PDF/60/2544. Thiourea and new derivatives: Mode of action and effects on the expression of the *Mycobacterium tuberculosis desA3* gene.

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ABSTRACT

Isoxyl, a thiourea (4, 4' diisoamyloxydiphenylthiourea) and new synthetic derivatives were investigated in vitro for their antimycobacterial activity against *Mycobacterium tuberculosis*. By employing the rapid microplate alamar blue assay, isoxyl was shown to be highly effective against Thai clinical isolate of *Mycobacterium tuberculosis* with MIC of 0.30-1.25 µg/ml. Four new derivatives also exhibited potent activity against *M. tuberculosis* with MIC in the range of 0.30-10.0 µg/ml. The selective mode of action of thiourea was demonstrated by whole cell labeling of *M. tuberculosis* with [1, 2 -C^{14}] acetate. Analysis of labeled fatty acids by thin layer chromatography (TLC) demonstrated the inhibitory effects on the synthesis of oleic acid, indicating a unique mechanism of action. The specificity of the inhibition on oleic acid synthesis pointed to a $\Delta 9$ stearoyl-desaturase as a drug target. The transcripts of the *M. tuberculosis desA3*, which encodes $\Delta 9$ stearoyl-desaturase, were detected by reverse transcription polymerase chain reaction (RT-PCR) demonstrating the expression and importance of enzymatic function. A new LightCycler real-time RT-PCR was developed for quantitative detection of mRNA of *M. tuberculosis desA3*. *M. tuberculosis* RNA could be extracted by the use of a modified commercially ready-to use guanidine-phenol extraction method. The single tube reaction of real-time RT-PCR was performed in glass capillary containing the SYBR Green I dye as a detection signal. The amplification of 341 nucleotide region of the *desA3* gene specific for *M. tuberculosis* by real-time RT-PCR demonstrated that *desA3* transcripts were diminished in cells exposed to thiourea. These results confirmed that thiourea is a novel antituberculosis agent, which had specific mechanism by inhibiting the synthesis of oleic acid and affected the expression of *desA3* in *M. tuberculosis*. We propose here that thioureas serve as a promising compound for future antituberculosis drug development and DesA3 is a new therapeutic target worthy for further study.

Keyword : Thiourea, oleic acid, tuberculosis, *desA3*

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PDF/60/2544 กลไกการออกฤทธิ์ของสารต้านเชื้อวัณโรค thiourea และอนุพันธ์ใหม่ และผลต่อการแสดงอกของยีน *desA3* ในเชื้อวัณโรค

