# Co-infection with HIV

6

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**Abstract**

This chapter describes the epidemiology, current spread, and clinical aspects of HIV/*Leishmania* co-infection and highlights the recently released guidelines of WHO on their management. It discusses the development of resistant *Leishmania* strains for existing anti-*Leishmania* drugs and the complexity of chemotherapy for *Leishmania*/HIV co-infection, which relies on the same drugs that are used in uncomplicated *Leishmania.* Additionally, prospects for future chemotherapeutic alternatives that target *Leishmania* and HIV and tackle both infections simultaneously are summarized.

## 6.1 Introduction

HIV/*Leishmania* co-infection was first reported in 1985, and since then, it has been reported in 35 countries with a prevalence ranging between 1 and 30% of cases of leishmaniasis, depending on the analyzed geographical areas. It is an expanding but significantly underestimated problem, as it mostly affects neglected populations. Two comprehensive reviews on epidemiology, immunology, and clinical features of HIV-*Leishmania* co-infection published with a decade in between permit a comparison of its progression and knowledge thereof [1, 2].

In 2009, the human immunodeficiency virus (HIV) affected 33.3 million people worldwide and caused 1.8 million deaths (see Fig. 6.1). Currently, 22.5 million of infected people live in sub-Saharan Africa which is where 69% of the 2.6 million new HIV infections in 2009 occurred. However, there are clear indications that

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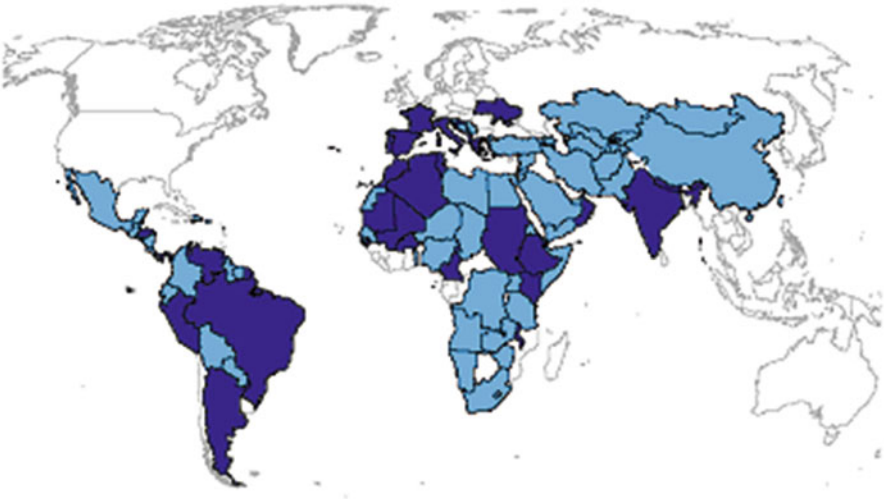

**Fig. 6.1** Countries with endemic leishmaniasis and with *Leishmania*-HIV co-infection. Dark blue: countries reporting HIV/Leishmaniasis co-infection. Light blue: leishmaniasis endemic countries. Source: http://www.who.int/leishmaniasis/burden/hiv_coinfection/burden_hiv_coinfection/en/index.html, accessed at 17/5/2011

suggest that the HIV epidemic in Africa and worldwide is stabilizing with 0.5 million less new infections in 2009 than at the peak of the epidemic 12 years ago. Nevertheless, HIV is concentrating and expanding within urban areas (http://www.unaids.org/documents/20101123_GlobalReport_Chap2_em.pdf).

Leishmaniasis is a hypoendemic disease in Southern Europe with less than 0.3 cases per 100,000 inhabitants. Co-infection was first reported in Spain, with most of the cases among HIV-positive intravenous drug users, some of them as an activation of asymptomatic infection when becoming immunosuppressed and others as a new infection when sharing *Leishmania*-infected needles [3]. After the introduction of antiretroviral therapy (ART) at the end of the 1990s, the number of new co-infected cases declined rapidly in all European countries [2, 4].

