

Abstract

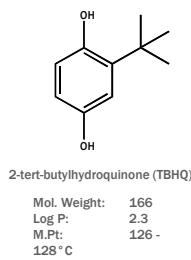
Issues concerning the overuse of topical antibiotics and the emergence of resistance have led to a need for novel antimicrobials for dermatological applications. Repositioning known chemical entities with a history of use in man offers a more effective development route in terms of time, money and risk. 2-tert-butylhydroquinone (TBHQ) is a sterically hindered lipophilic hydroquinone used extensively as an antioxidant, especially to inhibit peroxidation of unsaturated fats. The compound is a permitted direct food additive and a permitted cosmetic ingredient in both the US and EU. It therefore represents an excellent repositioning candidate. Experiments were carried out in order to evaluate its suitability as a topical anti-staphylococcal and anti-propionibacterial agent. *In vitro* tests showed that TBHQ was active against a panel of 10 antibiotic susceptible and resistant coagulase-negative staphylococci (MICs 0.98-7.8 mg/L) and 16 *Staphylococcus aureus* isolates including MRSA, VISA and GISA strains (MICs 1.95 - 7.8 mg/L). TBHQ was also active against strains of *Propionibacterium acnes* and *Propionibacterium granulosum* resistant to erythromycin, clindamycin and related antibiotics via target site mutation or methylation of 23S rRNA and/or to tetracyclines via target site mutation of 16S rRNA. MICs of TBHQ for 21 propionibacterial antibiotic susceptible and resistant isolates ranged from 1.95 to 15.6 mg/L; one isolate of *P. granulosum* showed reduced susceptibility (MIC 62.5 mg/L). Time to kill studies using suspensions of *P. acnes* NCTC737 in aqueous buffer showed that TBHQ at 31.25 mg/L is rapidly bactericidal. Current knowledge of its mode of action suggests that TBHQ affects multiple cellular processes including both direct effects due to TBHQ itself and indirect effects attributed to free radicals generated via oxidation to 2-tert-butylbenzoquinone. It is therefore unlikely that resistance would emerge during clinical use. TBHQ has physicochemical characteristics ideal for topical delivery and retains antimicrobial efficacy in the presence of salt and skin lipids. In view of its safety profile, physicochemical properties and antimicrobial potency, TBHQ represents a novel drug candidate for the local treatment of acne and/or the prevention of staphylococcal skin infections.

Background

Widespread antibiotic resistance in pathogens associated with prevalent dermatoses such as acne and atopic dermatitis is putting pressure on the dermatology research community to come up with alternative anti-infective solutions that minimise the need for oral or topical antibiotics.

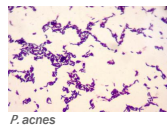
Repositioning known chemical entities with a history of use in man and favourable physicochemical properties offers an efficient route for the development of new topical antimicrobials.

2-tert-butylhydroquinone (TBHQ) is a sterically hindered lipophilic phenolic used extensively as an antioxidant to inhibit peroxidation of unsaturated fats in food¹ (E319, GRAS listed) and cosmetics².



Objective

To evaluate the suitability of TBHQ as a topical anti-staphylococcal and anti-propionibacterial agent.



Methods

Minimum Inhibitory Concentration (MIC) estimation by broth micro-dilution was used to quantify the antimicrobial activity of TBHQ.

Disc diffusion assays were employed to determine the effect of salt and lipid on activity.

Anti-staphylococcal activity was confirmed using 26 isolates representing various species of coagulase-negative staphylococci and *S. aureus* (including MRSA, VISA and GISA strains)

Anti-propionibacterial activity was confirmed using a panel of 24 antibiotic susceptible and resistant isolates of *Propionibacterium acnes* and *Propionibacterium granulosum*.

The bactericidal activity of TBHQ was investigated in time-to-kill studies using suspensions of *P. acnes* NCTC737 in aqueous buffer in which cell viability was determined using standard plating techniques.

