UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

POSGRADO EN CIENCIAS FARMACOBIOLÓGICAS

“Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosos”

Tesis para obtener el grado de:
Doctorado en Ciencias Farmacobiológicas

Presenta:
Jacobo Delgado Yolanda Monserrath

Director de Tesis: Dr. Bruno Rivas Santiago
Codirectora de Tesis: Dra. Gabriela Navarro Tovar

San Luís Potosí, S. L. P. Julio, 2025

Este proyecto se realizó en la Unidad de Investigación Biomédica de Zacatecas adscrito al Instituto Mexicano del Seguro Social (UIBMZ, IMSS), y en el Laboratorio de Síntesis y Fotoquímica de la Facultad de Ciencias Químicas (UASLP) en el periodo comprendido entre agosto del 2021 a junio del 2025, bajo la dirección del Dr. Bruno Rivas Santiago y la Dra. Gabriela Navarro Tovar, y fue apoyado por el fondo bajo el título “Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosis” con número de registro: R-2021-3301-012, de donde se obtuvieron recursos para la realización del trabajo.

El programa de Doctorado en Ciencias Farmacobiológicas de la Universidad Autónoma de San Luis Potosí pertenece al Sistema Nacional de Posgrados de Calidad (SNP) del SECIHTI, registro 003383. Número de la beca otorgada por SECIHTI CVU: 1009432

Los datos del trabajo titulado “Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosos” se encuentran bajo el resguardo de la Facultad de Ciencias Químicas y pertenecen a la Universidad Autónoma de San Luis Potosí.

DOCTORADO EN CIENCIAS FARMACOBIOLÓGICAS

REGISTRO DE TESIS DE DOCTORADO

GENERACIÓN AGOSTO 2021 - 2025

Nombre del Alumno Yolanda Monserrath Jacobo Delgado

Tema de Tesis "Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosos"

Línea de Investigación en la que se inserta la Tesis Desarrollo y evaluación de medicamentos

COMITÉ TUTELAR

	Nombre	Firma
CoDirector de tesis	Dr. Bruno Rivas Santiago	
CoDirectora de tesis	Dra. Gabriela Navarro Tovar	
Asesora del PCFB	Dra. Diana Patricia Portales Pérez	
Asesora del PCFB	Dra. Rosa del Carmen Milán Segovia	
Asesora externa	Dra. Ester Boix Borrás	

Trabajo de tesis vinculada con:

Convenio_____Servicio _____ Proyecto __X__ Grupo _____ Artículo _____

Financiamiento de Tesis otorgado por

Institución, organismo o asociación: Instituto Mexicano del Seguro Social

Nombre del Proyecto en su caso: "Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con Mycobacterium tuberculosis y su aplicación para el diseño de fármacos antituberculosos"

Monto: $100,000

Fecha de ejecución del proyecto: Agosto 2021 – Agosto 2025

Objetivo del proyecto: Identificar moléculas aprobadas por la FDA con capacidad de inducir a CEBPα para proponerlas como nuevos fármacos anti-micobacterianos

Participantes en el proyecto: M. en C. Yolanda Monserrath Jacobo Delgado, Dr. Bruno Rivas Santiago, Dra. Gabriela Navarro Tovar, Dra. Diana Patricia Portales Pérez, Dra. Rosa del Carmen Milán Segovia, Dra. Ester Boix Borrás, Dr. Edgar Rodríguez Lara

Nombre y Firma del Director de Tesis

Dra. Gabriela Navarro Tovar

Dr. Bruno Rivas Santiago

Dictamen de Aprobado

Comité Local de Investigación en Salud **3301**.
HOSP GRAL ZONA/MF 1 ZACATECAS

Registro COFEPRIS **17 CI 32 056 012**
Registro CONBIOÉTICA **CONBIOETICA 32 CEI 001 2017082**

FECHA **Miércoles, 01 de septiembre de 2021**

Dr. Bruno Tonatiuh Rivas Santiago

P R E S E N T E

Tengo el agrado de notificarle, que el protocolo de investigación con título **Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con Mycobacterium tuberculosis y su aplicación para diseño de fármacos antituberculosos** que sometió a consideración para evaluación de este Comité, de acuerdo con las recomendaciones de sus integrantes y de los revisores, cumple con la calidad metodológica y los requerimientos de ética y de investigación, por lo que el dictamen es **A P R O B A D O**:

Número de Registro Institucional
R-2021-3301-012

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

POSGRADO EN CIENCIAS FARMACOBIOLÓGICAS

“Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosos”

Tesis para obtener el grado de:

Doctorado en Ciencias Farmacobiológicas

Presenta:

Jacobo Delgado Yolanda Monserrath

SINODALES:

Presidenta: Dra. Diana Patricia Portales Pérez _____

Secretario: Dr. Sergio Zarazúa Guzmán _____

Vocal: Dra. Gabriela Navarro Tovar _____

Vocal: Dr. Bruno Rivas Santiago _____

Vocal: Dra. Rosa Del Carmen Milán _____

Vocal: Dr. Juan Valentín Trujillo Páez _____

SAN LUIS POTOSÍ, S. L. P. FECHA: Julio, 2025

INTEGRANTES DEL COMITÉ TUTORIAL ACADÉMICO

Dr. Bruno Rivas Santiago Director de tesis Unidad de Investigación Biomédica de Zacatecas, Instituto Mexicano del Seguro Social. Zacatecas, Zac.
Dra. Gabriela Navarro Tovar Codirectora de tesis Facultad de Ciencias Químicas de la Universidad Autónoma de San Luis Potosí, San Luis Potosí, S.L.P.
Dra. Diana Patricia Portales Pérez Asesora de Tesis Facultad de Ciencias Químicas de la Universidad Autónoma de San Luis Potosí, San Luis Potosí, S.L.P.
Dra. Rosa del Carmen Milán Segovia Asesora de Tesis Facultad de Ciencias Químicas de la Universidad Autónoma de San Luis Potosí, San Luis Potosí, S.L.P.
Dra. Ester Boix Borras Asesora de Tesis Departamento de Bioquímica y Biología Molecular de la Universidad Autónoma de Barcelona, Barcelona.

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ
Facultad de Ciencias Químicas
Centro de Investigación y Estudios de Posgrado
Posgrado en Ciencias Farmacobiológicas
Programa de Doctorado

Formato D5

Carta Cesión de Derechos

San Luis Potosí SLP a Junio / 27 / 2025

En la ciudad de *San Luis Potosí* el día *27* del mes de *Junio* del año *2025* La que suscribe *Yolanda Monserrath Jacobo Delgado* Alumna del programa de posgrado en *Ciencias Farmacobiológicas* adscrito a la *Universidad Autónoma de San Luís Potosí* manifiesta que es autora intelectual del presente trabajo terminal, realizado bajo la dirección de: *Dr. Bruno Rivas Santiago y la Dra. Gabriela Navarro Tovar* y cede los derechos del trabajo titulado *"Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con Mycobacterium tuberculosis y su aplicación para el diseño de fármacos antituberculosos"* a la **Universidad Autónoma de San Luis Potosí**, para su difusión con fines académicos y de investigación.

Los usuarios de la información no deben reproducir de forma total o parcial texto, gráficas, imágenes o cualquier contenido del trabajo sin el permiso expreso del o los autores. Éste, puede ser obtenido directamente con el autor o autores escribiendo a la siguiente dirección *yolandajacobo1996@gmail.com*. Si el permiso se otorga, el usuario deberá dar el agradecimiento correspondiente y citar la fuente del mismo.

_____

Nombre y firma del alumno

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ
Facultad de Ciencias Químicas
Centro de Investigación y Estudios de Posgrado
Posgrado en Ciencias Farmacobiológicas
Programa de Doctorado

Formato D28

Carta de Análisis de Similitud

San Luis Potosí SLP a 06 / 19 / 2025

L.B. María Zita Acosta Nava
Biblioteca de Posgrado FCQ

Asunto: Reporte de porcentaje de similitud de tesis de grado

Por este medio me permito informarle el porcentaje de similitud obtenido mediante Turnitin para la tesis titulada "Evaluación de la vía independiente de VDR como promotora de la respuesta inmune en la infección con *Mycobacterium tuberculosis* y su aplicación para el diseño de fármacos antituberculosos" presentada por el autor Yolanda Monserrath Jacobo Delgado. La tesis es requisito para obtener el grado de Doctorado en el Posgrado en Ciencias Farmacobiológicas. El análisis reveló un porcentaje de similitud de 24% excluyendo referencias y metodología.

Agradezco sinceramente su valioso tiempo y dedicación para llevar a cabo una exhaustiva revisión de la tesis. Quedo a su disposición para cualquier consulta o inquietud que pueda surgir en el proceso.

Sin más por el momento, le envío un cordial saludo.

A T E N T A M E N T E

_____

Coordinador Académico del Posgrado
en Ciencias Farmacobiológicas

Dedicatoria y Agradecimiento

Por siempre, a mi familia.

RESUMEN

La tuberculosis [TB], causada por el bacilo *Mycobacterium tuberculosis* (Mtb), se encuentra entre las enfermedades infecciosas con mayor mortalidad y morbilidad a nivel mundial, además los casos de TB resistente a antibióticos han ido en aumento. La respuesta inicial es la respuesta inmune innata, dentro de la cual destacamos la secreción del péptido antimicrobiano LL-37 por macrófagos y células del epitelio pulmonar. El calcitriol, metabolito activo de la vitamina D, tiene un efecto positivo en el control y resolución de la TB al inducir la expresión de LL-37. Por otra, parte se ha reportado la capacidad del factor nuclear CEBPα de inducir la expresión del mismo péptido en conjunto con la activación del receptor a vitamina D, además de promover la expresión de LL-37 mediante un efecto de estrés a retículo endoplásmico (RE). Sin embargo, se desconoce el papel de CEBPα durante la respuesta inmune en la infección por Mtb. El objetivo de este trabajo es evaluar la participación de CEBPα sobre la expresión de LL-37 durante la infección con Mtb y reposicionar moléculas que tengan como blanco terapéutico a CEBPα y evaluar su potencial efecto antituberculoso.

Palabras Clave

Tuberculosis, LL-37, estrés a retículo endoplásmico, CEBPα.

ABSTRACT

Tuberculosis [TB], caused by the bacillus *Mycobacterium tuberculosis* (Mtb), is among the infectious diseases with the highest mortality and morbidity worldwide, and cases of antibiotic-resistant TB have been increasing. The initial response is the innate immune response, within which we highlight the secretion of the antimicrobial peptide LL-37 by macrophages and pulmonary epithelial cells. Calcitriol, an active metabolite of vitamin D, has a positive effect on the control and resolution of TB by inducing the expression of LL-37. On the other hand, the ability of the nuclear factor CEBPα to induce the expression of the same peptide in conjunction with the activation of the vitamin D receptor has been reported, in addition to promoting the expression of LL-37 through an effect of stress on the endoplasmic reticulum (ER). However, the role of CEBPα during the immune response in Mtb infection is unknown. The objective of this work is to evaluate the participation of CEBPα in the expression of LL-37 during Mtb infection and to reposition molecules that therapeutically target CEBPα and evaluate their potential antituberculosis effect.

Key words

Tuberculosis, LL-37, endoplasmic reticulum stress, CEBPα**.**

ÍNDICE GENERAL

INTRODUCCIÓN

La tuberculosis [TB] es considerada la enfermedad infecciosa más letal a nivel global cuyo agente etiológico es el bacilo *Mycobacterium tuberculosis* (Mtb). Por otra parte, los casos de TB fármaco resistentes han ido en aumento. Aunque Mtb puede infectar cualquier órgano, la enfermedad pulmonar es la más común. La infección ocurre cuando un paciente con la enfermedad activa libera al bacilo en gotas de saliva a través del habla o estornudos. Los bacilos de Mtb ingresan por las vías respiratorias hasta alcanzar la parte inferior del árbol bronquial.

La respuesta inicial a la infección es la inmunidad innata, en donde destacamos la secreción del péptido antimicrobiano [AMP] LL-37 por células inmunes y no inmunes, como macrófagos y células del epitelio pulmonar, respectivamente. Además, son estas mismas células las primeras que entran en contacto con Mtb. Previamente, se ha reportado que el calcitriol, metabolito activo de la vitamina D, tiene un efecto positivo en el control y resolución de la TB al promover la expresión de LL-37, y de hecho, la vitamina D es considerando el principal inductor de LL-37.

Por otra parte, se ha documentado la capacidad del factor de transcripción nuclear CEBPα de inducir la expresión del mismo péptido en conjunto con la activación del receptor a vitamina D. Además, CEBPα es capaz de promover la expresión de LL-37 mediante un efecto de estrés a retículo endoplásmico (RE) incluso en ausencia de la vitamina D. Sin embargo, se desconoce el papel de CEBPα durante la respuesta inmune en la infección por Mtb y sí este factor de transcripción ayuda en la eliminación del bacilo.

ANTECEDENTES

De acuerdo al reporte global de la Organización Mundial de la Salud (OMS), la TB es la enfermedad infecciosa con mayor mortalidad y morbilidad a nivel global. La infección es causada por el bacilo *Mycobacterium tuberculosis* (Mtb). En el 2023 se registraron 10.8 millones de casos nuevos y 1.25 millones de muertes a causa de esta enfermedad. Además, en el Reporte Global de la TB del 2024 se hace especial énfasis en el incremento de los casos de infecciones con cepas fármaco resistentes. En el plan de intervención para poner fin a la TB propuesta por la OMS se esperaba una reducción del 20% de los casos de TB para el 2020; sin embargo, solo se logró una reducción del 9%, incluso se estima que aproximadamente un cuarto de la población mundial ha estado en contacto con Mtb (WHO, 2024). En este sentido la TB sigue siendo un riesgo latente y una crisis de salud pública mundial, por lo que son necesarias nuevas propuestas terapéuticas para combatir la infección.

La transmisión del bacilo de Mtb ocurre cuando pacientes con TB activa liberan aerosoles que lo contienen mediante tos, habla o estornudos. Estos aerosoles son inhalados por potenciales huéspedes hasta llegar a sus vías respiratorias. De esta forma el sistema inmune innato reconoce a Mtb y da inicio a diferentes mecanismos de protección. Se estima que solo el 30% de las personas expuestas al bacilo se infectan, y de estos solo el 10% desarrolla la enfermedad activa, mientras que el resto logra contener a la bacteria en una estructura denominada granuloma (Jilani et al., 2020). Estos datos reflejan la alta eficacia del sistema inmune innato para eliminar o contener a Mtb. Dentro de los mecanismos desencadenados por la respuesta inmune se encuentra la secreción de citocinas y quimiocinas encargadas de reclutar células inmunes al sitio de infección, así como la secreción de sustancias bactericidas. Entre estas últimas se destacan los péptidos antimicrobianos (AMPs), los cuales son moléculas clave en el control de la infección por Mtb (Maertzdorf et al., 2018). La alta eficiencia del sistema inmune para eliminar el bacilo pudiera ser manipulada o incrementada para promover la eliminación del bacilo.

Actualmente, en México el tratamiento contra la TB se describe en la “Norma Oficial Mexicana NOM-006-SSA2-2013 para la Prevención y Control de la Tuberculosis en la Atención Primaria a la Salud”. En un alto porcentaje de las personas con TB activa, la enfermedad es tratable y curable con el plan de tratamiento propuesto, sin embargo, alrededor del 10% de los casos no muestran una respuesta efectiva y aproximadamente la mitad de estos se han asociado a la infección con cepas fármaco resistentes, casos en los que la OMS establece que debe administrarse un tratamiento acortado. Este tratamiento consiste en una combinación de diversos fármacos antituberculosos cuya duración puede extenderse desde 9 hasta 12 meses, misma razón por la cual es común la aparición de diversos efectos adversos. Como consecuencia del tratamiento prolongado de TB existe una alta tasa de abandono, siendo este el principal motivo de fracaso en la terapia antituberculosa. El abandono terapéutico tiene consecuencias graves, como el desarrollo de resistencia bacteriana, reducción de las tasas de curación, continuación de la propagación de la infección y el deterioro físico de la salud del paciente (Molina-Rueda. et al., 2012). La aparición de farmacorresistencia en el bacilo, además de promover la toxicidad de los medicamentos e incrementar la tasa de mortalidad, también conduce al incremento en el costo de la terapia. Con el fin de disminuir los costos derivados de las complicaciones y efectos adversos en la terapia contra TB, se ha propuesto la combinación de los antibióticos actuales con fármacos que modulen la respuesta inmune del huésped, ya que se ha demostrado que ésta es capaz de controlar y eliminar la infección por sí misma.