Both visceral leishmaniasis (VL) and HIV are highly prevalent in East Africa, but VL is a disease of very isolated, remote areas in Ethiopia, Kenya, Somalia, Uganda, and Sudan where the prevalence of HIV is low. Migration and its consequences of malnutrition and poor housing have been identified as major factors in transmission of leishmaniasis [5]. In contrast with Europe, in Africa the lack of access to ART remains a major challenge, although patient coverage rose from 7% in 2003 to 42% in 2008 and in Eastern and Southern Africa to 48%. The prospects for co-infected patients with no access to ART are grim, as they will relapse after leishmaniasis treatment and eventually become unresponsive to leishmaniasis drugs.

Nowadays, Ethiopia has by far the highest prevalence of HIV/VL worldwide (15–30% of VL cases). Most cases occur in a selective group of male young workers that migrate every year from the Highland territories to the fertile lands in the

Northwest of the country (Humera) in order to harvest sesame and sorghum [6]. This region borders Eritrea and Sudan, both areas with a large presence of deployed soldiers, prostitution and HIV transmission, and also highly endemic for VL [7]. It has been shown that infected migrants disseminate leishmaniasis to non-endemic areas when returning home [8, 9].

In Southern Sudan, the number of HIV/VL co-infected patients rose sharply after the peace agreement was signed in 2005 and large-scale migration took place toward Jonglei and the Upper Nile states, well-known areas of leishmaniasis transmission. In 2008, a prevalence of 25% of co-infection among VL patients was found in a specific area of Southern Sudan [10]. The situation is expected to worsen due to the current VL epidemic in Southern Sudan, with more than 10,000 cases since September 2010 (http://www.who.int/leishmaniasis/Upsurge_kalaazar_Southern_Sudan.pdf) and almost 200,000 refugees that recently returned from North Sudan [11]. An additional 800,000 people are expected to return in the coming year after the outcome of the recent referendum for the independence of Southern Sudan. A great majority of these are expected to settle in the two abovementioned endemic states. A VL outbreak that occurred in the early 1990s claimed 100,000 lives in the same area [12].

In the Indian subcontinent (ISC), harboring 75% of the total burden of VL in the world, the number of co-infections is lower than in Africa, with reported figures of less than 1% of all VL cases, although this is disputed by specific studies that estimate an increase in prevalence not only in India but also in Nepal [13–15]. The reasons underlying this discrepancy may be related to a different pattern of transmission; while for HIV an urban pattern was shown, and confined to the South of India, *Leishmania* transmission is mostly rural and the areas with higher endemism are located in the Northern states (Bihar, Jharkhand, Uttar Pradesh, and West Bengal). Bangladesh and Nepal share this dual epidemiological pattern, and consequently the percentage of co-infection has remained low.

In South America, co-infected cases are only reported in Brazil at a low rate of 1:10,000, again, with two different transmission patterns that maintain the rate of co-infected cases at 2% of the total of infected VL patients [16].

CL-HIV co-infection has spread to a much lower extent than VL-HIV (i.e., 0.1% of the total CL cases [16]).

## 6.2 Clinical Manifestation of HIV/*Leishmania* Co-infection

VL as an opportunistic infection of HIV manifests as an uncontrolled infection with a very high parasite burden. Both HIV and *Leishmania* not only contribute separately to the impairment of the immune response targeting the same cells (macrophages) but also exert a synergistic deleterious effect on the host cells, increasing both virus replication and parasite multiplication [17] and favoring progression of the disease into AIDS [18]. Parasite distribution appears frequently not to be confined exclusively to the typically affected organs in immunocompetent patients but also disseminated into peripheral locations, such as the skin, gut, lungs,

peripheral blood, peritoneal fluid, etc. [19, 20]. This distribution may represent a challenge for current chemotherapy. Furthermore, the abundance of parasites in peripheral blood in these patients may increase the chances for transmission via sand flies [21], therefore contributing to the spread of drug-resistant strains, especially via anthroponotic transmission cycles in *Leishmania (L.) donovani.*

When compared with VL-HIV, clinical impairment of leishmaniasis in CL-HIV is much less severe; nevertheless, in an outbreak of *L. (L.) major* in Burkina Faso reported in 2000, CL-HIV patients showed more polymorphic lesions and required longer treatment [22].