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บทคัดย่อ

Isoxyl (4, 4 diisoamyloxydiphenylthiourea) และสารประกอบอนุพันธุ์ใหม่ของ thiourea ที่ได้จากการสังเคราะห์ มีฤทธิ์ต้านเชื้อวัณโรคโดยการทดสอบที่ให้ผลเร็วด้วย microplate alamar blue assay พบว่า isoxyl สามารถทำลายเชื้อวัณโรคที่แยกได้จากผู้ป่วยด้วยความเข้มข้นต่ำสุด 0.30-1.25 μg/ml และสารอนุพันธุ์ของ thiourea มีฤทธิ์ต้านเชื้อวัณโรคด้วยค่าความเข้มข้นต่ำสุด 0.30-10.0 μg/ml การศึกษากลไกการออกฤทธิ์ที่จำเพาะของสาร thiourea ทำโดยการติดฉลากสารเมตาโบไลท์ในขบวนการสังเคราะห์กรดไขมันในเชื้อวัณโรคด้วย [1, 2 C^{14}]acetate และวิเคราะห์กรดไขมันที่ถูกติดฉลากด้วย thin layer chromatography สาร thiourea และอนุพันธ์ใหม่มีฤทธิ์ยับยั้งการสังเคราะห์ oleic acid บ่งชี้ว่าเอนไซม์ Δ9 stearoyl-desaturase ซึ่งสังเคราะห์ oleic acid เป็นเป้าหมายที่สารออกฤทธิ์ การตรวจพบ transcript ของยีน *desA3* ที่ถอดรหัสให้เอนไซม์ Δ9 stearoyl-desaturase แสดงความสำคัญของหน้าที่เอนไซม์โดยยีนมีการแสดงออก ผลของสาร thiourea ต่อการแสดงออกของยีน *desA3* ศึกษาโดยการสกัด RNA ด้วยน้ำยา Trizol วิเคราะห์หาปริมาณของ *desA3* transcript ด้วย real-time reverse transcription PCR แบบปฏิกิริยาเดียวโดยใช้ primer ที่จำเพาะต่อเชื้อวัณโรค ตรวจพบผลผลิตปฏิกิริยาที่ใช้สาร SYBR green I ติดฉลากขนาด 341 base pair ของยีน *desA3* สาร thiourea มีผลทำให้ transcript ของยีน *desA3* ในเชื้อวัณโรคลดลง ผลการศึกษานี้ แสดงให้เห็นว่า สาร thiourea มีฤทธิ์ต้านเชื้อวัณโรคโดยมีกลไกการออกฤทธิ์ยับยั้งการสังเคราะห์ oleic acid ที่เฉพาะ สารมีผลทำให้การแสดงออกของยีน *desA3* ลดลง และเอนไซม์ DesA3 ในเชื้อวัณโรคมีคุณสมบัติเป็นเป้าหมายของยาต้านวัณโรค

คำหลัก : สาร thiourea, กรดไขมัน oleic, วัณโรค, ยีน *desA3*

INTRODUCTION

Identifying and understanding the functions of specific genes is a fundamental and essential step to find and validate new targets for drug design and development. Elucidation of such genes can be approached by using molecular genetics, biochemical analysis and enzyme inhibitors that affect the function of gene products. Such compounds or drugs may selectively inhibit specific targeted enzymes and thereby cause an accumulation of precursor(s) and depletion of product(s) leading to an identification of the mode of action of drugs and the specific gene that encodes the targeted enzyme.

A thiourea, isoxyl (thiocarlide; 4, 4′ diisoamyloxy diphenylthiourea), is known to be an effective anti-tuberculosis drug, active against a range of multidrug-resistant strains of *Mycobacterium tuberculosis*, and has been used clinically (Titscher, 1966; Urbancik, 1966; Urbancik, 1970). Little was known of its mode of action. Recently, it has been shown that exposure of *M. tuberculosis* to an inhibitory level of ISO caused an accumulation of stearic acid concomitant with the depletion of oleic acid. Synthesis of mycolic acid is also affected. The anti-bacterial effect of ISO was reversed by supplementing growth medium with oleic acid. The specificity of oleic acid inhibition pointed to a $\Delta 9$ stearoyl-desaturase, an enzyme that introduces one double bond at carbon 9 of stearic acid to form oleic acid as the drug target (Phetsuksiri, et al., 2003). Development of a cell-free assay for $\Delta 9$ desaturase activity allowed direct demonstration of the inhibition of oleic acid synthesis by ISO. The three putative fatty acid desaturases in the *M. tuberculosis* genome, *desA1*, *desA2*, and *desA3*, were cloned and expressed in *Mycobacterium bovis* BCG. Whole cell labeling demonstrated increased synthesis of oleic acid only in the *desA3* overexpressing strain and an increase in the minimal inhibitory concentration for ISO (Phetsuksiri, et al., 2003). The results validated $\Delta 9$ desaturase, DesA3, as a target of thiourea. In this study, antimycobacterial activities of thiourea and new derivatives against clinical isolate of *M. tuberculosis* were evaluated and the effects of thioureas on the synthesis of oleic acid and on the expression of the *desA3* gene, which is known to encode $\Delta 9$ desaturase in *M. tuberculosis* were explored. This is the extent of published work conducted on the new drug target discovery and evaluation of efficiency of the thioureas as anti-tuberculosis drugs worthy of further development.