Table 1. Susceptibility of staphylococci to TBHQ

| Staphylococcal species | Number of strains tested | MIC range (mg/L) | MBC range (mg/L) |
|--------------------------------------|--------------------------|------------------|------------------|
| <i>S. aureus</i> (MSSA) | 10 | 3.9 - 7.8 | 3.9 - 15.6 |
| <i>S. aureus</i> (EMRSA) | 3 | 1.95 - 3.9 | 3.9 |
| <i>S. aureus</i> (VISA - Mu3 & Mu50) | 2 | 3.9 | 7.8 |
| <i>S. aureus</i> (GISA - HO41340156) | 1 | 7.8 | 15.6 |
| <i>S. simulans</i> ATCC 27848 | 1 | 7.8 | 31.25 |
| <i>S. xylosus</i> ATCC 29971 | 1 | 7.8 | 15.6 |
| <i>S. cohnii</i> ATCC 29974 | 1 | 3.9 | 7.8 |
| <i>S. haemolyticus</i> ATCC 29970 | 1 | 3.9 | 7.8 |
| <i>S. warneri</i> ATCC 27836 | 1 | 3.9 | 7.8 |
| <i>S. capitis</i> ATCC 27840 | 1 | 3.9 | 3.9 |
| <i>S. hominis</i> ATCC 27844 | 1 | 1.95 | 3.9 |
| <i>S. auricularis</i> ATCC 33753 | 1 | 0.98 | 1.95 |
| <i>S. saprophyticus</i> NCTC 7292 | 1 | 3.9 | 7.8 |
| <i>S. epidermidis</i> NCTC 11047 | 1 | 3.9 | 7.8 |

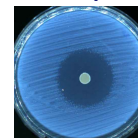
Table 2. Susceptibility of propionibacteria to TBHQ

| Propionibacterial species | Number of strains tested | Resistance genotype* | Resistance phenotype | MIC range (mg/L) | MBC range (mg/L) |
|---------------------------|--------------------------|----------------------|----------------------|------------------|------------------|
| <i>P. acnes</i> | 2 | None | None | 1.95 - 7.8 | 7.8 - 15.6 |
| <i>P. granulosum</i> | 2 | None | None | 3.9 | 7.8 |
| <i>P. avidum</i> | 1 | None | None | 3.9 | 3.9 |
| <i>P. acnes</i> | 2 | 1058/2058 | Tet/MLS | 3.9 | 7.8 |
| <i>P. acnes</i> | 2 | 1058/2059 | Tet/MLS | 3.9 - 7.8 | 7.8 - 15.6 |
| <i>P. acnes</i> | 2 | 1058 | Tet | 1.95 - 7.8 | 7.8 |
| <i>P. acnes</i> | 2 | EmX | MLS | 3.9 | 7.8 - 15.6 |
| <i>P. acnes</i> | 2 | 2058 | MLS | 3.9 | 7.8 |
| <i>P. granulosum</i> | 1 | EmX | MLS | 62.5 | 62.5 |
| <i>P. acnes</i> | 1 | 2059 | MLS | 3.9 | 7.8 |
| <i>P. granulosum</i> | 1 | 2058 | MLS | 7.8 | 7.8 |
| <i>P. granulosum</i> | 2 | 2059 | MLS | 15.6 | 15.6 - 31.25 |
| <i>P. acnes</i> | 2 | Unknown | Clin | 3.9 | 7.8 |
| <i>P. acnes</i> | 1 | 2057 | Ery | 3.9 | 7.8 |

Table 3. TBHQ retains activity in the presence of NaCl and lipid

| Organism tested | Zone of inhibition (mm) | | |
|-----------------------------|-------------------------|-------------|----------------|
| | Additive free control | 100 mM NaCl | 1% (v/v) lipid |
| <i>S. aureus</i> ATCC 29213 | 41.77 | 54.16 | 31.44 |
| <i>P. acnes</i> NCTC 737 | 9.95 | 19.48 | 10.37 |

Disk diffusion assays were carried out with 200µg TBHQ per disk on solid medium containing either NaCl or lipid at the concentrations shown