Without an adequate immune response, drugs lose, at least partially, their efficacy against *Leishmania* infection; even those compounds previously considered to be effective regardless of the strength of the immune response such as amphotericin-B (AMB). Co-infected patients relapse repeatedly after each treatment course and finally become unresponsive to all drugs used. Prognosis of VL-HIV is poor, although significantly better in patients (1) with a high $CD4^+$ count, (2) maintained under ART, and (3) having achieved parasitological or clinical cure after an initial episode of VL [23]. A drawback is the increased toxicity of antileishmanial drugs in co-infected patients, which negatively impairs prognosis, especially in case of pentavalent antimonials ($Sb^V$) [2, 24].

## 6.3 Risk for Drug Resistance in Co-infection

Resistance to antileishmanial drugs has only rarely been documented, except for resistance to $Sb^V$, widespread in the ISC due to their prolonged misuse [25, 26]. A detailed description of this situation can be found in Chap. 7. Resistance develops experimentally for all drugs, although in practical terms, miltefosine (MIL) and paromomycin are likely to be more prone to the development of resistant strains than AMB, not only because of their mechanism of interaction with the parasite but also because of the requirement for relatively long treatments, increasing the risk of low compliance [27, 28]. Indeed, after a decade of uncontrolled use of MIL in India and Nepal, the total failure rate for MIL reached up to 22% in a 12-month follow-up [29]. Whether this lack of response is due to resistant strains or not has yet to be determined, but this flags a new concern for the use of MIL which is thoroughly described in Chap. 4. On the other hand, although AMB-resistant strains have been described in vitro [30] and a decreased efficacy has been observed in co-infected patients after several treatment cycles [31, 32], no resistant AMB strains were found in these patients, and there is a nil record of resistant strains in the literature despite its constant use in leishmaniasis for many years. AMB resistance has been described for fungal infections in immune-suppressed patients [33].

In the ISC, combination therapy of two antileishmanial drugs in regimen with reduced dosages and duration was proven effective, and in theory, this is the most promising alternative to thwart the increasing trend of resistance [34]. However, for this strategy to be successful, adherence to therapy should be ensured at the primary healthcare level. This is a difficult task in practical terms during massive control

campaigns fueled by the need for decentralization of the treatment without proper funds to ensure directly observed treatment (DOT). Poor treatment compliance is another problem and may be worse in patients with a low education level. With no guaranteed compliance, the risk of developing resistant strains cannot be ruled out. For this reason and to expand the life span of the few existing medicines against leishmaniasis, it is highly recommended to use, in the ISC, an alternative regimen consisting of one single iv infusion of 10 mg/kg total dose of liposomal amphotericin-B (L-AMB) with a proven efficacy of >96% in India and an ascertained 100% compliance [35].

In co-infected patients, relapses predispose to the selection of resistant infectious strains. In foci where the source of infection consists of *Leishmania*-contaminated syringes, or those with anthroponotic transmission like East Africa and the ISC, there is a major risk for the spread of these resistant strains to other patients. Resistance can in theory easily appear in immune-compromised patients; a decreased susceptibility of parasite isolates to pentavalent antimonials has been demonstrated in a canine leishmaniasis model after only one treatment [36].

## 6.4 New WHO Recommended Treatment Guidelines for the Treatment of *Leishmania*/HIV Co-infected Patients

Considering that there are only few published clinical studies on the efficacy of treatments for HIV/VL co-infection outside the Mediterranean area, the Expert Committee on Leishmaniasis provided the following guidance on patient management [37].

Due to their efficacy, safety, and the absence of resistant strains until now, liposomal AMB formulations (L-AMB) constitute the first choice in the treatment of co-infected patients at a dose of 3–5 mg/kg infusions, daily or intermittently for a 10-dose schedule at days 1–5, 10, 17, 24, 31, and 38, up to a 40 mg/kg total dose. $Sb^V$ are more toxic for co-infected patients than for non-co-infected VL patients and require careful monitoring for pancreatitis and cardiotoxicity. $Sb^V$ should therefore only be used in areas where their efficacy is not yet decreased and liposomal AMB formulations are not available. MIL may be used as an alternative to antimonials as it was shown to be safer than antimonials and reasonably effective in co-infected patients [24].

