博士論文の要旨及び審査結果の要旨

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博士論文名	Lysocin E targeting menaquinone in the membrane of Mycobacterium tuberculosis is a promising lead compound for anti-tuberculosis drugs. （結核菌の細胞膜中のメナキノンを標的とするライソシンＥは、抗結核薬の有望なリード化合物である）
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博士論文の要旨

Background and purpose: Tuberculosis (TB) is one of the severe infectious diseases caused by Mycobacterium tuberculosis var. tuberculosis (Mtb). The recent WHO report ranks TB above HIV as a leading cause of mortality by infectious diseases, with more than 1.5 million attributed deaths in 2020. TB as a health security threat is mainly associated with the issue of multidrug-resistant TB (MDR-TB). Therefore, there is an urgent need to develop new anti-tuberculosis drugs with a novel mode of action to cure drug-resistant tuberculosis and shorten the chemotherapy period by sterilization of tissue infected with dormant bacteria. Lysocin E is an antibiotic that showed antibacterial activity against Staphylococcus aureus by binding with its menaquinone (Vitamin K2). This study aims to evaluate the anti-tuberculosis activities of lysocin E and decipher its mode of action.

Method: The in vitro anti-Mtb activities of lysocin E against Mtb were evaluated by determining the MIC and MBC values of the drugs and, checked its efficacy in combination with other drugs by checkerboard and kill kinetics assays. To study the drug mode of action, MK synthetase-conditional knockdown (CKD) Mtb strains (A+8 and A+110) was constructed by employing the TetR/tetO-inducible CRISPR-dCas9 interference specialized for mycobacteria, and the menA CKD of A+8 and A+110 strains were induced by incubating the strains with anhydrotetracycline (aTc). The CKD was confirmed by quantifying the mRNA of menA by real-time PCR. Then, the MK amount was quantified by using reverse-phase high-performance liquid chromatography (HPLC), and the effect of MK reduction on the susceptibility of CKD strains to drugs. Moreover, the effect of the drug on membrane disruption was evaluated by Flow Cytometry and electron microscopy analysis. The Lysocin E' effect on oxygen consumption and ATP synthesis was also evaluated. Finally, the efficacy of the drug was evaluated

in the THP1 cells-derived macrophage and mice infection models.
Results: Lysocin E has a high in vitro activity against both drug-susceptible and resistant Mtb, and dormant mycobacteria. Lysocin E minimum inhibitory concentration (MIC) or minimal bactericidal concentration (MBC) against replicating drug susceptible and resistant Mtb H37Rv was < 0.5 μg/ml. In the 21 days kill kinetics assay, lysocin E leads to 4 log10 CFU reduction over 7 days, which was superior to that of INH, RFP, and EMB alone, especially as the other drugs showed regrowth of Mtb after 7-, 10-, and 15 days, respectively. The MK synthetase-CKD Mtb strains showed a significant reduction of mRNA and MK amount in A+110 strain compared to the vector control. Following these observations, the susceptibility of the CKD strains to lysocin E was determined by CFU count. A+110 strain showed a threefold increment in CFU as compared to the vector control. These data suggest that MK is a target of lysocin E also in Mtb. It also caused Mtb membrane disruption and, inhibited oxygen consumption and ATP synthesis, even more pronounced than that of bedaquiline. Thus, the applicant has concluded that the high anti-tuberculosis activity of lysocin E is attributed to its synergistic effect of membrane disruption and respiratory inhibition. The efficacy of lysocin E in macrophage-infected Mtb was lower than the direct exposure in the media, probably due to its low ability to penetrate cells. However, lysocin E's efficacy against Mtb was still superior to that of streptomycin, causing a significant CFU reduction in mice and macrophage infection models, which showed its potential role as a choice for TB treatment.
Conclusions: Considering lysocin E's pharmacological safety, ability to kill mycobacteria at any growth state, a novel mode of action, and no antagonistic effect against anti-TB frontline drugs, it is a promising lead compound for the development of a new tuberculosis drug that cures drug-resistant and latent tuberculosis in a shorter period. However, our in vivo Mtb infection study was limited to 24 days of infection and two weeks of treatment only, and extending the treatment and study duration could have provided insight into the efficacy of lysocin E over a longer duration

審査結果の要旨

結核は、未だに世界で年間 150 万人以上が死亡する重大な細菌感染症である。従来の薬剤が効かない多剤耐性結核や休眠菌にも有効な、新しい作用機序を持った新規抗結核薬の開発が急務である。本学位論文において、申請者はLysobacter sp.が産生する抗菌物質ライソシンEの結核菌に対する有効性と、作用機序を評価した。その結果、ライソシンEは薬剤感受性結核菌だけでなく、多剤耐性及び休眠結核菌に対しても高い抗菌活性を示した。また、ライソシンEが結核菌細胞膜のメナキノンに結合し、膜障害と呼吸阻害の相乗効果で抗菌作用を発現すると結論づけた。一方で、マクロファージに感染した結核菌に対するライソシンEの有効性は低かった。しかしながら、その様な状況でも細胞感染モデルと動物感染モデルの両方でライソシンEの除菌効果は、既存薬ストレプトマイシンよりも高く、結核治療の選択肢としての潜在性が証明された。この様に本論文は、薬剤耐性や休眠菌の存在が結核治療の難渋・長期化に繋がっている現状に対し、新たな創薬の可能性を示した。本論文は、細胞内浸透性など今後の物質の改良の必要があるものの、作用機序、多剤耐性・休眠結核菌への有効性等の観点からライソシンEが抗結核創薬の有望なリード化合物である事を示しており、学位論文としての価値があると判断した。