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Review

Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells

Herbert Kolodziej [a,*], Albrecht F. Kiderlen [b]

[a] *Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany*
[b] *Robert Koch-Institut, Department of Infectious Diseases, Nordufer 20, D-13353 Berlin, Germany*

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Abstract

The antileishmanial and immunomodulatory potencies of a total of 67 tannins and structurally related compounds were evaluated in terms of extra- and intra-cellular leishmanicidal effects and macrophage activation for release of nitric oxide (NO), tumour necrosis factor (TNF) and interferon (IFN)-like activities. Their effects on macrophage functions were further assessed by expression analysis (iNOS, IFN-α, IFN-γ, TNF-α, IL-1, IL-10, IL-12, IL-18). With few exceptions, e.g., caffeic acid derivatives, these polyphenols revealed little direct toxicity for extracellular promastigote *Leishmania donovani* or *L. major* strains. In contrast, many polyphenols appreciably reduced the survival of the intracellular, amastigote parasite form in vitro. Upon activation, e.g., by immune response mediators such as IFN-γ, macrophages may transform from permissive host to leishmanicidal effector cells. Our data from functional bioassays suggested that the effects of polyphenols on intracellular *Leishmania* parasites were due to macrophage activation rather than direct antiparasitic activity. Gene expression analyses not only confirmed functional data, they also clearly showed differences in the response of infected macrophages when compared to that of noninfected cells. Conspicuously, infected macrophages showed augmented and prolonged activation of host defense mechanisms, indicating that parasitised macrophages were exquisitely predisposed or "primed" to react to activating molecules such as polyphenols. This promotive effect may be of special benefit, e.g., stimulation of the non-specific immune system selectively at the site of infection and when needed. Although these data provide the basis for an immunological concept of plant polyphenols for their beneficial effects in various infectious conditions, in vivo experiments are essential to prove the therapeutic benefits of polyphenolic immunomodulators.

Keywords: *Leishmania*; Polyphenols; Hydrolysable tannins; Proanthocyanidins; Macrophages; Antileishmanial activity; Immunomodulatory activity; Nitric oxide; Cytokines; Gene expression analysis

Contents

* Corresponding author. Tel.: +49 30 838 53731; fax: +49 30 838 53729.
E-mail address: kolpharm@zedat.fu-berlin.de (H. Kolodziej).

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1. Introduction

The leishmaniases comprise a group of diseases with extensive morbidity and mortality in most developing countries. They are caused by species of the genus *Leishmania* (Sarcomastigophora, Kinetoplastida) and range from self-healing cutaneous leishmaniasis (CL) to progressive mucocutaneous infections (MCL) to fatal disseminating visceral leishmaniasis (VL). While CL poses basically cosmetic problems, and MCL leads to painful disfiguration, social stigmatisation and often severe secondary infections, VL is generally lethal if left untreated. According to the World Health Organization, leishmaniasis currently affect some 12 million people and there are 2 million new cases per year and with growing tendency. Moreover, it is estimated that approximately 350 million people live at risk of infection with *Leishmania* parasites (Ashford et al., 1992). Leishmaniases are prevalent in 88 countries throughout the world in tropical to Mediterranean climate zones, including 22 in the New World and 66 in the Old World; of these, 72 are developing countries (Desjeux, 1996). CL is endemic in Iran, Saudi Arabia, Syria, Afghanistan and in some South American countries. More than 90% of the VL cases worldwide are registered in India, Bangladesh, Indonesia and Sudan. In Mediterranean Europe, poor-health communities and certain risk groups such as intravenous drug abusers sharing needles and immunodeficient persons (e.g., AIDS-patients) are strongly affected. *Leishmania*/HIV co-infections have increased in Mediterranean countries, where up to 70% of potentially fatal VL cases are associated with HIV infection, and up to 9% of AIDS cases suffer from newly acquired or reactivated VL (Alvar et al., 1997).

Protozoa of the genus *Leishmania* are obligate intracellular parasites of mononuclear phagocytes of vertebrate hosts (Alexander and Russell, 1992). The pathogen requires two different hosts to complete its biological cycle: an insect vector (sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World), and a vertebrate host (e.g., humans, rodents, dogs). To survive successfully and multiply within these two disparate biological environments, the parasites must undergo profound biochemical and morphological adaptations (Alexander et al., 1999). Within the insect vector, the parasite exists as extracellular, motile flagellate in the gut. During a blood meal, promastigotes are discharged in the bloodstream and are rapidly phagocytized by Langerhans cells, macrophages, monocytes, or, transiently, by neutrophils. Within these cells they reside in compartments originating from the plasma membrane, the phagosomes, and transform into nonmotile amastigotes. Lysosomes readily fuse with the phagosomes, but *Leishmania* amastigotes not only resist phagolysosomal enzymes, they also thrive and multiply within the acidic, hydrolase-rich parasitophorous vacuole. Massive amastigote multiplication leads to host cell disruption and release of amastigotes to infect newly recruited host cells. Though the parasite is sensitive to humoral defense mechanisms such as antibodies or the complement system, its intracellular habitat offers almost complete protection. Only if the macrophage is activated, the parasites may be killed and then degraded by the host cell. Ingestion of infected peripheral monocytes during the blood meal by a female sandfly completes the biological cycle.

Macrophage activation, i.e., conversion of a host to an effector cell, occurs both during natural (innate) and specific (adaptive) immune reactions to the infection in an immunocompetent host. It is induced by interferon (IFN)-γ, a cytokine that is released mainly by appropriately stimulated natural killer or T cells. Activation of microbicidal mechanisms in macrophages may also be achieved by their exposure to immunomodulating agents. Immunomodulatory activities have been shown for a number of plant extracts and natural products, providing a rational explanation for their medicinal