El reconocimiento de Mtb se da mayormente por el receptor tipo Toll (TLR)-2 que reconoce lipoproteínas de Mtb, y TLR-9 que censa DNA micobacteriano intracelular (Katalinic´-Jankovic et al., 2012). En la respuesta inmune innata destacamos la producción de mediadores solubles, entre ellos los AMPs, de gran importancia en el control de la infección por Mtb (Maertzdorf et al., 2018).

Los AMPs son moléculas clave de la inmunidad innata que forman una barrera soluble en diferentes epitelios, entre ellos en el epitelio respiratorio. La barrera soluble formada

evita la entrada de microorganismos potencialmente patógenos para el huésped (Michea et al., 2016). Las familias de AMPs de catelicidinas y defensinas poseen las propiedades antimicrobianas mejor descritas. Sus mecanismos de acción son diversos, entre ellos se destaca una atracción electrostática entre el péptido catiónico y los componentes aniónicos presentes en los microorganismos patógenos, lo que causa la inserción del péptido en la membrana y posteriormente la lisis del mismo. Sin embargo, los AMPs también tienen blancos intracelulares y actúan interrumpiendo procesos vitales en estos mismos microorganismos (Tellez & Castaño, 2010). LL-37 es la única catelicidina humana descrita, y se encuentra muy bien documentado su amplio espectro microbicida así como sus funciones inmunomoduladoras (Vandamme et al., 2012). Así mismo, se ha reportado que LL-37 es capaz de lisar la pared celular del bacilo de Mtb (Rivas-Santiago et al., 2006). Por otra parte, se ha demostrado que células presentes en el epitelio respiratorio y macrófagos alveolares secretan al AMP LL-37, lo que constituye un mecanismo clave durante en la infección por Mtb (Maertzdorf et al., 2018). Por ende, los AMPs representan una parte fundamental en la correcta activación del sistema inmune innato en pulmón. Sin embargo, a pesar de que el porcentaje de infección representa aproximadamente solo el 30% de los contactos, este número incrementa en poblaciones con bajos niveles séricos de vitamina D, mismas que muestran un peor pronóstico de la enfermedad (Talat et al., 2010).

El calcitriol, la forma activa de la vitamina D, es una prohormona derivada del colesterol con innumerables acciones debido a la amplia distribución de su receptor (Zuluaga Espinosa et al., 2011). Se ha reportado que el calcitriol influye genómicamente en células del sistema inmune y es conocido por sus diversos efectos inmunomoduladores. Entre estos se ha reportado que favorece el control de infecciones al promover efectos bactericidas mediante la inducción de AMPs por parte de los macrófagos, particularmente de la catelicidina LL-37 (Hernández Sánchez et al., 2011), cuyas concentraciones plasmáticas se han relacionado positivamente con el control de la infección de TB (Majewski et al., 2018). Adicionalmente, aquellos

pacientes con bajas concentraciones séricas de LL-37 son más susceptibles a desarrollar la infección activa de TB. Este efecto se asocia a una menor capacidad de lisis del bacilo, evidenciando la importancia del péptido durante la infección tuberculosa (Rahman et al., 2015). Anteriormente, nuestro grupo de investigación ha reportado una alta secreción de LL-37 por macrófagos alveolares infectados con la bacteria Mtb en las primeras 18 horas post infección. Además, la cantidad de péptido secretada depende de la multiplicidad de infección utilizada (Rivas-Santiago et al., 2008).

Considerando las propiedades inmunomoduladoras de LL-37, se encuentra documentado que este péptido modula la expresión de citocinas anti y pro-inflamatorias como un posible mecanismo para eliminar a Mtb en macrófagos infectados (Torres-Juarez et al., 2015). Resultados similares se han encontrado en modelos murinos de TB progresiva, en donde el homólogo de LL-37, denominado CRAMP, se encuentra considerablemente incrementado en células de epitelio pulmonar y macrófagos (Castañeda-Delgado et al., 2010).

Complementario a esto, estudios en humanos han demostrado una elevación en las concentraciones séricas de LL-37 en pacientes de TB pulmonar activa (Majewski et al., 2017; Majewski et al., 2018; Yamshchikov et al., 2010), y que, además, bajas concentraciones del mismo péptido se asocian a un peor pronóstico de la enfermedad e incluso a una mayor susceptibilidad a la infección por Mtb, efecto que pudiera explicarse por una menor capacidad de lisis del bacilo (Rahman et al., 2015). En conjunto los antecedentes demuestran que el AMP LL-37 está involucrado en la eliminación de Mtb como parte de la respuesta inmune innata.

La catelicidina LL-37 es secretada por una gran variedad de células, principalmente por células inmunes, aunque su expresión también puede ser inducida en células epiteliales, entre ellas células del epitelio respiratorio (Park et al., 2011; Sorensen et al., 2001). El gen CAMP codifica a la proteína LL-37, y su expresión es principalmente regulada por el calcitriol. El inicio de la expresión de LL-37 ocurre cuando el calcitriol se une a su receptor citoplasmático VDR (receptor a vitamina D), formando un

complejo que transloca al núcleo celular para unirse a los elementos de respuesta a la vitamina D (VDRE) que se localizan en la región promotora del gen CAMP (Vandamme et al., 2012). Por ello, poblaciones con bajos niveles séricos de vitamina D son más susceptibles a desarrollar TB activa (Nnoaham & Clarke, 2008; Talat et al., 2010).

Por otra parte, se ha identificado en células de epitelio pulmonar un sitio de unión para el factor de transcripción CEBPα dentro del promotor del gen CAMP, que se localiza de forma adyacente a los VDRE. Asimismo, se ha reportado que la activación de CEBPα promueve la expresión de LL-37, misma que se ve significativamente incrementada con la activación conjunta de CEBPα y VDR (Dhawan et al., 2015).

CEBPα forma parte de la familia de factores de transcripción nucleares C/EBP. Particularmente, CEBPα se ha estudiado por su papel durante la embriogénesis y la diferenciación celular, aunque también es capaz de modular la expresión de diferentes genes que contengan el motivo CCAAT en sus regiones promotoras (NCBI, 2021). Recientemente se describió que la activación de CEBPα puede ser inducida en queratinocitos en condiciones que causan estrés a retículo endoplasmático (RE) (Park et al., 2011), y que esta activación potencializa la expresión de LL-37 independientemente de la vitamina D (Dhawan et al., 2009). Esto fue confirmado mediante el uso de RNAs de silenciamiento (siRNAs) dirigidos a CEBPα, en donde se observó una menor transcripción del gen CAMP (Hau et al., 2013). Sin embargo, no hay reportes del papel de CEBPα durante la secreción de LL-37 en la infección por Mtb. Cabe destacar que la alta eficiencia del sistema inmune puede ser regulada para promover la eliminación bacteriana mediante el reposicionamiento de fármacos con la intención de promover la activación de genes que respalden los mecanismos de la inmunidad innata durante la infección por TB, como es CEBPα.

Por lo tanto, el objetivo de este trabajo fue evaluar la capacidad de *Mycobacterium tuberculosis* para inducir la expresión de CEBPα, así como su papel en el control de la infección en un modelo *in vitro*, para posteriormente reposicionar fármacos que activen a CEBPα y evaluar su potencial actividad antituberculosa.

JUSTIFICACIÓN

La tuberculosis es un importante problema de salud pública al ser la primera causa de muerte a nivel global por enfermedades infecciosas. Entre los mecanismos tempranos de la inmunidad innata frente a la infección por Mtb destacamos al AMP LL-37. El principal inductor descrito del péptido LL-37 es la forma activa de la vitamina D, el calcitriol, que ejerce esta función al unirse a los VDRE dentro del gen CAMP. Adicionalmente, en la literatura se ha reportado que CEBPα también induce al mismo péptido cuando la célula enfrenta condiciones de estrés a RE. Sin embargo, se desconoce si el bacilo de Mtb es capaz de promover la activación de CEBPα, y sí este factor promueve a su vez la secreción de LL-37 como un mecanismo de respuesta de las células infectadas.

Este estudio presentará evidencia sobre el papel de CEBPα en la infección con Mtb, que pudiera proponerlo como posible blanco farmacológico al inducir o potenciar la respuesta inmune y así promover la eliminación del bacilo o bien incrementar la sensibilidad del mismo a LL-37. El reposicionamiento de fármacos nos ayudaría a encontrar una molécula farmacológica que pudiera tener como blanco terapéutico a CEBPα y así tener una opción nueva para el tratamiento de la TB.

HIPÓTESIS

CEBPα potencializa la expresión y secreción del AMP LL-37 y su participación es necesaria en el control de la infección por Mtb en células de epitelio pulmonar y macrófagos derivados de monocitos humanos (hMDMs). Existen fármacos aprobados por la FDA (Administración de Alimentos y Medicamentos, por sus siglas en inglés) capaces de inducir la activación de CEBPα en células de epitelio pulmonar y hMDMs infectadas con Mtb.

OBJETIVOS

General

Evaluar el efecto de Mtb sobre la expresión de CEBPα, así como la participación de CEBPα en el control de la infección en un modelo *in vitro*. Reposicionar fármacos que activen CEBPα y evaluar su actividad antimicobacteriana.

Específicos

1. Evaluar la expresión del mRNA de CEBPα en células de epitelio pulmonar y hMDMs infectadas con Mtb.
2. Evaluar la expresión de la proteína LL-37 en células de epitelio pulmonar y hMDMs infectadas con Mtb.
3. Evaluar el crecimiento de Mtb en células de epitelio pulmonar y hMDMs tratadas con un inhibidor farmacológico de estrés a RE.
4. Identificar a través de herramientas bioinformáticas moléculas aprobadas por la FDa capaces de inducir la activación de CEBPα.
5. Evaluar la citotoxicidad de los fármacos seleccionados y determinar las concentraciones óptimas de estudio.
6. Acoplar las moléculas seleccionadas a nanopartículas (síntesis de liposomas).
7. Evaluar el crecimiento de Mtb en células de epitelio pulmonar y hMDMs tratadas con liposomas acoplados a las moléculas candidato.

ARTÍCULO

A new target for drug repositioning: CEBPα elicits LL-37 expression in a vitamin D-independent manner promoting Mtb clearance

Running title: CEBPα elicits LL-37 and promotes Mtb clearance

Yolanda M. Jacobo-Delgado [a, b], Adrián Rodríguez-Carlos [a], Alan Santos-Mena [a], Óscar E. González-Muñiz [a, b], Camelia Félix-Arellano [a], Gabriela Navarro-Tovar [b], Bruno Rivas-Santiago [a].

[a] Zacatecas Biomedical Research Unit, Mexican Social Security Institute. Interior de la Alameda #45 Colonia Centro ZIP code 98000., Zacatecas, Mexico

[b] Faculty of Chemical Sciences, Autonomous University of San Luis Potosi. Av. Manuel Nava #6, Zona Universitaria, ZIP code 78290, San Luis Potosi, S.L.P. Mexico.

YMJD. 0000-0003-2174-6277 – yolandajacobo1996@gmail.com
ARC. 0000-0002-3514-5299 - rdz.carlos09@hotmail.com
ASM. 0000-0002-4853-3063 - alan.santito@hotmail.com
OEGM. 0009-0000-7983-2587 - oscar_glzm@outlook.es
CFA. 0009-0001-0577-2056 - cameflx16@gmail.com
GNT. 0000-0003-3789-2324 - gnavarrotovar@gmail.com
BRS. 0000-0002-1521-1519 - rondovm@gmail.com

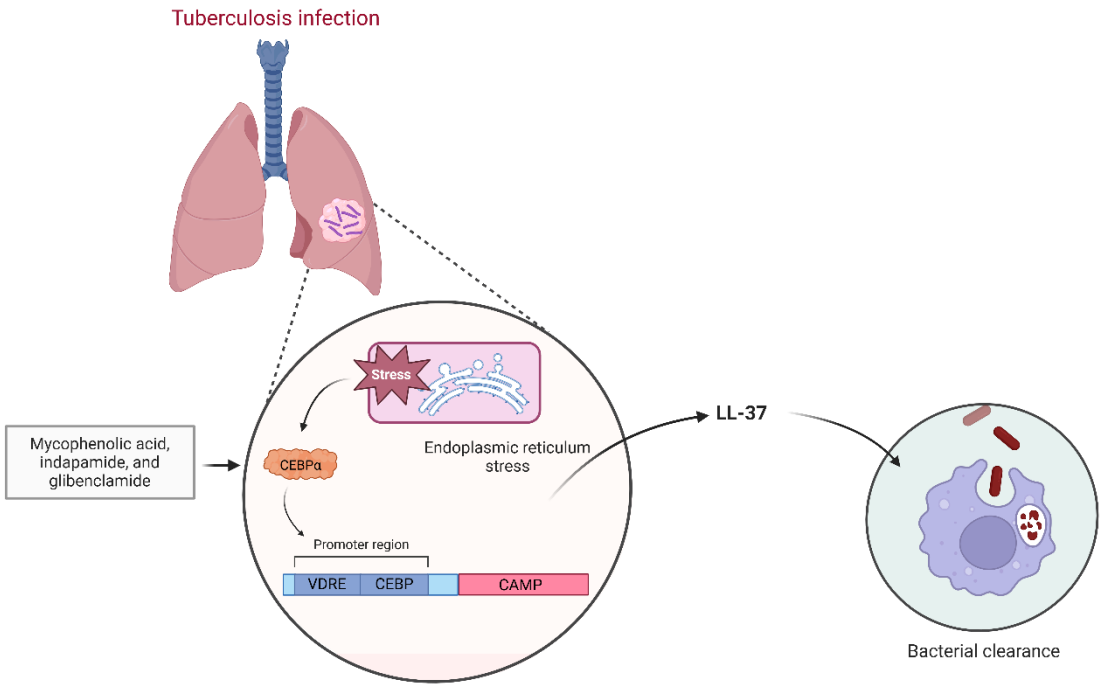
Correspondence and requests for reprints should be addressed to Bruno Rivas Santiago, Ph.D., Zacatecas Biomedical Research Unit, Mexican Social Security Institute. Zacatecas, Mexico. E-mail: rondovm@gmail.com

ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and is a growing public health problem worldwide. Within the innate immune response, we highlight the secretion of the antimicrobial peptide LL-37, which is crucial for Mtb elimination in infected cells. Previous reports have shown that CEBPα activation induces LL-37 independently of its main inducer, vitamin D, under endoplasmic reticulum (ER) stress. In this study, we report that infection with Mtb causes ER stress in pulmonary epithelial cells and macrophages. The stress induces the activation of CEBPα, which in turn promotes the LL-37 expression. Furthermore, the participation of CEBPα is necessary for the correct clearance of Mtb in an in vitro infection model. We identify candidate drugs (mycophenolic acid, indapamide, and glibenclamide) capable of activating CEBPα and promoting LL-37 through in silico assays. The effect of the drugs was corroborated by gene and protein expression analysis. Finally, we observed that treatment with these drugs improves bacterial clearance in infected cells. Our results lead us to suggest CEBPα as a potential therapeutic target as an adjuvant in the standard treatment of tuberculosis, seeking a reduction in treatment time, and thus a lower appearance of drug resistance.

KEYWORDS: Tuberculosis, innate immunity, antimicrobial peptides, CEBPα, drug repositioning.

