Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Gholamreza Zarrini, Zahra Bahari Delgosha, Kamyar Mollazadeh Moghaddam & Ahmad Reza Shahverdi (2010) Post-antibacterial effect of thymol, Pharmaceutical Biology, 48:6, 633-636, DOI: 10.3109/13880200903229098

To link to this article: https://doi.org/10.3109/13880200903229098

Published online: 30 Apr 2010.

Pharmaceutical Biology, 2010; 48(6): 633–636

informa
healthcare

ORIGINAL ARTICLE

Post-antibacterial effect of thymol

Gholamreza Zarrini[1], Zahra Bahari Delgosha[1], Kamyar Mollazadeh Moghaddam[2], and Ahmad Reza Shahverdi[3]

[1]Department of Animal Biology, Faculty of Natural Biology, University of Tabriz, Tabriz, Iran, [2]Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, and [3]Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Abstract

The antibacterial activity of thymol has been well established and reported in the scientific literature. Continued suppression of bacterial growth following limited exposure to antimicrobial compounds at different concentrations greater than or equal to the minimum inhibitory concentration level (MIC) and at concentrations less than the MIC can be used as an indicator of biological activity, and are respectively referred to as a post-antibacterial effect (PAE) and a post-antibiotic sub-MIC effect (PA-SME). In this study, the PAE and the PA-SME of thymol against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* were investigated. A spectrophotometric method was used to determine the PAE and the PA-SME of thymol against the selected test strains. Thymol exhibited a considerable PAE and PA-SME at MIC and sub-MIC concentrations against test strains. The greatest duration of both the PAE and the PA-SME was observed for thymol against *E. coli* and *P. aeruginosa*. The PAE and PA-SME times for *E. coli* were 12 and 8 h, respectively, and for *P. aeruginosa* were 11 and 7.5 h, respectively. The duration of the PAE and PA-SME observed for *S. aureus* and *B. cereus* was shorter than for Gram-negative strains.

Keywords: *Antibacterial activity; post-antibacterial activity; thymol*

Introduction

Increasing interest exists in the use of monoterpenes in natural medicine as antibacterial agents and resistance modulators (Moleyar & Narasimham, 1986; Baca et al., 2002; Ramsewak et al., 2003; Trombetta et al., 2005; Valero & Francés, 2006; Abdolpour et al., 2007; Rafii & Shahverdi, 2007). Thymol, a phenolic monoterpene (Figure 1), is found in the essential oils of different plants such as *Thymus vulgaris* L. (Laminaceae), *Monarda didyma* L. (Laminaceae), and *Monarda fistulosa* L. (Laminaceae) (Bhaskara et al., 1998; Johnson et al., 1998; Savickiene et al., 2002). Currently, this plant-based volatile compound is synthesized abundantly and used as a preservative or a potent antibacterial ingredient in food or pharmaceutical dosage forms (Manou et al., 2002; Burt, 2004). For example, thymol as a solution in oil of 1 or 2%, is applied to the respiratory passages by means of a spray in nasal catarrh. Also, an alcohol solution may be inhaled from hot water for bronchial and laryngitis care. Although the antibacterial activity of thymol has been studied extensively against different bacteria (Pharmaceutical Society of Great Britain, 1911; Tippayatum & Chonhenchob, 2007), the post-antibacterial activity of this compound is not yet understood. In this study, the post-antibacterial activity of thymol was investigated against *Escherichia coli* (ATCC 11345), *Staphylococcus aureus* (ATCC 29737), *Pseudomonas aeruginosa* (ATCC 12432), and *Bacillus cereus* (ATCC 16754). Continued suppression of bacterial growth following limited exposure to antimicrobial compounds at different concentrations [greater than or equal to the minimum inhibitory concentration (MIC) level] can be used as an indicator of biological

Address for Correspondence: Ahmad Reza Shahverdi, Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. PO Box: 14155/6451, Tehran, Iran. Tel: +98-21-6482706. Fax: +98-21-66461178. E-mail: shahverd@sina.tums.ac.ir

(Received 23 December 2008; revised 06 March 2009; accepted 13 April 2009)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2010 Informa UK Ltd
DOI: 10.3109/13880200903229098

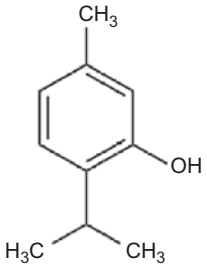

Figure 1. Chemical structure of thymol.

activity and is termed the post-antibacterial effect (PAE) (Odenholt-Tornqvist, 1993; Jacobs et al., 2003). Conversely, the post-antibacterial sub-MIC effect (PA-SME), which defines the effect of sub-inhibitory concentrations (less than the MIC level) of the agent, increases the effect of the agent and further decreases the dosing interval (Rotschafer et al., 1994).

