Microbial Pathogenesis 88 (2015) 103–108

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Simultaneous multi-parametric analysis of *Leishmania* and of its hosting mammal cells: A high content imaging-based method enabling sound drug discovery process

Claire-Lise Forestier [a, *], Gerald Frank Späth [b], Eric Prina [b], Sreekanth Dasari [a]

[a] INSERM U1095, URMITE-UMR CNRS 7278, Infectiopole Sud, University of Aix-Marseille, Marseille, France
[b] Institut Pasteur and CNRS URA2581, Unité de Parasitologie moléculaire et Signalisation, Paris, France

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form
18 August 2014
Accepted 21 October 2014
Available online 31 October 2014

Keywords:
Leishmania
Drug screening
Microscopy imaging

ABSTRACT

Leishmaniasis is a vector-borne disease for which only limited therapeutic options are available. The disease is ranked among the six most important tropical infectious diseases and represents the second-largest parasitic killer in the world. The development of new therapies has been hampered by the lack of technologies and methodologies that can be integrated into the complex physiological environment of a cell or organism and adapted to suitable *in vitro* and *in vivo Leishmania* models. Recent advances in microscopy imaging offer the possibility to assess the efficacy of potential drug candidates against *Leishmania* within host cells. This technology allows the simultaneous visualization of relevant phenotypes in parasite and host cells and the quantification of a variety of cellular events. In this review, we present the powerful cellular imaging methodologies that have been developed for drug screening in a biologically relevant context, addressing both high-content and high-throughput needs. Furthermore, we discuss the potential of intra-vital microscopy imaging in the context of the anti-leishmanial drug discovery process.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The protozoan parasite of the genus *Leishmania* is transmitted by blood-feeding female phlebotomine sandflies to a range of mammals, including humans, rodents and canids, causing several pathologies that are collectively known as leishmaniases. Human leishmaniases are present in over 98 countries, primarily in tropical and subtropical areas, and cause severe morbidity and mortality. More than 12 million people are currently affected by these diseases worldwide; 2 million new clinical cases are reported every year, and 350 million people are estimated to be at risk [1,2].

The *Leishmania* parasite is a digenetic organism that alternates between two distinct developmental stages during its life cycle: extracellular flagellated promastigotes and obligate intracellular non-motile amastigotes. Procyclic promastigote parasites reside in the midgut of the infected sandfly and mature into the highly infective, cell cycle-arrested metacyclic form during migration to the insect mouthpart [3,4]. During a blood meal, metacyclic promastigote parasites are inoculated into the dermis of the mammalian host, where they infect professional phagocytic cells, such as macrophages and dendritic cells. After uptake, metacyclic promastigotes differentiate into amastigotes within the host cell parasitophorous vacuole (PV); this form is the obligate intracellular stage of the *Leishmania* parasite [5]. It is believed that the rapid multiplication of amastigotes within the PV eventually leads to the rupture of the infected host cell. Thus, amastigotes are released into the environment and can re-invade surrounding host cells, causing the diverse pathologies associated with this disease [6].

Neither prophylactic nor therapeutic vaccines are currently available for the prevention of *Leishmania* infection and thus chemotherapy remains the most important element in the control of leishmaniasis [7–10]. However, all current treatments have several significant drawbacks, including high costs, serious toxic side effects, length of treatment, parenteral route of drug administration. Their efficacy is further limited by the emergence of resistant *Leishmania* parasites. These disadvantages limit the utilization of these treatments in endemic areas. The lack of vaccines and safe, affordable and efficient therapies for the prevention and/or treatment of leishmaniases and the emergence of drug-resistant parasites are serious impediments to the control of these diseases [11]. In addition, the AIDS epidemic, difficulties in the control of vectors, increase in international travel and population

* Corresponding author.
E-mail address: claire-lise.forestier@inserm.fr (C.-L. Forestier).

http://dx.doi.org/10.1016/j.micpath.2014.10.012
0882-4010/© 2014 Elsevier Ltd. All rights reserved.

displacement as a result of international conflicts led to a global rise in leishmaniasis cases [12]. Consequently, improved medical interventions and new therapies for patients suffering from leishmaniasis are urgently needed [12].

In this review we discuss how imaging-based methodologies are currently revolutionizing the field of anti-leishmanial drug discovery. These detection technologies have the capacity to provide multiple types of information and measurements of drug activity/toxicity against both *Leishmania* parasites and the mammalian cells that host these important pathogens. By allowing high-content analysis of *Leishmania*–host cell interactions, emerging microscopy imaging systems represent powerful and promising tools in the field of parasite pharmacology that will maximize our knowledge of screened compounds and significantly accelerate the drug discovery process that is urgently needed for leishmaniases.

2. *Leishmania*–host cell interplay in the drug discovery process

Despite the ability of *Leishmania* parasites to infect a variety of host cell types, including monocytes and fibroblasts, these parasites preferentially colonize and develop within typical professional phagocytic cells, such as long-lived macrophages, dendritic cells and short-lived neutrophils [13–17]. The paradox of *Leishmania* infection resides in the fact that while the parasite strictly requires macrophages for its development, these professional phagocytic cells are programmed to rapidly fight infectious agents and play a central role in innate immunity and the initiation of adaptive immunity.

