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REVIEW ARTICLE

Role of calcium channels in cellular antituberculosis effects: Potential of voltage-gated calcium-channel blockers in tuberculosis therapy

Lele Song [a,b], Ruina Cui [a], Yourong Yang [a], Xueqiong Wu [a,*]

[a] *Army Tuberculosis Prevention and Control Key Laboratory, Institute of Tuberculosis Research, The 309th Hospital of Chinese PLA, Beijing 100091, China*
[b] *BioChain (Beijing) Science and Technology, Inc., Beijing 100176, China*

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The immunity of human immune cells and their ability to inhibit *Mycobacterium tuberculosis* (MTB) are key factors in the anti-MTB effect. However, MTB modulates the levels and activity of key intracellular second messengers, such as calcium, to evade protective immune responses. Recent studies suggest that inhibiting L-type calcium channel in immune cells using either antibodies or small interfering RNA increases calcium influx, upregulates the expression of proinflammation genes, and reduces MTB burden. First, we will review the key factors in calcium-signaling pathway that may affect the immunity of immune cells to MTB infection. Second, we will focus on the role of calcium channels in regulating cellular immunity to MTB. Finally, we will discuss the possibility of using calcium-channel blockers as anti-MTB chemotherapy drugs to enhance chemotherapy effects, shorten treatment period, and overcome drug resistance.

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB). Although MTB may invade various organs, it mainly affects the lungs, causing pulmonary TB. In 2012, more than 20 million people worldwide were infected with MTB, including 8.6 million

* Corresponding author. Army Tuberculosis Prevention and Control Key Laboratory, Institute of Tuberculosis Research, The 309th Hospital of Chinese PLA, No. 17, Heishanhu Road, Haidian District, Beijing 100091, China.
E-mail address: wu-xueqiong@263.net (X. Wu).

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new cases and 1.3 million deaths.[1] The major challenges in the prevention and treatment of TB are the large amount of floating population and hidden infections, the slow progress in treating multidrug-resistant TB (MDR-TB), and the co-infected immune-compromised population.[2] At present, chemotherapy alone is not enough to cope with these challenges. Therefore, there is a necessity to develop new treatment methods, including the use of an integrated approach to develop new drugs, immunotherapy, and gene therapy.

It was found recently that during MTB infection, increase in calcium influx or release of calcium from intracellular calcium pool activates the intracellular calcium-signaling pathway, thereby activating the gene expression of anti-infection and immune-protective proteins in immune cells, especially macrophages.[3,4] This increase in calcium signaling enhances the phagocytic activity and the anti-MTB ability of immune cells, ultimately enhancing the anti-MTB ability of the whole immune system. As the key player in maintaining the intracellular calcium level, calcium channels have crucial roles in regulating the calcium-signaling pathway. Blocking of L-type calcium channel with verapamil, which is an L-type calcium channel-specific blocker, enhances the intracellular calcium level and triggers the downstream calcium-signaling pathway, ultimately activating the anti-infection gene expression.[3] We herein review the findings on immune cell calcium signaling during MTB infection, discuss the mechanism behind the anti-MTB effect of verapamil, assess the possibility of using verapamil as a candidate in combined chemotherapy, and propose future research directions.

Intracellular calcium-signaling pathway and its physiological significance

As a key intracellular second messenger, calcium has crucial physiological roles in muscle contraction, synaptic transmission and plasticity, cell motility, fertilization, cell growth, cell proliferation, and gene expression. It is also involved in regulating the enzyme activity, the ion-channel permeability, and cytoskeleton components.[5] The resting intracellular calcium concentration is usually maintained at 10—100 nmol/L. To maintain such low intracellular calcium levels, calcium is actively transported out of the cell or into the endoplasmic reticulum (ER), sarcoplasmic reticulum, and mitochondria. When calcium signaling is activated, calcium enters into the cell through cell-surface calcium channels and activates the ER calcium channels, which release more calcium from the intracellular calcium store. The specific signal may trigger the sudden increase of intracellular calcium levels to 500—1000 nmol/L.[6] The most common calcium-signaling pathway is the phospholipase C (PLC) pathway. Many cell-surface receptors, including G protein-coupled receptors and receptor tyrosine kinases, can activate the PLC pathway. The PLC hydrolyzes membrane phospholipids (phosphatidylinositol 4,5-bisphosphate) to generate inositol trisphosphate (IP_3) and diacylglycerol (DAG), which are two classic second messengers.[6] DAG activates protein kinase C (PKC), whereas IP_3 spreads to the ER and binds to the IP_3 receptor (IP_3R). The IP_3R is an ER calcium channel that is responsible for calcium release from the ER. Gene expression can then be activated by calcium mainly through the following: the ternary complex factor pathway, the serum response factor pathway, and the cyclic adenosine monophosphate (AMP) response element pathway. Gene expression is activated through different calcium-signaling pathways in different cell types.[7]

The possible calcium-signaling pathway in immune cells

The exact calcium-signaling pathway in immune cells is not clear. However, it may regulate cell function in the following manner (Fig. 1): the extracellular calcium enters into the cells through the cell-membrane calcium channel, including selective calcium channels, such as voltage-gated calcium channels (VGCCs), or nonselective calcium channels, such as purine receptor (P2X7), cyclic nucleotide-gated ion channels (CNGs), and canonical transient receptor potential channels (TRPCs). VGCC is activated by changes in membrane potential, P2X7 by adenosine triphosphate (ATP), CNG by cAMP, and TRPC by PLC or DAG. On the one hand, calcium entering into the cell can directly activate calmodulin (CaM) and PKC to produce a series of cascading effects.[8] Calcium activates downstream kinases through the mitogen-activated protein kinase (MAPK) pathway, and MAPK regulates the phosphorylation of several transcription factors, including *C-myc* gene, the MAPK-interacting kinase (Mnk), and cAMP response element binding protein (CREB).[9] MAPK regulates gene transcription by modulating the level and activity of transcription factors, which is very important for cell growth, differentiation, and apoptosis. On the other hand, calcium enters into cells and activates the calcium channels on the ER surface, such as the ryanodine receptor (RyR), to induce more calcium release from the ER (calcium-induced calcium release or CICR).[10] Meanwhile, Ras and Src participate in the synthesis of IP_3 in the presence of PLC, and activate the IP_3R on ER surface to release calcium. IP_3 activates the calcium-signaling pathway mainly through PLC and phosphoinositide 3-kinase, which activate a series of infection and cell differentiation-related transcription factors, such as nuclear factor of activated T cells and CREB.[6]

The role of ion channels in anti-TB therapy

The role of purine receptors in TB pathology and cellular immunity

Understanding the mechanism of TB pathology and cellular immunity from the ion-channel perspective is a new field of study. The current studies focus on the role of purine receptor P2X7 in TB infection and immunity, as well as on the anti-TB therapy targeting P2X7. Human P2X7 is a membrane ligand-gated ion channel widely distributed in immune cells.[11] It has two transmembrane domains and is activated by extracellular ATP.[12,13] In macrophages and myeloid cells, activation of P2X7 receptor by ATP induces K^+ efflux and Ca^{2+} influx, and triggers the processing and secretion of cytokines interleukin-1β (IL-1β), IL-18, and IL-12.[11] IL-12 synergizes with IL-18 or IL-1β for the production of interferon-γ from