GRAPHICAL ABSTRACT



Mycobacterium tuberculosis infection leads to endoplasmic reticulum stress, activating CEBPα. CEBPα induces LL-37 which promotes bacterial clearance. Mycophenolate acid, indapamide, and glibenclamide allow CEBPα activation and are proposed as antituberculosis drugs.

1. INTRODUCTION

Tuberculosis [TB], a disease caused by the bacillus *Mycobacterium tuberculosis* (Mtb), remains one of the most lethal infectious diseases globally, as reported by the World Health Organization (WHO). The 2022 WHO Global TB Report highlights a concerning rise in drug-resistant strains, underscoring the urgent need for novel therapeutic strategies (WHO, 2024). TB is primarily transmitted via inhalation of aerosols containing Mtb bacilli, which are expelled by individuals with active disease through talking, coughing, or sneezing. Upon entry into the respiratory tract, the host's innate immune system activates a series of protective responses. These immune defenses are highly effective, with approximately 70% of exposed individuals successfully clearing the bacteria. Among the 30% who become infected, only 10% progress to active disease (Jilani et al., 2020). Despite this, TB continues to cause approximately 10 million new cases annually (WHO, 2024). Given the remarkable efficiency of the innate immune system, enhancing these natural defense mechanisms represents a promising avenue for therapeutic intervention.

Upon entering the respiratory tract, Mtb initially encounters epithelial cells and alveolar macrophages, the latter serving as the primary host for the bacillus. Once recognizing Mtb, both cell types release soluble mediators of the innate immune response, including antimicrobial peptides (AMPs) (Maertzdorf et al., 2018). AMPs are potent bactericidal molecules that create a protective soluble barrier across various epithelial surfaces, including the respiratory tract. These peptides are characterized by short sequences of positively charged amino acids, and their antimicrobial action is primarily driven by electrostatic interactions between the peptide and the negatively charged components of the pathogen's membrane. This interaction allows AMPs to integrate into the pathogen's membrane leading to membrane disruption and subsequent lysis (Jacobo-Delgado et al., 2023).

Among the various families of AMPs, cathelicidins are particularly notable due to their broad-spectrum antimicrobial activity. LL-37, the only human cathelicidin, has been extensively documented for its ability to lyse the cell wall of Mtb (Deshpande et al., 2020). Furthermore, evidence suggests that individuals with lower serum concentrations of LL-37 are more susceptible to developing active TB, highlighting its critical role in host defense against Mtb (Rahman et al., 2015). In addition to its antimicrobial activity, LL-37 exhibits significant immunomodulatory properties, making it a promising target for therapeutic strategies aimed at enhancing bacterial clearance in TB-infected cells (Maertzdorf et al., 2018). Notably, LL-37 is an inducible peptide, and its expression can be stimulated by specific factors. The primary known inducer is calcitriol, the biologically active form of vitamin D. When calcitriol binds to its cytoplasmic receptor, the vitamin D receptor (VDR), it forms a complex with the retinoid X receptor (RXR). This complex translocates to the nucleus, where it binds to vitamin D response elements (VDREs) in the promoter regions of target genes. One such target is the CAMP gene, which encodes the LL-37 peptide due to the presence of a VDRE site within its promoter (Ismailova & White, 2022).

Vitamin D regulates a broad spectrum of genes in both immune and non-immune cells, particularly those involved in host defense mechanisms. Beyond the canonical vitamin D pathway, recent studies have demonstrated that the activation of the CCAAT/enhancer-binding protein alpha (CEBPα) transcription factor can also enhance the expression of LL-37 in epithelial cells (Dhawan et al., 2015). CEBPα belongs to the C/EBP family of transcription factors, recognizing the CCAAT motif within the promoter regions of its target genes. While much of the research on CEBPα has focused on its role in gene regulation during embryogenesis and cell differentiation (Ramji & Foka, 2002), evidence suggests that its activation in respiratory epithelial cells promotes the secretion of LL-37 even in the absence of vitamin D. Furthermore, CEBPα and VDR appear to act synergistically to increase LL-37 production, as silencing CEBPα with RNA interference significantly reduces the expression of the CAMP gene (Dhawan et

al., 2015; Hau et al., 2013). This points to a non-canonical pathway for LL-37 induction, independent of the vitamin D signaling axis.

Moreover, studies in human keratinocytes have shown that conditions causing endoplasmic reticulum [RE] stress activate CEBPα, leading to increased LL-37 expression, independent of vitamin D (Park et al., 2011). Given that ER stress is often associated with infectious processes (Bettigole & Glimcher, 2015), the involvement of CEBPα in the immune response to Mtb is an intriguing area for exploration. These findings suggest that CEBPα could be a viable pharmacological target for enhancing the innate immune response, particularly in the context of Mtb infection.

In this study, we propose to investigate whether Mtb infection induces CEBPα expression in epithelial cells and macrophages and to assess the impact of this pathway on bacterial clearance via LL-37 induction. Preliminary observations indicate that inhibition of CEBPα impairs the ability of infected cells to eliminate mycobacteria, underscoring its potential role in controlling TB. Based on these insights, we explored repositioning FDA-approved drugs for their potential to bind and activate CEBPα, evaluating their anti-tuberculous properties.

2. MATERIAL AND METHODS

Mycobacterium tuberculosis culture

Drug-sensitive *Mycobacterium tuberculosis* H37Rv strain (ATCC® 27294™) was cultured in 25 cm² polystyrene flasks with 10 mL of Middlebrook 7H9 medium (DIFCO, USA) supplemented with 10% oleic acid, albumin, dextrose, and catalase (OADC enrichment medium; Becton Dickinson, Franklin Lakes, NJ), 5% Tween 80, and 0.2% glycerol, and was incubated at 37°C under agitation. The strain was maintained in culture until it reached the logarithmic phase, which was determined by spectrophotometry by measuring the optical density at 600 nm. Previous studies have

reported that an absorbance of 0.762 corresponded to $1.33x10^8$ bacteria/mL (Peñuelas-Urquides et al., 2013). Working aliquots with a theoretical concentration of $2x10^8$ bacteria/mL were prepared and stored at -20°C. The CFU number used for in vitro infections was confirmed in each experiment by plating onto 7H10 agar.

Cell culture

The type 2 pneumocyte (T2P) cell line (A549 ATCC® CCL185™ Manassas, VA, USA) was grown with RPMI-1640 (Biowest, Nuaille, FR) supplemented with 10% FreeAdd 1X (Biowest, Nuaille, FR) and 100 µg/mL of antibiotic (penicillin 100X, Corning cellgro, Manassas, VA, USA). For the infection assay, the cell line was seeded in 24-well dishes plates (Corning cellgro, Manassas, VA, USA) with 1% FreeAdd 1X and maintained for 18 h in the presence of 5% CO_2 at 37ºC until infection with Mtb. The macrophages derived from human monocytes (MDM), were obtained according to the Declaration of Helsinki and approved by the National Committee of Ethics and National Commission of Scientific Research of the Mexican Institute of Social Security (IMSS). The procedure of macrophages isolation and differentiation was carried out according to previous reports (Jacobo-Delgado et al., 2021). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated using Lymphosep (Biowest, Nuaille, FR). 2.5×10^6 PBMC were cultured in RPMI medium using 24-well plates (Costar, Corning, NY, USA). After 2 h, non-adherent cells were removed. Subsequently, cells were differentiated for seven days with RPMI medium (Biowest, Nuaille, FR) supplemented with 10% of FreeAdd 1X. To assess macrophage phenotype, the CD68+ differentiation marker was evaluated using flow cytometry, obtaining a percentage of positive cells greater than 95%.

Infection and stimuli

The cells with their respective treatment were infected at a multiplicity of infection (MOI) of 5:1 (bacteria: cells) for 3 hours. After the infection period, cells were thoroughly washed at least twice with RPMI 1640 (Corning cellgro, Manassas, VA, USA) to remove non-internalized bacteria, and the corresponding treatment was reapplied until the

evaluation time was completed. At least three replicates of each treatment were performed in duplicate. The conditions evaluated were: 1) Control without infection; 2) Pharmacological induction of endoplasmic reticulum (ER) stress: treatment with tunicamycin 200 nM (Sigma-Aldrich, Missouri, USA) or thapsigargin 100 nM (Sigma-Aldrich, Missouri, USA) was for 6 hours. Both drugs were resuspended using absolute ethanol molecular biology grade (Sigma-Aldrich, Missouri, USA) as the vehicle. Subsequently, the supernatant was removed, and the cells were cultured in their respective medium for an additional 18 hours; 3) Infection with Mtb, as previously described; 4) Infection with Mtb + calcitriol: treatment with calcitriol (Sigma-Aldrich, Missouri, USA) at concentrations of $1x10^{-8}$M and $1x10^{-6}$M was for 18 hours, starting at the time of infection; 5) Infection with Mtb + calcitriol + tauroursodeoxycholic acid (TUDCA) 200 µM (Sigma-Aldrich, Missouri, USA): treatment with the ER stress inhibitor TUDCA was used for 6 hours prior to infection.

Evaluation of genetic expression by RT-qPCR

Once the time for each treatment was completed, the supernatant was removed, and 200 µL/well of trizol (Invitrogen, Auckland, New Zealand) was added to perform the total RNA extraction using the Chloroform: Isoamyl alcohol method. The obtained RNA was resuspended in 11 µL of diethylpyrocarbonate (DEPC)-treated water to proceed with cDNA synthesis. The synthesis was performed in the Applied Biosystems thermal cycler according to the specifications of the enzyme used. The cDNA obtained was quantified using the NanoDrop® ND-100 (NanoDrop Technologies, Inc, USA) at a wavelength of 260 nm to determine the concentration. Additionally, the 260/280 nm and 260/230 nm ratios were used to verify purity. Based on the concentrations of each sample, working aliquots a concentration of 75 ng/µL were prepared and stored at -20°C until use. To perform the amplification of the different genes, a cDNA intercalating dye, EvaGreen (Biotium, California, USA), and specific primers, shown in Table 1, were used. The amplification was done in the Lightcycler® 480 thermal cycler (Roche Life Science, USA). The analysis of the amplification curves to determine the crossing points (CP) was carried out using the Lightcycler 4.05 software (Roche Applied

Science) on the same thermal cycler. Finally, data obtained from three independent experiments in duplicate were normalized using the CP of the housekeeping gene HPRT. Relative expression was then determined using the 2-ΔΔCt method, followed by the appropriate statistical analysis (Livak & Schmittgen, 2001).

Table 1. Primer sequences evaluated

Gene	Protein	Reverse primer	Forward primer
HPRT	**Hypoxanthine Phosphoribosyltransferase 1**	**5' – ACC TGG TTC ATC ATC ACT AAT CAC – 3'**	**5' – GAC CGG TTC TGT CAT GTC G – 3'**
CEBPα	**CCAAT - enhancer binding protein α**	**5' – GTC TGG GTC CCC ATC CAT – 3'**	**5' – TCG GAT GCT AAC CTC TAC CG – 3'**

Protein analysis by Western Blot

Treated cells were lysed with RIPA buffer (10 mM Tris-HCl pH 8, 1 mM EDTA, 0.5 mM EGTA, % Triton x-100, 0.1% Deoxycholate, 0.1% SDS y 140 mM NaCl), then protein extract was quantified with Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, USA #15045), following the manufacturer's instructions. Cellular proteins (30 µg) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes, which were blocked with 10% non-fat milk in PBST (PBS-Triton X-100 0.5%) for 1 h at room temperature. Monoclonal antibodies used to analyze LL-37 protein expression (mouse, Santa Cruz USA, (D-5): sc-166770 1:200) and anti-mouse (rabbit Merck USA, 06-599, 1:10,000). Densitometric analysis was performed using the Image Analysis software (Thermo Fisher Scientific), and adjusted with the loading control (β-actin, Sigma-Aldrich St. Louis USA #A5316 Mouse, 1:3000).

Intracellular Mtb growth assay

At the end of the treatment times for the infected cells, the supernatants were discarded, and the cells were lysed with SDS (Sigma-Aldrich, Germany) for 10 minutes. Subsequently, the action was neutralized with Middlebrook 7H9 medium (DIFCO, USA) containing 20% BSA (Sigma-Aldrich, Germany). The bacteria obtained from cell lysis were subjected to 4 serial dilutions (1:10) from each well in triplicate on Middlebrook 7H10 agar (DIFCO, USA). Finally, they were incubated for 21 days at 37°C. After the incubation period, the number of colony forming units per milliliter (UFCs/mL) in each condition was counted and averaged. The results were compared between groups to determine if there was variation in mycobacterial growth among the different treatments.

Molecular docking

The chemical structure of thapsigargin and tunicamycin was entered in SMILES format (Simplified Molecular Input Line Entry System) into the ZINC15 database website (https://zinc15.docking.org) to search for other structurally similar molecules. As a search and selection parameter for candidates, a Tanimoto similarity index ≥ 0.60 was considered. Subsequently, we searched for FDA-approved (Food and Drug Administration, USA) items using predetermined filters. Once the candidate molecules were selected, the affinity of the ligand of interest was confirmed using the SEA (Similarity Ensemble Approach, http://sea.bkslab.org/) program. These results were also confirmed through molecular docking analysis using Autodock Vina software (http://vina.scripps.edu/), using the binding affinity of the prototype molecule as a reference value and drugs with the ability to bind to the nuclear factor CEBPα were sought. The crystallized protein was searched in the PDB (Protein Data Bank), and it was then analyzed using the UCSF Chimera free access software (https://chimeratool.com/) to visualize the molecular structure and related data. The DoGSiteScorer software (https://openebench.bsc.es/tool/dogsitescorer) was then used to predict an activation site in the CEBPα molecule without interfering with the DNA binding site. For drug selection, molecular docking analysis was performed using

Autodock Vina software (http://vina.scripps.edu/) to evaluate the docking of various queried compounds that, using predefined filters, had been previously approved by the FDA. Molecules with the highest Z score in the same Autodock Vina software were selected. Once the candidate molecules were selected, the affinity of the ligand of interest for the CEBPα target was confirmed using the SEA program. Finally, each molecule was evaluated considering the available information, commercial availability, and cost of the compound, as well as various scientific backgrounds, including published articles, described activity, related adverse effects, and the physical and chemical characteristics of each compound. The cytotoxicity of each compound was evaluated for each kind of cell used in the present study using the Guava ViaCount Assay (Luminex, Austin Tx, USA), which distinguishes between viable and non-viable cells based on the differential permeability of DNA-binding dyes.

Immunocytochemistry Evaluation

Cells were fixed in the Chamber Slide™ System (Thermo Fisher Scientific, USA # 154453), and the endogenous peroxidase was quenched with 0.03 % H_2O_2 in absolute methanol. Then, the slide was washed and blocked with PBS supplement with 12 % human pool serum. Later, slides were incubated for 18 h at room temperature with mouse anti-LL-37 (Santa Cruz, USA, (D-5): sc-166770 1:200) and rinsed and incubated with donkey anti-mouse immunoglobulin G (IgG) biotin-labeled antibody (Santa Cruz, CA, USA). Bound antibodies were detected with avidin-peroxidase (Biocare, Medical, Concord, CA, USA) and counterstained with hematoxylin. For visualization, we use an image analyzer (Axiovert Vision Release 4.8.2 Software, Zeiss, GER).

Statistical Analysis

The statistical analysis was carried out using GraphPad Prism 5.0 software (San Diego, CA, USA). Initially, it was determined whether the data were parametric or non-parametric using the Kolmogorov-Smirnov test. For parametric data, an ANOVA was performed followed by Tukey's post-test, while for non-parametric data, the Kruskal-

Wallis test was used with Dunn's post-test. A value of $p < 0.05$ was considered statistically significant for all analyses.

Ethics approval

All studies were conducted in accordance with the Helsinki Declaration. All experimental protocols were approved by Mexican Institute of Social Security Use Committee (No-1912). Approval register R-2021-3301-012.