Secondary prophylaxis has shown to prolong survival by reducing the number and severity of relapses in co-infected patients, especially in those with $CD4^+$ counts lower than 200 cells/μL. It also reduces the possibility of transmission of resistant parasites. In zoonotic VL, *Leishmania* parasites are transmitted by the sand fly, from patients only to dogs, and not to humans, meaning that secondary prophylaxis can be completed with any drug, as there is no risk of spread of resistant strains. Based on the experience collected for zoonotic leishmaniasis in the Mediterranean basin, WHO-recommended prophylaxes include L-AMB (3–5 mg/kg/day) administered once every 3 weeks for 12 months and $Sb^V$ (20 mg $Sb^V$/kg/day every 3–4 weeks) or pentamidine (4 mg/kg/day [300 mg for an adult] every 3–4 weeks).

In anthroponotic foci, where resistant parasites may be transmitted in absence of any animal reservoir within the cycle, it is strongly recommended not to use secondary prophylaxis with medicines used in mainstream therapy regimes for primary attacks [2, 38]. This protocol reduces the options to pentamidine, which is not used anymore for treating primary VL. However, the efficacy of secondary prophylaxis has not yet been ascertained in any anthroponotic foci.

Drug resistance may appear in *Leishmania*/HIV co-infected patients after consecutive relapses despite maintenance therapy with ART and secondary prophylaxis. Combination regimens are not yet studied in co-infected patients. All these data suggest that it is extremely urgent to invest in research into new options for treatment and prophylaxis.

## 6.5 Perspectives in HIV-*Leishmania* Chemotherapy

No doubt, combination of ART with classical leishmanicidal drugs with minimal euthymic character, that is, as independent as possible of the immune status of the host, like liposomal formulations of AMB, is the golden standard for the next medium-range future. An educated guess for the future, taking into account the current status of the chemotherapy pipeline, is that improvement in chemotherapy will likely come from improvement of current leads or from better formulations that will enable drugs to reach the anatomical locations that harbor *Leishmania* amastigotes in HIV patients. Furthermore, independent advances for both therapies will have a real and positive impact on infection when used in combination.

Perusing the literature, an appealing approach seems to be the development of drugs active on both HIV and *Leishmania*, not necessarily addressing the same or homologous target. Their optimization may be problematic in terms of preserving their activity on both microorganisms.

Although scarce, there are several examples and early proofs of concept for this approach. Leishmanicidal activity of specifically designed HIV drugs, like inhibitors of HIV aspartyl proteinase, has been tested, following a chemotherapeutical "piggy-back" approach, and new molecules with antileishmania and antiviral activities have been discovered by high-throughput screening. Examples for these two new trends ensue.

### 6.5.1 Inhibitors of Aspartyl Proteinases

The HIV aspartyl proteinases involved in the maturation of viral proteins are inhibited by specific inhibitors (HIV-PIs) and act in combination with viral reverse transcriptase inhibitors in ART. Their application has led to a tremendous reduction in the severity and incidence of AIDS, including co-infections with *Leishmania* [39–41].

The leishmanicidal effects of HIV-PI's were first reported by Savoia et al [42]. The rationale for their use is the inhibition of some proteasomal activities by HIV-PI, together with the leishmanicidal activity described for other human proteasomal inhibitors [42].

Although incomplete, there is a growing awareness of the activity of HIV-PIs on different *Leishmania* developmental stages, compiled in Table 6.1.

The following conclusions can be inferred from this table:

**Table 6.1** Leishmanicidal activity of HIV-proteinase inhibitor (HIV-PI)

| Ref | HIV-PI[a] | *Leishmania* system and HIV-PI inhibition | | | | Comments |
|---|---|---|---|---|---|---|
| | | Species (strain) | Stage assayed[b] | | | |
| | | | Promastigote | Axenic amastigote | Intracellular amastigote[c] | |
| [42] | IDV | *L. (L.) major* | $IC_{50}$ = 8.3 ± 0.9 μM | | | |
| | SQV | LRC-L137 | $IC_{50}$ = 7.0 ± 0.7 μM | | | |
| | IDV | | 70% at 50 μM | | | |
| | SQV | *L. (L.) infantum* MHOM/TN/80/IPT1 | 67% (50 μM) | | | |
| [44] | NFV | *L. (L.) infantum* | <5% (25 μM) | 77% (25 μM) | 79.9% (25 μM) | Data for MDM[d] amastigote infection |
| | RTV | MHOM/MA/67/ITMAP-263 | <5% (25 μM) | 83% (25 μM) | 43.7% (25 μM) | |
| | SQV | | <5% (25 μM) | 0% (25 μM) | 61.5% (25 μM) | Strain resistant to $Sb^V$ |
| | | *L. (L.) donovani* | | | 92.4% (25 μM) | |
| | NFV | (9518) | <5% (25 μM) | | 52.6% (25 μM) | |
| | RTV | | <5% (25 μM) | | 50.1% (25 μM) | |
| | SQV | | <5% (25 μM) | | | |
| [46] | NFV | *L. (L.) amazonensis* | $IC_{50}$ = 15.1 ± 1.1 μM | | 86% at 50 μM | IND, SQV $IC_{50}$s > 50 μM. |
| | LPV | MHOM/BR/77/LTB0016 | $IC_{50}$ = 16.4 ± 0.8 μM | | 80% at 50 μM | |
| | APV | | $IC_{50}$ = 16.4 ± 0.8 μM | | | |
| [45] | | *L. (L.) donovani* (9518) | | 66% (12.5 μM) | | |
| [43] | NFV | *L. (L.) infantum* | $IC_{50}$ = 14.1 ± 0.2 μM | | 64% (10.5 μM) | |
| | SQV | (MCAN/VE/98/IBO-78) | $IC_{50}$ = 55.1 ± 6.5 μM | | 34% (10 μM) | |
| | NFV | *L. (L.) donovani* | $IC_{50}$ = 14.1 ± 3.9 μM | | | |
| | SQV | MHOM/IN/80/DD | $IC_{50}$ = 51.9 ± 3.4 μM | | | |
| | NFV | *L. (L.) mexicana* | $IC_{50}$ = 9.9 ± 0.5 μM | | 74% (10.5 μM) | |
| | SQV | MHOM/VE/80/NR | $IC_{50}$ = 42.1 ± 7.3 μM | | 43% (10 μM) | |
| | NFV | *L. (L.) amazonensis* | $IC_{50}$ = 13.4 ± 3.0 μM | | | |
| | SQV | IFLA/BR/67/PH8 | $IC_{50}$ = 40 ± 1.2 μM | | | |
| | NFV | *L. (V.) braziliensis* | $IC_{50}$ = 14.6 ± 0.4 μM | | | |
| | SQV | MHOM/BR/75/M2903 | $IC_{50}$ = 36 ± 0.35 μM | | | |
| | NFV | *L. (L.) major* | $IC_{50}$ = 13.4 ± 2.5 μM | | | |
| | SQV | MHOM/SU/73/5-ASKH | $IC_{50}$ = 46.9 ± 1.5 μM | | | |
| | NFV | *L. (L.) pifanoi* | | $IC_{50}$ = 9.9 ± 1.4 μM | | |
| | SQV | MHOM/VE/60Ltrod | | $IC_{50}$ = 15.2 ± 2.7 μM | | |

[a] Abbreviations for HIV-PI: IDV.- Indinavir, LPV.- Loponavir , NFV.- Nefinavir, RTV.- Ritonavir, SQV.- Saquinavir.

[b].-Percentage of inhibition of the expressed parameter at (HIV-PI concentration)

[c].- Expressed as inhibition percentage for macrophage:parasite association index.

[d].- MDM.- monocyte derived macrophage

1. There is a strong variation in leishmanicidal activities depending both on the HIV-PI and the species of *Leishmania* tested [42, 43].
2. When a given HIV-PI was tested in parallel on different *Leishmania* species, the efficacy for those causative of CL was scarcely higher than for those producing VL [42, 43].
3. Within a given *Leishmania* species, variation of HIV-PI among different strains is low [42, 43], including those resistant to $Sb^V$ [44].
4. $IC_{50}$s were higher for *L. (L.) infantum* strains isolated from patients with previous ART therapy [43]; in fact nelfinavir (NFV) resistance is induced by growing the parasites under drug pressure [45].
5. Efficacy of HIV-PIs on macrophages infected with *Leishmania* is maintained regardless of HIV co-infection [43];
6. HIV-PIs kill *Leishmania* at much higher concentrations (micromolar range) than those required for inhibition of viral replication (nanomolar range).

