Acta Derm Venereol (Stockh) 1994; 74: 347–350

Dicarboxylic Acids Affect the Growth of Dermatophytes *In vitro*

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Azelaic acid is a dicarboxylic acid with known antimycotic activity. In this study we have used an agar dilution technique to test the effect of six other dicarboxylic acids (sebacic, undecanedioic, dodecanedioic, tridecanedioic, tetradecanedioic and hexadecanedioic acid, 10^{-4}–10^{-2} mol/l, pH 5.5) on in vitro growth of *Trichophyton* (*T.*) *rubrum*, *T. mentagrophytes* and *Microsporum* (*M.*) *canis*. Furthermore, the fungicidal activity of 10^{-2} mol/l undecanedioic and sebacic acid was tested using a *T. rubrum* growth assay. Undecanedioic acid proved fungistatic at 10^{-2} mol/l for all species and fungicidal for *T. rubrum*. A minor fungistatic effect on *T. rubrum* and *T. mentagrophytes* was also seen with the other acids at this concentration. *M. canis* was inhibited only by high concentrations of four acids, whereas low concentrations of all six agents resulted in enlarged thallus diameters. We conclude that among dicarboxylic acids fungistatic activity is not limited to azelaic acid. Undecanedioic acid appears promising for further investigations. *Key words: fungi; undecanedioic acid; in vitro antimycotic activity; Trichophyton; Microsporum.*

(Accepted March 4, 1994.)

Acta Derm Venereol (Stockh) 1994; 74: 347–350.

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Antimycotic properties of fatty acids have been known for a long time (1–4). Dicarboxylic acids are closely related to saturated fatty acids due to their similar chemical structure ($HO_2 \cdot C(CH_2)_n \cdot CO_2H$). Interestingly, azelaic acid, which is a medium length dicarboxylic acid, has also been shown to have antibacterial (5, 6) as well as antimycotic (5, 7) effects in vitro. Its antimycotic activity is pH-dependent (7) and may be due to its ability to damage mitochondria of eukaryotic cells (8–10). Although such an interference with fungal growth may not be specific for azelaic acid, little is known about antimycotic properties of other dicarboxylic acids. Therefore, the objective of this study was to examine the effects of dicarboxylic acids other than azelaic acid on dermatophytes.

MATERIAL AND METHODS

Fungistatic effects

Three different strains each of *Trichophyton* (*T.*) *rubrum*, *T. mentagrophytes* and *Microsporum* (*M.*) *canis* were used, which were isolated from patients and identified by their typical morphology on Sabouraud glucose agar (11). Dicarboxylic acids of different chain lengths ($HO_2 \cdot C(CH_2)_n \cdot CO_2H$; with n ranging from 8 to 14) were purchased from Sigma Chemical Co. Deisenhofen, Germany (sebacic acid ($n = 8$) and tetradecanedioic acid ($n = 12$)), Fluka Chemie AG Buchs, Switzerland (undecanedioic acid ($n = 9$)), Merck-Schuchardt Hohenbrunn, Germany (dodecanedioic acid ($n = 10$)), and Aldrich Chemie Steinheim, Germany (tridecanedioic acid ($n = 11$) and hexadecanedioic acid ($n = 14$)).

An agar dilution technique was used to determine the fungistatic effects of the dicarboxylic acids. Agar plates were composed as follows: neopeptone (Difco) 2%, glucose 2%, purified ethanol 1.0%, dicarboxylic acid 10^{-4}, $10^{-3.5}$, 10^{-3}, $10^{-2.5}$, or 10^{-2} mol/l, agar-agar 1.8%; all plates were buffered at pH 5.5 with 0.02 molar NaH_2PO_4 plus citric acid or NaOH, depending on the concentration of dicarboxylic acid incorporated. After autoclaving (120°C, 20 min), chloramphenicol 40 mg/l and penicillin 40000 U/l were added. Control plates were of equal composition except that no dicarboxylic acids were incorporated.

Punch biopsies (3 mm diameter) taken 3 mm centripetally from the progressing margins of fresh thalli grown for 8–14 days on Sabouraud agar plates (Becton-Dickinson) were used as standardized inocula by putting them upside down onto the test agar plates. After 14 days of incubation at 26°C, thallus diameters on the test plates were measured. For statistical evaluations the Student's t-test was performed and p values ≤ 0.05 were considered significant.