References

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- Cosmetic Ingredient Review. Final report on the safety assessment of tert-butylhydroquinone. *J Am Coll Toxicol* 1986; 5: 329-51.
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- Li HY et al. NF-E2 related factor 2 activation and heme oxygenase-1 induction by tert-butylhydroquinone protect against deltamethrin-mediated oxidative stress in PC12 cells. *Chem Res Toxicol* 2007; 20(9): 1242-51.
- van Esch GJ. Toxicology of tert-butylhydroquinone (TBHQ). *Food Chem Toxicol* 1986; 24(10-11):1063-5.

Table 4. Effects of various lipids on the activity of TBHQ versus *P. acnes*

| Lipid | Concentration per disc (µg) | Mean zone size (mm ±SD) | Zone increase (mm) | Interaction |
|------------------|-----------------------------|-------------------------|--------------------|-------------|
| Palmitic acid | 400 | 23.0 ± 0.5 | -0.3 | 0 |
| Arachidonic acid | 400 | 30.0 ± 0.3 | 6.7 | + |
| Oleic acid | 400 | 22.2 ± 0.5 | -1.1 | 0 |
| Linoleic acid | 400 | 26.9 ± 1.4 | 3.6 | 0 |
| Squalene | 400 | 24.7 ± 1.9 | 1.4 | 0 |
| Triolein | 400 | 25.0 ± 0.3 | 1.7 | 0 |
| TBHQ control | 800 | 23.3 ± 0.8 | n/a | n/a |

Interaction: 0 = <5 mm increase, + = >5 mm increase, - = >5 decrease Lipids alone produced no zones of inhibition, except arachidonate (13.5 mm)

Figure 1. TBHQ is bactericidal against *P. acnes*

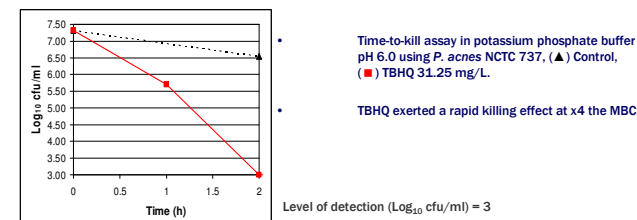


Table 5. Spontaneous resistance to TBHQ in *P. acnes* does not occur

| Days incubation | Fusidic acid control at 10 × MIC | | TBHQ at 10 × MIC | |
|-----------------|----------------------------------|----------------------------|------------------|-----------------------------|
| | No. of colonies | Mutation rate | No. of colonies | Mutation rate |
| 7 | 130 | 1 in 2.9 × 10 ⁷ | 0 | <1 in 3.8 × 10 ⁹ |
| 12 | 640 | 1 in 5.9 × 10 ⁶ | 0 | <1 in 3.8 × 10 ⁹ |

- Initial cell density was 3.8 × 10⁹ colony forming units per ml.
- P. acnes* PRP-026 was used. This is a clinical isolate resistant to erythromycin and clindamycin.
- Fusidic acid was used as a control because mutational resistance to this antibiotic is known to occur at high frequency.

Summary & Conclusions

- TBHQ affects multiple cellular processes including both direct effects due to TBHQ itself and indirect effects attributed to free radicals generated via oxidation to TBBQ³. It is therefore unlikely that resistance would emerge during clinical use.
- TBHQ has physicochemical characteristics ideal for topical delivery to sebaceous follicles and retains antimicrobial efficacy in the presence of salt and skin lipids.
- As a lipophilic anti-oxidant, TBHQ may prevent oxidation of sebaceous and epidermal lipids *in vivo*. It is also an inducer of heme oxygenase⁴, a redox sensitive stress protein with anti-inflammatory effects. These activities may complement its antimicrobial activity *in vivo* and enhance its therapeutic effectiveness.
- In view of its safety profile⁵, physicochemical properties and antimicrobial potency, TBHQ represents a novel drug candidate for the local treatment of acne and prevention of staphylococcal skin infections.