3. RESULTS

Mycobacterium tuberculosis elicits CEBPα expression through ER stress

Our results showed that Mtb infection significantly promotes CEBPα expression in T2P and MDMs (Figure 1; panels A and B, respectively). This effect is lost when cells are stimulated with TUDCA. Besides, pharmacological inducers of ER stress (thapsigargin and tunicamycin) significantly promote CEBPα expression, although to a lesser extent than Mtb infection. These results suggest that Mtb is a strong inducer of CEBPα in infected cells and that the induction is through ER stress. Interestingly, observed effects are influenced by vitamin D in MDMs but not in T2P. When MDMs are cultured with fetal bovine serum (FBS), infection with Mtb promotes CEBPα expression but not in the same amount compared to those cultured in FBS-free conditions (Figure S1). The total composition of FBS is not completely characterized however, vitamin D is present within its components, previous reports described that this vitamin has intense activity on the human monocyte genome during their differentiation into macrophages (Seuter et al., 2016). Our results show that in the absence of vitamin D, compensatory mechanisms are activated in MDMs, including CEBPα. To eliminate the influence of vitamin D, FBS culture was excluded from all experiments, ensuring that the results are vitamin D-independent.

CEBPα promotes LL-37 secretion during *Mycobacterium tuberculosis* infection

We observed that LL-37 secretion during Mtb infection occurs even in the absence of vitamin D in T2P and MDMs (Figure 2; panels A and B, respectively) that is significantly increased when vitamin D is added exogenously. We previously reported that TUDCA treatment inhibits CEBPα expression and similarly inhibits LL-37 secretion in both cell types, even in cells that received exogenous vitamin D (Figure 2; panels A and B, respectively). These results strongly suggest that LL-37 is induced in infected cells even in vitamin D-free conditions, most likely derived from the increase in CEBPα expression as a direct effect of Mtb infection.

CEBPα activation correlates with increased Mtb clearance

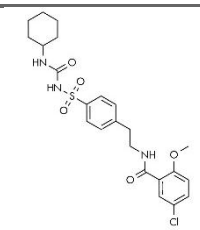
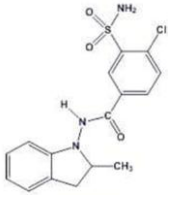
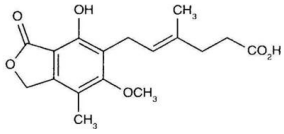
Then we evaluated whether increased CEBPα correlates with enhanced Mtb clearance in infected cells. We first observed an enhanced mycobacterial elimination when T2P and MDMs (Figure 3; panels A and B, respectively) were infected and stimulated with vitamin D (VD). However, the VD effect was significantly lost when the inhibitor TUDCA was added in both cell types. We propose that the effect is due to the inhibition of CEBP and the consequent decrease in LL-37 peptide production, thus CEBP is a potential therapeutic target for the adjuvant treatment of TB.

FDA drugs with CEBPα binding potential

Considering the therapeutic potential of CEBPα to promote an antimicrobial state during Mtb infection, we decided to search in silico for FDA-accessible drugs with high binding and activation potential for the transcription factor. First, we obtained the CEBPα crystallized structure and identified the serine residues associated with its activation and the DNA binding site (Figure 4; panels A and B, respectively). Molecular docking assays were directed to these serine residues, seeking not to cause steric hindrance at the DNA binding site. The crystallized structures of all FDA-approved drugs available in the ZINC15 database (https://zinc15.docking.org) were obtained, yielding 1,200 molecules. From the molecular docking results, we selected the three molecules with the highest binding strength (ΔG Kcal/mol) to any of the specific serine

residues: glyburide, indapamide, and mycophenolic acid (Table 2). The interaction of the crystallized drugs with the serine residues of interest is shown in Figure 4, panels D-E. Cytotoxicity in T2P cells and MDMs was discarded to 2-10 μM indapamide, 100-5,000 nM glibenclamide, and 100 - 2,000 nM mycophenolic acid (Figure S2).

Table 2. Characteristics of drugs selected by molecular docking

ZINC ID	FDA-approved drug	Structure	Reported activity	ΔG Kcal/mol	Amino acid residues
537808	Glibenclamide		Increase insulin secretion from beta cells by closing ATP-sensitive potassium channels	-5.8	Ser 299
601305	Indapamide		Inhibits the Na+/Cl- cotransporter in the distal tubule of nephrons	-5.8	Ser 277
1758	Mycophenolic acid		Selective noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH)	-5.3	Ser 277

Mycophenolic acid, indapamide and glibenclamide promote CEBPα expression

After in silico analysis, we next evaluated the capacity of the candidate molecules to promote CEBPα expression. The three molecules were evaluated in T2P and MDMs cultures, results showed that mycophenolic acid and indapamide promoted the expression of the target transcription factor in both cell types (Figure 5; panels A-D). whereas, glibenclamide have activity only on T2P cells (Figure 5; panels E-F).

CEBPα increase correlates with LL-37 expression

We further assessed whether the upregulation of CEBPα correlated with increased expression levels of LL-37. The results demonstrated that all selected molecules significantly enhanced the expression of this antimicrobial peptide in T2P cells (Figure 6, panel A). However, in MDMs, glibenclamide had no effect (Figure 6, panel B), likely because it did not promote CEBPα expression in this cell type (Figure 5, panel F). Immunocytochemistry and Western Blot confirmed that LL-37 protein levels were indeed induced by the molecules selected in silico in both cell types, with vitamin D serving as a positive control for LL-37 induction and tunicamycin as a control for CEBPα (Figure 6, panel C-E). Therefore, mycophenolic acid, indapamide, and glibenclamide showed potential as antituberculosis agents by promoting an antibacterial state via LL-37 induction.

CEBPα-activating drugs promote Mtb clearance in infected cells

Finally, we investigated the potential anti-tuberculous activity of the selected molecules in T2P cells and MDMs infected with Mtb. In T2P cells, only the highest concentrations of mycophenolic acid and indapamide promoted bacterial clearance, whereas in MDMs, all three candidate molecules—mycophenolic acid, indapamide, and glibenclamide—sustained an antibacterial state during infection (Figure 7; panels A and B, respectively). These findings suggest that the alternative pathway for LL-37 induction via the transcription factor CEBPα is critical during Mtb infection. Activation of this pathway, independent of vitamin D, enhances bacterial clearance during tuberculosis infection. Consequently, drugs that target CEBPα activation are proposed as adjuvants in TB treatment, with the potential to reduce treatment duration and, thus, minimize the risk of adverse effects or therapeutic non-compliance.

4. DISCUSSION

Despite advances in time and the appearance of novel pathogenic viruses and bacteria, tuberculosis continues to rank among the leading causes of mortality from infectious diseases globally. Although *Mycobacterium tuberculosis* exhibits a high rate of transmission, 70% of individuals exposed to the bacteria successfully clear the infection through robust activation of the immune system, supporting the rationale for host-directed therapies aimed at enhancing antibacterial responses. In this study, we investigate the role of CEBPα as an alternative pathway in the induction of LL-37 during Mtb infection.

Previous studies have demonstrated that CEBPα activation can occur under endoplasmic reticulum (ER) stress conditions induced by pharmacological agents such as thapsigargin and tunicamycin (Park et al., 2011). However, a wide range of external stressors, including pathogenic infections, can also trigger ER stress. Recent findings have shown that Mtb infection induces apoptosis in infected macrophages through ER stress as a mechanism for promoting its dissemination (Xu et al., 2022). In line with this, our research has revealed an upregulation of CEBPα expression via ER stress, in Mtb-infected type 2 pneumocytes (T2P) and human monocyte-derived macrophages (MDMs).

Low or early levels of ER stress are associated with the activation of the unfolded protein response (UPR) to regulate the inflammatory response and inhibit apoptosis (Hsieh et al., 2022). Additionally, it has been proposed that the UPR and NFkB pathways converge through the action of different transcription factors (Schmitz et al., 2018). NFkB is considered a master regulator of innate immunity (Barnabei et al., 2021), and we propose that CEBPα is induced upon activation of the UPR pathway during early Mtb infection avoiding cell death, thus CEBPα may impact the immune response mainly through NFkB, whether other nuclear receptors are involved, need to be further elucidated.

In line with these findings, CEBPα activation has been reported to correlate with increased LL-37 expression in human keratinocytes (Park et al., 2011). A similar response was observed in pulmonary epithelial cells, where CEBPα activation in conjunction with vitamin D led to increased LL-37 levels. Calcitriol, the active form of vitamin D, is a key inducer of LL-37. Notably, a binding site for CEBPα has been identified within the vitamin D response elements (VDRE) of the CAMP gene promoter, which encodes LL-37 (Dhawan et al., 2015). In the present study, we observed an upregulation of LL-37 in both T2P and MDMs infected with Mtb following CEBPα activation, even in the absence of vitamin D, which adds to the present knowledge the fact that LL-37 can contribute to immunity even in the absences of calcitriol which has been widely described as its main inducer. The increased expression of LL-37 was sufficient to promote the elimination of the bacilli within the infected cells. These findings suggest that CEBPα plays a critical role in controlling Mtb infection, as it promotes an antibacterial state even in the absence of key nutritional factors such as vitamin D.

Furthermore, we demonstrated that Mtb-infected cells supplemented with vitamin D showed a reduced ability to clear the bacilli when CEBPα was inhibited using tauroursodeoxycholic acid, as described elsewhere (Park et al., 2011). This suggests that while vitamin D is a major inducer of LL-37, its full effect may depend on the combined actions with other molecules such as CEBPα. Based on these results, we propose that the induction of CEBPα could be a potential adjuvant in first-line TB treatments. CEBPα is widely expressed in various tissues, including the respiratory system (Nerlov, 2007) making it a promising target for enhancing Mtb clearance in the lungs, the primary site of infection. Leveraging the benefits of pharmacological repositioning (reviewed elsewhere (Jourdan et al., 2020)) we aimed to explore FDA-approved compounds for their potential to activate CEBPα.

The regulation pathways of CEBPα during adipogenesis and myeloid differentiation have been extensively studied. However, the precise mechanism of its activation and translocation to the nucleus is not fully described yet. Previous studies have shown that CEBPα can be activated by interacting with other transcription factors, such as CEBPβ and NFkB (Ramji & Foka, 2002; Yamamoto et al., 2002). It is likely that after binding, the structural changes discover hidden specific sequences that promote its translocation to the nucleus (Zuo et al., 2006). These findings support our proposal that CEBPα might alter the immune response through NFkB, nonetheless further studies need to be carry out to elucidate it.

The deacetylation of histone H3 inhibits CEBPα activation in leukemia. Therefore, histone deacetylase inhibitors (iHDAC) can restore its activity (Song et al., 2015). Previously, our group reported that iHDACs modulate defensins and cathelicidins expression (Rodríguez-Carlos et al., 2021), and the overall effect may be due to CEBPα activation in addition to chromatin decompaction. On the other hand, it has been observed that the activation of hormone receptors can deplete corepressors associated with CEBPα, promoting adipogenesis (Zuo et al., 2006). Thus, the total mechanisms that regulate CEBPα are complex, but the evidence points out that the repression mechanisms are mainly related to epigenetic modifications.

Similarly other studies have reported that phosphorylation of CEBPα can attenuate its activation. In particular, phosphorylation of Ser 248, Ser 277, and Ser 299 by protein kinase C (PKC) promotes a lower DNA binding capacity and therefore a decreased activity (Mahoney et al., 1992; Ramji & Foka, 2002). Based on this, in the present study, we directed the molecular docking assays to the above-mentioned serine residues to create a physical impediment between the amino acid and the enzyme and allow the activation of CEBPα to persist, without disrupting the DNA binding site. Therefore, we sought to consider all FDA-approved molecules. The candidates were selected for their binding affinity to CEBPα and their accessibility to position them as low-cost adjuvant treatments.

Treatment of T2P and MDM cells with the drugs selected from the *in silico* analysis—mycophenolic acid, indapamide, and glibenclamide—resulted in increased expression of CEBPα, which was correlated with an upregulation of LL-37, the antimicrobial peptide of interest which was further confirmed using immunocytochemistry assays. Furthermore, treatment with the three selected drugs candidates enhanced Mtb clearance in infected MDMs, while significant clearance in infected T2P cells was observed only with the highest doses of mycophenolic acid and indapamide, these concentrations have none cytotoxic activity.

Mycophenolic acid is an immunosuppressant used for almost 60 years to prevent rejection during organ transplants. However, in the 1990s it was replaced by the prodrug mycophenolate mofetil, mainly due to the large number of adverse effects associated with the former. Its immunosuppressive activity is specific for B and T lymphocytes at high doses (Strathie Page & Tait, 2015). In contrast, the drug also showed antibacterial, antifungal, and antiviral properties by inhibiting purine synthesis in lower concentrations (Silverman-Kitchin et al., 1997; Siebert et al., 2018). Besides, modified molecules of mycophenolic acid have been successfully shown antibacterial activity (Siebert et al., 2018), however, their potential antituberculosis role is unknown. In another report, treatment with mycophenolic acid promoted the elimination of *Leishmania tropica* in infected macrophages, presumably by inhibiting guanosine nucleotide synthesis (Berman & Webster, 1982), and it is unclear whether the drug has any therapeutic target in human cells. Our results strongly suggest that mycophenolic acid interacts with CEBPα to avoid its inhibition, the effect of which is reflected in the induction of LL-37 by CEBPα, thereby establishing an anti-tuberculosis environment in an in vitro model. It should be noted that the doses evaluated in the present study (<500 nM) are way below mycophenolic acid immunosuppressive effect (10 uM) to avoid adverse effects.

Indapamide, a thiazide diuretic commonly used to treat arterial hypertension, has no documented effects on the immune system. In fact, one of its rare adverse effects is neutropenia, which can increase susceptibility to infections (Aziz & Rajbhandari, 2023). However, antihypertensive therapy in general has been linked to an enhancement of innate immunity, potentially due to its role as a modulator of vascular function (Fonseca et al., 2015). This study is the first to report that indapamide promotes an antibacterial state by inducing the expression of LL-37 and is effective in clearing Mtb in infected cells, following activation of CEBPα.

Glibenclamide is used in diabetes mellitus treatment by increasing insulin secretion by pancreatic beta cells (Trivedi & Chaturvedi, 2023). Its anti-inflammatory effect is widely documented, which is reflected in lower mortality in patients with diabetes mellitus and bacterial sepsis (Koh et al., 2011; Koh et al., 2013). Glibenclamide also promotes an antimicrobial state in macrophages infected with *Leishmania donovani* (Rub et al., 2019), whether the drug induces the immune response in the infected cells is unknown. Besides, glibenclamide acts in synergy with retinoic acid in macrophages to establish an anti-inflammatory environment and promote healing (Lin et al., 2018). Previously, our research group demonstrated that retinoic acid is a potential anti-tuberculosis agent by regulating the inflammatory response and inducing antimicrobial peptides in an *in vitro* model of Mtb infection (Jacobo-Delgado et al., 2021). Similarly, it has been reported that glibenclamide shows synergy with first-line anti-tuberculosis drugs by enhancing bactericidal activity (Trivedi & Chaturvedi, 2023). Altogether with our results lead us to propose glibenclamide as an adjuvant TB treatment by boosting the innate immune response through various pathways: synergy with vitamins, anti-TB drugs, and CEBPα activation.

In conclusion, our findings demonstrate that activation of CEBPα promotes the expression of LL-37 via a non-canonical pathway, leading to the clearance of Mtb in an *in vitro* model of infected cells. We showed that mycophenolic acid, indapamide, and glibenclamide induce LL-37 expression through CEBPα activation, suggesting their

potential use alongside standard TB therapies to reduce drug-related side effects (Figure 8).

AUTHOR CONTRIBUTIONS: Y.M.J.D., G.N.T., and B.R.S., conceptualization, investigation, formal analysis and methodology; Y.M.J.D., A.R.C., A.S.M., O.E.G.M., and C.F.A., data curation, software, validation and visualization; G.N.T., and B.R.S., funding acquisition, project administration, resources and supervision; Y.M.J.D. and B.R.S., Writing – original draft and writing – review and editing.