Fungicidal effects

T. rubrum was exposed to 10^{-2} mol/l sebacic and undecanedioic acid for an assessment of fungicidal effects. For this purpose, 3 mm-punch biopsies obtained from fresh *T. rubrum* thalli grown on Sabouraud agar plates (Becton-Dickinson) were brought into a broth containing neopeptone (Difco) 2%, glucose 2% and purified ethanol 1.0%, buffered at pH 5.5. After incubation for 10 days at 26°C, the mycelia were thoroughly rinsed with sterile saline and transferred into fresh nutrient broth, now supplemented with 10^{-2} mol/l sebacic or undecanedioic acid (pH 5.5). In this milieu the mycelia remained exposed to the dicarboxylic acids for 1, 2, 4 or 8 h at 26°C. Thereafter the mycelia were again washed with sterile saline, and each mycelial pellet was used to inoculate a separate Sabouraud agar plate (Becton-Dickinson) at one point. After incubation for 14 days at 26°C, all plates were checked for fungal growth, and colony diameters were measured.

Arithmetic means of colony diameters were compared to control values, which were obtained with mycelia treated in the same way except that the dicarboxylic acids were omitted from the second broth used. The Student's t-test was used for statistical evaluations, with $p \leq 0.05$ accepted as significant.

RESULTS

Fungistatic effects

There were no relevant differences between the responses of individual strains belonging to the same species. Therefore, the results are summarized for the distinct species (Tables I–II). Furthermore, supplementation with dicarboxylic acids had no apparent influence on macroscopic thallus morphology (growth rate excepted) or pigmentation of any of the species tested.

Trichophyton rubrum (Table I)

All dicarboxylic acids tested were inhibitory for *T. rubrum* at 10^{-2} mol/l. At this concentration, no growth at all occurred with undecanedioic acid, and only minimal growth of some colonies was seen with tridecanedioic and tetradecanedioic acid. The fungistatic effect of all acids was clearly dose-dependent, being undetectable or only minimal with 10^{-4} mol/l.

Trichophyton mentagrophytes (Table I)

This species was almost completely inhibited by undecanedioic acid 10^{-2} mol/l. All other dicarboxylic acids also reduced growth of *T. mentagrophytes* at this concentration, but to a much lesser

 ISSN 0001-5555

Table I. *Inhibition of Trichophyton rubrum and Trichophyton mentagrophytes by dicarboxylic acids*

Acid	Concentration (mol/l)					
	0	10^{-2}	$10^{-2.5}$	10^{-3}	$10^{-3.5}$	10^{-4}
Sebacic a.	35.2 (28.7)	5.4*** (22.7***)	31.5*** (30.4***)	32.8*** (28.3)	32.3*** (28.1***)	32.4 (26.1***)
Undecanedioic a.	35.2 (28.7)	0*** (1.6***)	22.4*** (26.3***)	32.9*** (27.6*)	34.9 (27.2***)	35.9** (27.6*)
Dodecanedioic a.	35.2 (28.7)	24.9*** (23.6***)	26.0*** (22.2***)	27.8*** (22.6***)	35.3 (25.4***)	35.7 (26.4***)
Tridecanedioic a.	35.2 (28.7)	1.1*** (16.1***)	14.5*** (19.1***)	24.6*** (19.5***)	34.1** (23.6***)	36.5** (25.6)
Tetradecanedioic a.	35.2 (28.7)	0.8*** (21.0***)	27.8*** (22.5***)	30.6*** (24.5***)	32.5*** (27.1**)	32.7*** (24.3***)
Hexadecanedioic a.	35.2 (28.7)	28.7*** (25.5***)	30.8*** (25.5***)	32.4*** (27.0***)	32.9*** (26.3***)	33.7*** (27.5**)

Arithmetic means of thallus diameters in mm (n of controls is 33 for *T. rubrum* and 29 for *T. mentagrophytes*); diameters obtained with *T. mentagrophytes* are given in parentheses. The numbers of readings (n) for the different concentrations of the various acids range from 29 to 36 for *T. rubrum* and from 26 to 30 for *T. mentagrophytes*. Significant differences from control values are marked by * ($p \leq 0.1$), ** ($p \leq 0.01$), or *** ($p \leq 0.001$). a. = acid.

degree; tridecanedioic acid 10^{-2} mol/l reduced thallus diameters to 56% of the control value, and with the other acids still 79–89% of control diameters were measured. The inhibitory effect of all acids was small (< 18%) at concentrations below 10^{-3} mol/l, although statistically significant in most cases.

