In vitro and in vivo killing of ocular Demodex by tea tree oil

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Aims: To compare the in vitro killing effect of different agents on Demodex and to report the in vivo killing effect of tea tree oil (TTO) on ocular Demodex.

Methods: Survival time of Demodex was measured under the microscope. Sampling and counting of Demodex was performed by a modified method.

Results: Demodex folliculorum survived for more than 150 minutes in 10% povidone-iodine, 75% alcohol, 50% baby shampoo, and 4% pilocarpine. However, the survival time was significantly shortened to within 15 minutes in 100% alcohol, 100% TTO, 100% caraway oil, or 100% dill weed oil. TTO’s in vitro killing effect was dose dependent. Lid scrub with 50% TTO, but not with 50% baby shampoo, can further stimulate Demodex to move out to the skin. The Demodex count did not reach zero in any of the seven patients receiving daily lid scrub with baby shampoo for 40–350 days. In contrast, the Demodex count dropped to zero in seven of nine patients receiving TTO scrub in 4 weeks without recurrence.

Conclusions: Demodex is resistant to a wide range of antiseptic solutions. Weekly lid scrub with 50% TTO and daily lid scrub with tea tree shampoo is effective in eradicating ocular Demodex.

The organism Demodex folliculorum is found in the eyelash follicle and Demodex brevis burrows deep in sebaceous and meibomian glands. Although their pathogenic role remains unsettled, efforts have been made to eradicate ocular Demodex in patients presenting with blepharitis. Junk et al.2 reduced the Demodex count in half after 1 month of lid scrub with baby shampoo combined with topical 2% metronidazole gel. In a case report, Fulk and Clifford3 used topical 1% mercuric oxide ointment to bring the Demodex count from 10 mites in eight lashes to one in four lashes in 3 weeks. Subsequently, they used topical 4% pilocarpine gel to decrease the Demodex count from 2.4 per lash to 0.9 per lash in 2 weeks.4 Judged from the fact that the aforementioned treatments do not eradicate ocular Demodex in one life cycle—that is, 14–18 days,5 we questioned their capability of killing Demodex in vivo.

We thus surveyed a number of potential agents and discovered that tea tree oil (TTO) is a promising acaricide. TTO, a natural essential oil steam distilled from the leaf of Melaleuca alternifolia, has long been used as an aboriginal traditional medicine in Australia for wounds and cutaneous infection. It has antibacterial,6–9 antifungal,9–13 anti-inflammatory,14 15 and acaricidal effects.16 Shampoo containing TTO has been used to kill lice as well as nits,17 and 2.5% TTO gel is well tolerated and non-toxic for treating dental plaque and chronic gingivitis.18 Here, we further showed how weekly lid scrub with 50% and daily lid scrub with TT shampoo could eradicate ocular Demodex in 1 month in seven of nine patients.

MATERIALS AND METHODS

Materials
Ten percent povidone iodine, 75% and 100% alcohol, mineral oil, 10% SDS, and TTO were from Sigma (Milwaukee, WI, USA); 4% pilocarpine solution was from Bausch & Lomb (Tampa, FL, USA), caraway oil and dill weed oil were from Essential Oil Company (Portland, Oregon, USA), TT shampoo was from Kato Sales, Inc (Altamonte Springs, FL, USA). TTO was diluted with mineral oil into different concentrations.

In vitro killing

Because there is a high prevalence of Demodex in patients with cylindrical dandruff (CD),19 lashes with CD were chosen for the in vitro killing study. Under the microscope, if the eliplated lash contained live Demodex, different solutions were added. The movement of Demodex body and legs was continuously observed for 150 minutes. The survival time (ST) was defined as the time when the solution was added to the time when the movement ceased (fig 1). The average ST was compared among different solutions. The value 150 minutes was used to denote as no effect, and after that time, we also noted that the Demodex body gradually deformed and shrunk (data not shown). Because Demodex at an earlier stage of life was more vulnerable, we only tested adult Demodex that had four pairs of well developed legs and a stumpy body. Because Demodex buried in CD might preclude the killing effect, we only tested Demodex organisms in which more than half of the body was exposed to the test solution.