FUNDING STATEMENT. This work was supported by fundings from Mexican Institute for Social Security-IMSS (R-2021-3301-012). YMJD had a scholarship by CONAHCyT (CVU: 1009432).

DATA AVAILABILITY STATEMENT. The data that support the findings of this study are available from the corresponding author [BRS] upon reasonable request.

CONFLICT OF INTEREST DISCLOSURE. The authors declare no competing interests, and all data generated during this study are included in this published article.

FIGURES

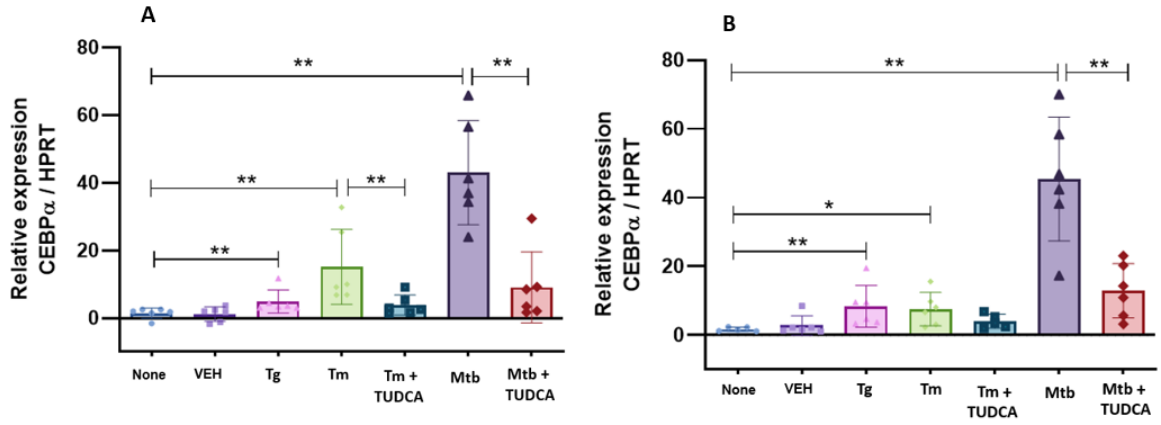


Figure 1. **Mycobacterium tuberculosis infection increases the expression of CEBPα through endoplasmic reticulum (ER) stress.** Cells were treated as indicated above and CEBPα relative expression was evaluated by RT-qPCR 24 h post-stimulation in T2P and MDMs (Panel A and B, respectively). VEH: vehicle; Tm: tunicamycin (200 nM); Tg: thapsigargin (100 nM); TUDCA: tauroursodeoxycholic acid (200 μM). Graphs show mean ± SD from at least 3 independent experiments by duplicate. *p < 0.05; **p < 0.01.

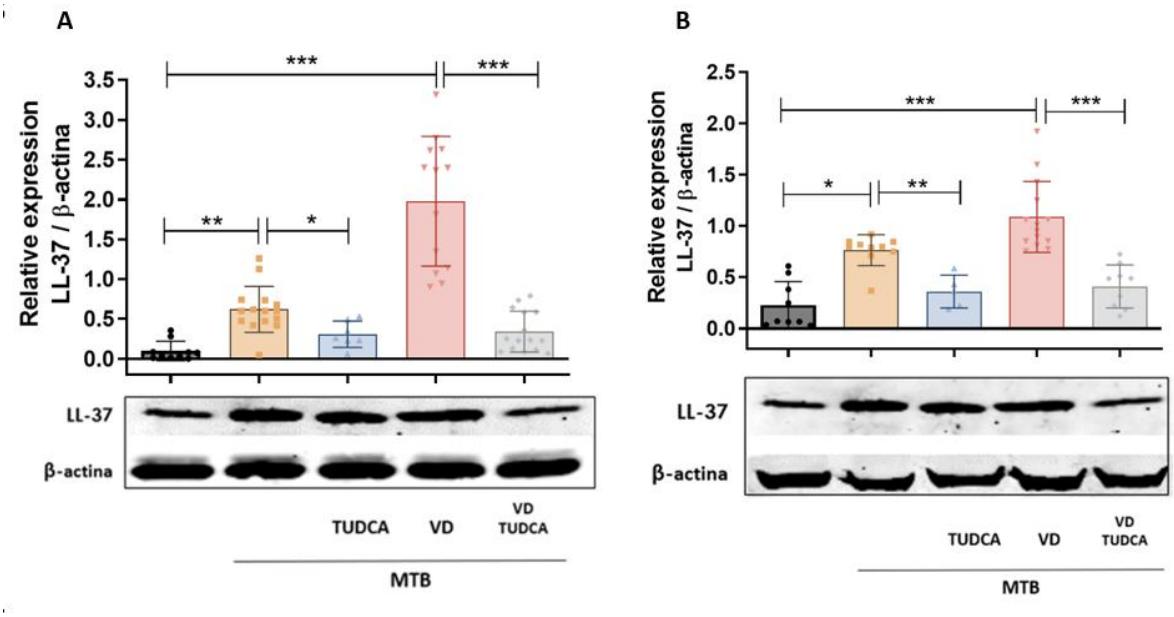


Figure 2. **CEBPα promotes LL-37 secretion during Mycobacterium tuberculosis infection.** Cells were treated as indicated above and LL-37 expression was evaluated by Western Blot 48 h post-stimulation in T2P and MDMs (Panel A and B, respectively). TUDCA: tauroursodeoxycholic acid (200 μM); VD: Vitamin D ($1x10^{-6}$M); MTB: Mycobacterium tuberculosis. Graphs show mean ± SD from at least 3 independent experiments by duplicate. *p < 0.05; **p < 0.01; ***p<0.001.

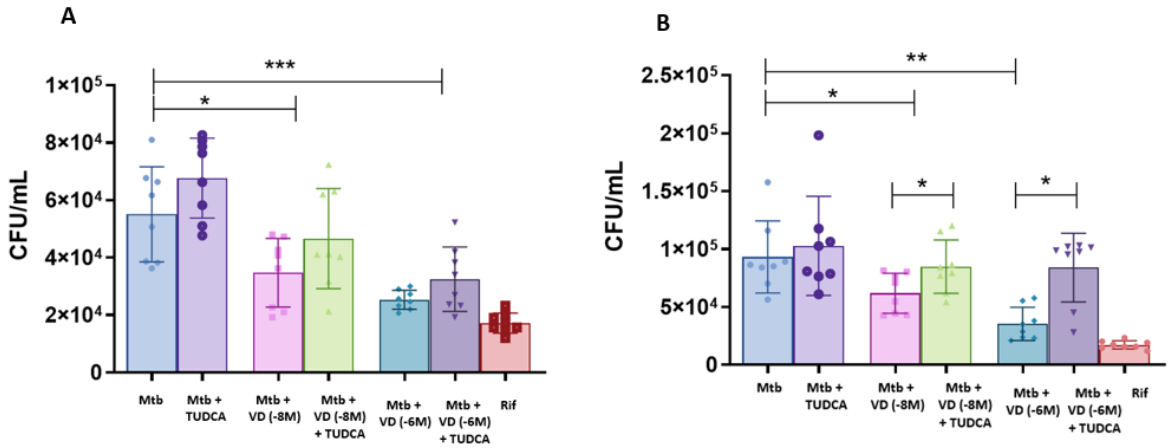


Figure 3. **CEBPα activation correlates with increased Mycobacterium tuberculosis clearance.** Cells were treated as indicated above, after treatment cells were lysed and seeded on 7H10 agars 48 h after stimulation, and colony-forming units per milliliter (CFU/mL) were made in T2P and MDM (Panel A and B, respectively). CFU/mL were counted 21 days later. Rif: rifampicin; TUDCA: tauroursodeoxycholic acid; VD: Vitamin D (-6M and -8M refers to the molar concentration); Mtb: Mycobacterium tuberculosis. Graphs show mean ± SD from at least 3 independent experiments by duplicate. *p < 0.05; **p < 0.01; ***p<0.001.

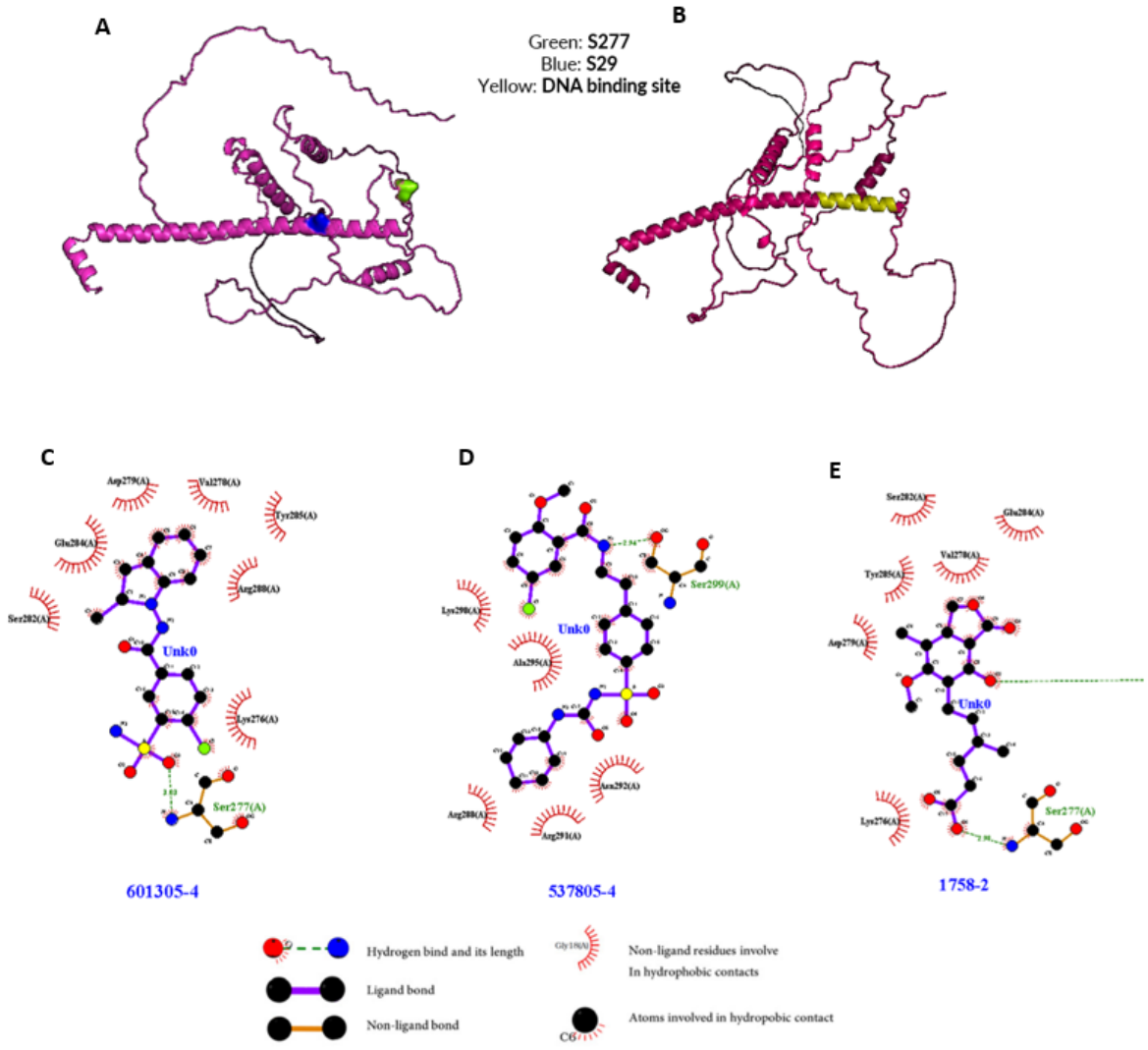


Figure 4. **FDA drugs with CEBPα binding potential.** Crystallized structure of CEBPα, the serine residues associated with its activation are marked in green and blue, and the DNA binding site is marked in yellow (Panel A and B, respectively). Three-dimensional model of the interactions between CEBPα and indapamide, glyburide, and mycophenolic acid (Panel C, D and E, respectively).

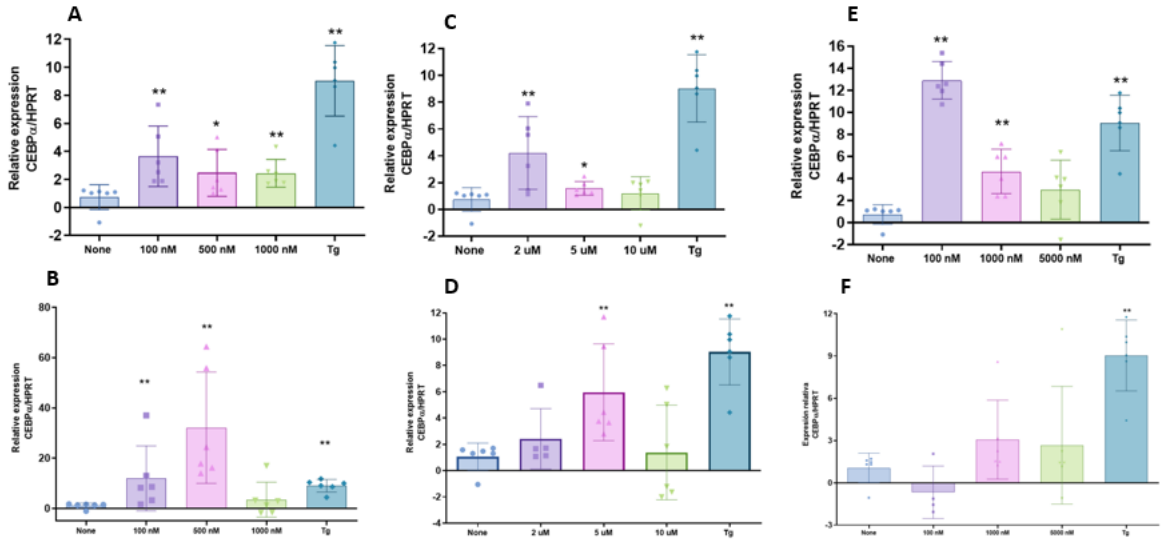


Figure 5. **Mycophenolic acid, indapamide and glibenclamide promote CEBPα expression.** Cells were treated as above and relative CEBPα expression was assessed by RT-qPCR 24 h after stimulation. Expression promoted by mycophenolic acid in T2P and MDMs (Panel A and B, respectively), indapamide in T2P and MDMs (Panel C and D, respectively), and glibenclamide in T2P and MDMs (Panel E and F, respectively). Tg: thapsigargin. Graphs show mean ± SD of at least 3 independent experiments in duplicate. *$p < 0.05$; **$p < 0.01$.