Materials and methods

Antibacterial susceptibility and PAE and PA-SME assays

Susceptibility tests were conducted by the standard broth dilution method in accordance with the National Committee for Clinical Laboratory Standards (2008) in Müller–Hinton broth (MHB) with an inoculum of approximately 10^5 colony-forming units (CFU)/mL. Standard test strains listed in Table 1 were used. The MHB was supplemented with thymol *(Sigma* Aldrich, Steinheim, Germany), concentrations ranging from 100 to 800 µg/mL. Data are reported as MIC values, the lowest concentration of thymol inhibiting visible growth after 24 h of incubation at 37°C.

The PAE and PA-SME of thymol were determined separately by the following method (Odenholt-Tornqvist, 1993). The test strains were incubated separately in the presence of thymol (at MIC and one-half MIC concentrations) for 90 min. Subsequently, tested compounds were removed by centrifugation of the culture followed by two washes with 10 mL of thymol in pre-warmed MHB. The pellets were re-suspended in 30 mL of thymol-free, pre-warmed MHB to determine the PAE and PA-SME. MHB culture media were inoculated under the same conditions to constitute a thymol-free control. All culture media were incubated at 37°C for 24 h. The optical densities (ODs) of culture media were measured at 600 nm at different intervals (0–48 h after re-suspension). The PAE and PA-SME periods were calculated according to Odenholt-Tornqvist (1993), i.e., the time required for antibiotic-treated cultures to reach 50% of the OD_{max} of the control culture, minus the time required for the control culture to reach the same point (Odenholt-Tornqvist, 1993).

Table 1. The antibacterial effect and post-antibacterial duration of thymol against different test strains.

		Post-antibacterial duration (h)	
Test strain	MIC (µg/mL)	MIC level	Sub-MIC level
Staphylococcus aureus (ATCC 29737)	200	7.5	6.5
Escherichia coli (ATCC 11345)	400	12	8
Pseudomonas aeruginosa (ATCC 12432)	400	11	7.5
Bacillus cereus (ATCC 16754)	100	7	6

Results and discussion

The MICs of thymol for different test strains are reported in Table 1. Different test strains exhibited varied susceptibility to thymol. The greatest susceptibility was observed for thymol against the Gram-positive bacteria *B. cereus* (MIC = 100 µg/mL) and *S. aureus* (MIC = 200 µg/mL). The antibacterial effect of thymol is well known and reported in the literature, and this research confirmed previous results (Tippayatum & Chonhenchob, 2007). The effects of thymol at different concentrations (MIC and one-half MIC) on the reduction of bacterial growth of the test strains after a short exposure time are illustrated in Figure 2. The durations of the PAE and PA-SME of thymol against test strains at the MIC and one-half MIC concentrations are also reported in Table 1. The greatest durations of PAE and PA-SME were observed for thymol against *E. coli* and *P. aeruginosa*. The PAE and PAE-SME times for *E. coli* were 12 and 8 h, respectively, and for *P. aeruginosa* were 11 and 7.5 h, respectively (Table 1).

The durations of the PAE and the PA-SME observed for *S. aureus* and *B. cereus* were less than observed for Gram-negative strains (Table 1). The antibacterial activity of thymol against different test strains has been reported in the literature (Tippayatum & Chonhenchob, 2007), but the PAE and the PA-SME of this natural product has not been investigated previously. To the best of our knowledge, based on a survey of the literature, this is the first finding on the PAE and PA-SME effects of thymol. Determination of the PAE is an important factor that influences optimal antimicrobial dosing intervals. Conversely, antibacterial agents without a PAE usually require more frequent administration than agents exhibiting PAEs. In this study, the post-antibacterial effect of thymol at concentrations of MIC (PAE) and sub-MIC (PA-SME) were investigated against different test strains.

