{-3} to 10^{-2} mol/l, dicarboxylic acids from C_8 to C_{13} are capable of inhibiting cell respiration, and that they are competitive

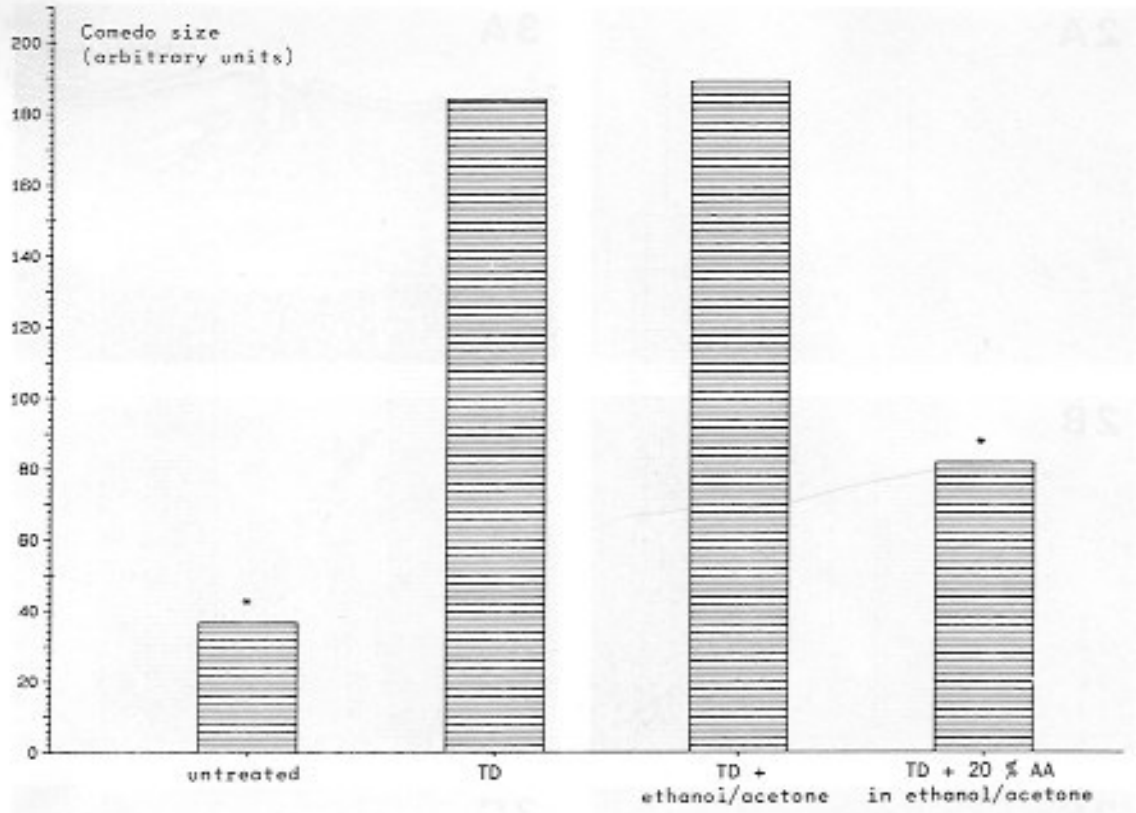


Fig. 4. Influence of a daily (except Saturday and Sunday) local treatment with 200 µl of azelaic acid (AA) 20% solution in ethanol/acetone over a 2-week period. Values are a median of 10 animals per group and 20 comedones per animal. *Significant difference vs. the tetradecane-treated (TD) controls ($\alpha = 0.05$, Kruskal-Wallis test).

inhibitors of mitochondrial oxido-reductases such as NADH-dehydrogenase, succinic-acid-dehydrogenase and reduced ubiquinon-cyt-c-oxido-reductase.

The question of whether the inhibition of mitochondrial oxido-reductases accounts for the effect observed in vivo, remains open, however.

Moreover, follicular epithelial hyperplasia is considered to be a pathogenetic factor in acne. Tetradecane-induced comedo formation in the rabbit ear was taken as the model. A significant reduction in the follicular epithelial hyperplasia which follows daily treatment over a period of 14 days with 200 µl of 20% azelaic acid solution or azelaic acid cream could be demonstrated by low-magnification photographs of ear skin (Fig. 2A–C) and the corresponding histological pictures (Fig. 3A–C).