Microsporum canis (Table II)

M. canis did not grow at all with 10^{-2} mol/l undecanedioic acid. Smaller but still significant suppressive effects (reduction to 56–82% of control values) were seen with sebacic, tridecanedioic and tetradecanedioic acid at this concentration.

In contrast, supplementation with the same concentration (10^{-2} mol/l) of dodecanedioic and hexadecanedioic acid stimulated a significant increase of thallus diameters. With 10^{-2} mol/l of hexadecanedioic acid, the length of thallus diameters reached 149% of the control colonies. Furthermore, lower concentrations (< 10^{-3} mol/l) of all acids, including undecanedioic acid, resulted in colony diameters larger than those of control cultures.

Fungicidal effects (Fig. 1)

Undecanedioic and sebacic acid, which were both shown to have fungistatic effects, were also tested for their fungicidal activity. *T. rubrum* was exposed to 10^{-2} mol/l of these agents for 1, 2, 4 and 8 h. Exposure to control buffer for the same time intervals did not significantly affect colony numbers or thallus diameters of *T. rubrum* in subsequent cultures.

Undecanedioic acid caused a time-dependent fungicidal effect on *T. rubrum*, as determined by thallus diameters in subsequent cultures. Their reduction was already significant after exposure of the mycelia to 10^{-2} mol/l undecanedioic acid for 1 h only and increased continuously with extended times of exposure (Fig. 1; $p \leq 0.001$ after 2 h). Furthermore, within 8 h, 8 out of 9 thalli were destroyed by undecanedioic acid.

The fungicidal activity of sebacic acid was less pronounced. The mycelia were not definitely destroyed but increasingly impaired in their subsequent growth after exposure to 10^{-2} mol/l sebacic acid for 2 h or longer, as reflected by smaller colony diameters (Fig. 1) than in controls ($p \leq 0.001$ after 2 h).

Table II. *Inhibition/stimulation of Microsporum canis by dicarboxylic acids*

Acid	Concentration (mol/l)					
	0	10^{-2}	$10^{-2.5}$	10^{-3}	$10^{-3.5}$	10^{-4}
Sebacic a.	23.0	12.8***	19.5***	24.6***	25.8***	26.3***
Undecanedioic a.	23.0	0***	6.4***	20.1***	26.7***	25.9***
Dodecanedioic a.	23.0	25.0*	34.7***	34.6***	32.8***	35.4***
Tridecanedioic a.	23.0	18.9***	21.2*	28.6***	30.9***	31.2***
Tetradecanedioic a.	23.0	15.9***	19.6***	23.1	23.6	25.6
Hexadecanedioic a.	23.0	34.3***	33.3***	25.6***	25.6***	28.1***

Arithmetic means of thallus diameters in mm (n of controls = 33). The numbers of readings (n) for the different concentrations of the various acids range from 27 to 36. Significant differences from control values are marked by * ($p \leq 0.05$), or *** ($p \leq 0.001$). a. = acid.

