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Transcriptional Responses to Cinnamaldehyde in *Mycobacterium tuberculosis*

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Abstract

Background: Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*.
Objectives: Development of the drug resistance is becoming a threat to disease control, which underscores need for new agents targeting *M. tuberculosis*.
Materials and Methods: In this study, analysis of gene expression was performed using real-time polymerase chain reaction (RT-PCR).
Results: Results of the current study showed that the minimum inhibitory concentration value of cinnamaldehyde against *M. tuberculosis* was 200 μg/mL. Moreover, RT-PCR data showed that a total of 25 genes were regulated by the cinnamaldehyde. Of these, 12 genes were up-regulated, and 13 genes were down-regulated.
Conclusions: Cinnamaldehyde is a pattern to expand the new anti-TB drugs, because the targets of the cinnamaldehyde are different from those of anti-tubercular agents.

Keywords: Antimycobacterial Activity, Cinnamaldehyde, Medicinal Plant, *Mycobacterium tuberculosis*

1. Background

The tuberculosis (TB) disease is still among world's leading infectious diseases (1). Although accessible antimicrobial agents are effective at eradicating infections, there are issues with emergence of drug-resistant *M. tuberculosis* strains (2). Additionally, increased incidence of HIV infection has been shown to be associated with an increased mortality rate (3).

Some plants have been proven to be the sources of useful drugs. Cinnamaldehyde (Figure 1) has been elicited from several plants, such as *Cinnamomum cassia*. The cinnamaldehyde has been used in traditional medicine and has been demonstrated to suppress the growth of *Clostridium botulinum* (4), and *Staphylococcus aureus* (5). A few studies have reported on mechanisms underlying the effects of antimycobacterial agents (6).

2. Objectives

The aim of this study was to determine antimycobacterial effects of cinnamaldehyde.

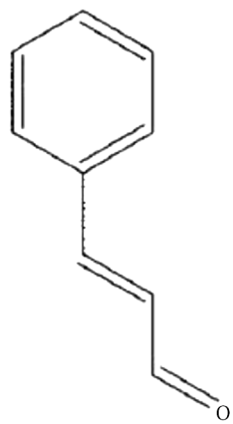

Figure 1. Chemical Formula of Cinnamaldehdye

3. Materials and Methods

3.1. Bacterial Strains and Reagents

The cinnamaldehyde was supplied by Sigma-Aldrich chemical company. *Mycobacterium tuberculosis* was ob-

tained from Zabol University of Medical Sciences.

3.2. Growth Curve of Mycobacterium tuberculosis With Different Concentrations of the Cinnamaldehyde

Mycobacterium tuberculosis was grown in a flask (100 mL) containing the Middlebrook 7H9 medium. Then, 1 mL of culture was placed in each of tubes and exposed to different concentrations of the cinnamaldehyde.

3.3. Cell Culture and Treatment by Quantitative Real-Time Polymerase Chain Reaction

Mycobacterium tuberculosis was grown in the Middlebrook 7H9 broth. The drug treatments were carried out by adding stock solution to one of the cultures.

3.4. Quantification of Gene Expression by Real-Time Polymerase Chain Reaction

Real-time PCR reactions were carried out using a Rotor-Gene 6000. Primers used are shown in Table 1. Analyses were carried out using the relative expression software tool (REST[a]) software as described by Pfaffl et al. (7).

Table 1. Primers Used in the Quantitative Real-Time Polymerase Chain Reaction Studies

Gene Name	Sense Primer	Antisense Primer
frdA	ACGAGCACAACAAAGGTACGA	AGGTGCAGCAGGTCTAGATTGA
frdB	CTTGAATCTCGGTGAAAGAC	ACCGATTTGACCAGATCATC
frdC	AGGAGATGCTGACTGAGAT	CTAGCGCCTCATTAAGATT
frdD	GCAACCCGATCACCAAGCTAGAT	CATGGTCGAGCACGAACCGCATC
narJ	CACCCACTACGCCAATATCT	AGCGGTACCACACTGACAC
narI	GAGTCCTCGTCGGAGATATG	GAACCCGAGGTGAATATATC
narH	GTTTGTGGGTTGACTATG	CGGTTTGCCATACATTGAAG
narG	GCTAGTGATCGAACACAA	CAACCCCAAGTACAACA
pks3	TCCACACTCGCTACTAATAG	TATCCACCAGTACAGCACAT
papA3	CTTTGAGGTTGTCGCGACAC	CGACGAACACCAGCATAAT
Mmpl10	ACTCGGCGTATATCTGAAGG	CTGTCCATACGGGTCAAAGT
rpsH	GAGCGTCAAGTCAGTATAGA	CAACTGATAGGTGGCGTCGT
rpsS	CAAGGCTAAAACTCACACGA	GGACTTAACCCAACATATAA
rplW	CTGCATCTGCGACTAAGTA	TCGATGGACAAGGTGTAGAA
rplT	ACCTTCGAGGACCTGAAATT	CCTCCACCACAACTACGTAT
rplO	GCAAGACAAGTGCTGATCAA	GAAGCGCTCAAAGAAACAAC
rplD	GTGGACGGCGATTGAAAATT	AGCTGGCTGGGTGAACGC
rplB	GGTAGCCAGCAAATAATACT	GCCAAGATGGTGTAGAGAAT
infC	AGACCGTCGTCATAGAACAATAG	TTGGTCTCGTAATCGAGATAGT
rho	AGGTCATCCAGCAGTATATG	TTGGTCACCGAAATAGAAGA
argR	GGTGTTTGTGTTGTCAATAC	TAGCGCATCAGGATGAACAA
argJ	GGATGACCTGCATAAGCAAT	GAAGCGCTCAAAGATACGTC
argG	CCAAATGGCCAGATATCAAC	CGAGCACCTGTTGTTAGTG
argF	CTACCACCGCCGTCTATATA	CCCTCTTCCACTGACATGAT
argD	GCGATGTATCAGTCAGTAAG	GCATTGGCGACTACAATG

4. Results

The bacteria were exposed to the different concentrations of cinnamaldehyde. The results in Figure 2 showed that 200 μg/mL of the cinnamaldehyde was the minimum inhibitory concentration (MIC) of the cinnamaldehyde, which inhibited the growth of *M. tuberculosis*. Therefore, *M. tuberculosis* was exposed to 200 μg/mL of the cinnamaldehyde in order to promote an alteration in gene expression of bacteria.