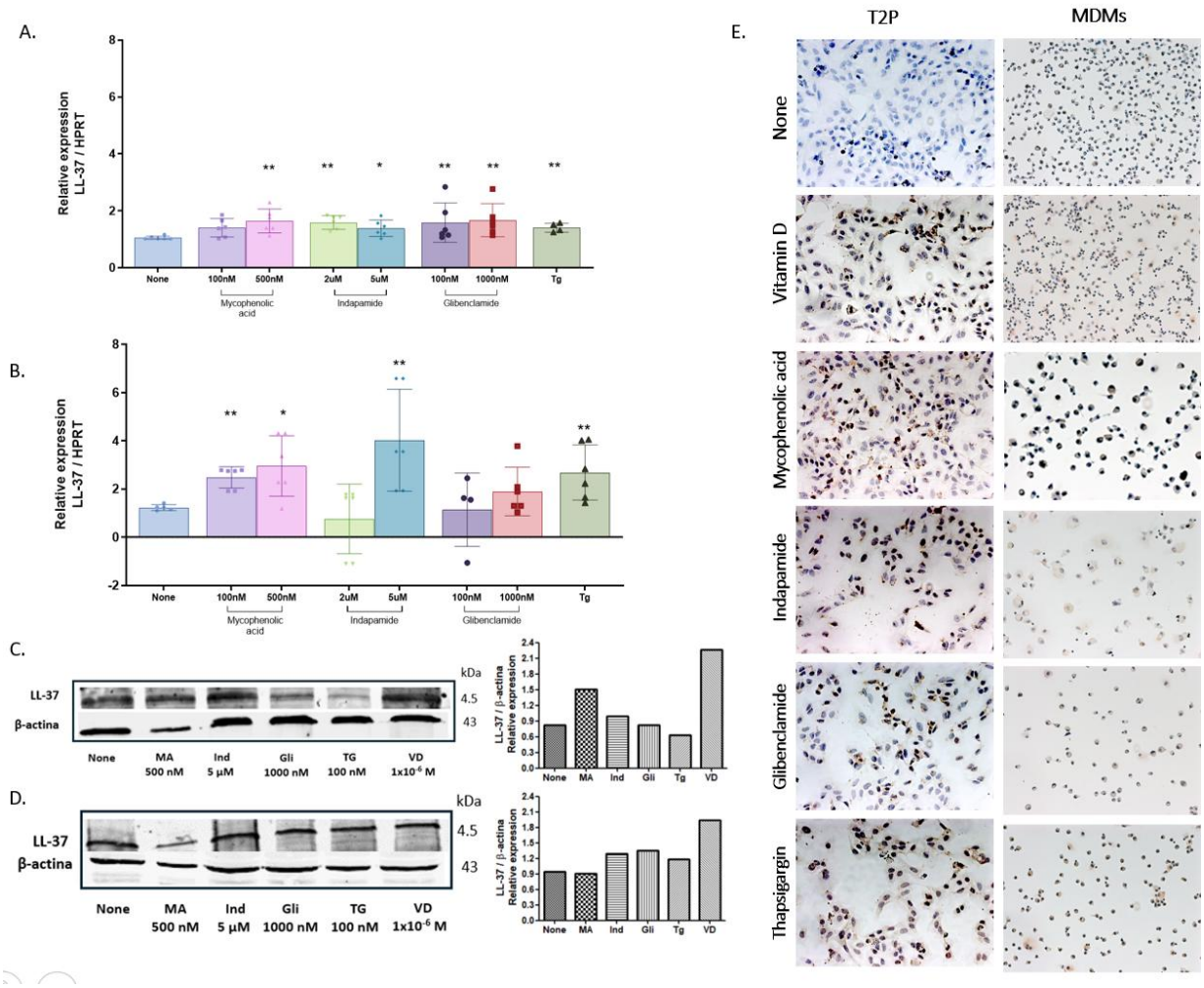


Figure 6. **CEBPα upregulation correlates with LL-37 expression.** Cells were treated as mentioned above and relative LL-37 expression was assessed by RT-qPCR and Western Blot 24 h after stimulation. Expression promoted by mycophenolic acid, indapamide, and glibenclamide in T2P and MDMs by RT-qPCR (Panel A and B, respectively) and Western Blot in T2P and MDMs (Panel C and D, respectively). Protein was assessed by immunocytochemistry assays in T2P and MDMs (Panel E). Tg: thapsigargin. Graphs show mean ± SD of at least 3 independent experiments in duplicate. *p < 0.05; **p < 0.01.

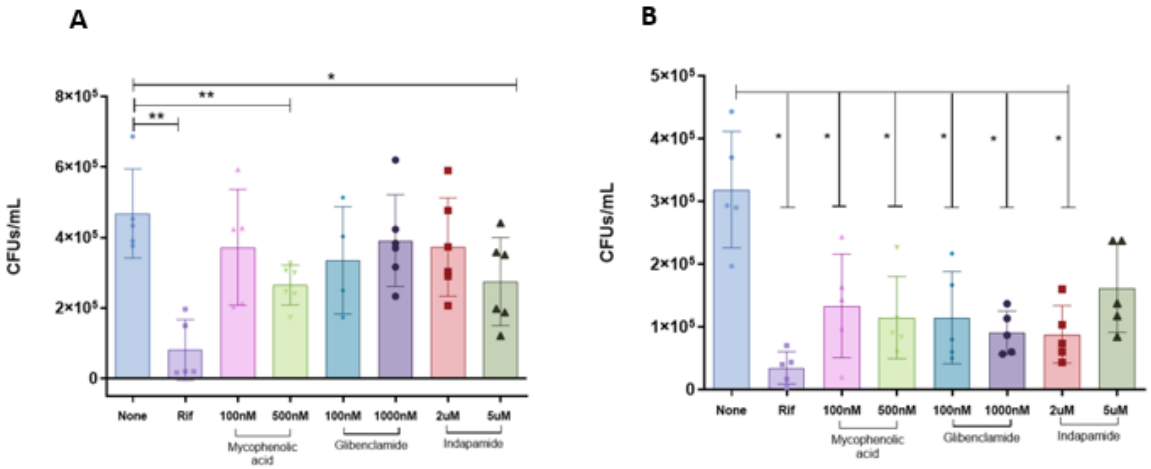


Figure 7. **CEBPα-activating drugs promote Mtb clearance in infected cells.** Cells were treated as indicated above, after treatment cells were lysed and seeded on 7H10 agars 48 h after stimulation, and colony-forming units per milliliter (CFU/mL) were made in T2P and MDM (Panel A and B, respectively). CFU/mL were counted 21 days later. Rif: rifampicin. Graphs show mean ± SD from at least 3 independent experiments by duplicate. *p < 0.05; **p < 0.01.

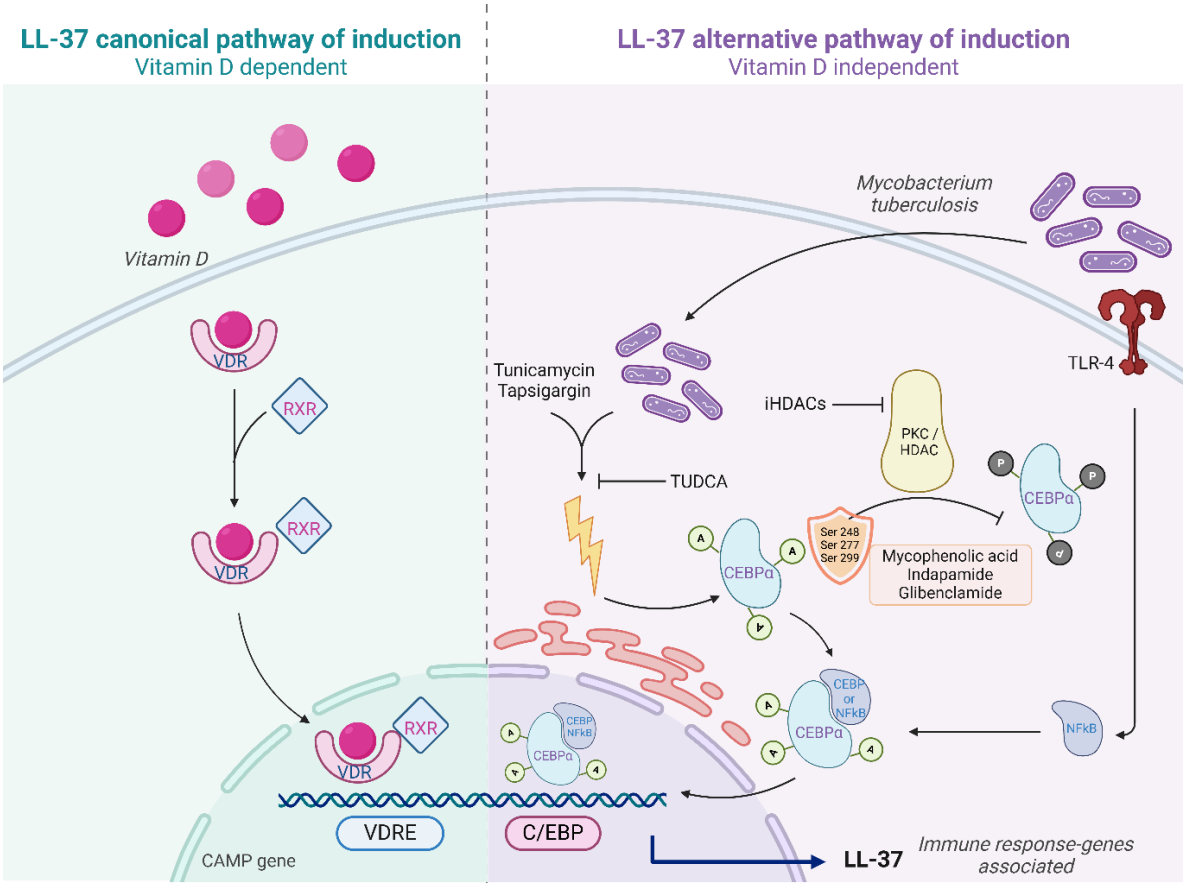


Figure 8. **LL-37 Induction Pathways.** The described canonical pathway depends on vitamin D. The vitamin diffuses through the cell membrane, then binds to its receptor VDR and forms a complex with the RXR receptor. The complex translocates to the nucleus and binds to the VDRE in the promoter region of the CAMP gene that codes for the LL-37 peptide. The alternative pathway is independent of vitamin D, where LL-37 is expressed under endoplasmic reticulum (ER) stress. Stress can be induced pharmacologically using tunicamycin or thapsigargin. In the present study, we demonstrate that infection with Mtb causes ER stress. As a product of stress, one of the factors activated for cell survival is CEBPα, which is normally acetylated. Acetylated CEBPα might bind with other factors of the same family, such as CEBPβ, or with other transcription factors such as NFkB. The formed complexes translocate to the nucleus and bind to CEBP response sites located in the CAMP promoter region, adjacent to

VDRE. HDACs promote CEBPα inhibition, as does PKC by phosphorylating serines Ser 248, Ser 277, and Ser 299. Herein, we described three molecules (mycophenolic acid, indapamide, and glibenclamide) that protect serines from being phosphorylated, thus allowing CEBPα activation thus inducing LL-37 during Mtb infection therefore promoting bacterial clearance. *VDR: Vitamin D receptor. RXR: Retinoid X receptor. VDRE: Vitamin D response elements. TUDCA: tauroursodeoxycholic acid. HDAC: Histone deacetylase. iHDACs: Histone deacetylase inhibitors.*

Figure S1

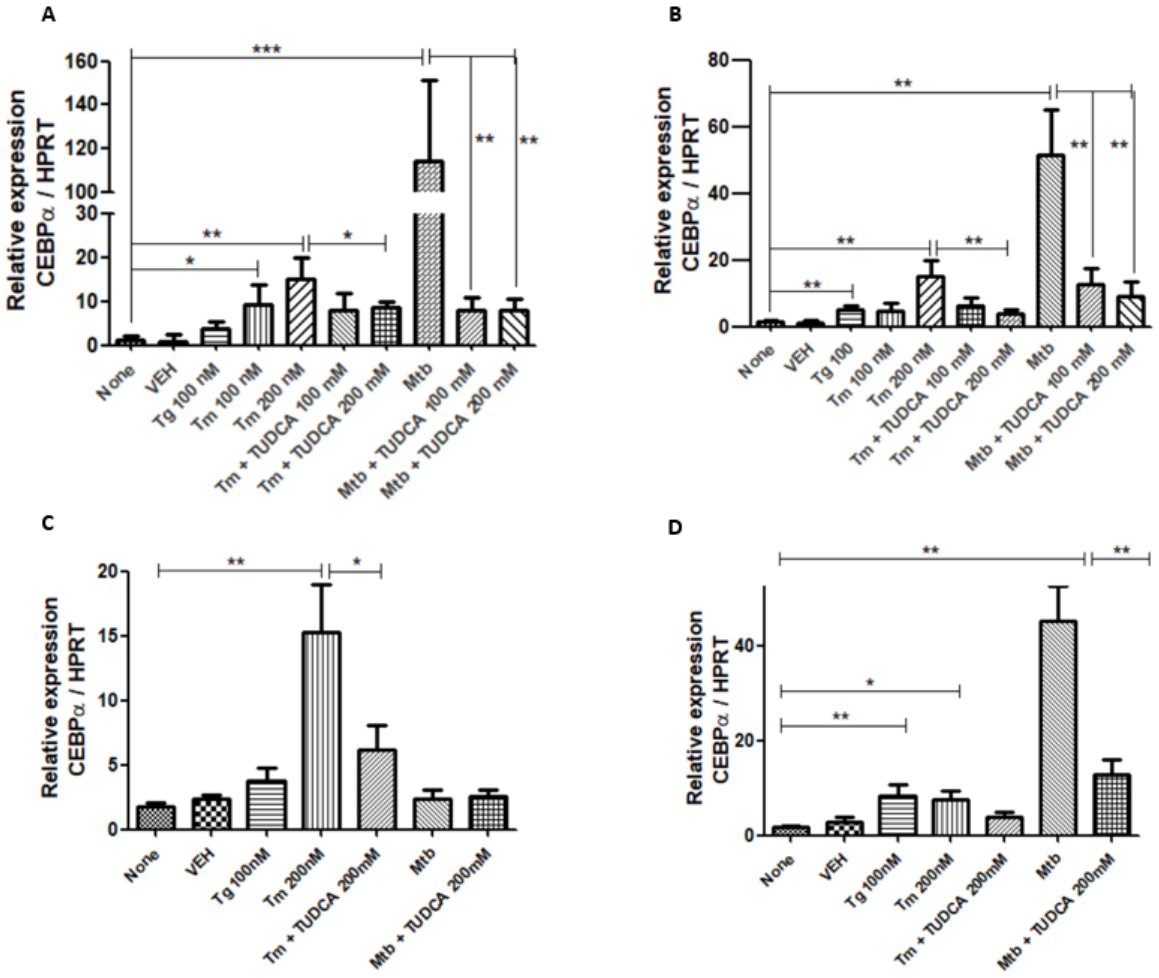
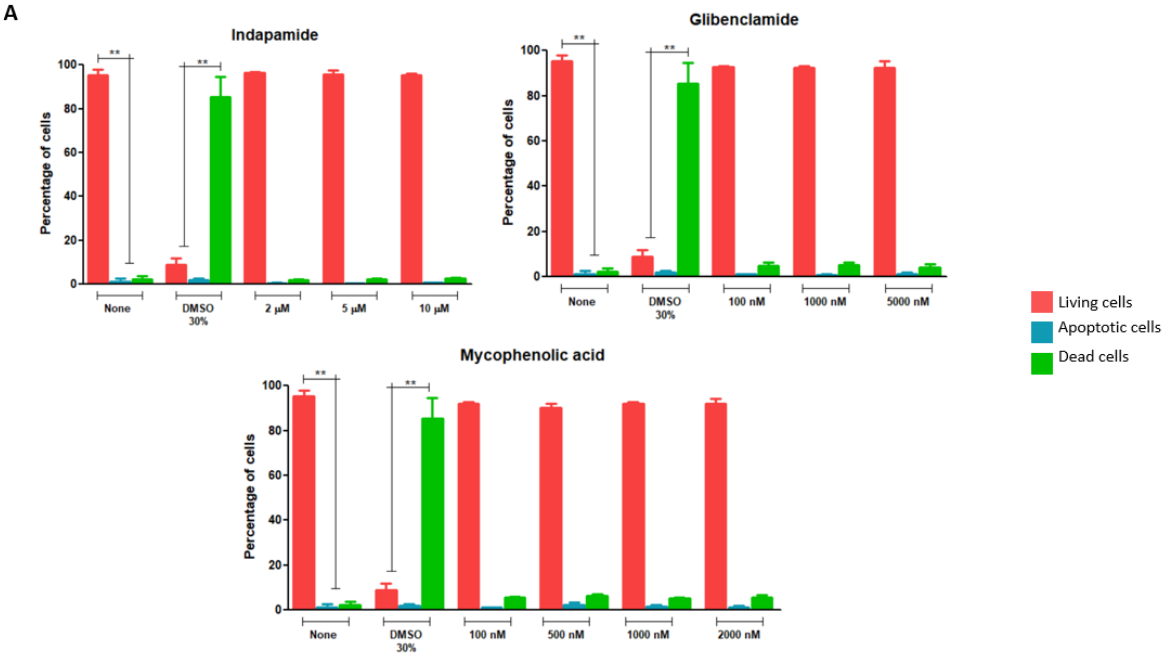


Figure S1. **FBS influences CEBPα expression in Mycobacterium tuberculosis-infected cells.** Infection increases the expression of CEBPα through endoplasmic reticulum (ER) stress. Cells were treated as indicated and CEBPα relative expression was evaluated by RT-qPCR 24 h post-stimulation in T2P with or FBS (Panel A and C, respectively) and MDMs with or FBS (Panel B and D, respectively). To note, FBS contains unknown vitamin D concentrations. VEH: vehicle; Mtb: Mycobacterium tuberculosis; Tm: tunicamycin; Tg: thapsigargin; TUDCA: tauroursodeoxycholic acid. Graphs show mean ± SD from at least 3 independent experiments by duplicate. *p < 0.05; **p < 0.01; ***p<0.001.