The results of a quantitative morphometric analysis of this effect is given in Fig. 4.

This effect seems to be specific, since treatment with pimelic acid ($HOOC\text{-}(CH_2)_5\text{-}COOH$) in the same concentration had no effect (Fig. 5).

As azelaic acid is applied topically in high concentration (a 20% cream) it is assumed that it exerts its therapeutic effect in acne by an antimicrobial, probably bacteriostatic, effect on acne-relevant microorganisms such as *Propionibacterium acnes* and, in addition, by a strong comedolytic effect. It is not very likely that azelaic acid acts via the inhibition of sebaceous gland activity, though this is a matter which requires study, employing appropriate investigative techniques.

Toxicology

In numerous studies in acute, chronic and reproduction toxicology, in investigations on the mutagenic and sensitizing potential, and in local tolerance studies (Table III), it was demonstrated that azelaic acid is not toxic.

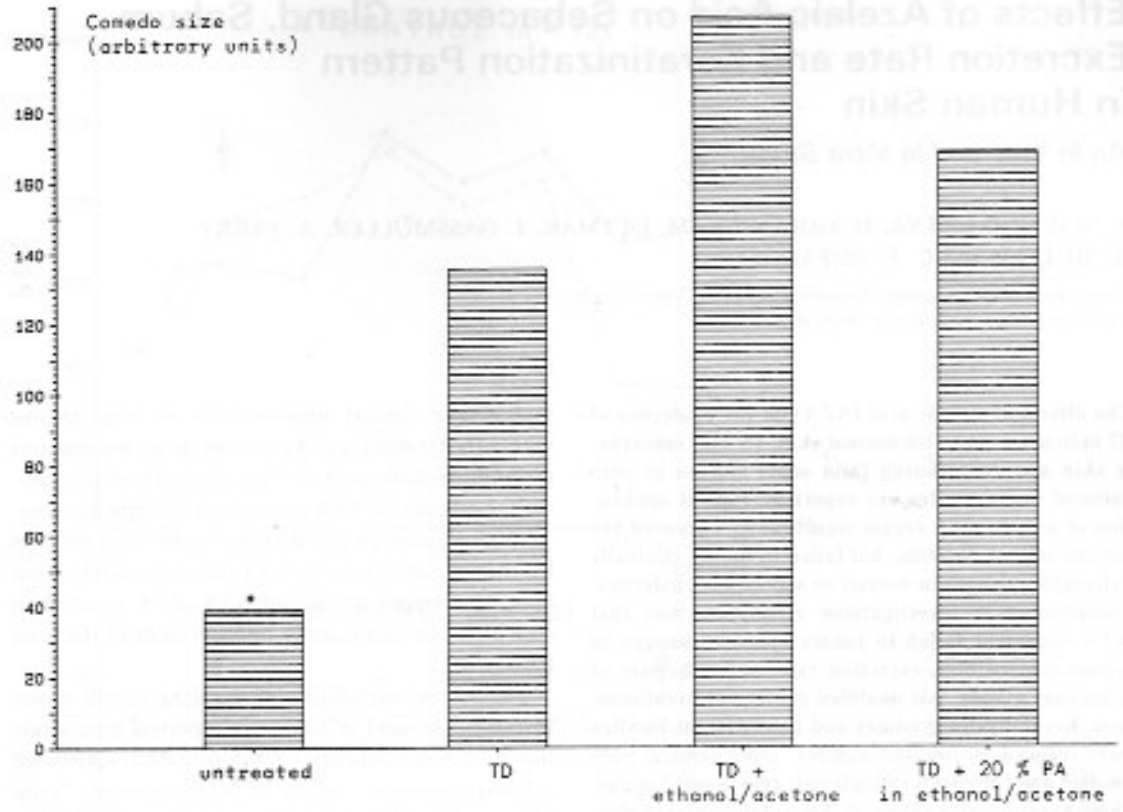


Fig. 5. Influence of a daily (except Saturday and Sunday) local treatment with 200 µl of pimelic acid (PA) 20% solution in ethanol/acetone over a 2-week period. Values are a median of 10 animals per group and 20 comedones per animal. *Significant difference vs. the tetradecane-treated (TD) controls (α=0.05, Kruskal-Wallis test).

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