Patients

This study followed the tenets of Helsinki Declaration. Patients with CD at the lashes or who complained of ocular irritation without identifiable causes were recruited. All patients had received routine complete eye examination and external photography, and were found to have ocular Demodex (see below). They were not using topical or systemic anti-inflammatory and antibacterial medications. Seven patients had practised lid scrub with diluted baby shampoo at least once daily.

Lash sampling and Demodex counting

The method of lash sampling and Demodex counting is detailed in a recent report.20

Lid scrub with TTO

In the office, after we administered a drop of 0.5% oxybuprocaine (proparacaine), a cotton tip wetted in 50% TTO was used to scrub the lash roots from one end to the other as one stroke. A total of six strokes were applied to each lid. A dry cotton tip was then used to remove excess TTO from the lid margin. Because TTO scrub not only loosened CD but also stimulated Demodex to move out to the skin (see Results), a dry cotton tip was used to remove CD 5 minutes later. After...
another 5 minutes, the second lid scrub with TTO was applied in the same manner. Following 10 minutes of rest, the third TTO lid scrub was applied. If there was any irritation, the eye was rinsed with non-preserved saline. This office scrub was repeated weekly until the Demodex count reached 0 for two consecutive visits.

**Home lid scrub and other instructions**

At home, the patient was asked to mix 0.5 ml of TT shampoo with tap water in both middle fingers. With eyes closed, the lids were massaged with a medium pressure for 3–5 minutes. The skin was then rinsed with clean water and dried with a towel. Such home lid scrub was practised twice daily for 1 month and then once daily thereafter. In addition, the patient was also instructed to discard used facial make-up, and wash the hair, the face, nostrils, the external ear and the neck with TT shampoo daily. The bedding and pillow cases were washed with hot water and dried in a heated dryer immediately after the first office scrub, and once a week thereafter. If the spouse also complained of similar eye irritation, the same home lid scrub was also practised.

**Statistical analysis**

The data were reported as means (SD), and analysed by MicroSoft Excel (MicroSoft, Redmont WA, USA). The data between groups were evaluated by two tailed *t* test where *p* < 0.05 was considered statistically significant.

**RESULTS**

**In vitro killing effect**

We first surveyed the effect of a number of agents listed in table 1 on D folliculorum, and noted that 50% baby shampoo, 100% mineral oil, 75% alcohol, 10% povidone-iodine, or 4% pilocarpine could not kill Demodex in 150 minutes. In contrast, 100% alcohol killed them in 3.9 (SD 1.2) minutes. Because Demodex is frequently trapped in the CD, which consists of cellular debris made of lipids and keratins, we also examined whether a pretreatment with such a detergent as 10% SDS or 50% baby shampoo might promote the killing effect of other agents. The results showed that none of these pretreatments for 30 minutes could facilitate the killing by subsequent application of either 10% povidone-iodine or 75% alcohol. In contrast, 100% of TTO, caraway oil or dill weed oil
Table 1  Survival time of Demodex folliculorum in different solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>ST (minutes)</th>
<th>10% PI</th>
<th>75% Alc</th>
<th>MO</th>
<th>4% Pilo</th>
<th>Mix 1</th>
<th>Mix 2</th>
<th>Mix 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST (minutes)</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>DN</td>
<td>9</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>100% Alc</td>
<td>3.9 (1.2)</td>
<td>150</td>
<td>34.7 (4.3)</td>
<td>14.8 (9.5)</td>
<td>3.7 (0.8)</td>
<td>4.4 (2.3)</td>
<td>14.0 (8.3)</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td>21</td>
<td>16</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

ST, survival time; DN, number of Demodex tested; BS, baby shampoo; MO, mineral oil; PI, povidone-iodine; Alc, alcohol; Mix 1, 50% BS in water for 30 minutes followed by 10% PI; Mix 2, 10% SDS in water for 30 minutes followed by 10% PI; Mix 3, 10% PI for 30 minutes followed by 75% Alc; TTO, tea tree oil; CWO, caraway oil; DWO, dill weed oil; Pilo, pilocarpine.

killed these mites in 3.7 (0.8), 4.4 (2.3), and 14.0 (8.3) minutes, respectively. Furthermore, the killing effect of TTO was dose dependent—that is, being 14.8 (9.5), 34.7 (4.3), and 150 minutes for 50%, 25%, and 10% of TTO, respectively (p<0.01); 50% TTO and 10% TTO also exerted a similar killing of D brevis (not shown).