Thus, a real impact of HIV-PIs on the *Leishmania* burden with their current dosing scheme, aside from improvement due to HIV recession, can only be explained if the macrophage may concentrate HIV-PIs up to toxic levels for intracellular parasites. In fact both axenic and intracellular parasites are more susceptible to HIV-PIs than promastigotes [44].

*Leishmanicidal targets for HIV-PIs.* At first sight, the logical mechanism for HIV-PIs is the inhibition of aspartic proteinase activities in *Leishmania*. Using typical substrates and conditions, this activity and its inhibition by NFV have been evidenced in lysates of *L. (L.) mexicana* and *L. (L.) infantum* [43, 46]. Furthermore, characterization of this aspartic proteinase activity was carried out for *L. (L.) mexicana* [47]. Additional targets, perhaps as a consequence of a prior proteinase inhibition, are suggested by (1) inhibition of karyokinesis by NFV in bi- and polynuclear *L. (L.) mexicana* promastigotes [43] and (2) appearance of plasma membrane blebbings and mitochondria swelling assessed on parasites treated with HIV-PIs at their respective $IC_{50}$ [46]. This last observation seems to be related to an apoptosis-like process induced by NFV on *L. (L.) donovani* axenic amastigotes, evidenced by mitochondrial depolarization and release of endonuclease G, together with induction of oxidative stress [45].

The use of HIV-PIs as leishmanicidal agents in the absence of *Leishmania*/HIV co-infection is questionable; first, there is a large gap in active concentrations for anti-HIV and anti-*Leishmania* effects; second, HIV-PIs are not exempt from toxic side effects, especially at HIV-PI concentrations required for leishmanicidal activity setup in vitro, and *Leishmania* resistance can be easily induced [43, 45]. Finally, oxidative stress induced by NFV is mostly precluded by episomal overexpression of the *gsh1* gene [45], encoding for γ-glutamylcysteinyl synthase, the enzyme responsible for the limiting step in the synthesis of glutathione, immediate precursor of trypanothione, the ultimate responsible for thiol redox in the metabolism in *Leishmania*. As such, inhibition of glutathione synthesis reverts $Sb^V$ resistance [48], so possible cross-resistance between antimonials and HIV-PIs may occur; against this pessimistic statement, we must pinpoint that NFV was active on a *L. (L.) donovani*

$Sb^V$-resistant strain [44] and, secondly, discrepancy between mechanisms of $Sb^V$ resistance raised in vitro with those from clinical field isolates is not unusual: inhibition of glutathione biosynthesis did not improve $Sb^V$ susceptibility in field isolates of *L. (V.) panamensis* resistant to $Sb^V$ [49]; in the same trend, in transcriptomics for *L. (L.) donovani* strains resistant to $Sb^V$ in Nepal, mRNA levels for γ-glutamylcysteinyl synthase were decreased [50].

Another important issue is the higher expression of virulence factors in parasites treated with sublethal concentrations of HIV-PI, as leishmaniolysin or cysteine proteinase b reported for *L. (L.) amazonensis* [46].

Altogether, HIV-PIs may have a side-lethal activity on *Leishmania*. Nevertheless, there are several concerns. Apparently, there is a risk of easy induction of resistance, toxic side effects, and induction of virulence factors. Additional studies are needed in order to highlight the clinical relevance of this approach and balance its advantages and disadvantages; furthermore, it will be worthwhile to test novel HIV-PIs for their leishmanicidal activity. In conclusion, an educated guess is that the intrinsic leishmanicidal effect of HIV-PIs in patients is much less relevant than the effect caused by improvement in their immune response caused by the inhibition of HIV proliferation. As such, their usefulness as straightforward new leishmanicidal agents ranks much lower than that of current leishmanicidal drugs in non-HIV co-infected *Leishmania* patients.