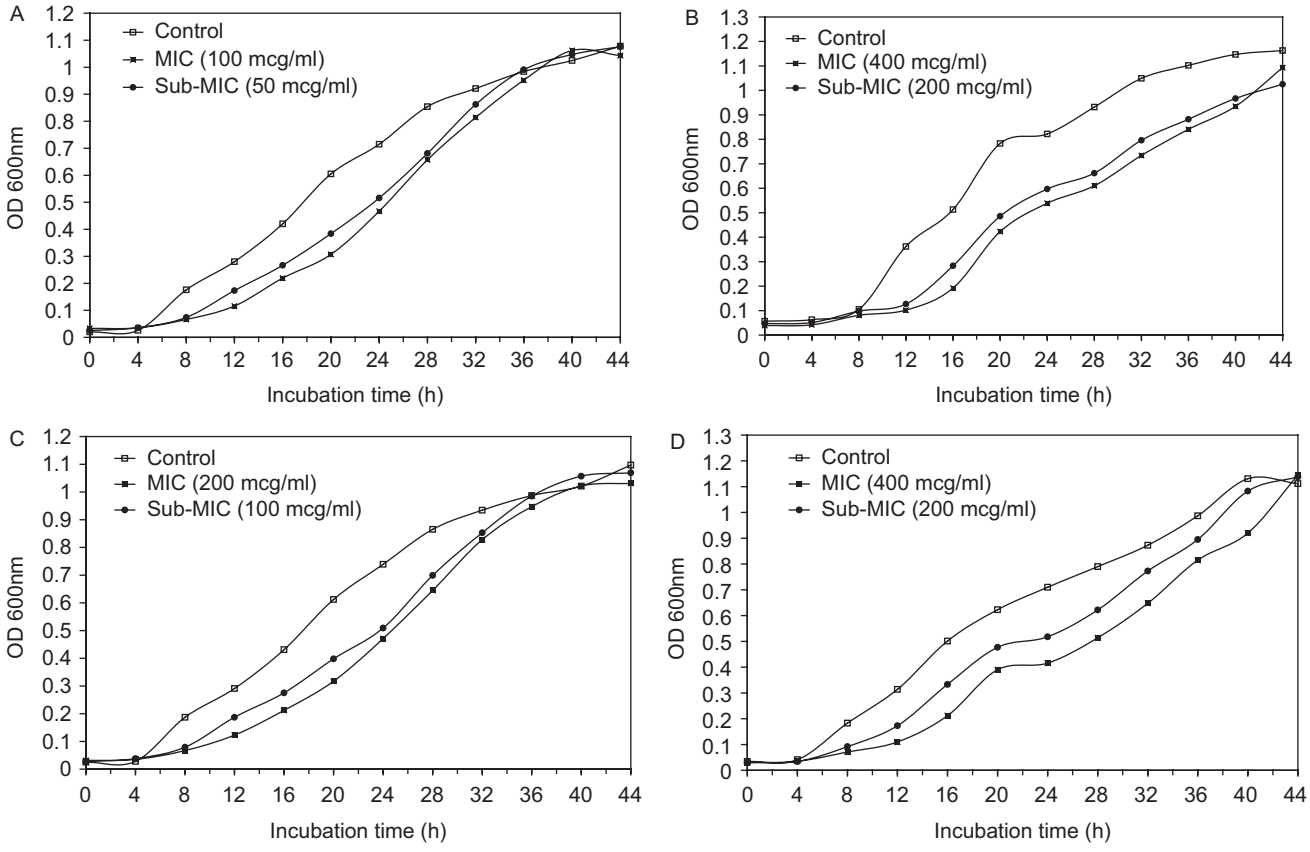

Figure 2. The post-antibacterial effect and post-antibiotic sub-MIC effect of thymol against (A) *Bacillus cereus*, (B) *Pseudomonas aeruginosa*, (C) *Staphylococcus aureus*, and (D) *Escherichia coli*.

Thymol exhibited considerable PAE and PA-SME effects at the concentrations tested against the selected strains.

Declaration of interest

This work was supported by the Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

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