In vivo killing by lid scrub with diluted baby shampoo

In seven patients who had been practising daily home lid scrub with diluted baby shampoo for more than 1 month, we found out that the Demodex counts did not reach zero (fig 2). Cases 3, 4, and 5 maintained a constant number of Demodex after 40–350 days of lid hygiene. Cases 1 and 7 actually showed an increase of Demodex after 110–350 days. Only cases 2 and 6 showed a decrease of Demodex after 64 and 125 days of lid hygiene, respectively, but never reached zero.

In vivo killing by lid scrub with TTO

We recently confirmed that lashes with CD are pathognomonic for ocular Demodex. It is thus important to get rid of CD, which harbours Demodex. In a patient presenting with symmetrical signs of CD in both eyelids, lid scrub with 50% TTO in the right eye and with 50% baby shampoo in the left eye for 5 minutes equally cleaned off CD (fig 3A and B). However, Demodex was found on the skin surface adjacent to the lashes (fig 3C, arrows), and a Demodex tail was seen protruding from the lash root (fig 3C, arrowheads) in the TTO treated eye, but not in the baby shampoo treated eye (fig 3D). Furthermore, the skin in the eye receiving TTO was redder than that receiving baby shampoo. These findings suggested that lid scrub with TTO did not merely mechanically remove CD from the lash root, but might attract mites to move out to the skin.

To examine this possibility, we epilated lashes with diffuse CD (fig 4A) and found that D folliculorum clustered around the lash follicle (fig 4D). After office lid scrub with 50% TTO, the lashes were free of CD (fig 4B). Interestingly, tails were protruding from the lash root (fig 4B, arrow). When these lashes were epilated, D folliculorum was found on the lash trunk close to the skin surface—that is, away from the lash follicle (fig 4E). When such lash was rotated by a forceps before epilation, we noted several mites migrating along the lash trunk (fig 4F and 4G). If no home lid scrub was subsequently practised, CD returned in 1 week (fig 4C). If the lashes were scrubbed with baby shampoo, simple mechanical rotation did not stimulate Demodex to move out (not shown). These results indicated that lid scrub with 50% TTO, but not baby shampoo, indeed stimulated Demodex to migrate from CD embedded inside the skin.

Because lid scrub with 50% TTO did not kill mites buried deep in the skin, and because if no lid scrub was carried out at home, CD quickly returned in 1 week, we advocated that weekly office lid scrub with 50% TTO be followed by daily home lid scrub with TT shampoo in nine patients (fig 5). The results showed that the Demodex count dropped to zero in 3 weeks in five patients and in 4 weeks in another two patients without any recurrence 1 month later. These seven patients were younger (59.86 (8.7) year old), and had a mean count of 7.9 (4.1) before treatment. In the remaining two patients, cases 1 and 2, the count did not reach zero, and showed a rebound 35 days and 14 days after treatment, respectively. These cases were 68 and 81 years old, and had a higher count of 26 and 16, respectively, before treatment. The rebound count was less than the previous one, and the interval between rebounds was 2–3 weeks.

**DISCUSSION**

Applying our recently modified sampling method, we detected Demodex in 100% (n = 32) of patients presenting CD at eye lashes, and in 22% (n = 23) of those with clean lashes. In a randomly selected general population (n = 206), the incidence of Demodex on lids and the nose is 4% in those younger than 19 years old, 30% in those between 20 and 80 years old, and 47% in those older than 80 years old. However, no research has convincingly demonstrated whether a minimal number of mites sampled must be present in order to produce symptoms.