## 6.6 High-Throughput Screening for New Anti-HIV and Anti-*Leishmania* Leads

Medium- and high-throughput screening of compounds produced by combinatorial chemistry [51, 52], massive screening of natural products [53–55], or new leads produced by academic groups constitute an important source for promising antileishmanial drugs. The screening of the same series of compounds for anti-pathogenic protozoa and anti-HIV activities nowadays is not infrequent, although the number of groups that specifically focus on a co-treatment philosophy is, in contrast, rather scarce [56].

In many cases for a single drug endowed with both leishmanicidal and antiviral activities, the concentration required for effectiveness on both infections is beyond the threshold of patient cytotoxicity, precluding their use as a single drug for co-therapy; in a series of acrinidone derivatives, 2-(benzothiazol-2-ylamino)-10H-acridin-9-one showed an $IC_{50}$ against *Leishmania* of 3 μM; nevertheless, the anti-HIV activity was higher ($IC_{50} = 27.9$ μM) and quite close to cytotoxic values for mammalian cells [57]. A reduced number of compounds with anti-*Leishmania* and anti-HIV activities have gone upstream in the pipeline and gone past the stage of initial in vitro tests. For example, the group of Figadère in the Université de Paris-Sud has synthesized more than 200 2-substituted quinolines, and some have both anti-*Leishmania* and anti-HIV activities [56, 58]. A major advantage of these compounds is their druggability including possible oral administration. These

compounds have been successfully tested in murine models for CL and VL [59, 60], but not for anti-HIV activity.

Marine products are an endless and mostly untapped source for anti-HIV and anti-*Leishmania* compounds [54, 61–64], and a reduced number are active in both diseases, such as the semisynthetic derivatives of curcuphenol, a sesquiterpene isolated from the sponge *Myrmekioderma styx* [65], which has better leishmanicidal than anti-HIV activity, but both in the micromolar concentration range. Manzamine A and 8–hydroxymanzamine, belonging to the growing family of β-carboline alkaloids, were isolated from sponges from the *Acanthostrongylophora* genus and display remarkable anti-*Leishmania* and anti-HIV activities [66–68].

Very often, the complexity of natural products impairs their chemical synthesis; in such cases, improvement of the antiviral and leishmanicidal activities can be achieved through semisynthetic methods, modifying the natural structure of the compound instead of synthesizing it from scratch. An example of this methodology is illustrated by isoaaptamine, a molecule isolated from sponges of the genus *Hymeniacidon*. Its 9-O-4-ethylbenzoyl derivative showed a sixfold improved anti-*Leishmania* activity compared to the non-acylated natural form while preserving its anti-HIV activity [69].

Anti-HIV and anti-*Leishmania* activities have also been described for marine peptides. Mollamides are cyclic hexapeptides containing a thiazoline group isolated from the tunicate *Didemnum molle* [70]; mollamide B showed a moderate anti-HIV activity, whereas its leishmanicidal effect is threefold higher on a molar basis. Animal antimicrobial peptides and their artificial surrogates may act simultaneously on both pathogens, suggesting their putative future use in co-infections, but this is now only at its very first stage of development.

A caveat for lead optimization is that in many cases, mechanism of actions and targets of anti-HIV and anti-*Leishmania* activity may differ greatly; therefore, it will be unlikely that their optimization will lead to parallel benefits for both targeted microorganisms. An exception will be those modifications not affecting drug-target interaction but the pharmacokinetics or pharmacology of the drug.

## 6.7 Concluding Remarks

*Leishmania* chemotherapy in HIV co-infected patients is much more complex than chemotherapy for uncomplicated *Leishmania* infections alone and relies mostly on the same drugs. The major determining factor on outcome is the reduction of the HIV burden by antiretroviral chemotherapy. Due to the reciprocal detriment effect of both infections on the immune system, the use of parasiticidal and highly effective liposomal AMB appears to be the most reliable treatment for VL/HIVE co-infected patients. There are prospects for a single drug tackling both infections simultaneously, but research in this direction is in a very early stage and hampered by a lack of financial support or capacity to assay the same compound for both anti-HIV and anti-*Leishmania* activity. In order to develop and optimize leads and create

a chemotherapeutic alternative for co-infected patients, a strong research effort will have to be made.

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