Thus, the success of metacyclic promastigote and amastigote parasites in invading and establishing intracellular residence within their typical host cells depends on the ability of the parasite to escape the antimicrobial function of macrophages. The mechanisms by which *Leishmania* counteracts such a harsh intracellular environment have attracted considerable attention, primarily because such studies can lead to the identification of critical steps that might serve as targets for new anti-leishmanial drugs and pharmacological interventions [18]. The dissection of the mechanisms that are used by *Leishmania* to manipulate macrophages can serve as a starting point for the design of new drugs or therapeutic strategies against the disease. This rationale constitutes the basic principle of the target-based drug discovery approach, which is a methodology that has dominated drug discovery for the past 10–15 years [19,20]. This approach is based on the assumption that the disruption of a single gene or molecular mechanism is a key event in pathogen development and disease ontogeny. This strategy requires substantial work to first identify and subsequently validate a target that is involved in the disease process prior to the initiation of screening for compounds that may block this specific target [19]. While the target-based approach appears to be coherent, the results generated using this approach have been primarily unsuccessful [21].

The emerging idea in the field of parasitic and infectious diseases is that the target-based approach might be more relevant if the causative agent is targeted as a whole and monitored in assays using pertinent *in vitro* and/or *in vivo* biological infectious models. These phenotype-based assays rely on the selection of a specific phenotype, such as the absence or reduction of parasites within host cells, organs or tissues, with the goal of screening compounds that will interfere with *Leishmania* growth and survival within host cells either directly or indirectly by modifying macrophage functions that are required for intracellular parasite multiplication. In this context, the cellular microscopic imaging-based approach constitutes a promising tool that would allow the visualization of host cell infection by *Leishmania* and enable the integration of biological complexity into drug screens.

3. Classical methods for anti-leishmanial drug screening

Primary drug screening procedures against the *Leishmania* parasite were classically performed *in vitro* using promastigotes and/or amastigotes under axenic culture conditions. Parasite sensitivity to drug treatment can be determined using simple methods that evaluate parasite number via cell counting [22] or measurements of the activity of the ornithine decarboxylase enzyme [23]. Colorimetric or fluorometric assays are also commonly used for the quantification of parasite growth, including assays based on the mitochondrial reduction of Alamar Blue and redox indicator resazurin [24–27] or the tetrazolium salt MTT, XTT and MTS [28–30], assays based on the measurement of ATP production by viable parasites using an ATP-bioluminescence system [31] and assays that employ transgenic parasites expressing different reporter genes, such as the fluorescent protein GFP, chloramphenicol acetyl transferase (CAT), β-galactosidase, alkaline phosphatase, β-lactamase and firefly luciferase [32–35].

The main advantage of this reporter gene technology over other classical screening methods lies in the capacity of this technology to provide information concerning drug activity against *Leishmania* parasites that establish residence and multiply within target cells. Drug activity against *Leishmania* parasites within macrophages has been evaluated primarily microscopically by determining the percentage of infected cells and the number of amastigotes per cell via the examination of up to 300 macrophages using Giemsa, nucleus-specific dyes, such as Hoechst or DAPI, or fluorescent transgenic parasites [36]. Flow cytometry in combination with the use of fluorescent parasites facilitates the analysis of the impact of a drug on *Leishmania*–host cell interactions by quantifying the infection rate and screening a large number of cells in a short time, with up to five parameters measured simultaneously for each cell [37–43]. In contrast to previous classical methods that were performed using axenic parasites, these two recently developed technologies offer the capacity to provide information on a more complex level, in *in vitro* biological infectious systems that focus on *Leishmania*-infected host cells. However, these methods have several limitations, such as the inability to generate high-content information at high spatial and temporal resolution for both parasite and host cells and the inability to screen a large number of drug candidates.

4. High-content analysis of *Leishmania*–macrophage interactions *in vitro*

Recent advances in microscopy imaging technologies combined with powerful methodologies for image analysis have allowed for i) the visualization of *Leishmania*–host cell interactions with high spatial resolution, ii) the simultaneous assessment of a variety of host cell features in correlation with compound potency/toxicity and iii) the quantification of multiple drug-mediated effects on both intracellular parasites and host cells. Thus, microscopy imaging-based high-content screening assays represent a novel tool in the field of parasite pharmacology. These assays possess unique capacities i) to be applied to relevant *in vitro* models of *Leishmania* infection and ii) to be combined with automated handling of biological materials, image acquisition and data storage and analysis to increase throughput, as required in the drug testing process.

A consideration of the role of host cells in the modulation of a compound's therapeutic efficiency is critical as well as the influence of a drug on the physiology of host cells. Because *Leishmania* is an obligate intracellular parasite, the permeability of the host cell

Download English Version:

https://daneshyari.com/en/article/3416434

Download Persian Version:

https://daneshyari.com/article/3416434

Daneshyari.com