Consistent with our earlier report, we noted that the Demodex count in all seven patients receiving daily lid hygiene with baby shampoo never reached zero in a period between 40 and 350 days—that is, beyond one life cycle (fig 2). These results, together with those published earlier, strongly imply that the conventional treatment eradicate Demodex, a notion also supported by the finding that 50% baby shampoo did not kill Demodex in vitro (table 1). It should also be noted that 75% alcohol and 10% povidone-iodine, respectively.
agents known to kill most microbes, could not kill *Demodex* in vitro (table 1). Because previous incubation with 50% baby shampoo or 10% SDS, detergents dissolving oil, before 10% povidone-iodine still could not kill *Demodex* (table 1), we suspect that *Demodex* is resistant to povidone-iodine. Although 4% pilocarpine gel was used to treat *Demodex*, it did not kill *Demodex* in 150 minutes (table 1).

Norr21 tested 45 different agents and found only a few could kill *Demodex* within several minutes—absolute alcohol, ether, xylol, benzene, Danish (sulfur-containing) ointment, dill weed oil, and caraway oil. We also noted that 100% alcohol killed *Demodex* in 3.9 (1.2) minutes. Furthermore, 100% of dill weed oil and caraway oil—that is, essential oils, also killed *Demodex* in 14 (8.3) and 4.4 (2.3) minutes, respectively (table 1). Unfortunately, these agents are not amenable for clinical use because of their intrinsic toxicity (irritation) to the eye.

We were encouraged by TTO’s killing effect, which resembled that of caraway oil and exhibited a clear dose-dependent relation (table 1). Although the killing effect of TTO on lice is thought to be mediated by anti-cholinesterase activity,22 it cannot explain its killing effect on nits. TTO might cause contact dermatitis and allergy,23 24 but the dermal LD50 exceeds 5 g/kg in rabbits, and undiluted TTO does not produce phototoxic effects on the skin of hairless mice.25 TTO patch test does not cause adverse reactions.26 These results encouraged us to use TTO to treat ocular *Demodex*. TT shampoo is commercially available for treating head lice without an adverse effect.

Although both effectively cleaned CD, TTO, but not baby shampoo, stimulated *Demodex* to migrate from the CD buried inside the skin (figs 3 and 4). However, if daily lid scrub were not followed, CD returned in 1 week (fig 4). Because mechanical agitation to the lash following TTO scrub was sufficient to stimulate *Demodex* to migrate out to the skin, we advocate daily lid scrub following each office lid scrub with TTO. Because neither 50% baby shampoo nor TT shampoo, of which the concentration of TTO is less than 10%, could kill...
Demodex in vitro (table 1), we believe that home lid scrub acts by arousing Demodex to move out and eliminates them before mating.

Using the proposed scrub regimen, the Demodex count was dramatically reduced in 1 week and reached zero in 1 month without recurrence in seven of nine patients (fig 5). The two patients in whom treatment failed were older, started with a higher Demodex count, and rebound in an interval of 2–3 weeks. Norn,21 and our recent studies19 have noted that patients with a higher Demodex count tend to be older, suggesting that Demodex infestation, if untreated, tends to be worsened with age because of continuous propagation by mating. We thus speculate that higher Demodex infestation produces more CD inside and outside the lash follicle, and with more resistance to TTO. If this interpretation were correct, we predict that it is easier to treat Demodex at an early stage. Alternatively, the failure might be caused by re-infestation by mites migrating from other places of the body or from the spouse. Because the rebound count was less than the previous one, we speculate that continuous lid scrub might eventually eradicate Demodex.

Lid scrub with 50% TTO did generate variable degrees of irritation in some patients, but could be minimised if caution is exercised to avoid spilling into the eye. We were encouraged by the finding that patients’ symptoms were relieved, ocular surface inflammation was resolved, and the lipid tear film stability improved in these patients (in
preparation). Collectively, these findings strongly suggest that ocular Demodex infestation might indeed be pathogenic and warrants further controlled studies.

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