Figure S2



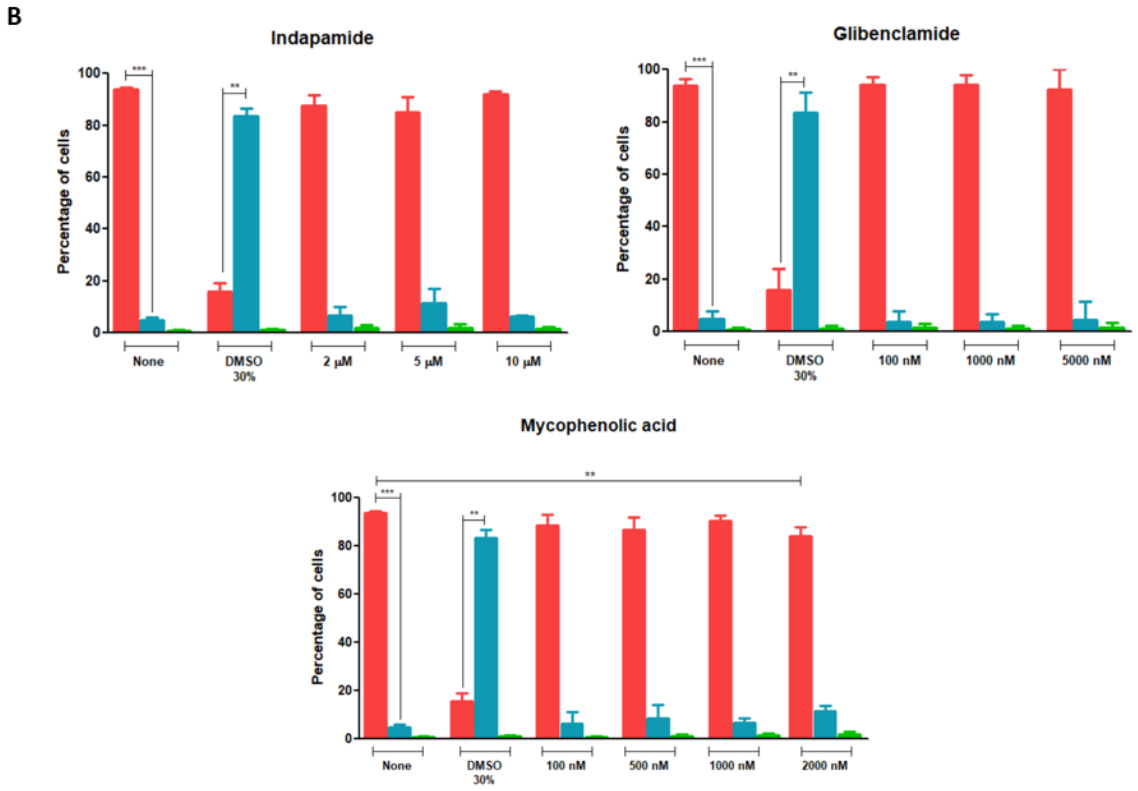


Figure S2. **Determination of safe concentrations for the selected drugs.** Cells were treated as indicated and viability was assessed by flow cytometry 48 h post-stimulation in T2P and MDMs (Panel A and B, respectively). DMSO: dimethyl sulfoxide. Graphs show mean ± SD from at least 3 independent experiments by duplicate. **p < 0.01; ***p<0.001.

CONCLUSIONES

Estos resultados reportan por primera vez que la infección por *Mycobacterium tuberculosis* induce estrés a RE, lo que lleva a la activación del factor de transcripción CEBPα. La activación de CEBPα promueve la expresión del AMP LL-37, que a su vez correlaciona con la eliminación de la micobacteria. Encontramos que existen fármacos FDA accesibles que inducen la activación de CEBPα, y con ello la expresión de LL-37. Los fármacos seleccionados, ácido micofenólico, indapamida y glibenclamida, no son citotóxicos para los modelos celulares utilizados en la ventana de concentraciones evaluadas. Reportamos que los fármacos seleccionados inducen la expresión relativa de CEBPα (ácido micofenólico 100 y 500 nm, glibenclamida 100 y 1000 nm e indapamida 2 y 5 μM) y esto correlaciona también con un incremento en la expresión de LL-37. Finalmente, el tratamiento de las moléculas seleccionadas en células infectadas con Mtb promueven la eliminación del bacilo. Por lo tanto, estos resultados nos llevan a sugerir a CEBPα como un potencial blanco terapéutico para el tratamiento de la TB.

BIBLIOGRAFÍA

Aziz, S., & Rajbhandari, S. (2023). Not So Innocent Indapamide. *Journal of Ayub Medical College Abbottabad*. *35* (2), 329-331. https://doi.org/10.55519/jamc-02-10426

Barnabei, L., Laplantine, E., Mbongo, W., Rieux-Laucat, F., & Weil, R. (2021). NF-κB: At the Borders of Autoimmunity and Inflammation. *Frontiers in immunology*. *12*: 716469. https://doi.org/10.3389/fimmu.2021.716469

Berman, J. D., & Webster, H. K. (1982). In vitro effects of mycophenolic acid and allopurinol against Leishmania tropica in human macrophages. *Antimicrobial Agents and Chemotherapy*. *21* (6), 887-891. https://doi.org/10.1128/aac.21.6.887

Bettigole, S. E., & Glimcher, L. H. (2015). Endoplasmic reticulum stress in immunity. *Annual Review of Immunology*. *33:* 107-138. https://doi.org/10.1146/annurev-immunol-032414-112116

Castañeda-Delgado, J., Hernández-Pando, R., Serrano, C. J., Aguilar-Leon, D., Leon-Contreras, J., Rivas-Santiago, C.,…Rivas-Santiago, B. (2010). Kinetics and cellular sources of cathelicidin during the course of experimental latent tuberculous infection and progressive pulmonary tuberculosis. *Clinical and Experimental Immunology*. 161 (3), 542-550. https://doi.org/10.1111/j.1365-2249.2010.04199.x

Deshpande, D., Grieshober, M., Wondany, F., Gerbl, F., Noschka, R., Michaelis, J., & Stenger, S. (2020). Super-Resolution Microscopy Reveals a Direct Interaction of Intracellular Mycobacterium tuberculosis with the Antimicrobial Peptide LL-37.

International Journal of Molecular Sciences. *21* (18), 6741. https://doi.org/10.3390/ijms21186741

Dhawan, P., Wei, R., Sun, C., Gombart, A. F., Koeffler, H. P., Diamond, G., & Christakos, S. (2015). C/EBPα and the Vitamin D Receptor Cooperate in the Regulation of Cathelicidin in Lung Epithelial Cells. *Journal of Cellular Physiology*. *230* (2), 464-472. https://doi.org/10.1002/jcp.24729

Dhawan, P., Weider, R., & Christakos, S. (2009). CCAAT Enhancer-binding Protein α Is a Molecular Target of 1,25-Dihydroxyvitamin D3 in MCF-7 Breast Cancer Cells. *Journal of Biological Chemistry*. 284 (5), 3086 - 3095. https://doi.org/10.1074/jbc.M803602200

Fonseca, H. A., Fonseca, F. A., Lins, L. C., Monteiro, A. M., Bianco, H. T., Brandão, S. A.,…Izar, M. C. (2015). Antihypertensive therapy increases natural immunity response in hypertensive patients. *Life Sciences*. 143 (15), 124-130. https://doi.org/10.1016/j.lfs.2015.10.030

Hau, C. S., Kanda, N., Noda, S., Tatsuta, A., Kamata, M., Shibata, S.,…Tada, Y. (2013). Visfatin enhances the production of cathelicidin antimicrobial peptide, human β-defensin-2, human β-defensin-3, and S100A7 in human keratinocytes and their orthologs in murine imiquimod-induced psoriatic skin. *The American Journal of Pathology*. 182 (5), 1705-1717. https://doi.org/10.1016/j.ajpath.2013.01.044

Hernández Sánchez, F., Herrera Barrios, M. T., & Torres Rojas, M. (2011). Role of vitamin D in infection with Mycobacterium tuberculosis: Evidence for its protective role. *NCT Neumologia y Cirugia de Torax*. 70 (4):252-260. https://www.medigraphic.com/pdfs/neumo/nt-2011/nt114e.pdf

Hsieh, P. C., Peng, C. K., Liu, G. T., Kuo, C. Y., Tzeng, I. S., Wang, M. C.,…Huang, K. L. (2022). Aqueous Extract of Descuraniae Semen Attenuates Lipopolysaccharide-Induced Inflammation and Apoptosis by Regulating the Proteasomal Degradation and IRE1α-Dependent Unfolded Protein Response in A549 Cells. *Frontiers in immunology*. *13*: 916102. https://doi.org/10.3389/fimmu.2022.916102

Ismailova, A., & White, J. H. (2022). Vitamin D, infections and immunity. *Reviews in Endocrine and Metabolic Disorders*. 23: 265-277. https://doi.org/10.1007/s11154-021-09679-5

Jacobo-Delgado, Y. M., Rodríguez-Carlos, A., Serrano, C. J., & Rivas-Santiago, B. (2023). Mycobacterium tuberculosis cell-wall and antimicrobial peptides: a mission impossible? *Frontiers in immunology*. 14: 1194923. https://doi.org/10.3389/fimmu.2023.1194923

Jacobo-Delgado, Y. M., Torres-Juarez, F., Rodríguez-Carlos, A., Santos-Mena, A., Enciso-Moreno, J. E., Rivas-Santiago, C.,…Rivas-Santiago, B. (2021). Retinoic acid induces antimicrobial peptides and cytokines leading to Mycobacterium tuberculosis elimination in airway epithelial cells. *Peptides. 142*: 170580. https://doi.org/10.1016/j.peptides.2021.170580

Jilani, T., Avula, A., Zafar Gondal, A., & Siddiqui, A. (2020). *Active tuberculosis*. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK513246/

Jourdan, J. P., Bureau, R., Rochais, C., & Dallemagne, P. (2020). Drug repositioning: a brief overview. *Journal of Pharmacy and Pharmacology*. *72* (9), 1145-1151. https://doi.org/10.1111/jphp.13273

Katalinic´-Jankovic, V., Furci, L., & Cirillo, D. M. (2012). Microbiology of Mycobacterium tuberculosis and a new diagnostic test for TB. *European Respiratory Monograph.* Monograph chapter 1-13. https://doi.org/10.1183/1025448x.10022311

Koh, G. C., Maude, R. R., Schreiber, M. F., Limmathurotsakul, D., Wiersinga, W. J., Wuthiekanun, V.,…Peacock, S. J. (2011). Glyburide is anti-inflammatory and associated with reduced mortality in melioidosis. *Clinical Infectious Diseases*. *52* (6), 717-725. https://doi.org/10.1093/cid/ciq192

Koh, G. C., Weehuizen, T. A., Breitbach, K., Krause, K., de Jong, H. K., Kager, L. M.,…Wiersinga, W. J. (2013). Glyburide reduces bacterial dissemination in a mouse model of melioidosis. *PLOS Neglected Tropical Diseases*. *7* (10), e2500. https://doi.org/10.1371/journal.pntd.0002500

Lin, Y. W., Liu, P. S., Pook, K. A., & Wei, L. N. (2018). Glyburide and retinoic acid synergize to promote wound healing by anti-inflammation and RIP140 degradation. *Scientific Reports*. *8*: 834. https://doi.org/10.1038/s41598-017-18785-x

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 25 (4), 402-408. https://doi.org/10.1006/meth.2001.1262

Maertzdorf, J., Tönnies, M., Lozza, L., Schommer-Leitner, S., Mollenkopf, H., Bauer, T. T., & Kaufmann, S. H. E. (2018). Mycobacterium tuberculosis Invasion of the Human Lung: First Contact. *Frontiers in immunology*. *9*: 1346. https://doi.org/10.3389/fimmu.2018.01346

Mahoney, C. W., Shuman, J., McKnight, S. L., Chen, H. C., & Huang, K. P. (1992). Phosphorylation of CCAAT-enhancer binding protein by protein kinase C attenuates site-selective DNA binding. *Journal of Biological Chemistry*. 267 (27), 19396-19403. https://pubmed.ncbi.nlm.nih.gov/1527059/

Majewski, K., Agier, J., Kozlowska, E., & Brzezinska-Blaszczyk, E. (2017). Serum level of cathelicidin LL-37 in patients with active tuberculosis and other infectious diseases. *Journal of Biological Regulators and Homeostatic Agents*. 31 (3): 731-736. https://pubmed.ncbi.nlm.nih.gov/28956425/

Majewski, K., Agier, J., Kozlowska, E., & Brzezinska-Blaszczyk, E. (2018). Status of cathelicidin IL-37, cytokine TNF, and vitamin D in patients with pulmonary tuberculosis. *Journal of Biological Regulators and Homeostatic Agents*. 32 (2), 321-325. https://pubmed.ncbi.nlm.nih.gov/29685013/

Michea, M. A., Briceño, C., Alcota, M., & González, F. E. (2016). Antimicrobial peptides and lipid mediators: Their role in periodontal diseases. *Revista Clínica de Periodoncia, Implantología y Rehabilitación Oral.* 9 (3), 231-237 https://doi.org/10.1016/j.piro.2016.03.003

Molina-Rueda, M. J., Martin-Vivaldi, J. A., & Molina-Rueda F. (2012). Treatment of tuberculosis: Which patients, and why do they abandon it? *Atención Primaria.* 44 (11), 635-688. https://doi.org/10.1016/j.aprim.2012.04.006

NCBI. (2021). *CEBPA CCAAT enhancer binding protein alpha [Homo sapiens (human)]*. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=1050

Nerlov, C. (2007). The C/EBP family of transcription factors: a paradigm for interaction between gene expression and proliferation control. *Trends in Cell Biology*. 17 (7), 318-324. https://doi.org/10.1016/j.tcb.2007.07.004

Nnoaham, K., & Clarke, A. (2008). Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysi. *International Journal of Epidemiology*. 37 (1), 113-119. https://doi.org/10.1093/ije/dym247

Park, K., Elias, P. M., Oda, Y., Mackenzie, D., Mauro, T., Holleran, W. M., & Uchida, Y. (2011). Regulation of cathelicidin antimicrobial peptide expression by an endoplasmic reticulum (ER) stress signaling, vitamin D receptor-independent pathway. *Journal of Biological Chemistry*. 286 (39), 34121-34130. https://doi.org/10.1074/jbc.M111.250431

Peñuelas-Urquides, K., Villarreal-Treviño, L., Silva-Ramírez, B., Rivadeneyra-Espinoza, L., Said-Fernández, S., & de León, M. B. (2013). Measuring of Mycobacterium tuberculosis growth. A correlation of the optical measurements with colony forming units. *Brazilian Journal of Microbiology*. 44 (1), 287-289. https://doi.org/10.1590/s1517-83822013000100042

Rahman, S., Rehn, A., Rahman, J., Andersson, J., Svensson, M., & Brighenti, S. (2015). Pulmonary tuberculosis patients with a vitamin D deficiency demonstrate low local expression of the antimicrobial peptide LL-37 but enhanced FoxP3+ regulatory T cells and IgG-secreting cells. *Clinical Immunology*. 156 (2), 85-97. https://doi.org/10.1016/j.clim.2014.12.003

Ramji, D. P., & Foka, P. (2002). CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochemical Journal.* 365 (3), 561-575. https://doi.org/10.1042/bj20020508

Rivas-Santiago, B., Hernández-Pando, R., Carranza, C., Juarez, E., Contreras, J. L., Aguilar-Leon, D.,…Sada, E. (2008). Expression of cathelicidin LL-37 during Mycobacterium tuberculosis infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. *Infection and Immunity*. 76 (3), 935 - 941. https://doi.org/10.1128/IAI.01218-07

Rivas-Santiago, B., Sada, E., Hernández-Pando, R., & Tsutsumi, V. (2006). Antimicrobial peptides in the innate immunity of infectious diseases. *Salud Pública de México*. 48 (1), 62-71. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0036-36342006000100010&lng=es&tlng=.