Real-time PCR was used to analyze gene expression in *M. tuberculosis*. Overall, there were 25 genes differentially regulated by the cinnamaldehyde. Among these, 12 genes exhibited a significant increase in the transcription and 13 exhibited a significant decrease in the transcription (Table 2).

5. Discussion

Four genes of frdA, frdB, frdC and frdD were down-regulated by 3.1-fold, 3.4-fold, 3.2-fold and 3.3-fold, respectively. The frdA genus has been found to be up-regulated in the *M. tuberculosis* in (7) as well as in studies that investigated the behaviour of the *M. tuberculosis* grown under carbon starvation (8). Studies on the behaviour of Mycobacterium phlei found that fumarate reductase (FRD) activity increased fourfold when the bacteria were grown under the low-oxygen conditions (9, 10). Fumarate reductase has reported to be a successful target in treatment of protozoan infections using a variety of compounds (11).

Nitrate reductase is a membrane-bound molybdenum-containing complex (12). Absorption of nitrogen into the mycobacterial metabolism is important for survival of *M. tuberculosis* (13). It is suggested that the narGHJI mediates nitrate absorption in *M. tuberculosis* (14).

The pks3 and mmpL10 genes were up-regulated by 3.9-fold and 4.0-fold, respectively. Suppression of papA3 gene expression from the *M. tuberculosis* resulted in loss of penta-acylated trehalose (PAT) (15-17). Pks3 is a polyketide synthase that is included in PAT biosynthesis of *M. tuberculosis* (18-20).

The rpsH and rpsS genes were up-regulated by 3.5-fold and 3.3-fold, respectively. Initiation factor infC was up-regulated by 3.7-fold and the transcription level of gene

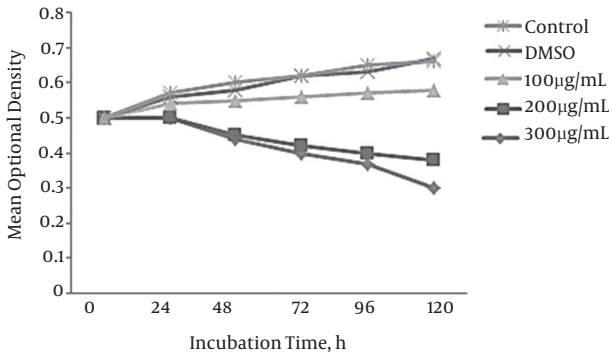

Figure 2. *Mycobacterium tuberculosis* Exposed to Different Concentrations of Cinnamaldehdye

rho was also elevated by 3.4-fold. A work indicated that Clostridium difficile challenge to several concentrations of antibiotics resulted in a general up-regulation of translation machinery (21, 22).

L-arginine metabolism has indicated to be important for the *M. tuberculosis* (23). Some genes such as argR, argJ, argG, argF and argD were inhibited by 3.5-fold, 3.2-fold, 3.1-fold, 3.1-fold and 3.4-fold, respectively. Suppression of gene expression argR in Legionella pneumophila can affect gene transcript levels predicted to encode terminal steps of L-arginine biosynthesis (24). In Listeria monocytogenes, mutation of argD led to a decreased replication rates in Caco-2 cells (25). In *Corynebacterium glutamicum*, genes encoding L-arginine biosynthesis proteins showed a decreased expression in ammonium-limited chemostat cultures (26, 27).

5.1. Conclusions

The findings of the current study show that the cinnamaldehyde has potential antimycobacterial properties.

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Footnote

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Table 2. Real-Time Polymerase Chain Reaction Analysis of Gene Expression[a]

Gene Name	Description	Fold Change
frdA	Fumarate reductase subunit A	-3.1
frdB	Fumarate reductase subunit B	-3.4
frdC	Fumarate reductase subunit C	-3.2
frdD	Fumarate reductase subunit D	-3.3
narJ	Nitrate respiration	-3.3
narI	Nitrate respiration	-3.0
narH	Nitrate respiration	-3.5
narG	Nitrate respiration	-3.2
pks3	Biosythesis of polyacyltrehalose	+3.9
papA3	Biosythesis of polyacyltrehalose	+3.4
Mmpl10	Biosythesis of polyacyltrehalose	+4.1
rpsH	Ribosome proteins	+3.5
rpsS	Ribosome proteins	+3.3
rplW	Ribosome proteins	+3.3
rplT	Ribosome proteins	+3.5
rplO	Ribosome proteins	+3.2
rplD	Ribosome proteins	+3.2
rplB	Ribosome proteins	+3.9
infC	Translation initiation factor IF-3	+3.7
rho	Ribosome proteins	+3.4
argR	Arginine biosynthesis	-3.5
argJ	Arginine biosynthesis	-3.2
argG	Arginine biosynthesis	-3.1
argF	Arginine biosynthesis	-3.1
argD	Arginine biosynthesis	-3.4

[a] +, Increase; and -, reduction.

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