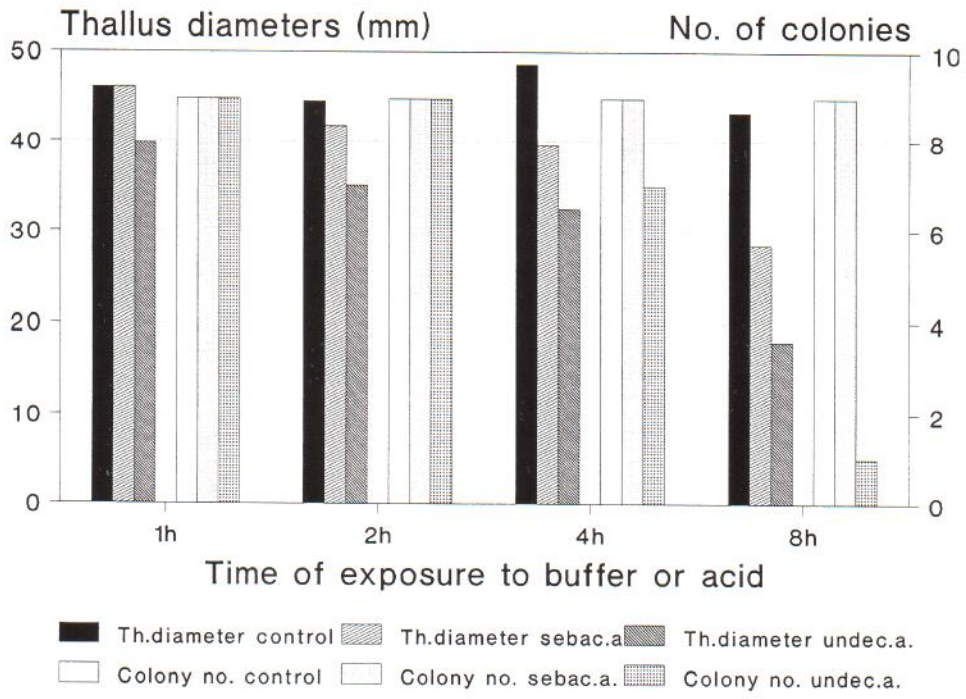

Fig. 1. Fungicidal effects of sebacic and undecanedioic acid. Effect of mycelial incubation with buffer (controls) and 10^{-2} mol/l sebacic and undecanedioic acid for 1, 2, 4 and 8 h on thallus diameters (in mm) and colony numbers determined after subsequent culture on Sabouraud agar for 14 days at 26°C. Thallus diameters shown are arithmetic means (n = 9). However, after 4 h of incubation with undecanedioic acid only 7 colonies proliferated (n = 7), and only 1 thallus survived after incubation with undecanedioic acid for 8 hours (n = 1). Th.diameter: thallus diameter; Sebac.a.: sebacic acid; Undec.a.: undecanedioic acid.

DISCUSSION

Our results show a pronounced inhibitory effect of several dicarboxylic acids on the growth of dermatophytes in vitro. This is a new observation, since except for azelaic acid (7) no such activity of dicarboxylic acid has been described (2). Similar to fatty acids and their related derivatives (2), an acidic pH is essential for the antifungal activity of dicarboxylic acids. This has already been reported for azelaic acid (7), and pilot experiments to the present study revealed a corresponding impact of pH on the activity of the dicarboxylic acids used in this investigation (data not shown).

Undecanedioic acid was most effective in our study. At a concentration of 10^{-2} mol/l and pH 5.5, this substance suppressed the growth of *T. rubrum, M. canis* and *T. mentagrophytes* and was fungicidal for *T. rubrum*. Correspondingly, in a recent comparison of saturated fatty acids with 7–13 carbon atoms, undecanoic acid proved to be most inhibitory for dermatophytes (4). Dodecanedioic acid and hexadecanedioic acid were least effective in our study, with only low inhibitory activities towards *T. rubrum* and *T. mentagrophytes* and none at all towards *M. canis*.

The fungistatic concentration reported for azelaic acid is $3 \cdot 10^{-2}$ mol/l at pH 4.9 (7), which is slightly higher than that of undecanedioic acid. In comparison, undecylenic acid, an unsaturated fatty acid still in use as an antimycotic agent, was found fungistatic for *Trichophyton interdigitale* at a concentration of only $2.2 \cdot 10^{-4}$ mol/l (pH 6.5) (2). Therefore, the antifungal mechanism of unsaturated fatty acids may differ from that of dicarboxylic acids. Since azelaic acid appears to interfere with mitochondrial functions (8–10), the pH-dependent antimycotic principle of dicarboxylic acids is likely to differ from that of therapeutically used inhibitors of mycotic ergosterol synthesis (12, 13). An inhibition of fungal cellular respiration by fatty acids was reported previously (14).

As an unexpected result, colony diameters of *M. canis* were found to increase with low concentrations ($\leq 10^{-3}$ mol/l) of all dicarboxylic acids. Pleomorphism was not observed. Hexadecanedioic acid and dodecanedioic acid exerted a stimulatory effect on *M. canis* even at the highest concentration (10^{-2} mol/l), whereas the other acids were suppressive at this molarity. A possible explanation may be that, compared to *T. rubrum* and *T. mentagrophytes, M. canis* is less susceptible to dicarboxylic acids. *M. canis* may even utilize dicarboxylic acids as nutrients, if their concentration is kept below fungistatic levels; a similar degradation of fatty acids by dermatophytes was reviewed previously (4). Differences of enzymatic activities between *M. canis* and other dermatophytes (15) may explain such divergent responses to dicarboxylic acids.

Despite these findings, dicarboxylic acids do not seem to play a role as natural cutaneous antifungal factors, since in contrast to a variety of fatty acids (16), dicarboxylic acids have not been identified as physiological epidermal constituents. However, out of the acids tested in this study, undecanedioic acid may be considered as a promising candidate for further analysis of its therapeutic efficacy, similar to azelaic acid (5, 6, 17).

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