Rodríguez-Carlos, A., Jacobo-Delgado, Y. M., Santos-Mena, A. O., & Rivas-Santiago, B. (2021). Modulation of cathelicidin and defensins by histone deacetylase inhibitors: A potential treatment for multi-drug resistant infectious diseases. *Peptides*. *140*: 170527. https://doi.org/10.1016/j.peptides.2021.170527

Rub, A., Shaker, K., Kashif, M., Arish, M., Dukhyil, A. A. B., Alshehri, B. M.,…Amir, K. (2019). Repurposing Glyburide as Antileishmanial Agent to Fight Against Leishmaniasis. *Protein & Peptide Letters*. 26 (5), 371-376. https://doi.org/10.2174/0929866526666190301114012

Schmitz, M. L., Shaban, M. S., Albert, B. V., Gökçen, A., & Kracht, M. (2018). The Crosstalk of Endoplasmic Reticulum (ER) Stress Pathways with NF-κB: Complex Mechanisms Relevant for Cancer, Inflammation and Infection. *Biomedicines*. *6* (2), 58. https://doi.org/10.3390/biomedicines6020058

Seuter, S., Neme, A., & Carlberg, C. (2016). Epigenome-wide effects of vitamin D and their impact on the transcriptome of human monocytes involve CTCF. *Nucleic Acids Research*. *44* (9), 4090-4104. https://doi.org/10.1093/nar/gkv1519

Siebert, A., Wysocka, M., Krawczyk, B., Cholewiński, G., & Rachoń, J. (2018). Synthesis and antimicrobial activity of amino acid and peptide derivatives of mycophenolic acid. *European Journal of Medicinal Chemistry*. 143 (1), 646-655. https://doi.org/10.1016/j.ejmech.2017.11.094

Silverman-Kitchin, J. E., Pomeranz, M. K., Pak, G., Washenik, K., & Shupack, J. L. (1997). Rediscovering mycophenolic acid: A review of its mechanism, side effects, and potential uses. *Journal of the American Academy of Dermatology*. 37 (3): 445-449. https://doi.org/10.1016/S0190-9622(18)30747-3

Song, G., Wang, L., Bi, K., & Jiang, G. (2015). Regulation of the C/EBPα signaling pathway in acute myeloid leukemia (Review). *Oncology Reports*. 33 (5), 2099-2106. https://doi.org/10.3892/or.2015.3848

Sorensen, O. E., Follin, P., Johnsen, A. H., Calafat, J., Tjabringa, G. S., Hiemstra, P., & Borregaard, N. (2001). Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood*. 97 (12), 3951–3959. https://doi.org/10.1182/blood.v97.12.3951

Strathie Page, S. J., & Tait, C. P. (2015). Mycophenolic acid in dermatology a century after its discovery. *Australasian Journal of Dermatology.* 56 (1), 77-83. https://doi.org/10.1111/ajd.12259

Talat, N., Perry, S., Parsonnet, J., Dawood, G., & Hussain, R. (2010). Vitamin D Deficiency and Tuberculosis. Progression. *Emerging Infectious Diseases.* 16 (5), 853-855. https://doi.org/10.3201/eid1605.091693

Tellez, G. A., & Castaño, J. C. (2010). Antimicrobial peptides. *Infectio*. 14 (1), 55-67 https://doi.org/10.1016/S0123-9392(10)70093-X

Torres-Juarez, F., Cardenas-Vargas, A., Montoya-Rosales, A., González-Curiel, I., Garcia-Hernandez, M., Enciso-Moreno, J.,…Rivas-Santiago, B. (2015). LL-37 Immunomodulatory Activity during Mycobacterium tuberculosis Infection in Macrophages. *Infection and Immunity*. 83 (12), 4495 - 4503. https://doi.org/10.1128/IAI.00936-15

Trivedi, P., & Chaturvedi, V. (2023). Interactive effect of oral anti-hyperglycaemic or anti-hypertensive drugs on the inhibitory and bactericidal activity of first line anti-TB drugs against M. tuberculosis. *PLoS One*. *18* (11), e0292397. https://doi.org/10.1371/journal.pone.0292397

Vandamme, D., Landuyt, B., Luyten, W., & Schoofs, L. (2012). A comprehensive summary of LL-37, the factotum human cathelicidin peptide. *Cellular Immunology*. 280 (1), 22-35. https://doi.org/10.1016/j.cellimm.2012.11.009

WHO. (2024). *Global tuberculosis report 2024*. https://www.k.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024

Xu, P., Tang, J., & He, Z. G. (2022). Induction of Endoplasmic Reticulum Stress by CdhM Mediates Apoptosis of Macrophage During Mycobacterium tuberculosis Infection. *Frontiers in Cellular and Infection Microbiology*. *12*: 877265. https://doi.org/10.3389/fcimb.2022.877265

Yamamoto, H., Kurebayashi, S., Hirose, T., Kouhara, H., & Kasayama, S. (2002). Reduced IRS-2 and GLUT4 expression in PPARgamma2-induced adipocytes derived from C/EBPbeta and C/EBPdelta-deficient mouse embryonic fibroblasts. *Journal of Cell Science*. 115 (18), 3601-3607. https://doi.org/10.1242/jcs.00044

Yamshchikov, A. V., Kurbatova, E. V., Kumari, M., Blumberg, H. M., Ziegler, T. R., Ray, S. M., & Tangpricha, V. (2010). Vitamin D status and antimicrobial peptide cathelicidin (LL-37) concentrations in patients with active pulmonary tuberculosis. *The American Journal of Clinical Nutrition*. 92 (3), 603-611 https://doi.org/10.3945/ajcn.2010.29411

Zuluaga Espinosa, N. A., Alfaro Velásquez, J. M., Balthazar González, V., Jiménez Blanco, K. E., & Campuzano Maya, G. (2011). Vitamin D: new paradigms. *Medicina y Laboratorio*. 17: 211-246. https://www.medigraphic.com/pdfs/medlab/myl-2011/myl115-6b.pdf

Zuo, Y., Qiang, L., & Farmer, S. R. (2006). Activation of CCAAT/Enhancer-binding Protein (C/EBP) α Expression by C/EBPβ during Adipogenesis Requires a Peroxisome Proliferator-activated Receptor-γ-associated Repression of HDAC1 at the C/ebpα Gene Promoter. *Journal of Biological Chemistry*. 281 (12), 7960-7967. https://doi.org/https://doi.org/10.1074/jbc.M510682200

ANEXOS

Artículos publicados

Artículos de revisión
Vol. 61
Núm. 5

Uso potencial de liposomas en el tratamiento contra la tuberculosis

Potential use of liposomes in tuberculosis treatment

Yolanda Monserrath Jacobo-Delgado[1a], Gabriela Navarro-Tovar[2b], Bruno Rivas-Santiago[1c]

Resumen

La tuberculosis se ubica entre las enfermedades infecciosas con mayor mortalidad y morbilidad a nivel mundial, por detrás de la actual pandemia de COVID-19. Puede afectar a cualquier órgano, aunque la principal forma de infección es respiratoria. La correcta activación de la respuesta inmune logra eliminar o contener a la bacteria en un estado de latencia; sin embargo, la enfermedad activa es progresiva y debe ser tratada bajo estricta supervisión. El tratamiento para la tuberculosis es prolongado y consiste en una combinación de varios antifímicos; por lo tanto, se asocia a la aparición de una gran diversidad de efectos adversos. Estos efectos son la principal causa de abandono terapéutico, que a su vez facilita la aparición de cepas farmacorresistentes. De ahí la importancia de desarrollar nuevas estrategias terapéuticas con el objetivo de disminuir la dosis del fármaco o bien su tiempo de administración. Para lograr estos objetivos se ha propuesto el uso de nanovehículos, que son sistemas de liberación de fármacos controlados y dirigidos. Específicamente, los liposomas son formulaciones que presentan ventajas al ser administrados por vía respiratoria, ya que esta facilita el alcance a la mucosa respiratoria y a los pulmones, que es el principal órgano afectado en la infección por tuberculosis. En la presente revisión se analiza el uso de nanovehículos como sistemas efectivos de entrega de fármacos, así como las formulaciones que se encuentran en estudio. También se proponen perspectivas para la aplicación de la nanotecnología en el desarrollo de nuevos tratamientos farmacológicos para la tuberculosis.

Abstract

Tuberculosis is among the infectious diseases with the highest mortality and morbidity worldwide, behind the COVID-19 pandemic. It can affect any organ, although the respiratory infection is the most common. The correct activation of the immune response eliminates or contain the bacteria; however, the active disease is progressive and must be treated under strict supervision. Treatment for tuberculosis is prolonged and consists of a combination of several antibiotics associated with a wide variety of adverse effects. These effects are the main cause of therapeutic abandonment, which facilitates the appearance of drug-resistant strains. Hence the importance of developing new therapeutic strategies to reduce the dose of the drug or its administration time. To achieve these objectives, the use of nano-vehicles, which are controlled and directed drug release systems, has been proposed. Specifically, liposomes are formulations that have advantages when administered by the respiratory route since they facilitate the reach of the respiratory mucosa and the lungs, which are the main organs affected by tuberculosis. This review analyzes the use of nano-vehicles as effective drug delivery systems and the formulations under study. Perspectives for the application of nanotechnology in the development of new pharmacological treatments for tuberculosis are also proposed.

[1]Instituto Mexicano del Seguro Social, Hospital General de Zona No. 1 "Dr. Emilio Varela Lujan", Unidad de Investigación Biomédica de Zacatecas. Zacatecas, Zacatecas, México
[2]Universidad Autónoma de San Luís Potosí, Facultad de Ciencias Químicas, Posgrado en Ciencias Farmacobiológicas. San Luis Potosí, San Luis Potosí, México

ORCID: 0000-0003-2174-6277[a], 0000-0003-3789-2324[b], 0000-0002-1521-1519[c]

Palabras clave
Tuberculosis
Liposomas
Farmacorresistencia Microbiana

Keywords
Tuberculosis
Liposomes
Drug Resistance, Microbial

Fecha de recibido: 07/02/2022

Fecha de aceptado: 03/04/2023

Comunicación con:
Yolanda Monserrath Jacobo Delgado
yolandajacobo17@gmail.com
492 201 8721

Cómo citar este artículo: Jacobo-Delgado YM, Navarro-Tovar G, Rivas-Santiago B. Uso potencial de liposomas en el tratamiento contra la tuberculosis. Rev Med Inst Mex Seguro Soc. 2023;61(5):661-9. doi: 10.5281/zenodo.8316467

Microbial Pathogenesis 205 (2025) 107586

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

A new target for drug repositioning: CEBPα elicits LL-37 expression in a vitamin D-independent manner promoting Mtb clearance

Yolanda M. Jacobo-Delgado [a,b], Adrián Rodríguez-Carlos [a], Alan Santos-Mena [a], Óscar E. González-Muñiz [a,b], Camelia Félix-Arellano [a], Gabriela Navarro-Tovar [b], Bruno Rivas-Santiago [a,*]

[a] Zacatecas Biomedical Research Unit, Mexican Social Security Institute. Interior de la Alameda #45 Colonia Centro 98000, Zacatecas, Mexico
[b] Faculty of Chemical Sciences, Autonomous University of San Luis Potosí. Av. Manuel Nava #6, Zona Universitaria, 78290, San Luis Potosí, . Mexico

ARTICLE INFO

Keywords:
Tuberculosis
Innate immunity
Antimicrobial peptides
CEBPα
Drug repositioning

ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and is a growing public health problem worldwide. Within the innate immune response, we highlight the secretion of the antimicrobial peptide LL-37, which is crucial for Mtb elimination in infected cells. Previous reports have shown that CEBPα activation induces LL-37 independently of its main inducer, vitamin D, under endoplasmic reticulum (ER) stress. In this study, we report that infection with Mtb causes ER stress in pulmonary epithelial cells and macrophages. The stress induces the activation of CEBPα, which in turn promotes the LL-37 expression. Furthermore, the participation of CEBPα is necessary for the correct clearance of Mtb in an in vitro infection model. We identify candidate drugs (mycophenolic acid, indapamide, and glibenclamide) capable of activating CEBPα and promoting LL-37 through in silico assays. The effect of the drugs was corroborated by gene and protein expression analysis. Finally, we observed that treatment with these drugs improves bacterial clearance in infected cells. Our results lead us to suggest CEBPα as a potential therapeutic target as an adjuvant in the standard treatment of tuberculosis, seeking a reduction in treatment time, and thus a lower appearance of drug resistance.

1. Introduction

Tuberculosis [TB], a disease caused by the bacillus *Mycobacterium tuberculosis* (Mtb), remains one of the most lethal infectious diseases globally, as reported by the World Health Organization (WHO). The 2022 WHO Global TB Report highlights a concerning rise in drug-resistant strains, underscoring the urgent need for novel therapeutic strategies [1]. TB is primarily transmitted via inhalation of aerosols containing Mtb bacilli, which are expelled by individuals with active disease through talking, coughing, or sneezing. Upon entry into the respiratory tract, the host's innate immune system activates a series of protective responses. These immune defenses are highly effective, with approximately 70 % of exposed individuals successfully clearing the bacteria. Among the 30 % who become infected, only 10 % progress to active disease (Jilani, A et al. Updated 2023 Jan 6). Despite this, TB continues to cause approximately 10 million new cases annually [1]. Given the remarkable efficiency of the innate immune system, enhancing these natural defense mechanisms represents a promising avenue for therapeutic intervention.

Upon entering the respiratory tract, Mtb initially encounters epithelial cells and alveolar macrophages, the latter serving as the primary host for the bacillus. Once recognizing Mtb, both cell types release soluble mediators of the innate immune response, including antimicrobial peptides (AMPs) ([2]et al., 2018). AMPs are potent bactericidal molecules that create a protective soluble barrier across various epithelial surfaces, including the respiratory tract. These peptides are characterized by short sequences of positively charged amino acids, and their antimicrobial action is primarily driven by electrostatic interactions between the peptide and the negatively charged components of the pathogen's membrane. This interaction allows AMPs to integrate into the pathogen's membrane leading to membrane disruption and subsequent lysis [3].

* Corresponding author. Zacatecas Biomedical Research Unit, Mexican Social Security Institute, Zacatecas, Mexico.
E-mail addresses: yolandajacobo1996@gmail.com (Y.M. Jacobo-Delgado), rdz.carlos09@hotmail.com (A. Rodríguez-Carlos), alan.santito@hotmail.com (A. Santos-Mena), oscar_glzm@outlook.es (Ó.E. González-Muñiz), cameflx16@gmail.com (C. Félix-Arellano), gnavarrotovar@gmail.com (G. Navarro-Tovar), rondovm@gmail.com, bruno.rivas@imss.gob.mx (B. Rivas-Santiago).

https://doi.org/10.1016/j.micpath.2025.107586
Received 2 December 2024; Received in revised form 27 February 2025; Accepted 12 April 2025
Available online 18 April 2025